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Synthesis of new derivatives of Nortopsentin with potential inhibitory activity against GSK-3β

Dr
MARIA ROSALIA FERRARO

PhD COORDINATOR
PROF. PATRIZIA DIANA

SUPERVISORS
PROF. GIROLAMO CIRRINCIONE
DOTT.SSA BARBARA PARRINO

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INTRODUCTION

Glycogen synthase kinase-3 (GSK-3) has became one of the most attractive therapeutic targets for the treatment of diabetes,[1-4] inflammation,[5,6] multiple neurological diseases including Alzheimer,[7-10] amyotrophic lateral sclerosis (ALS),[11] Parkinson,[12,13] stroke,[14,15] bipolar disorders[16-18] and it is known as an important component of different signaling pathways involved in the regulation of cell fate, protein synthesis, cell mobility, proliferation and survival.[19-23]

GSK-3 is a multifunctional serine / threonine (Ser / Thr) kinase, originally found in mammals, and homologues have been found in all eukaryotes. There are two mammalian GSK-3 isoforms, encoded by distinct genes: GSK-3α (51 kDa) and GSK-3β (46 kDa). The two isoforms are highly conserved within their kinase domain but greatly differ at the C-terminal domain (only 36% of identity), while the α isoform additionally contains an extension N-terminal glycine-rich. Despite the high degree of similarity (they share 85% overall sequence homology, including 98% amino acid sequence identity within their kinase domains),[24] the two isoforms of GSK-3 are not functionally identical and they have different substrate specificities in the brain and as well as in other tissues.[25] Several studies show that GSK-3β knock-out mice die around embryonic day 16 due to liver degeneration caused by hepatocyte apoptosis.[26] Furthermore, GSK-3β activity was essential for NF-kappaB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation induced by TNF-α (tumor necrosis factor alpha) in hepatocytes. In contrast, GSK-3α knock-out mice are viable but exhibited enhanced glucose and insulin sensitivity and reduced fat mass. GSK-3α knock-out mice elicited metabolic and neuronal developmental abnormalities,[27,28] also demonstrating the involvement of GSK-3α in age-related diseases and in promotion the production of β-amyloid peptide and formation of senile plaques in Alzheimer's disease models.[29]

Thus, GSK-3 isoforms exhibit tissue-specific physiologically important functions which may not be overlapping and may be different. Most biochemical studies have focused on GSK-3β demonstrating its role in differentiation, cell growth, cell cycle progression and apoptosis. It is known, in fact, that the dysregulation of GSK-3β has been implicated in tumorigenesis and cancer progression.[30,31]

GSK-3β is composed of 12 exons in humans and 11 exons in mice with the ATG start codon located within exon 1 and the TAG stop codon found in the terminal exon. The gene product is a protein of 46 kDa consisting of 433 amino acids in the humans and 420 amino acids in the mice. GSK-3β is similar to other Ser / Thr kinases and consists of three distinct structural domains. The N-terminal domain is comprised of the first 134 amino acid residues and it forms a 7-strand β-barrel motif. A small linker region consists of the amino acid residues 135-151 and it connects the N-terminal domain to the central α-helical domain, which in turn is composed of the amino acid residues 152-342. The ATP binding site lies at the interface of the N-terminal and the central α-helical domains. The C-terminal domain consists of amino acid residues 343-433 and it is outside of the classical Ser / Thr kinase core fold; these amino acid residues form a helix-loop domain that interacts with the core α-helical domain. The p53 association region and the basic domain region are both located in the N-terminal domain (Figure 1).[32]

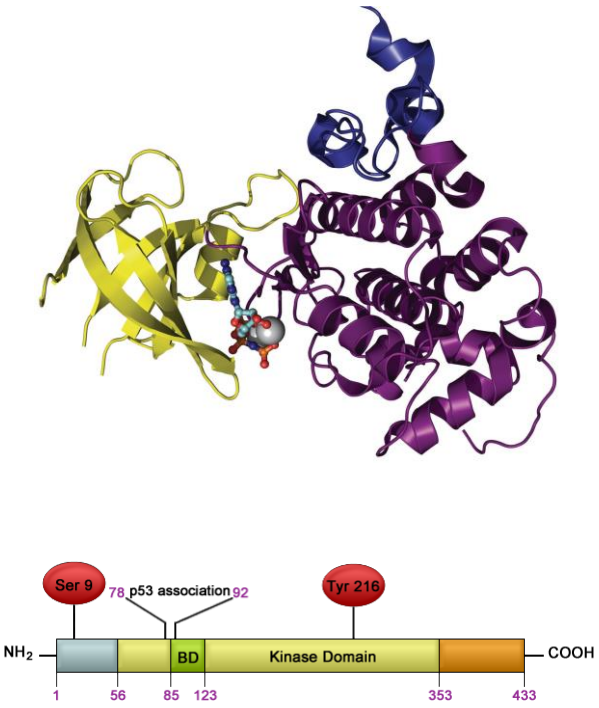


Figure 1. Structure of glycogen synthase kinase-3β.

A minor (~15% of total) splice variant of GSK-3β, termed GSK-3β2, has recently been identified, which contains a 13 amino acids insert within the kinase domain. An antibody selective for the novel splice insertion polypeptide revealed that GSK-3β2 is localized primarily to neuronal cell bodies, unlike unspliced GSK-3β that is also found in neuronal processes. Given the location of the insert within a highly conserved sequence, the splice-encoded peptide likely forms a loop / hook, it may allow differential binding of the splice variant to scaffolding proteins exposing the isoform to a distinct subset of target proteins.[33,34] However, GSK-3β1 and GSK-3β2 have regulation mechanisms and ATP binding properties similar, being both phosphorylated to a similar extent at the regulatory sites, serine 9 (Ser 9) and tyrosine 216 (Tyr 216), and showing identical sensitivities to the ATP-competitive inhibitors.[35]

GSK-3β has traditionally been considered as a predominantly cytosolic protein, however, it was recently shown that smaller but much more active pools of GSK-3β are present in the nucleus and mitochondria,[36] as well as in other subcellular compartments, which its levels and / or activation state can be regulated by localized signaling activities. Unlike most protein kinases, GSK-3β is constitutively active in resting cells and undergoes a rapid and transient inhibition in response to a number of external signals. GSK-3β activity is regulated by site-specific phosphorylation: full activity of GSK-3β generally requires phosphorylation at Tyr 216 residue, located in the kinase domain, and conversely, phosphorylation at amino-terminal Ser 9 residue leads to its inactivation through proteosomal degradation. GSK-3β is subjected to multiple regulatory mechanisms and phosphorylation of Ser 9 is probably the most important. Several kinases are capable of mediating this modification, including p70 S6 kinase (p70S6K),[37] extracellular signal-regulated kinases (ERKs), p90Rsk (also called MAPKAP kinase-1), protein kinase B (also called Akt), certain isoforms of protein kinase C (PKC),[38] and cyclic AMP-dependent protein kinase (protein kinase A).[39,40]·Insulin treatment inactivates GSK-3β, an effect that is associated with Ser 9 phosphorylation, and Akt was identified as the major kinase mediating this response to insulin.[41] Akt is activated by a signaling cascade involving phosphatidylinositol-3-OH kinase (PI3K), 3-phosphoinositide-dependent kinase-1, and likely other kinases as well, notably integrin-linked kinase that may also directly phosphorylate GSK-3β. Stimulation of certain receptors, such as the insulin receptor (insulin-like growth factor 1 receptor, IGF-IR), activates this signaling cascade leading to activation of Akt, which in turn, it phosphorylates Ser 9 of GSK-3β with inhibition of its activity. Some studies report that potential lethal stressors can cause failure of Akt activation with an increase in the active form of GSK-3β[11] which plays positive roles in cell proliferation and its aberrant expression promotes various tumor types including melanoma, glioblastoma, and lung, breast, ovarian, prostate, colon, liver, stomach and pancreatic tumors.[42-45]

Moreover, GSK-3β activity is often shut off after exposure to mitogenic / growth factors, in fact, epidermal growth factor (EGF), platelet derived growth factor (PDGF) and other growth factors also induce the phosphorylation of GSK-3β (Ser 9) through the PI3K pathway as well as through induction of the Raf-MEK-ERK-p90Rsk1 signaling pathway.[46]

Elucidation of the crystal structure of GSK-3 has helped to reveal how the phosphorylation of GSK-3β at Ser 9 results in its inhibition. When Ser 9 of GSK-3β is phosphorylated, a primed pseudosubstrate is created which folds and binds the positively-charged pocket of the kinase, formed by basic residues Arg 96 (R96), Arg 180 (R180) and Lys 205 (K205). This folding prevents the phosphorylation of substrates as the catalytic groove is blocked. This is a competitive mechanism of inhibition and if elevated concentrations of the primed substrates are present, they will out-compete the pseudosubstrate and the substrates will be phosphorylated.[47] Arg 96 of GSK-3β is a critical component of the positive pocket which binds primed substrates. Mutation of Arg 96 (R) to Ala 96 (A) (R96A) has consequences on the ability of GSK-3β to phosphorylate primed versus unprimed substrates. Priming of substrates usually occur N+4 from the site where GSK-3β phosphorylates the substrate. The R96A mutation disrupts the GSK-3β pocket of positive charge and prevents the phosphorylation of the primed substrates, that can not longer bind, resulting in the maintenance of enzyme activity and the phosphorylation of unprimed substrates even when GSK-3β is phosphorylated at Ser 9.[25,48]

In opposition to the inhibitory modulation of GSK-3β that occurs by serine phosphorylation, tyrosine phosphorylation of GSK-3β increases the enzymatic activity by providing access to the catalytic site of the protein.[49] Stimulation of GSK-3β (Tyr 216) is reported to be mediated by GSK-3β itself (intramolecular autophosphorylation leads to the stabilization of the enzyme),[50,51] by proline-rich tyrosine kinase 2 (PYK2),[52] a calcium-dependent tyrosine kinase, and by Fyn, a member of the Src tyrosine family that regulates important processes of growth and cell motility.[53]

PYK2 is a member of the focal adhesion kinase (FAK) family and it is most abundant in the central nervous system and hematopoietic cells; it is activated by intracellular calcium increases such as those induced by G-protein receptor activation, membrane depolarization, stress stimuli, and growth factors and it has been implicated in intracellular signaling events, in synaptic plasticity, neurite outgrowth and apoptosis.[52] PYK2 controls lysophosphatidic acid-induced activation of GSK-3β, which regulates the phosphorylation of microtubule-associated proteins with consequent regulation of the neuronal architecture. PYK2-mediated activation of GSK-3β is initiated by phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis and may serve to destabilize microtubules during actomyosin-driven neurite retraction, demonstrating the role of this kinase in neuronal development.[54]

In addition, PYK2 stabilizes the β-catenin by phosphorylation of GSK-3β (Tyr 216), and the dysregulation of expression of FAK / PYK2 causes increased activity of kinase with promotion of Wnt / β-catenin pathway, accumulation of β-catenin and intestinal tumorigenesis.[55]

The Src family kinases (SFKs), which include Fyn, cSrc, Yes and Lyn, are also required for the phosphorylation of the Tyr 216 residue of GSK-3β and their dysregulation leads to increased kinase activity, found in highly aggressive prostate cancer cells. It has been demonstrated that the treatment with GSK-3β inhibitors decreases cell survival, proliferation, migration, invasion and micrometastasis of prostate cancer cells as well as of many other types of cancer, in which GSK-3β acts as a tumor promoter.[53] GSK-3β is also subjected to the regulation of mitogen-activated protein kinase like MEK 1/2, and to the regulation of different apoptotic stimuli (heat shock proteins and staurosporine). They induce Tyr 216 phosphorylation on GSK-3β with a dynamic increase of its nuclear levels, favoring in this manner the interaction with its nuclear substrates.[43]

The activity of GSK-3β may also be regulated by phosphorylation of other amino acid residues that are part of the protein, for example, p38 mitogen-activated protein kinase (p38MAPK) phosphorylates the Thr 390 residue of the C-terminal domain reducing its activity as well as contributing to the canonical Wnt signaling and to the regulation of kinase substrates, such as β-catenin. This is of particular interest because the Thr 390 amino acid residue is not conserved in GSK-3α and thus it provides a potential GSK-3β isoform specific regulation.[56,57] In addition, ERK phosphorylates the residue Thr 43 of GSK-3β, resulting in its subsequent phosphorylation at Ser 9 by p90Rsk and in its inhibition.[58] Finally, protein phosphatases (eg, PP2A, PP1) have important roles in regulation of GSK-3β activity by removing the phosphate on Ser 9 as well as other regulatory residues.[59]

The majority of GSK-3β substrates exhibit an absolute requirement for prior phosphorylation by another kinase at a "priming" residue, located at C-terminal level differing from the site of subsequent phosphorylation by GSK-3β. GSK-3β-catalyzed phosphorylation of these substrates occurs at the fourth serine or threonine residue N-terminal to the primed site (pS/T_1-XXX-pS/T_2), where the first pS/T_1 (Ser or Thr) is the target residue, X is any amino acid (but often Pro), and the last pS/T_2 is the site for priming phosphorylation. Thus, the primed Ser / Thr is recognized by the positively charged "binding pocket" on GSK-3β, formed by basic residues Arg 96 (R96), Arg 180 (R180), and Lys 205 (K205), which facilitates the correct orientation of the substrate within the active site of the kinase (Figure 2).[23]

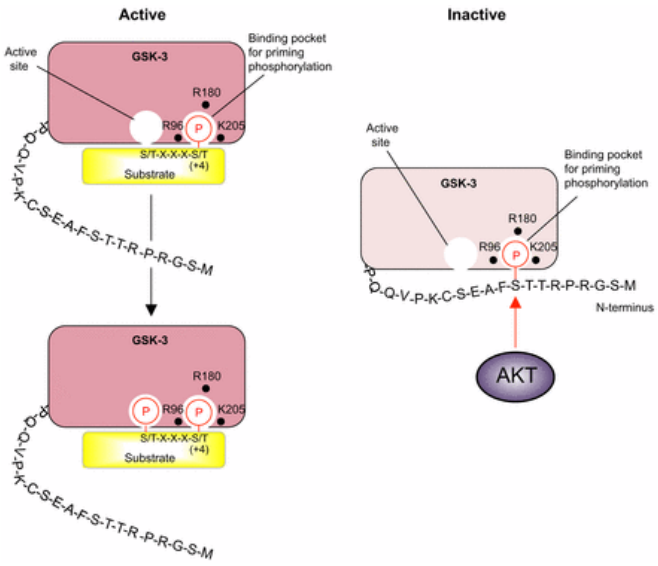


Figure 2. Regulation of GSK-3β.

Several protein kinases have been shown to act as priming enzymes for GSK-3β phosphorylation, including cyclin-dependent kinase 5 (CDK-5), protease-activated receptor 1 **(**PAR-1), casein kinase-1 (CK1), casein kinase-2 (CK2), protein kinase C (PKC), 5' adenosine monophosphate-activated protein kinase (AMPK). In the case of several substrates, the residue phosphorylated by GSK-3β acts to prime an additional Ser / Thr residue N-terminal to it. This can lead to a zippering effect where multiple residues become phosphorylated by GSK-3β. However, not all substrates require prior phosphorylation for recognition by GSK-3β, including members of the myc family of transcription factors. This category of substrates can contain acidic residues or peptide conformations that may be recognized by GSK-3β, thus substituting for the effect of the priming phosphate.[46]

More than forty proteins are substrates of GSK-3β, and these proteins have roles in a wide spectrum of cellular processes, including glycogen metabolism, transcription, translation, cytoskeletal regulation, cell differentiation, proliferation and apoptosis.

GSK-3β plays an important role in the degradation of the cyclin D1 through phosphorylation of the Thr 286 residue and in the degradation of the cyclin E through phosphorylation of the Thr 380 residue, thus promoting cell cycle progression. Cyclin D1 is synthesized early in G_1 phase and it plays a key role in the initiation and progression of this phase, as well as, in the G_1 / S transition of the cell cycle.[60] During G_1 phase, cells need to make a decision whether advancing toward another division or withdrawing from the cell cycle into the quiescence phase (G_0) in response to extracellular signals. The point at which this decision is made is called a restriction point. Cyclin D1 acts as a mitogenic signal sensor and forces cells to enter the proliferative cycle from G_0 phase.

Cyclin D1 forms a complex with the cyclin-dependent kinases (CDKs) 4 and 6 and functions as a regulatory subunit of them. The cyclin D1 / CDK4-6 complexes are phosphorylated by CDK-activating kinase (CAK) leading to their activation and translocation to the nucleus. The main role of the cyclin D1 / CDK4-6 complexes in cell cycle control is the phosphorylation of retinoblastoma protein (Rb) mediation. Rb serves as a gatekeeper of the G_1 phase, since Rb inhibits cell cycle progression through its ability to repress E2F transcriptional activity. Partially phosphorylated Rb still binds to E2F, but the transcription of some genes, such as cyclin E, induces its activation. Cyclin E binds to and activates CDK2, resulting in the phosphorylation and release of Rb from E2F, thereby E2F induces the expression of target genes whose functions are important for S phase entry and passage through the restriction point leads to DNA synthesis. When cells enter S phase, cyclin D1 is rapidly degraded by ubiquitin-proteasome-system and this proteolysis is triggered by phosphorylation made by GSK-3β, that leads to association of cyclin D1 with CRM1 (Chromosomal Maintenance 1, also known as Exportin 1), a nuclear protein that mediates the export of proteins from nucleus to cytoplasm to initiate their proteasomal degradation.[61]

In addition to phosphorylate cyclin D1 (Thr 286), GSK-3β collaborates with CDK2 to control the levels of cyclin E, because the regulation of cell cycle requires stringent controls on the amount and timing of cyclin E - CDK2 activity and hence on the amount and timing of cyclin E expression that it attains a maximum level in late G_1 phase and a minimum in M and early G_1 phases. GSK-3β and CDK2 kinases participate in cyclin E phosphorylation on Thr 380 and Ser 384, respectively, resulting in its nuclear export and degradation, so promoting the entry of the cell into S phase (Figure 3).[61,62] Thus, phosphorylation and proteolytic turnover of D1 and E cyclins and their subcellular localization during the cell division cycle are linked to the activity of GSK-3β,[63] whose aberrant expression with abnormalities in the regulation is involved in the development of cancer.[61,64] In particular, GSK-3β has been implicated to play roles in cancers which are resistant to chemo-, radio-, and targeted therapy.[65] Thus, targeting GSK-3β may be a means to overcome the resistance of these cancers to certain chemotherapeutic drugs, radiation and small molecule inhibitors.[30,31,66]

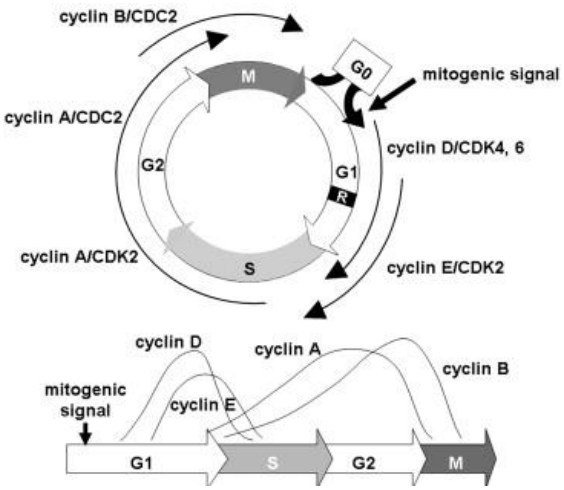


Figure 3. Schematic representation of the mammalian cell cycle and its regulatory molecules. The cell cycle progresses through four sequential phases, gap 1 (G_1), synthesis (S), gap 2 (G_2) and mitosis (M) phases. Passage through the cell cycle is controlled by cyclin / cyclin-dependent kinase (CDK) complexes and the timely activation of CDKs by the binding of partner cyclins is required. Cyclins are the regulatory subunits of CDKs, and each cyclin exhibits a characteristic pattern of expression and degradation. In lower panel, protein levels of cyclins throughout the progression of the cell cycle are shown. CDK, cyclin-dependent kinase; R, restriction point.

In addition to the activity carried out on D1 and E cyclins, GSK-3β can also phosphorylate cyclin-dependent kinase inhibitor p21 (Thr 57), resulting its proteasomal degradation.[67] p21 is a cell cycle regulatory protein that mediates various biological activities primarily by binding to and inhibiting the kinase activity of CDK2 and CDK1, leading to growth arrest at specific stages in the cell cycle. Furthermore, by binding to Proliferating Cell Nuclear Antigen (PCNA), p21 interferes with PCNA-dependent DNA polymerase activity, thereby inhibiting DNA replication and modulating various PCNA-dependent DNA. Its expression is tightly controlled by the tumor suppressor protein p53, through which p21 mediates the p53-dependent cell cycle G_1 phase arrest and might also promote apoptosis in response to a variety of stress stimuli.[68,69]

GSK-3β has important regulatory roles on other transcription factors involved in cell cycle regulation, and the inhibition of this kinase causes cell cycle arrest at G_1 phase with subsequent activation of cyclin-dependent kinase inhibitor p27,[70] a protein that prevents the activation of cyclin E - kinase 2 complex and regulates the passage of the G_1 / S restriction point.[71]

Moreover, GSK-3β through cooperation with mTOR (mammalian target of rapamycin) positively regulates the activity of p70 S6 Kinase (p70S6K), a Ser / Thr kinase involved in cell survival, growth and proliferation by regulating protein synthesis, cell metabolism, glucose homeostasis, insulin sensitivity, cell cycle, and gene transcription.[72]

GSK-3β, together with the yeast protein Mck1, inhibits the activity of the major mitotic cyclin - kinase complex cyclin B - CDK 1, that accumulates before of the anaphase and once inhibited in telophase, cells can exit mitosis and enter into the next cell cycle.[73] GSK-3β is also involved in the regulation of Activator protein 1 (AP-1),[74] a heterodimeric transcription factor complex composed by a Jun family member and a FOS family member that it binds to the TRE DNA sequence (5′-TGAGTCA-3′) and regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, bacterial and viral infections and it also controls a number of cellular processes including differentiation, proliferation, and apoptosis.[75]

Moreover, GSK-3β has the ability to regulate c-Myc activity through phosphorylation at Thr 58 residue, thereby regulating c-Myc rapid proteolysis by the ubiquitin pathway. The c-Myc protein is a basic / helix-loop-helix / leucine-zipper (bHLHZip) transcription factor that, together with its dimerization partner Max (also known as Myc-associated factor X), binds specific E-box sequences and exerts its oncogenic effects through regulation of genes involved with growth and proliferation cell. Thr 58 is a major phosphorylation site that strongly impacts on c-Myc functions and it is a mutational hotspot in Burkitt's and other aggressive human lymphomas, indicating that Thr 58 phosphorylation regulates the oncogenic potential of c-Myc. In addition to the effect on c-Myc proteolysis, phosphorylation induced by GSK-3β at Thr 58 alters the subnuclear localization of c-Myc, enhancing its localization to discrete nuclear bodies. Thus, c-Myc functions are strictly dependent by GSK-3β activity[76] and a failure of the GSK-3β regulation results in a dysregulation of the c-Myc, frequently overexpressed in breast cancer,[77] as well as in a variety of human cancers.[78,79]

GSK-3β positively regulates NFκB-mediated gene transcription and cell survival.[80] In a resting state, NFκB (p65 / p50) heterodimer is generally inactive and forms a complex with its inhibitory proteins IkBα (inhibitor kappa B-cells alpha) in the cytoplasm. GSK-3β regulates the activity of IkB kinase (IKK) and the activation of the latter leads to phosphorylation of IkBα, resulting in its proteasomal degradation, liberation and translocation of NF-kB into nucleus where it regulates gene expression involved in cell survival.[81] This explains the use of GSK-3β inhibitors in colon and pancreatic cancers, where it has been observed an uncontrolled activity by NF-kappa B,[82] whose role has been documented in the development of different solid malignancies and, considering its important role in immune cell function, hyperactive NF-κB is also frequently associated with the development of leukemia and lymphoma.[83] GSK-3β also regulates the activity of Snail, the transcription factor which represses E-cadherin transcription, a structural protein involved in the maintenance of the mechanical tissue balance. Phosphorylation of Snail made by GSK-3β leads to its cytoplasmic translocation with E-cadherin increased expression, generally found in oral

cancer.[84] In addition, stabilization of Snail protein, by increased GSK-3β phosphorylation, has also been observed during the epithelial-mesenchymal transition (EMT) in different types of solid tumors.[85,86]

GSK-3β positively modulates Notch receptor signaling, through direct phosphorylation of its intracellular domain (NICD), protecting the latter from proteasome degradation.[87] Notch Intracellular Domain (NICD) not degraded, is activated and it translocates into the nucleus, thereby regulating the expression of genes involved in cell proliferation, differentiation and survival. Dysregulation of the Notch signaling pathway, due to an over-expression of GSK-3β, was found in various diseases, such as T-cell leukemia, breast, prostate, colorectal and lung cancers as well as central nervous system (CNS) malignancies.[88]

Even if GSK-3β is a well-known downstream target of IGF-IR, throught PI3K-Akt pathway, as previously said, the same GSK-3β stimulates IGF-IR expression. This effect is due to the fact that GSK-3β activates the transactivation activity of O subfamily of forkhead / winged helix transcription factors (FOXO) that, in turn, directly bind to the IGF-IR promoter.[89] Once activated, phosphorylated IGF-1R recruits and activates signaling adaptor proteins, including IRS-1, IRS-2, and Shc. The first activates PI3K-Akt pathway, silencing GSK-3β and increasing anti-apoptotic effects; the last activates Ras-MAPK pathway, promoting gene expression and cell growth, events favored by GSK-3β. There is therefore a regulatory balance between GSK-3β and IGF-1R and the lack of this regulation causes tumorogenesis and tumoral progression. As a matter of fact, it has been demonstrated that IGF-1R is highly expressed in a wide variety of human cancers, and GSK-3β knockdown or GSK-3β inhibitors lead to a decrease in cellular proliferation and suppress IGF-IR-induced cell growth,[89] also bypassing in some cases the problem of the resistance to classic anticancer therapy.[90] GSK-3β stabilizes the expression of certain anti-apoptotic B-cell lymphoma-2 (Bcl-2) family members, in fact, it regulates BCL2L12 anti-apoptotic signaling which is expressed at high levels in glioblastoma.[91]

Glioblastoma is the most frequent malignant brain tumor that usually does not respond to currently available cancer treatments. The current standard therapy for newly diagnosed glioblastoma is the surgical resection of the tumor, followed by chemotherapy and radiation. However, it has been shown that pre-treatment with low doses of GSK-3β inhibitors potentiates the cytocidal effect of ionizing radiation in glioblastoma cells and at the same time protects normal hippocampal neurons from apoptosis induced by radiation. Therefore, inhibition of GSK-3β provides a double benefit for patients with glioblastoma treated with radiation, attenuating tumor proliferation and protecting brain tissue from decay and allowing its repair.[92]

Several data suggest that at the base of radioprotection mechanisms by GSK-3β inhibitors, there is the cytoplasmic MDM2 (Mouse double minute 2 homolog, also known as E3 ubiquitin-protein ligase Mdm2) accumulation, followed by MDM2-dependent p53 degradation, thus resulting in the regulation of p53 functions and p53-dependent cellular responses, with subsequently decrease of radiation-dependent apoptosis.[93] Moreover, GSK-3β binds directly to tumor suppressor protein, p53, a transcription factor that is activated by a wide variety of cellular stresses, notably including conditions that cause DNA damage. Important for GSK-3β-p53 binding appear to be two domains of p53, the basic domain (BD) and activation domain 1 (AD1), that have regulatory influences on its association with GSK-3β at the N-terminal domain. The activity of p53 is regulated both at the nuclear and mitochondrial levels by GSK-3β and inhibition of this kinase stabilizes its association with p53, thus contributing to the transcriptional and apoptotic actions of p53 and suggesting that mitochondrial GSK-3β is involved in the mitochondrial apoptotic signaling pathway.[94]

Further, indirectly stimulating the transcription of Bcl-2-associated X protein (Bax), a key component of the intrinsic apoptotic cascade through the regulation of the p53 activity, GSK-3β can directly phosphorylate Bax on Ser 163, thus modulating Bax expression and function at the transcriptional and post-translational levels, respectively, to promote mitochondrial apoptosis.[95] GSK-3β also regulates the expression of the myeloblastosis transcription factor (c-Myb), which can form a complex with the lymphoid enhancer-binding factor 1 (LEF-1) transcription factor and binds the promoter regions of BCL2 and BIRC5 (survivin) genes which lead to the prevention of apoptosis. Therefore, GSK-3β inactivation reduces the expression of c-Myb by promoting its ubiquitination-mediated degradation, thereby inhibiting the expression of c-Myb-dependent anti-apoptotic genes Bcl2 and survivin, with increase the levels of caspase-3 and promotion of apoptosis.[96]

Another target of GSK-3β is the pro-apoptotic protein Bcl-2-like protein 11, commonly called Bim. Inhibition of GSK-3β activity, in this case, promotes an apoptotic response specifically in pancreatic cancer cells, induces an increase of Bim expression, caspase-3 and -7 activation as well as poly adenosine diphosphate ribose polymerase (PARP) cleavage through activation of the cascade c-Jun N-terminal kinase (JNK)-cJun, thus promoting the death ligand-induced apoptotic response.[21]

GSK-3β activity on pro- and anti- apoptotic proteins leads to investigate how this target acts on regulation of apoptotic mechanisms. GSK-3β has paradoxical effects on apoptosis, as it promotes cell death caused by the intrinsic apoptotic pathway, but inhibits the death receptor-mediated extrinsic apoptotic pathway.[43]

Both apoptotic patways culminate in the activation of a family of intracellular cysteine proteases called caspase, that are classified as initiator caspases (caspases-8, -9, and -10) and effector caspases (caspases -3, -6, and -7), which can disrupt entire cells within a few minutes of their activation, leading to collapse and to cell death (Figure 4).[97]

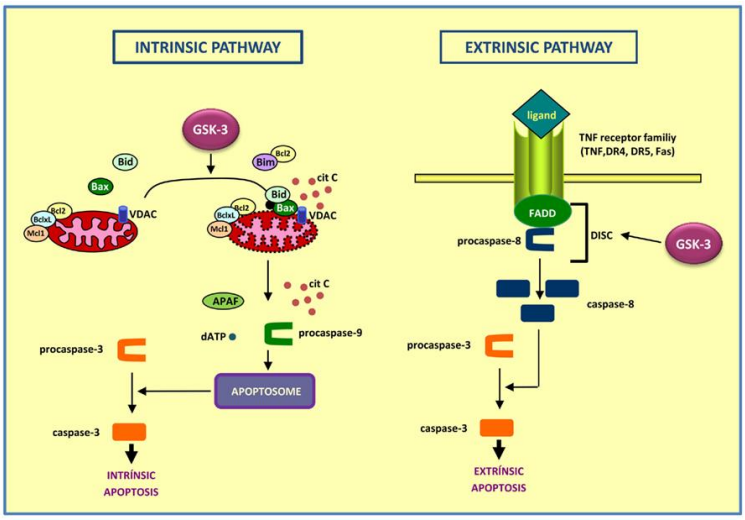


Figure 4. Intrinsic and Extrinsic Apoptocic Signaling Pathways and GSK-3β Activity.

The intrinsic apoptotic signaling pathway causes the disruption of mitochondria, leading to cell destruction and can be induced by numerous stimuli that cause cell damage, such as DNA damage, oxidative stress and endoplasmic reticulum (ER) stress. GSK-3β promotes this cascade by facilitating signals that cause disruption of mitochondria and by regulating transcription factors that control the expression of proteins involved in apoptotic signaling.[43]

The extrinsic apoptotic pathway, conversely, is mediated by the activation of cell-surface death domain-containing receptors (DRs) and initiates apoptosis by activating caspase-8. DRs belong to the tumor necrosis factor (TNF) family of receptors that contain conserved intracellular death domains that are critical for the initiation of extrinsic apoptotic signaling. Among the most well-known death receptors are TNF receptor 1 (TNF-R1), Fas (also called CD95 or Apol), death receptor 4 (DR4) and DR5. The activated death receptors bind to the cytoplasmic proteins FADD (Fas-associated death domain protein) and procaspase-8 (or procaspase-10) to form a complex known as the death-inducing signaling complex (DISC). DISC formation can allow autoactivation of caspase-8, which then leads to the activation of effector caspases and thereby triggering entrinsic apoptosis (Figure 4).[97]

GSK-3β inhibits the death receptor-mediated extrinsic apoptotis pathway by preventing activation of the initiator caspase-8, and this upstream anti-apoptotic site of GSK-3β action thwarts its pro-apoptotic actions, that are targeted downstream at the level of the mitochondria by blocking the signal from reaching that stage of the apoptosis cascade.[43] The caspase-8, generally, is not involved in intrinsic apoptotic signaling, so its inhibition by GSK-3β does not influence the intrinsic apoptotic cascade. Thus there is a clear segregation of the actions of GSK-3β on these two apoptotic signaling cascades, which allows GSK-3β to effectively regulate each pathway individually and in opposite directions. Intrinsic and extrinsic apoptotic signaling are normal mechanisms used to rid organisms of unwanted cells, the former in response to cell damage and the latter in response to external signals. Aberrant apoptosis can occur in association with a wide variety of diseases, especially well-documented for abnormally high apoptosis in neurodegenerative diseases and deficient apoptosis in cancer. Thus, since GSK-3β is an important component of both apoptotic pathway, GSK-3β-regulating drugs may provide a powerful approach to attenuate apoptosis in neurodegenerative diseases or to eliminate proliferative cancer cells. GSK-3β inhibitors may be useful in cancer therapies, also in conjunction with agents that activate death receptors, since the latter are preferentially expressed in cancer cells, so this combination may prove to be particularly efficient for killing tumor cells.[23,43,49,97]

GSK-3β is also an important regulator of the Wnt / β-catenin signaling pathway, since it regulates the levels of β-catenin, a key molecule involved in cell normal development as well as cancer progression. Different pools of β-catenin exist at various subcellular locations, for example one pool of β-catenin is associated with cadherins at the cell-cell junctions while another pool is free and localized in the cytosol and nucleus where it plays important roles in regulating gene expression. Normally, this pool of free β-catenin is maintained at a low level through the rapid turnover by GSK-3β. The Wnts are a family of secreted, cysteine-rich and glycosylated protein ligands that signal by activating the Frizzled family of membrane-bound receptors. Wnt signal transduction causes nuclear translocation of β-catenin and ultimately results in the activation of genes regulated by the T-cell factor (TCF) / lymphoid enhancer factor (LEF) family of transcription factors. In the absence of Wnt signals, free cytoplasmic β-catenin is incorporated into a cytoplasmic complex that includes adenomatous polyposis coli (APC), axin / conductin, casein kinase-1α (CK1α) and GSK-3β. These kinases phosphorylate specific amino terminal residues of the β-catenin (Ser 45 phosphorylated by CK1α that primes β-catenin for subsequent phosphorylation by GSK-3β at Ser 33, Ser 37, Thr 41), causing its ubiquitination and subsequent degradation by the proteasome.

In the presence of Wnt ligand, it interacts with the transmembrane receptor Frizzled (Fz) and its co-receptor LRP5 or LRP6 (low-density lipoprotein receptor-related protein 5 or 6) and the formation of Wnt-Fz-LRP5 / 6 complex along with the recruitment of scaffolding proteins Dishevelled (Dvl) results in phosphorylation of LRP5 / 6 by the GSK-3β; axin binds to the cytoplasmic portion of LRP5 / 6 leading to inhibition of the multiprotein complex which it is part of and preventing the phosphorylation of β-catenin by GSK-3β (GSK-3β-binding protein (GBP), also known as, frequently rearranged in advanced T-cell lymphomas (FRAT), may regulate the binding of GSK-3β to axin), in turn also inactivated through Wnt / Dvl signal transduction. This leads to the stabilization of β-catenin that not being degraded it translocates to the nucleus and interacts with many transcription factors including those of family TCF / LEF, leading to activate the responsive genes of the Wnt pathway (Figure 5).[98,99]

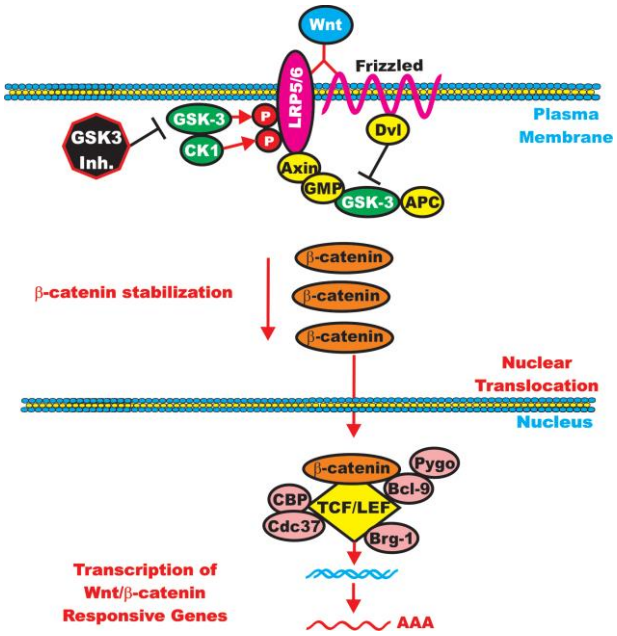


Figure 5. Wnt / β-catenin signaling pathway.

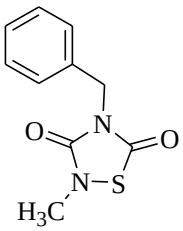
GSK-3β has two roles in the regulation of Wnt / β-catenin signaling. GSK-3β, on the one hand, phosphorylates β-catenin leading to its degradation, and on the other hand, phosphorylating the LRP5 / 6 co-receptor, it leads to the stabilization and activation of β-catenin with growth mechanisms and resistance to apoptosis by activating c-myc and cyclin D1 genes. Since GSK-3β is a kinase with a specific recognition motif present in substrate proteins that it phosphorylates, mutations can occur in certain genes which prevent GSK-3β-mediated phosphorylation of the proteins they encode. The mutant proteins may have altered half-lifes as compared to the WT proteins as they may not be subject to proteasomal degradation.

Genetic mutations changing the GSK-3β phosphorylation sites Ser 33, Ser 37, Ser 45 and Thr 41 present in β-catenin prevent the ability of GSK-3β to phosphorylate β-catenin and inhibit its proteasomal degradation. Likewise mutations at Ser 45 of β-catenin prevent CK1α from performing the priming phosphorylation, thus GSK-3β is unable to phosphorylate the other residues of β-catenin. Thus mutations at GSK-3β phosphorylation sites and its dysregulation lead to the promotion of tumor processes.[99] This explains the use of GSK-3β inhibitors in regulating the levels of β-catenin, since an increase of the same is often seen in basal cell carcinoma, in colorectal cancer and in leukemia.[100-102]

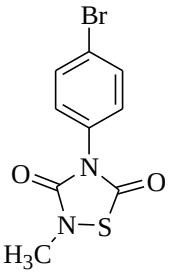
Many efforts have been made in the discovery and development of GSK-3β inhibitors in the last years as it is a very active field of research for academic centres and pharmaceutical companies. Some GSK-3β inhibitors have synthetic origin, others derive directly or indirectly from small molecules of natural origin. However, pharmacological inhibitors of GSK-3β can be classified into following three categories[103]:

- Non-ATP-competitive inhibitors
- ATP-competitive inhibitors
- Substrate competitive GSK-3β inhibitors

Small heterocyclic thiadiazolidinones (TDZD) were the first family of compounds reported as non-ATP-competitive GSK-3β inhibitors, including compound **1** and **2** with IC_{50} = 2.0 μM and 1.1 μM, respectively. TDZD also showed activity against various kinases including PKA, PKC, CK-2, and CDK1 / cyclin B.[103]

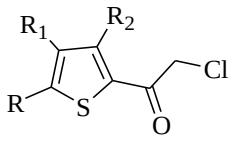


1



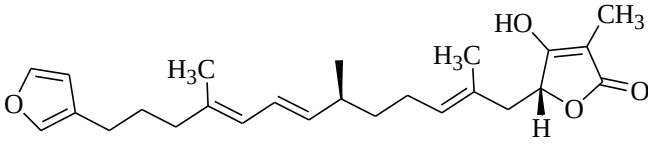
2

Halomethylketone (HMK) derivatives recently revealed as the first irreversible inhibitors of the second family of non-ATP-competitive GSK-3β inhibitors. In this case, inactivation of the enzyme is due to the formation of an irreversible covalent sulphurcarbon bond between the key Cys 199 located at the entrance of the ATP site of GSK-3β and the HMK moiety. Various HMK's **3** were used as pharmacological tools for the study of GSK-3 physiology and pathology in different cell models.[103]



3

Palinurin **4** and tricantin **5** metabolites also belong to the class of non-ATP-competitive GSK-3β inhibitors and were mainly isolated from the Mediterranean sponges *Ircinia dendroides*. Palinurin **4** showed inhibitory activity against GSK-3β with an IC_{50} value of 4.5 μM and Tricantin **5** has been found to inhibit recombinant human GSK-3β with an IC_{50} value of 7.5 μM.[103]



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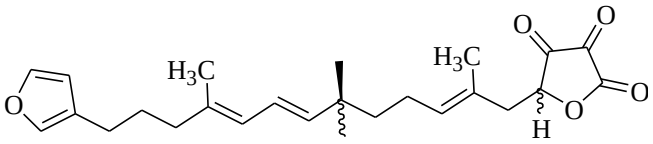
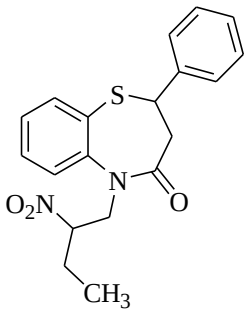
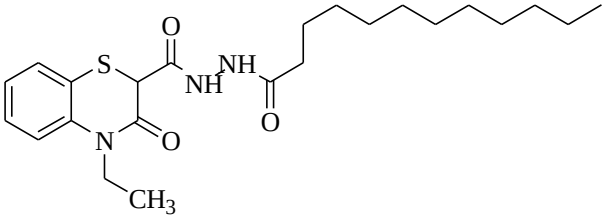


5

Benzothiazepinones (BTZs), others non-ATP-competitive GSK-3β inhibitors, include compound **6** and **7**, that were found to exhibit inhibitory activity against GSK-3β with IC_{50} values of 25 μM and 8.0 μM, respectively.[103]



6

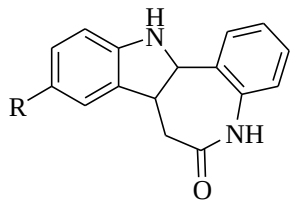


7

The category of ATP-competitive inhibitors consists of small metal cations, as lithium, that was the first "natural" GSK-3 inhibitor discovered. Lithium is a mood stabilizer, which has been used in the treatment of bipolar disorders. Lithium inhibits GSK-3β directly by competition with magnesium ions and indirectly via enhanced serine phosphorylation and autoregulation. Numerous studies have investigated therapeutic activity of lithium in various neuronal systems and verified its profound effect in neuroprotection against a variety of insults in apoptotic and brain injury paradigms.[103]

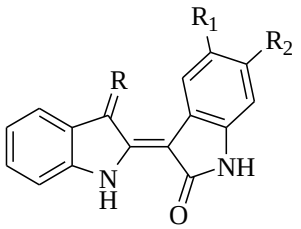
Paullones, particularly alsterpaullone **8** and kenpaullone **9**, the benzazepinone derivatives are potent ATP-competitive inhibitors of GSK-3β, with IC_{50} values within nanomolar range. The *in silico* interactions of alsterpaullone **8** and GSK-3β include two hydrogen bonds with Val 135 and one interaction between the nitro group and the side chain amino group of Lys 85.[104]

Kenpaullone **9** with a 9-halogen substitution pattern was reported to display improved inhibitory activity toward GSK-3β, considering paullones as selective and potent GSK-3β inhibitors.[103]



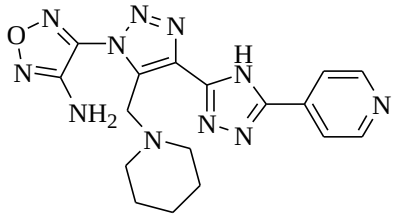
8 R = NO_2
9 R = Br

Indirubines are bis-indole compounds found in a variety of natural sources. The most well-known compound of this family is indirubin **10**, identified initially for the treatment of leukemia, and is a potent dual inhibitor of both CDKs (50-100 nM) and GSK-3β (5-50 nM), through an ATP-competitive mechanism. Also compounds **11-13** displayed remarkable inhibition against GSK-3β within the low nanomolar range. In particular, the introduction of bromine in compound **13** improved the activity for GSK-3β inhibition.[103]

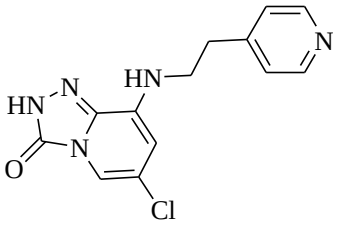


10 R = O, R_1 = R_2 = CH
11 R = NOH, R_1 = I, R_2 = CH
12 R = NOH, R_1 = R_2 = Cl
13 R = NOH, R_1 = CH, R_2 = Br

Triazole derivatives are part of ATP-competitive GSK-3β inhibitors. In particular, compounds **14** and **15** showed inhibitor activity against GSK-3β with IC_{50} values of 0.28 μM and 0.111 μM, respectively.[103]

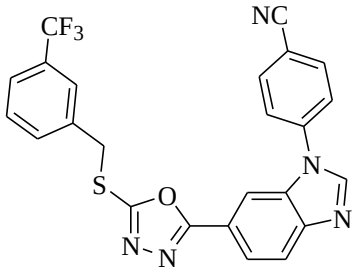


14

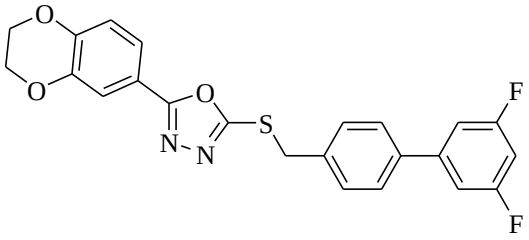


15

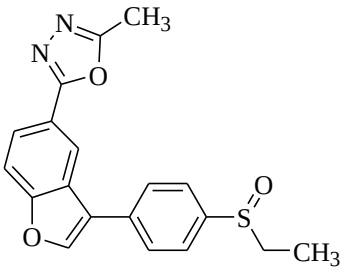
Oxadiazole derivatives also have been reported as ATP-competitive GSK-3β inhibitors. In fact, 1,3,4-oxadiazoles conjugated with fused heterocyclic ring such as benzodioxole, benzofuran, benzimidazole, benzothiazole, imidazopyridine and indazole have been found to exhibit potential GSK-3β inhibition. Among these, the compounds **16-18** showed inhibitor activity against GSK-3β with IC_{50} values in the range of nanomolar.[103]



16

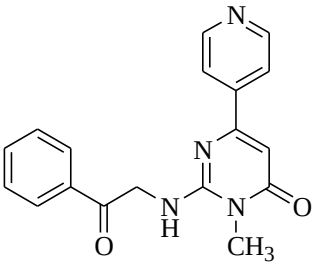


17

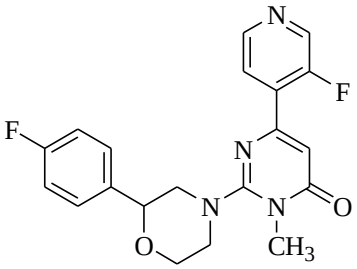


18

Furthermore, the category of ATP-competitive inhibitors includes also other potent GSK-3β inhibitors based on purine or pyrimidine motif, as the compounds **19,20**, which inhibit GSK-3β within the nanomolar concentration range.[103]

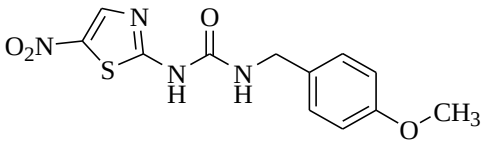


19



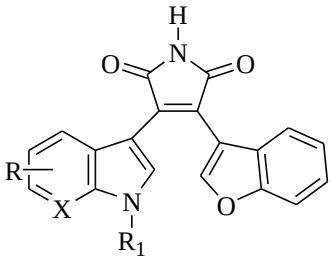
20

The thiazole AR-A014418 **21** is a selective ATP binding site competitor and showed inhibitor activity against GSK-3β with IC_{50} values in the nanomolar range.[103] It induces mitotic spindle dysfunction and chromosomal instability, resulting in the mitotic catastrophe, that is an alternative mechanism for the anticancer effects of GSK-3β inhibitors in cancer cells resistant to apoptosis.[105]



21

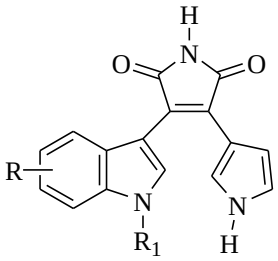
However, although in the literature there are numerous compounds with documented inhibitory activity against GSK-3β, maleimide derivatives **22-25** having different alkyl, aminoalkyl and hydroxyalkyl chains on the indole, 7-azaindazole and 7-azaindole portions, represent an interesting class of potent and highly selective ATP-competitive GSK-3β inhibitors, with IC_{50} values from micro to nanomolar range.[82,103,104,106-108]



benzofuranyl-indolyl-maleimides

22

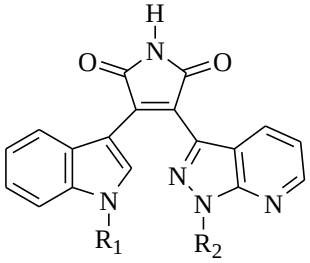
R = H, 5-Br, 5-F
R_1 = $-CH_3$, $-(CH_2)_2OH$, $-(CH_2)_3OH$, $-(CH_2)_2NH_2$
$-(CH_2)_3NHCH_3$, $-(CH_2)_2OCH_3$
X = CH, N



3-aryl-4-pyrrolyl-maleimides

23

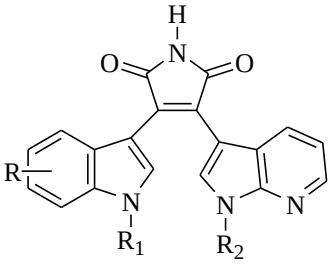
R = H, 5-OCH_3, 6-Br, 6-F, 6-Cl
R_1 = $-(CH_2)_n-N(CH_2)_4O$, $-(CH_2)_3-N(CH_2)_5$
$-(CH_2)_3OH$, $-(CH_2)_3-N(CH)_3N$
n = 2, 3, 4



7-azaindazolyl-indolyl-maleimides

24

R_1 = $-(CH_2)_3-N(CH_2)_4O$, $-(CH_2)_3-N(CH)_3N$
R_2 = $-(CH_2)_n-N(CH_2)_4O$, $-(CH_2)_3-N(CH_2)_5$
$-(CH_2)_3-N(CH_2)_4NCH_3$, $-(CH_2)_n-N(CH)_3N$
n = 2, 3, 4

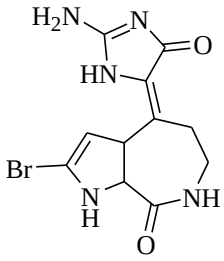


7-aza-bisindolyl-maleimides

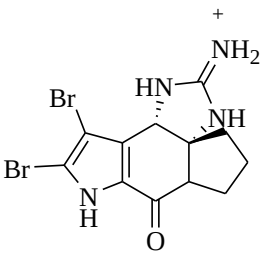
25

R = H, 5-Br, 5-F
R_1 = $-CH_3$, $-(CH_2)_8CHOH$, $-(CH_2)_8O_2NC_2H_5$
R_2 = $-(CH_2)_8CHOH$, $-(CH_2)_8O_2NC_2H_5$, $-(CH_2)_9O_2NCH_3$

Moreover, the compound **26-28**, isolated from marine sponges, represent an other interesting class of ATP-competitive GSK-3β inhibitors and exhibit their activity also towards other protein kinases. In particular, compound **28** prevents cell proliferation and induces apoptosis, demonstrating its ability to enter cells and to interfere with the activity of kinases important for cell division and cell death.[103]



26



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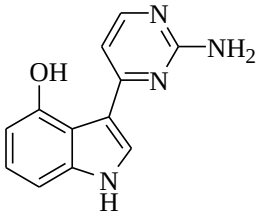
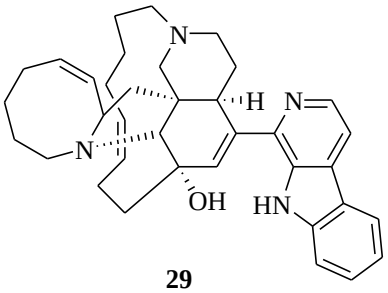


28

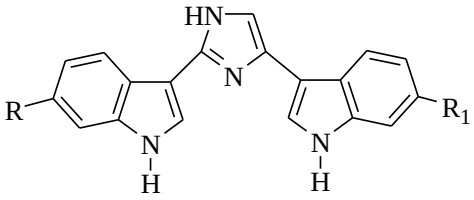
Manzamine alkaloids are β-carboline polycyclic compounds coming from Indo-Pacific sponges with potential therapeutic interest in CNS disorders. Manzamine A **29**, is a substrate competitive inhibitor of GSK-3β and CDK5 with IC_{50} values of 10 and 1.5 μM, respectively.[103]

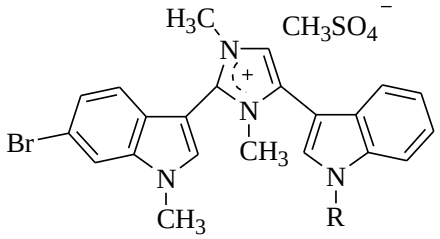


29

A plethora of GSK-3β inhibitors have been discovered in recent years and most of these, besides to inhibiting the GSK-3β, showed inhibitory activity against other kinases. Therefore, the discovery of new, selective and non-toxic GSK-3β inhibitors remains a challenge for pharmaceutical chemistry.
Marine organisms are a highly valuable resource for pharmacologically active compounds, especially of kinase inhibitors and therefore possess a potential role in the discovery and development of new drugs suitable for the treatment of human diseases. In particular, marine alkaloids with the bis-indole structure have received a considerable attention because of their potent biological activity such as antitumor, antiviral, antimicrobial and anti-inflammatory agents.[109-112]

Nortopsentins A-C **30-32**, group of bis-indole alkaloids having an imidazole as spacer, were the first isolated by Howard from *Spongosorites ruetzleri* and *Halichondria*. The subsequent catalytic hydrogenation of nortopsentin A-C **30-32** led to nortopsentin D **33**, while the methylation of nortopsentin B **31** with dimethyl sulfate in the presence of potassium carbonate in acetone afforded trimethyl and tetramethyl nortopsentins B **34,35**, as $MeSO_4^-$ salt. Nortopsentins A-C **30-32** exhibited *in vitro* cytotoxicity against P388 leukemia cells with GI_{50} values of 7.6, 7.8, 1.7, μg/ml, respectively. They also showed antifungal activity against *Candida albicans* (MIC 3.1, 6.2, 12.5 μg/ml). The *N*-methylated derivatives of nortopsentin B **34,35** showed *in vitro* a significant improvement in the citotoxycity against P388 cells compared to that showed by the parent compound (0.9 and 0.34 μg/ml respectively).[113-115]

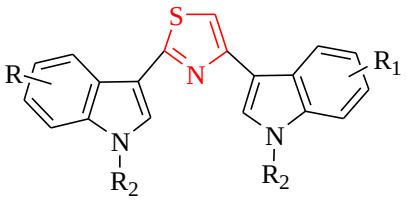




30 **Nortopsentin A** R = R_1 = Br
31 **Nortopsentin B** R = Br, R_1 = H
32 **Nortopsentin C** R = H, R_1 = Br
33 **Nortopsentin D** R = R_1 = H

34 R = H
35 R = CH_3

Due to the considerable biological activities shown and its low availability, nortopsentin has become an interesting "lead compound" for the search of new compounds of pharmaceutical interest. Among the several analogues reported in the literature, significant is the cytotoxic activity showed by 2,4-bis(3'-indolil)thiazoles **36-46** in which the imidazole central ring of the natural compound was replaced by a thiazole one. These compounds, tested by the National Cancer Institute (NCI, Bethesda MD) showed cytotoxic activity against a wide number of human cancer cell lines, as shown in table 1.[114-115]



	R	R_1	R_2
36	H	H	H
37	H	6-Br	H
38	6-Br	H	H
39	6-Br	6-Br	H
40	H	5-Br	H

	R	R_1	R_2
41	5-Br	5-Br	H
42	6-Br	6-OCH_3	H
43	6-Br	5-Br	H
44	6-OCH_3	6-Br	H
45	5-Br	6-Br	H
46	H	H	CH_3

Table 1. Cancer cell growth inhibitory activity of compounds **36-46** in *vitro*.

Cell line	Cytotoxicity (GI_{50} in µM)										
	36	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Leukemia											
CCRF-CEM	ND	14.6	10.9	10.1	2.11	2.40	27.7	2.58	2.66	2.99	>100
HL-60(TB)	ND	ND	ND	0.95	2.43	2.76	ND	3.76	4.13	3.86	>100
K-562	3.27	18.8	5.61	4.69	1.96	1.94	15.2	2.13	1.74	2.25	11.2
MOLT-4	5.31	19.9	31.2	5.80	1.41	1.75	23.0	1.55	2.95	2.26	14.1
RPMI82226	ND	19.4	12.2	11.4	1.97	1.95	27.1	2.24	2.03	1.84	20.8
Non-Small Cell Lung Cancer											
NCI-226	>100	14.4	24.4	3.3	2.10	3.14	45.4	2.48	2.24	7.23	32.2
NCIH322M	>100	16.7	18.9	18.4	2.01	1.99	70.8	2.51	3.09	2.99	24.5
NCI-H460	>100	16.1	7.31	16.4	2.55	1.93	23.2	2.31	1.58	2.00	26.5
EKVX	>100	15.6	29.5	28.6	ND	ND	32.1	0.48	0.29	0.55	ND
Colon Cancer											
HCT-15	>100	15.2	17.8	8.50	1.81	2.51	7.54	2.70	0.81	2.84	>100
SW-620	>100	16.5	25.6	12.5	1.52	2.14	7.00	3.57	5.50	2.89	59.4
CNS Cancer											
SF-295	33.6	14.6	9.23	4.81	1.98	2.71	73.5	4.56	ND	ND	23.5
SF-268	ND	18.3	32.1	ND	1.52	2.44	26.0	2.69	13.8	1.80	58.2
SNB-19	>100	17.8	41.8	17.2	2.11	3.75	>100	2.60	10.5	3.34	42.6
U21	>100	17.9	28.1	15.3	2.10	2.30	25.0	3.34	5.07	3.00	32.4
Ovarian Cancer											
IGROV1	8.14	13.0	30.5	14.4	1.85	1.70	81.5	2.96	4.61	2.43	27.0
OVCAR-5	>100	16.1	37.1	23.6	1.96	2.14	42.7	2.16	3.44	2.35	95.9
Renal Cancer											
786-0	>100	18.0	19.9	15.9	2.28	1.95	27.4	1.50	3.89	2.21	17.2
A498	>100	17.0	25.7	23.2	1.92	2.48	89.4	2.19	7.43	2.40	12.6
RXF 393	ND	22.4	7.62	18.4	1.81	1.69	11.9	2.42	2.03	1.66	14.1
Prostate Cancer											
PC-3	>100	15.6	16.9	15.3	2.02	2.57	10.4	4.16	3.49	2.81	21.9
DU-145	>100	18.8	14.9	18.4	2.29	2.61	>100	3.74	5.46	2.03	42.9
Breast Cancer											
MCF7	>100	16.7	27.2	6.46	2.13	2.70	54.1	0.88	4.36	3.82	45.5
MDAMB435	33.1	14.9	25.6	4.34	2.53	2.27	7.70	4.54	14.6	3.97	26.4
MDA-N	83.0	19.2	31.5	2.94	1.88	2.09	8.27	2.86	6.84	3.77	24.9
T-47D	>100	24.8	23.9	16.2	3.27	2.90	59.3	3.33	4.12	1.76	28.1
BT-549	>100	18.3	73.8	41.1	2.71	10.6	67.3	1.24	1.46	1.41	56.3
Melanoma											
LOX IWVI	21.8	15.5	6.55	11.3	1.97	1.69	64.1	2.32	2.41	1.87	36.4
MALME3M	>100	16.9	>100	ND	1.28	1.72	1.72	1.45	1.50	2.83	19.0
M14	>100	14.2	32.4	10.6	1.61	1.63	>100	2.86	5.49	2.77	26.7
SKMEL2	>100	ND	37.5	68.8	1.81	1.98	19.1	3.25	7.30	2.91	38.8
SKMEL28	>100	14.2	7.74	32.0	3.02	3.05	>100	9.84	1.36	4.96	61.5
SKMEL5	ND	14.9	23.7	28.5	1.76	1.63	>100	2.82	5.13	3.36	26.9

ND= Not Determined

In the research lab where I carried out my PhD thesis, different analogues of nortopsentin has been synthesized, in which the imidazole ring of the natural compound was replaced by other five-membered heterocycles, such as thiophene **47**,[116] pyrazole **48**,[117] furan **49**,[118] isoxazole **50**,[118] and pyrrole **51**[119] (Figure 6). Some of them showed remarkable antiproliferative activity in cancer cells, often with GI_{50} values at submicromolar level.

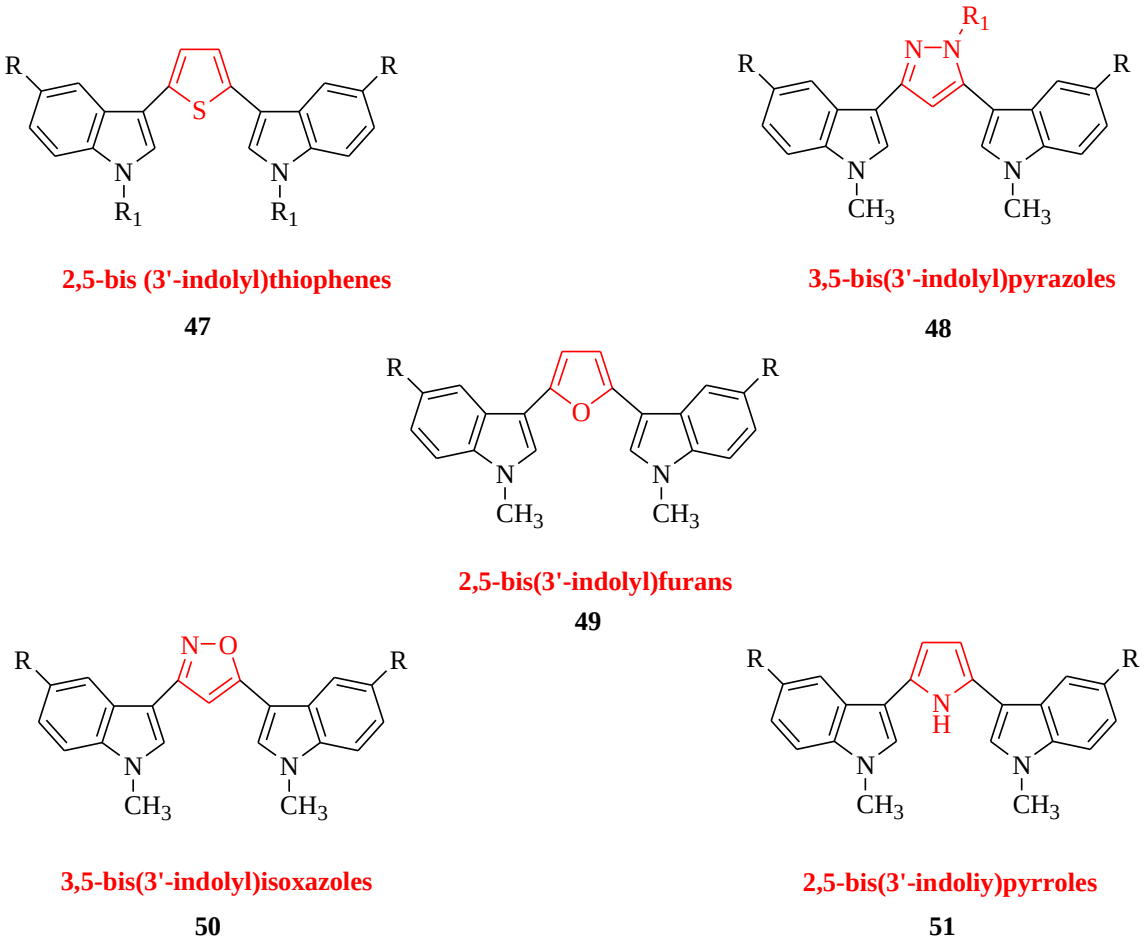
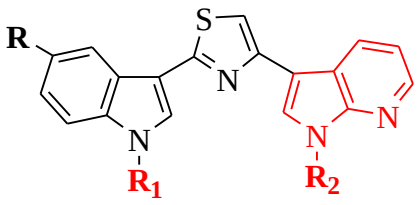


Figure 6. Nortopsentin Analogues.

Considering the interesting antitumor activity of 2,4-bis(3'-indolil)thiazoles **36-46** reported, the attention was also focused on the synthesis of indolyl-7-azaindolyl thiazoles **52a-n**, **53a-j**, **54a-j** (Table 2) in order to verify whether the introduction of a nitrogen atom in the 7 position on the indole portion led to an increase of the antitumor activity.[120]



3-[2-(1*H*-indol-3-yl)-1,3-thiazol-4-yl)-1*H*-7-azaindoles

52a-n, 53a-j, 54a-j

Table 2. 3-[2-(1*H*-indol-3-yl)-1,3-thiazol-4-yl]-1*H*-pyrrolo[2,3-*b*]pyridines **52a-n**, **53a-j**, **54a-j**.

Compd	R	R_1	R_2	Yields	Compd	R	R_1	R_2	Yields
52a	H	CH_3	H	85%	**53d**	Cl	Boc	H	90%
52b	CH_3	CH_3	H	70%	**53e**	Br	Boc	H	80%
52c	OCH_3	CH_3	H	90%	**53f**	H	Boc	CH_3	92%
52d	Cl	CH_3	H	78%	**53g**	CH_3	Boc	CH_3	53%
52e	Br	CH_3	H	60%	**53h**	OCH_3	Boc	CH_3	90%
52f	F	CH_3	H	95%	**53i**	Cl	Boc	CH_3	90%
52g	H	CH_3	CH_3	75%	**53j**	Br	Boc	CH_3	72%
52h	CH_3	CH_3	CH_3	60%	**54a**	H	H	H	99%
52i	OCH_3	CH_3	CH_3	85%	**54b**	CH_3	H	H	95%
52j	Cl	CH_3	CH_3	57%	**54c**	OCH_3	H	H	99%
52k	Br	CH_3	CH_3	55%	**54d**	Cl	H	H	99%
52l	F	CH_3	CH_3	91%	**54e**	Br	H	H	54%
52m	F	H	H	87%	**54f**	H	H	CH_3	71%
52n	F	H	CH_3	60%	**54g**	CH_3	H	CH_3	99%
53a	H	Boc	H	85%	**54h**	OCH_3	H	CH_3	90%
53b	CH_3	Boc	H	60%	**54i**	Cl	H	CH_3	99%
53c	OCH_3	Boc	H	90%	**54j**	Br	H	CH_3	90%

All the synthesized indolyl-azaindolyl thiazoles **52a-n**, **53a-j** and **54a-j** were submitted to the NCI in order to evaluate their biological activity as antitumor agents. Biological screening were performed according to the NCI protocol, at the 10^{-5} M dose for the *in vitro* disease-oriented antitumor screenings against a panel of about 60 human tumor cell lines. Compounds **52a**, **52d**, **52f**, **52g**, **52l-n**, **53f**, **54b-f** were selected by the NCI for full evaluation at five concentration levels (10^{-4}-10^{-8} M), and showed antiproliferative activity with GI_{50} values from micro to sub-micromolar range (Table 3).[120]

Table 3. In *vitro* inhibition of cancer cell lines growth by compounds **52a**, **52d**, **52f**, **52g**, **52l-n**, **53f**, **54b-f**.

Compd	N° of cell line tested	N° of active cell lines	GI_{50} (µM)	
			Range	MG_MID
52a	59	56	0.84-0.60	0.70
52d	59	59	0.85-0.66	0.73
52f	60	60	0.87-0.72	0.75
52g	59	59	0.83-0.72	0.76
52l	55	55	0.82-0.72	0.77
52m	23	14	0.80-0.69	0.69
52n	58	58	0.82-0.63	0.75
53f	59	59	0.78-0.68	0.72
54b	48	3	0.82-0.74	0.61
54c	58	58	0.79-0.62	0.73
54d	59	59	0.77-0.71	0.74
54e	59	59	0.77-0.70	0.74
54f	60	60	0.86-0.70	0.79

The five most active compounds, **52f**, **54f**, **52g**, **52l**, and **52n**, were further tested by Istituto Nazionale dei Tumori (Fondazione IRCCS, Milano) against two additional cell lines, STO and MesoII, derived from human Diffuse Malignant Peritoneal Mesothelioma (DMPM), a tumor type not included in the NCI panel. After seventy-two hours of exposure to increasing concentrations of each compound, compounds **52f**, **54f**, and **52l** showed a dose-dependent inhibition of cell proliferation in both cellular models and they did not interfere with the proliferation of normal cells (W138) (Table 4).[120]

Table 4. Cytotoxic activity of compounds **52f**, **54f**, **52g**, **52l**, **52n**, in DMPM and normal cells.

Compd	GI_{50} (μM)[a]		
	STO	MesoII	WI38
52f	0.49±0.07	25.12±3.06	>100
54f	0.33±0.07	4.11±0.22	>100
52g	0.61±0.14	16.77±1.99	18.76±3.21
52l	0.43±0.11	4.85±0.64	>100
52n	0.54±0.09	13.27±0.74	15.44±3.87

[a]Data are reported as GI_{50} values (concentration of drug required to inhibit growth by 50%) determined by MTS assay after 72 h of continuous exposure to each compound. The data represents mean values ± SD of at least three independent experiments.

As many nortopsentin analogues exhibited the cellular effects by inhibiting of kinase activity, in order to study the mechanism of action, compounds **52f**, **54f** and **52l** were tested on several protein kinases (CDK1, CDK5, EGFR, FGFR1, RET, MET, KIT, JAK2, PKCA, PKCB, CHKI, MAPK12, GSK, PKA, GSK-3α, GSK-3β). Results showed that those compounds markedly repressed CDK1 activity with GI_{50} values (0.89±0.07, 0.86±0.04, 0.75±0.03) comparable to those reported for two well-known CDK1 inhibitors, roscovitine and purvalanol A (0.73±0.06, and 0.59±0.08, respectively) and they decreased CDK1 activity in a time-dependent way. These compounds were also active against GSK-3β protein, but at higher concentrations (Table 5).[120]

Table 5. In *vitro* Kinase inhibitory activity of derivatives **52f**, **54f**, **52l**.

	GI_{50} (μM)[a]				
Protein kinase	**52f**	**54f**	**52l**	**Roscovitine**	**Purvalanol A**
CDK1	0.89±0.07	0.86±0.04	0.75±0.03	0.73±0.06	0.59±0.08
GSK-3β	42.18±3.28	35.68±1.69	40.18±2.94	>50	>50

[a]Concentration of drug required to inhibit by 50% (GI_{50}) the activity of CDK1 e GSK-3β. Values represent the mean values ± SD of three independent experiments.

Moreover, it has been assessed the phosphorylation status of histone H1, a specific substrate for CDK1, in STO cell lines, in order to confirm the ability of these three derivatives to inhibit CDK1 activity in living cells; the obtained results showed a substantial and time dependent reduction of kinase activity (Figure 7 A-B).[120]

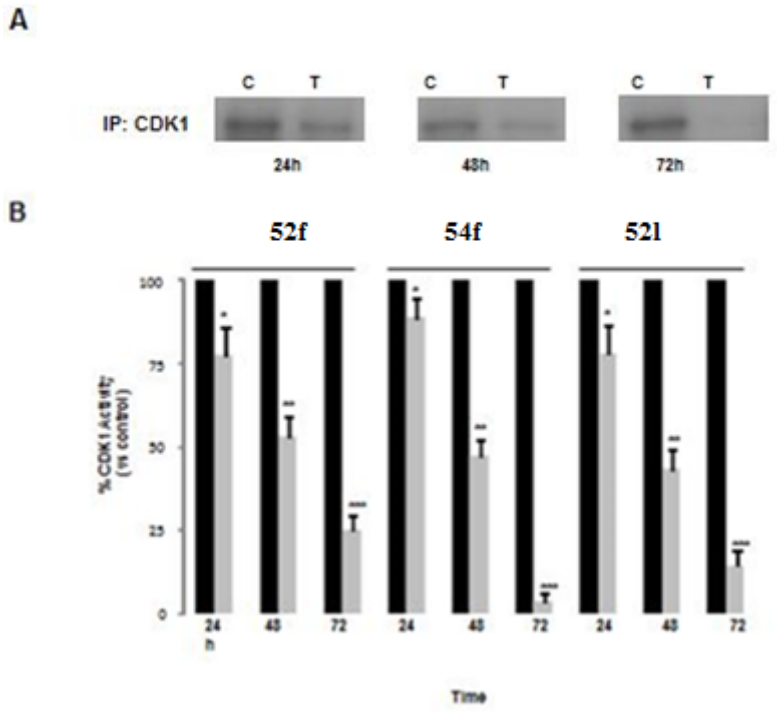


Figure 7. Effect of **52f**, **54f**, and **52l** derivatives on CDK1 kinase activity in DMPM cells. (A) Representative kinase assay illustrating the CDK1 activity in STO cells at different intervals after exposure to 1% (v/v) DMSO (control cells; C) or to derivative **54f** (GI_{50}; T). (B) Densitometric quantification of CDK1 activity in STO cells exposed to derivatives **52f**, **54f**, and **52l** for 24, 48, and 72 h. CDK1 activity was achieved by immunoprecipitation. Data are reported as the percentage of CDK1 activity in cells exposed to derivatives **52f**, **54f**, and **52l** (gray column) compared with DMSO-treated cells (black column) and represent the mean values ± SD of at least three independent experiments. ***p <0.001, **p < 0.01, *p < 0.05.

These compounds were also tested in STO and MesoII cells to observe the importance of nortopsentin analogue treatment on cell cycle progression. Compound **52f**, **54f**, **52l** induced a time-dependent accumulation of cells in the G_2 / M phase, and a concomitant decrease of the percentage of cells in the G_1 phase. It was also observed a marched growth in the number of cells with apoptotic morphology (in terms of chromatin condensation and DNA fragmentation) and a significant increase in the activity of caspase -9 and -3 (Figure 8).[120]

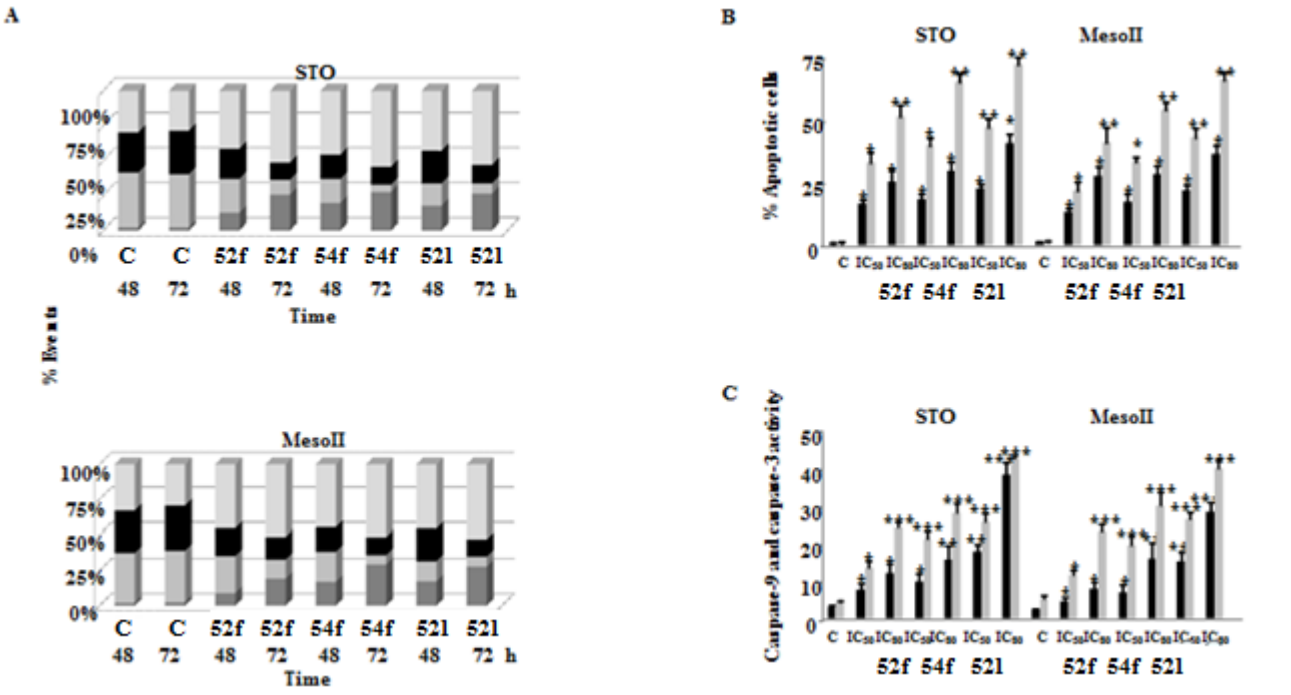


Figure 8. Effect of **52f**, **54f**, and **52l** derivatives on cell-cycle progression and apoptosis induction. (A) STO and MesoII cells were exposed to 1% (v/v) DMSO (control cells; C) or to derivatives **52f**, **54f**, and **52l** (GI_{50}) for 48 and 72 h. Data are reported as the percentage of cells in sub-G_1 (dark gray), G_1 (light gray), S (black), and G_2 / M (white) phases and represent the mean values of three independent experiments; SDs were always within 5%. (B) The percentage of cells with apoptotic morphology was assessed by fluorescence microscopy after exposure of DMPM cells to 1% (v/v) DMSO (control cells; C) or to derivatives **52f**, **54f**, and **52l** at 48 h (black column) and 72 h (gray column) after treatment. Data are expressed as mean values ± SD of at least three independent experiments. $^{**}p < 0.001$, $^{*}p < 0.01$. (C) The catalytic activity of caspases was assessed after 72 h of exposure of the DMPM cells to 1% (v/v) DMSO (control cells; C) or to derivatives **52f**, **54f**, and **52l**. Caspase-9 (black column) and caspase-3 (gray column) catalytic activity was determined in vitro by hydrolysis of the fluorogenic substrates (LEHD-pNA and DEVD-pNA, respectively). Data are expressed as mean values ± SD of at least three independent experiments. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.02$.

It was previously demonstrated that DMPM chemoresistance was principally caused by the dysregulation of apoptotic pathway, through the over-expression of members of the inhibitors of apoptosis protein family (IAP), such as survivin,[121] a multifunctional protein involved in cell division and apoptosis and selectively overexpressed in most human cancers.[122] It has been demonstrated that to exert its functions, survivin needs to be physically associated with CDK1 and phosphorylated on the Thr 34 residue by CDK1 / Cyclin B1 complex.[122]

It was investigated the effect of these compounds on survivin activation. Immunoblotting experiments demonstrated that 1*H*-pyrrolo[2,3-*b*]pyridine derivatives induced apoptosis in DMPM cells through a relevant and time-dependent decrease of levels of the active form of survivin, phosphorylated in Thr 34 (Figure 9).[120]

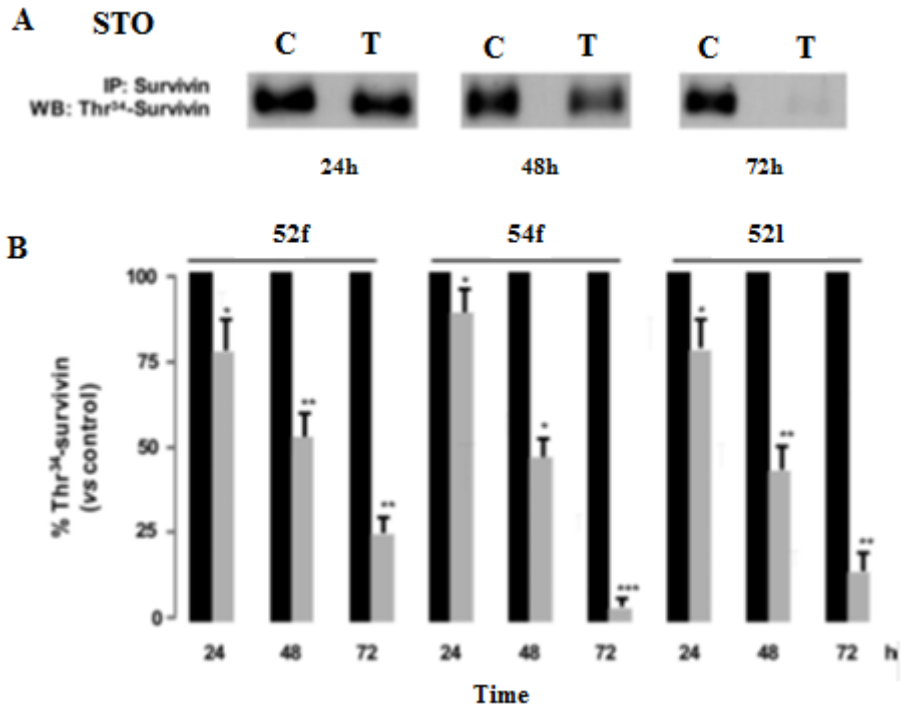


Figure 9. Effect of **52f**, **54f**, and **52l** derivatives on survivin phosphorylation. (A) Representative Western blotting illustrating the survivin phosphorylation status in STO cells after exposure to 1% (v/v) DMSO (control cells; C) or to derivative **54f**, (GI_{50}; T). (B) Densitometric quantification of surviving phosphorylation levels in STO cells exposed to **52f**, **54f**, and **52l** for 24, 48, and 72 h. The phosphorylation of survivin on the Thr 34 residue was evaluated on STO cells treated with 1% (v/v) DMSO (control cells; black column) or to derivatives **52f**, **54f**, and **52l** (IC_{50}; gray column) by Western immunoblotting. Survivin was immunoprecipitated using the antihuman survivin antibody and analyzed with the antibody to phosphorylated Thr 34. Data are expressed as mean values ± SD of at least three independent experiments. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

The cytotoxic effect of the most active compound **54f** was also investigated in DMPM cells alone or in combination with the taxan paclitaxel. At the beginning STO and MesoII cells were treated only with the taxan for 24 hours, and then they were also exposed for 72 hours to derivative **54f**. At all concentrations, it was observed a synergistic cytotoxic effect to inhibit DMPM cell survival. After the combination of two drugs, an inhibition of cell proliferation bigger than that estimated by simple addition / amount of the effects of the two agents it was detected (Figure 10A).

A higher catalytic activity of caspase-3 was identified in cells treated with the two compounds than in those exposed to each single agent (Figure 10B).[120]

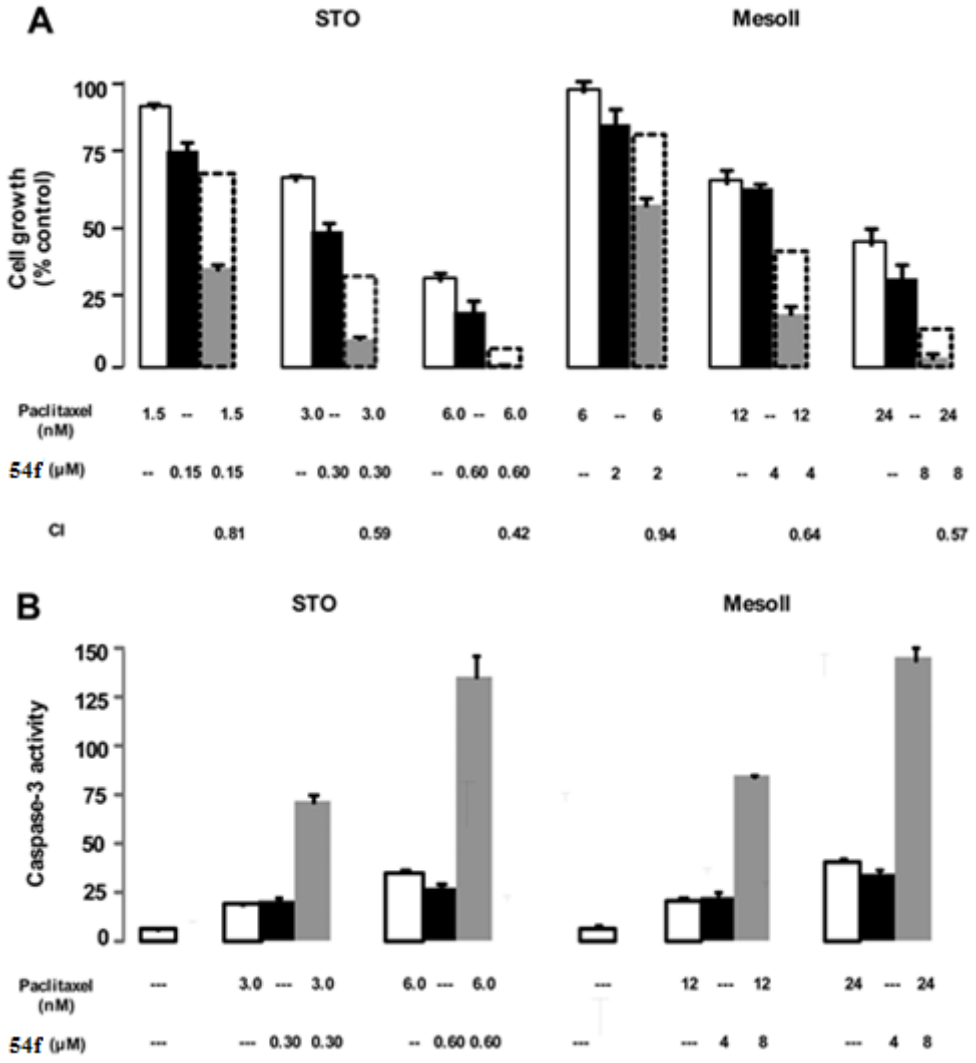


Figure 10. Cytotoxic effect of **54f** derivative in combination with paclitaxel in DMPM cells. (A) The cytotoxic effect of paclitaxel and **54f** derivative, alone or in combination, was assessed by MTS assay. The dashed lines represent the expected additive effect of the combination, calculated as the product of the effects of the individual drugs. Data are expressed as mean values ± SD of at least three independent experiments. CI was calculated according to Chou and Talalay.[123] (B) Caspase-3 catalytic activity was determined in vitro by the hydrolysis of the specific fluorogenic substrate (DEVD-pNA). Data are expressed as mean values ± SD of at least three independent experiments. *$p < 0.001$.

The antitumoral activity of **52f**, **54f**, and **52l** derivatives was then investigated on STO cells xenotransplanted in athymic nude mice and after the treatment with each compound it was possible to observe a marked tumor growth inhibition (Figure 11).[120]

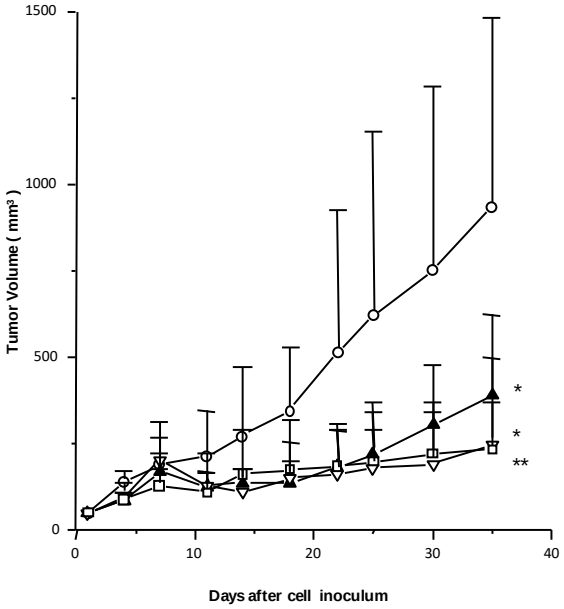


Figure 11. Activity of **52f**, **54f**, and **52l** derivatives on STO cells xenotransplanted on athymic mice. Drugs were administered ip at 25 (**52f** and **52l**) or 50 (**54f**) mg/kg qd×4−5/w×3w, starting from the day after the injection. **p < 0.01, *p < 0.05.

In particular, at the end of the experiment, a statistically significant tumor volume inhibition (TVI) compared with the control (73%, 58% and 75%, for **52f**, **54f** and **52l** derivatives, respectively) was observed (Table 6). In addition, two complete responses were observed in each treatment group, with the disappearance of tumor induced by treatment. Moreover, the compounds were well tolerated without any appreciable sign of toxicity (Table 6).[120]

Table 6. Activity of derivatives **52f**, **54f** and **52l**, on STO cells xenotransplanted in athymic nude mice.

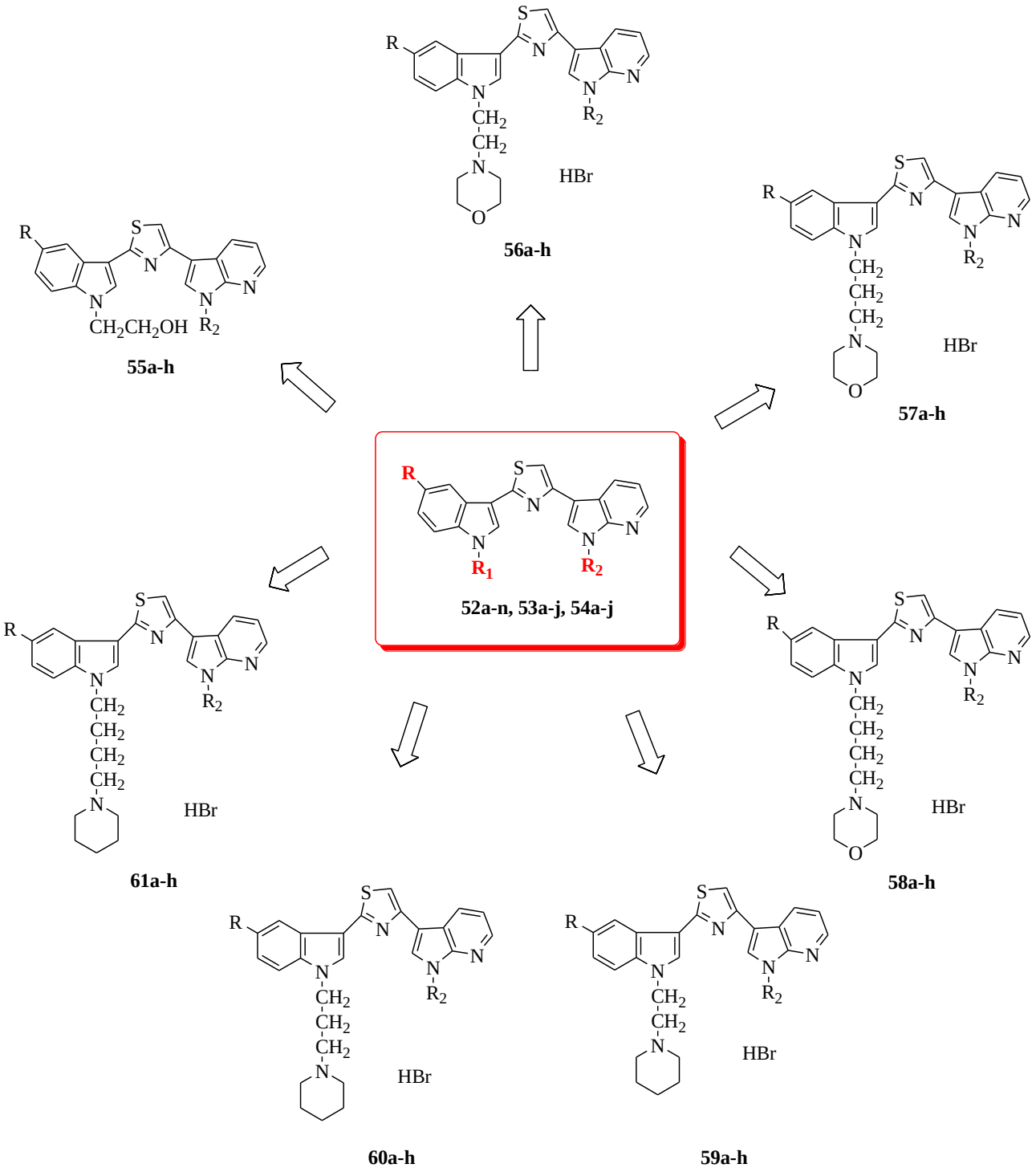
Compd	TVI (%)[a]	CR[b]	BWL (%)[c]	TOX[d]
52f	73*	2/8	4	0/8
54f	58*	2/8	7	0/8
52l	75**	2/8	1	0/8

[a]Tumor volume inhibition (%) in treated vs control mice, determined 17 days after the end of drug treatment (day 35). [b]Complete response, disappearance of tumor induced by treatment. [c]BWL, body weight loss induced by treatment (%). [d]Toxic death on treated animals. **p <0.01, *p < 0.05.

AIM OF THE WORK

Considering the interesting biological results shown by indolyl-7-azaindolyl thiazoles of type **52-54**, and considering the importance of alkyl, aminoalkyl and hydroxyalkyl chains of maleimide derivatives **22-25** against GSK-3β, the aim of my PhD project was the synthesis of new analogues 7-azaindolyl thiazoles, characterized by the presence of different chains on the indole ring, and the evaluation of their potential cytotoxic activity and possible selectivity against GSK-3β.
In particular, seven new series have been synthesized:

- 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1,3-thiazol-2-yl]-1*H*-indol-1-yl}ethanols **55a-h**
- 3-{2-[1-(2-Morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **56a-h**
- 3-{2-[1-(3-Morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **57a-h**
- 3-{2-[1-(4-Morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **58a-h**
- 3-{2-[1-(2-Piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **59a-h**
- 3-{2-[1-(3-Piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **60a-h**
- 3-{2-[1-(4-Piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **61a-h**


R
S
N
N
N
R2
CH2
CH2
N
O
HBr
56a-h
R
S
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N
N
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CH2
CH2
N
O
HBr
57a-h
R
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HBr
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S
N
N
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CH2CH2OH
R2
55a-h
R
S
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N
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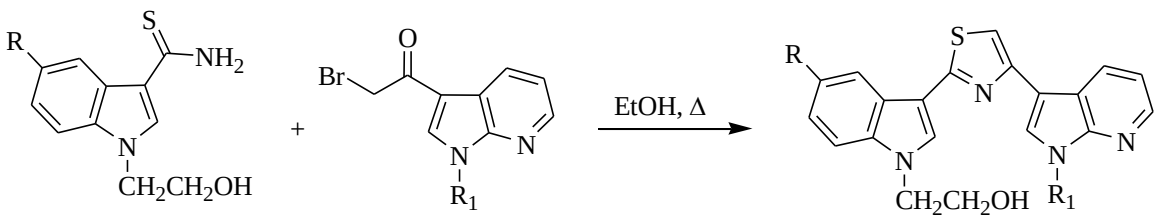
R1
R2
52a-n, 53a-j, 54a-j

RESULTS AND DISCUSSIONS: CHEMISTRY

The synthetic pathway of new 7-azaindolyl thiazoles **55a-h**, **56a-h**, **57a-h**, **58a-h**, **59a-h**, **60a-h**, **61a-h** (Tables 7-13), involves an Hantzsch reaction between two key intermediates: carbothioamides **62a-d**, **75a-d**, **79a-d**, **84a-d**, **88a-d**, **92a-d**, **95a-d**, and 3-bromoacetyl-7-azaindoles **63a,b** (Schemes 1, 6, 8, 10, 12, 14, 16).

In particular, 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1,3-thiazol-2-yl]-1*H*-indol-1-yl}ethanols **55a-h** were prepared from reaction of carbothioamides **62a-d** and 3-bromoacetyl compounds **63a,b**. These key intermediates, reacted in ethanol under reflux from 30 minutes to 2 hours, gave after crystallization, the desired 7-azaindolyl thiazoles **55a-h** (Scheme 1), in excellent yields (90-98%) (Table 7).

Scheme 1.



62a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

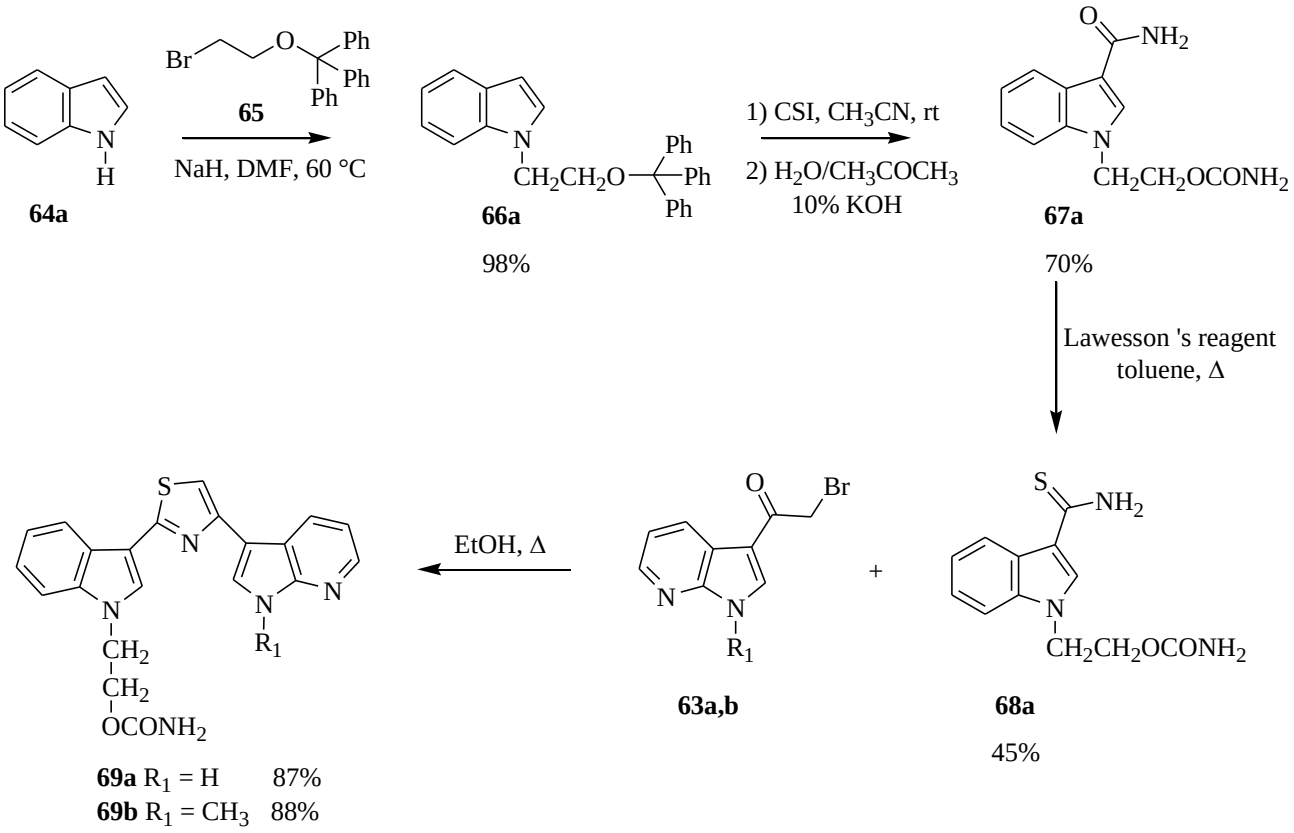
55a-h

Table 7. 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-1,3-thiazol-2-yl]-1*H*-indol-1-yl}ethanols **55a-h**.

Compd	R	R_1	Yield %
55a	H	H	**98**
55b	H	CH_3	**98**
55c	Br	H	**90**
55d	Br	CH_3	**90**
55e	F	H	**98**
55f	F	CH_3	**98**
55g	OCH_3	H	**98**
55h	OCH_3	CH_3	**98**

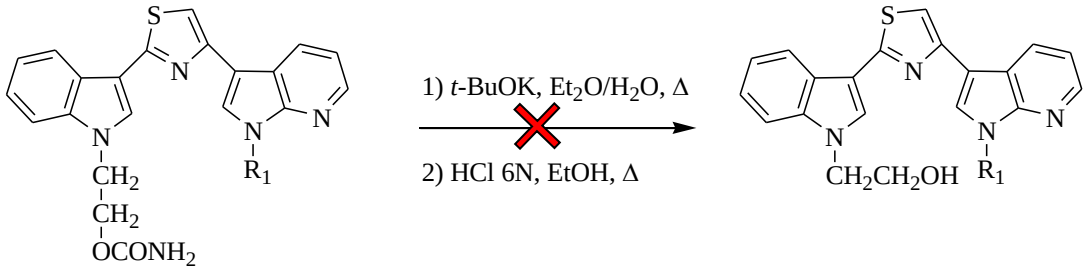
For the synthesis of carbothioamides of type **62** it was initially proceeded reacting indole **64a** with 1-bromo-2-trityloxy-ethane **65**[124], in the presence of sodium hydride (NaH) as a base and dimethylformamide (DMF) as reaction solvent, to get derivative **66a**. The reaction was heated to 60 °C for 3 hours to obtain derivative **66a** in excellent yield (98%). This latter was then dissolved in acetonitrile and reacted with chlorosulfonyl isocyanate (CSI), at room temperature for 30 minutes. The resulting intermediate, treated with a solution of acetone and water and subsequent basification with potassium hydroxide solution (10% KOH), afforded derivative **67a** (70%) in which deprotection and carbamoylation of the side chain occurred, leading to introduction of the amide function also in position 3 of the indole. Despite the original purpose, the obtained intermediate was used and reacted with 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (Lawesson's reagent), in toluene under reflux. The reaction gave carbothioamide **68a**, which was reacted with 3-bromoacetyl-7-azaindoles **63a,b** in ethanol under reflux, affording thiazoles **69a,b** (Scheme 2).

Scheme 2.



In order to achieve our original goal, thiazoles **69a,b** were subjected to hydrolysis using both basic and acid conditions to obtain the desired thiazoles of the type **55**. Unfortunately, these reactions showed only negative results (Scheme 3).

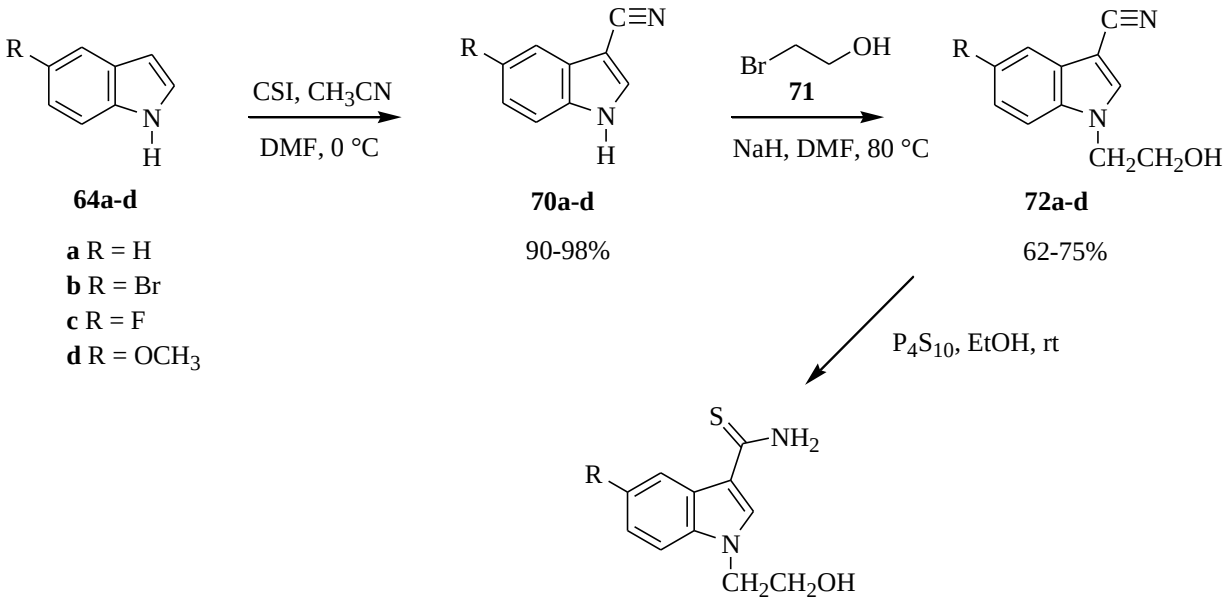
Scheme 3.



69a,**b** **55a**,**b**

On this basis, it was decided to change the synthetic strategy as shown in the scheme 4. The commercially available indole derivatives **64a-d** were reacted with CSI and DMF in acetonitrile at 0 °C, leading to the cyano derivatives **70a-d** in excellent yields (90-98%). Then, these latter were reacted with 2-bromoethanol **71**, using NaH as a base and DMF as reaction solvent. The reaction mixture was heated at 80 °C affording derivatives **72a-d** (62-75%), which were then reacted with phosphorus pentasulfide (P_4S_{10}) in ethanol. The reaction was conducted at room temperature allowing the synthesis of the desired key intermediate carbothioamides **62a-d** (70-80%).

Scheme 4.



64a-d

a R = H
b R = Br
c R = F
d R = OCH_3

70a-d

90-98%

72a-d

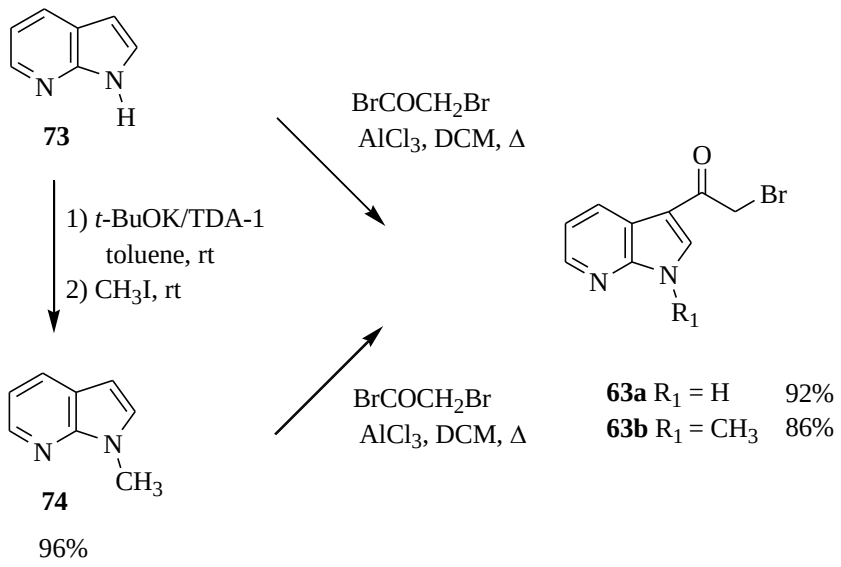
62-75%

62a-d

70-80%

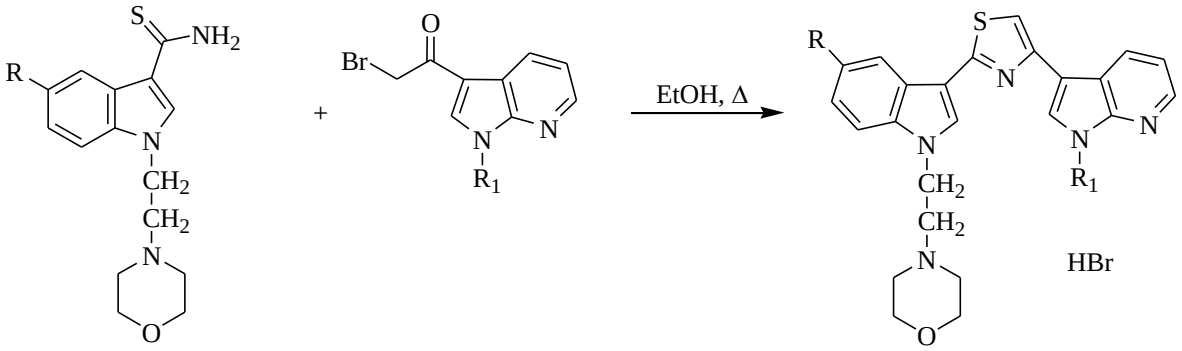
The 3-bromoacetyl-7-azaindole intermediates **63a,b** were synthesized in excellent yields (86-92%), starting from the commercial available 7-azaindole **73** and its methyl derivative **74** by acylation reaction with bromoacetyl bromide in the presence of aluminum chloride in dichloromethane (DCM) under reflux. The derivative **74** was obtained in good yield (96%) through the methylation of the 7-azaindole **73**, using potassium *t*-butoxide as a base, tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (1 or 2 drops) as phase transfer catalyst and methyl iodide (CH_3I) as methylating agent in toluene at room temperature (Scheme 5).[125]

Scheme 5.



3-{2-[1-(2-Morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **56a-h** were synthesized from carbothioamides **75a-d** and 3-bromoacetyl compounds **63a,b**. The key intermediates were reacted in ethanol for 30 minutes under reflux. After crystallization with ethanol, the desired 7-azaindolyl thiazoles **56a-h** were isolated as hydrobromide salts (Scheme 6) in excellent yields (70-98%) (Table 8).

Scheme 6.



75a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

56a-h

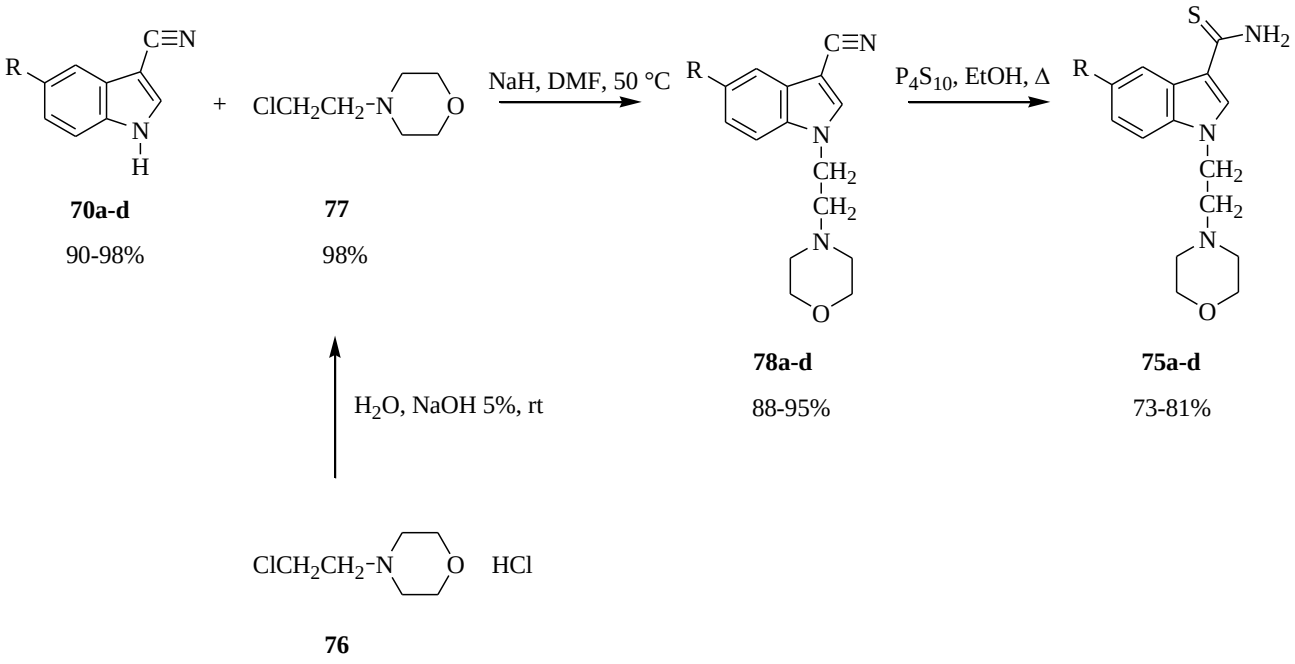
Table 8. 3-{2-[1-(2-Morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **56a-h**.

Compd	R	R_1	Yield %
56a	H	H	**70**
56b	H	CH_3	**80**
56c	Br	H	**81**
56d	Br	CH_3	**98**
56e	F	H	**95**
56f	F	CH_3	**98**
56g	OCH_3	H	**92**
56h	OCH_3	CH_3	**93**

In this case carbothioamides **75a-d** were prepared as shown in the scheme 7.

Once synthesized derivatives **70a-d** were reacted with 4-(2-chloroethyl)morpholine **77**[126], which was synthesized in excellent yield (98%) from the commercially available 4-(2-chloroethyl)morpholine hydrochloride **76**. The latter was solubilized in distilled water and treated with an aqueous solution of sodium hydroxide (NaOH) at 5%. Reaction between compounds **70a-d** and **77** performed at 50 °C, using NaH as a base and DMF as reaction solvent, led to the synthesis of intermediates **78a-d** (88-95%) which were then reacted with P_4S_{10} in ethanol under reflux affording carbothioamides **75a-d** (73-81%).

Scheme 7.



70a-d
90-98%

77
98%

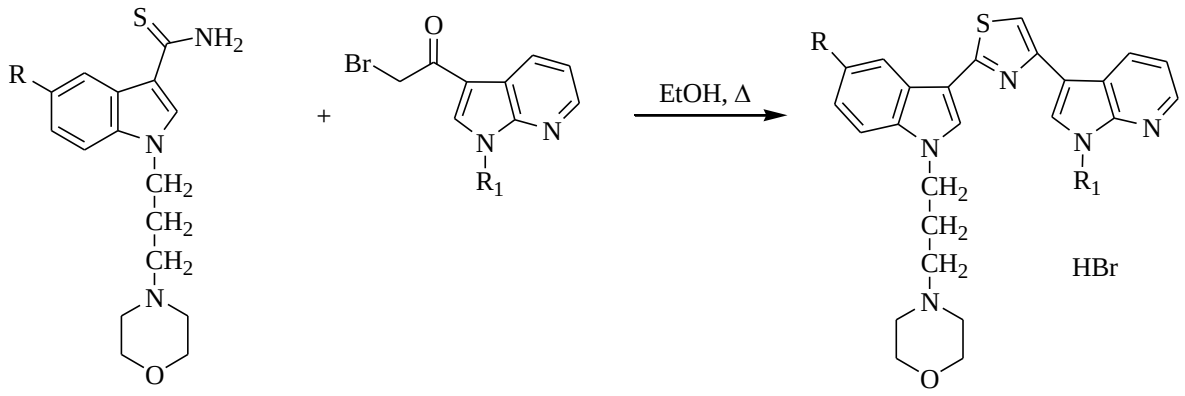
78a-d
88-95%

75a-d
73-81%

76

3-{2-[1-(3-Morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **57a-h** were synthesized from carbothioamides **79a-d** and 3-bromoacetyl compounds **63a,b**, reacted in ethanol under reflux (30 minutes - 1 hour). Also in this case the desired 7-azaindolyl thiazoles **57a-h** were isolated as hydrobromide salts (Scheme 8) in excellent yields (80-98%) (Table 9).

Scheme 8.



79a-d

a R = H
b R = Br
c R = F
d R = OCH_3

63a,b

a R_1 = H
b R_1 = CH_3

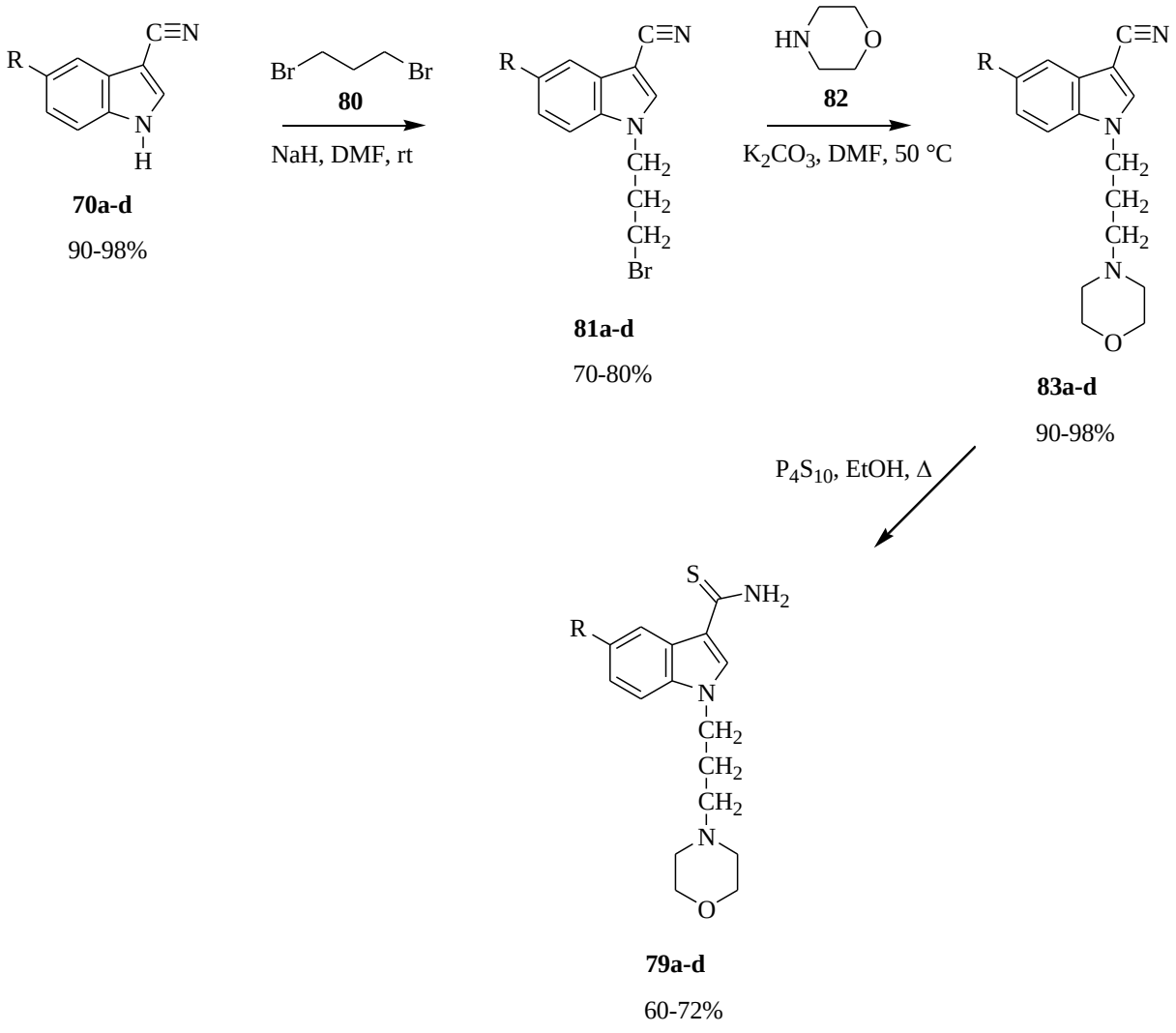
57a-h

Table 9. 3-{2-[1-(3-Morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **57a-h**.

Compd	R	R_1	Yield %
57a	H	H	**80**
57b	H	CH_3	**80**
57c	Br	H	**87**
57d	Br	CH_3	**90**
57e	F	H	**85**
57f	F	CH_3	**98**
57g	OCH_3	H	**98**
57h	OCH_3	CH_3	**98**

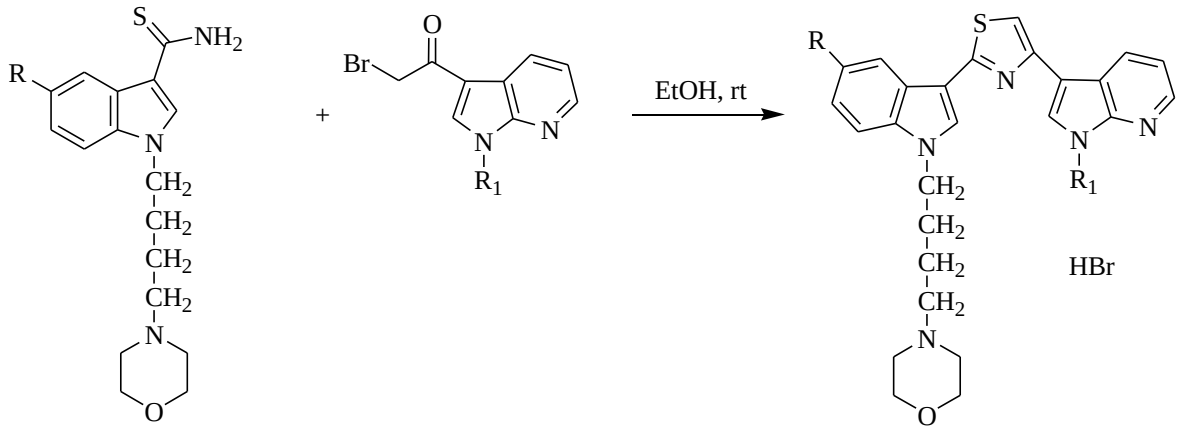
Carbothioamides **79a-d** were also obtained from derivatives **70a-d**, which were reacted in this case with 1,3 dibromopropane **80**. The reaction, carried out at room temperature, afforded derivatives **81a-d** (70-80%), which were subsequently solubilized in DMF and treated with morpholine **82** and K_2CO_3. The reaction heated at 50 °C led to the synthesis of derivatives **83a-d** in excellent yields (90-98%). These intermediates were then converted in the desired carbothioamides **79a-d** (60-72%) using the same synthetic procedure previously described (Scheme 9).

Scheme 9.



3-{2-[1-(4-Morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **58a-h** (70-95%) (Table 10) were obtained from reaction of carbothioamides **84a-d** and 3-bromoacetyl compounds **63a,b** performed in ethanol at room temperature for 24 hours (Scheme 10).

Scheme 10.



84a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

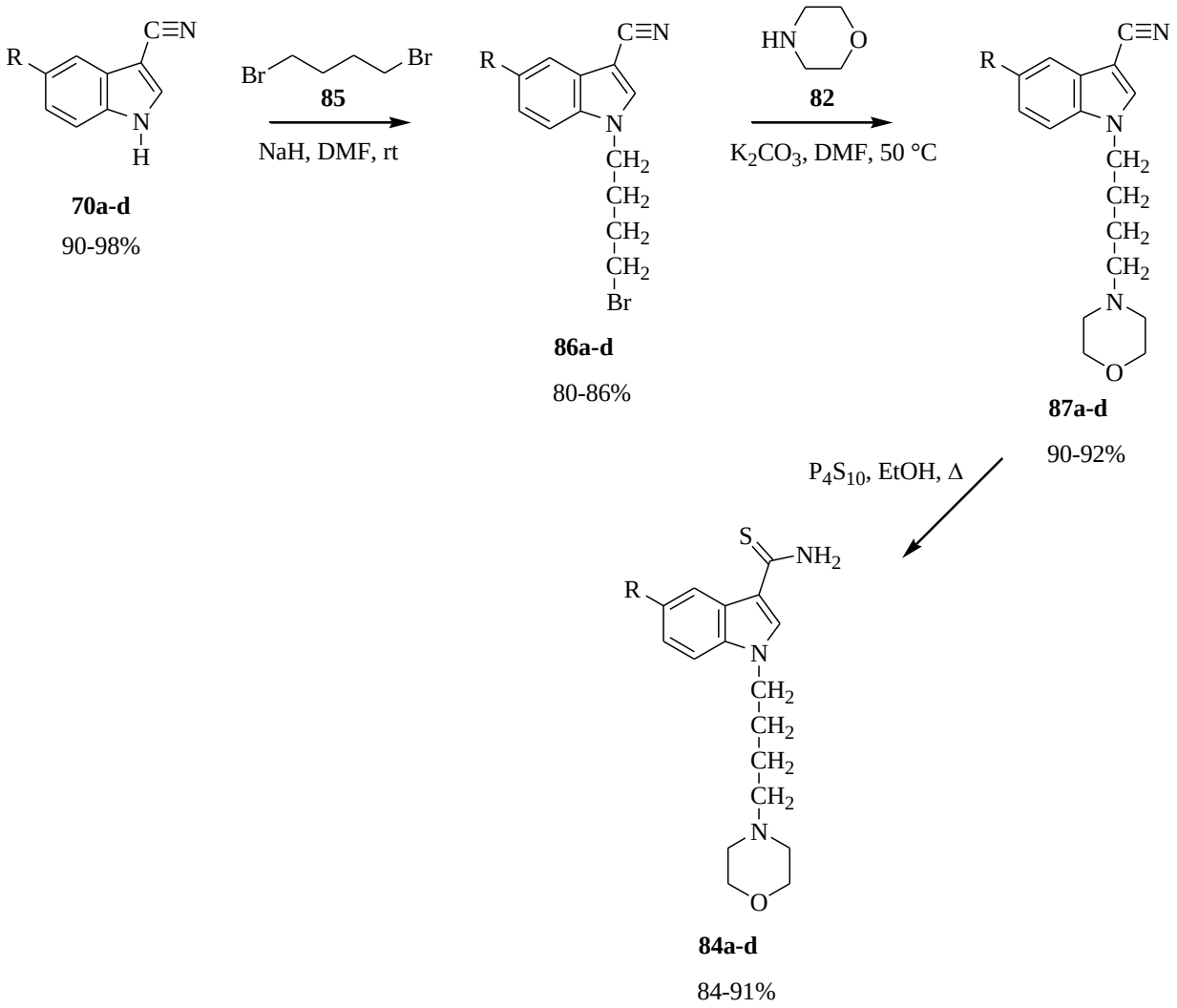
58a-h

Table 10. 3-{2-[1-(4-Morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **58a-h**.

Compd	R	R_1	Yield %
58a	H	H	**82**
58b	H	CH_3	**72**
58c	Br	H	**70**
58d	Br	CH_3	**74**
58e	F	H	**92**
58f	F	CH_3	**95**
58g	OCH_3	H	**76**
58h	OCH_3	CH_3	**88**

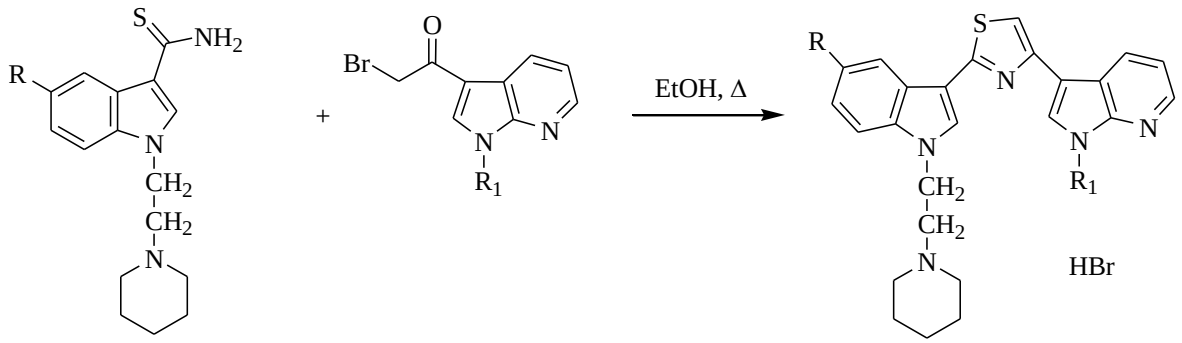
Carbothioamides **84a-d** were obtained in good yields (84-91%) using the same route described for the synthesis of carbothioamides of type **79**. In this case derivatives **70a-d** were reacted with 1,4 dibromobutane **85** affording derivatives **86a-d** in good yields (80-86%). These latter were then dissolved in DMF and treated with morpholine **82** giving derivatives **87a-d** (90-92%) which were finally reacted with P_4S_{10} in ethanol (Scheme 11).

Scheme 11.



3-{2-[1-(2-Piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **59a-h** were prepared from key intermediates **88a-d** and **63a,b**. Reaction of carbothioamides **88a-d** and 3-bromoacetyl-7-azaindole compounds **63a,b** in ethanol under reflux for 30 minutes gave, after crystallization, the desired 7-azaindolyl thiazoles **59a-h** as hydrobromide salts (Scheme 12) in very good yields (70-91%) (Table 11).

Scheme 12.



88a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

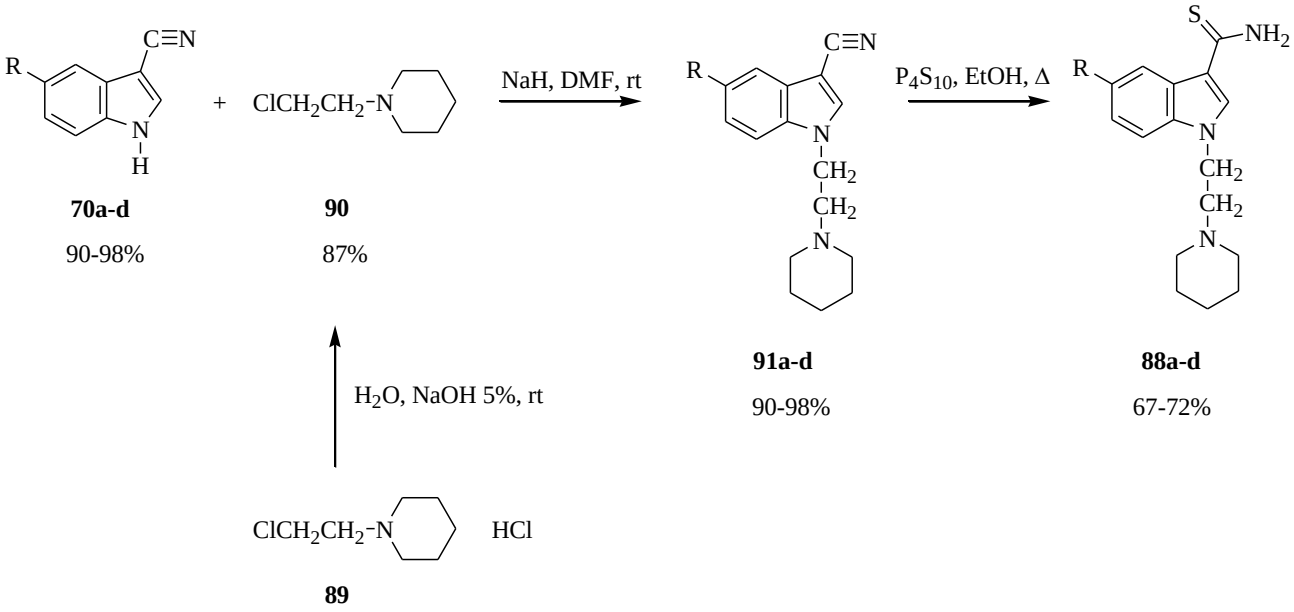
59a-h

Table 11. 3-{2-[1-(2-Piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **59a-h**.

Compd	R	R_1	Yield %
59a	H	H	**86**
59b	H	CH_3	**70**
59c	Br	H	**71**
59d	Br	CH_3	**78**
59e	F	H	**86**
59f	F	CH_3	**91**
59g	OCH_3	H	**80**
59h	OCH_3	CH_3	**91**

Carbothioamides **88a-d** were obtained starting the synthesis from reaction of derivatives **70a-d** and **90** which gave derivatives **91a-d** in excellent yields (90-98%). Compound **90**[127] (87%) synthesized from the commercially available 1-(2-chloroethyl)piperidine hydrochloride **89**, which was solubilized in distilled water and treated with an aqueous solution of sodium hydroxide (NaOH) at 5%. Once obtained, derivatives **91a-d** were reacted with P_4S_{10} in ethanol under reflux affording the desired key intermediates **88a-d** (67-72%) (Scheme 13).

Scheme 13.



70a-d
90-98%

90
87%

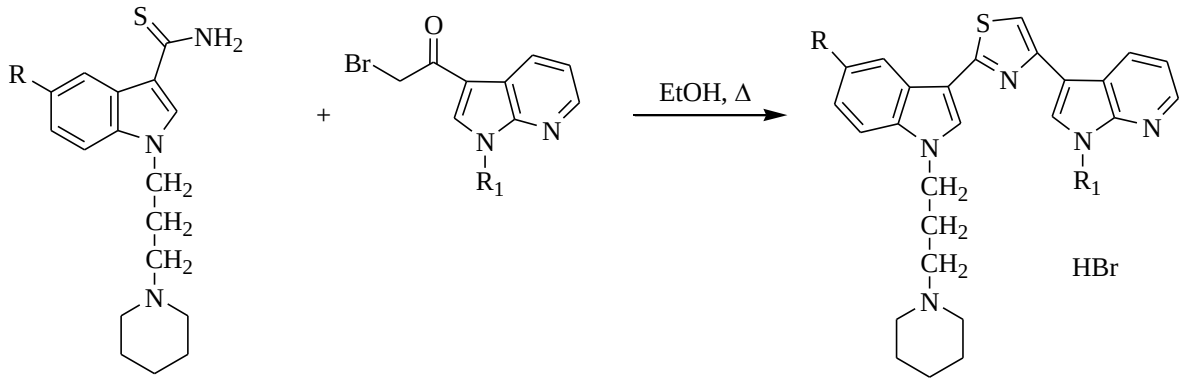
91a-d
90-98%

88a-d
67-72%

89

3-{2-[1-(3-Piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **60a-h** (56-98%) (Table 12) were synthesized from carbothioamides **92a-d** and 3-bromoacetyl compounds **63a,b**, reacted in ethanol under reflux for 30 minutes (Scheme 14).

Scheme 14.



92a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

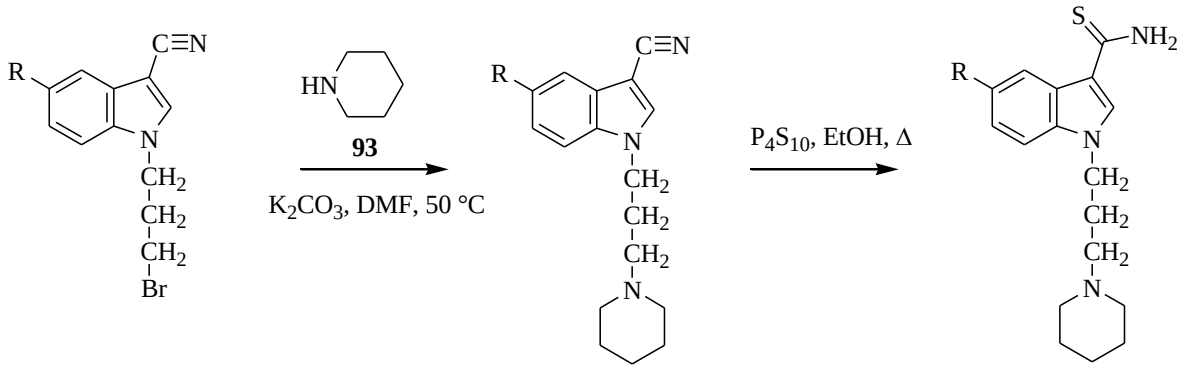
60a-h

Table 12. 3-{2-[1-(3-Piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **60a-h**.

Compd	R	R_1	Yield %
60a	H	H	**64**
60b	H	CH_3	**56**
60c	Br	H	**66**
60d	Br	CH_3	**68**
60e	F	H	**87**
60f	F	CH_3	**98**
60g	OCH_3	H	**82**
60h	OCH_3	CH_3	**65**

The key intermediates **92a-d** were synthesized starting from derivatives **81a-d**, whose synthesis was previously described in scheme 9. These latter were reacted with piperidine **93** affording derivatives **94a-d** in good yields (70-93%). The subsequent reaction of these intermediates with P_4S_{10} in ethanol under reflux gave the desired carbothioamides **92a-d** (80-88%) (Scheme 15).

Scheme 15.



81a-d

70-80%

94a-d

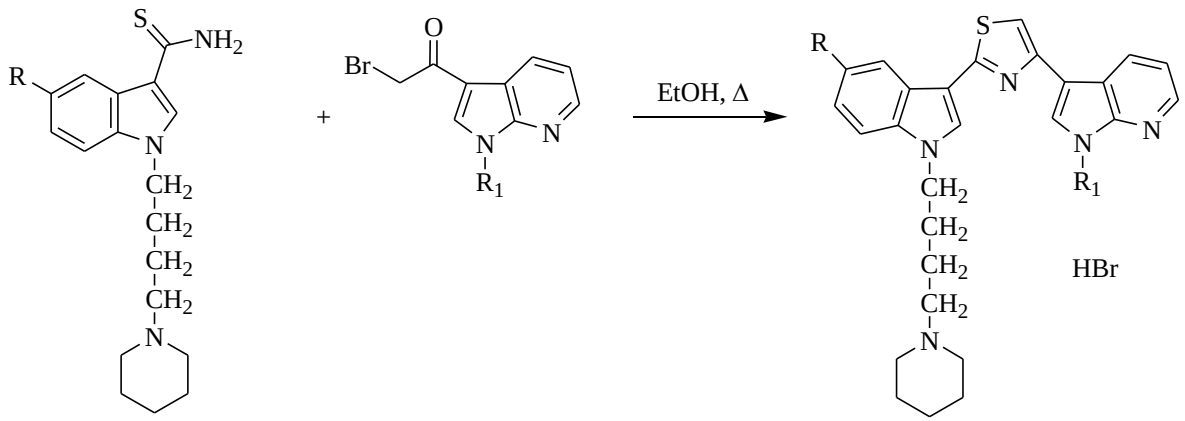
70-93%

92a-d

80-88%

3-{2-[1-(4-Piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **61a-h** were synthesized from carbothioamides **95a-d** and 3-bromoacetyl-7-azaindoles **63a,b**. The reaction, performed in ethanol, was heated under reflux for 30 minutes giving, after crystallization, the desired 7-azaindolyl thiazoles **61a-h** as hydrobromide salts (Scheme 16) in good yields (57-83%) (Table 13).

Scheme 16.



95a-d
a R = H
b R = Br
c R = F
d R = OCH_3

63a,b
a R_1 = H
b R_1 = CH_3

61a-h

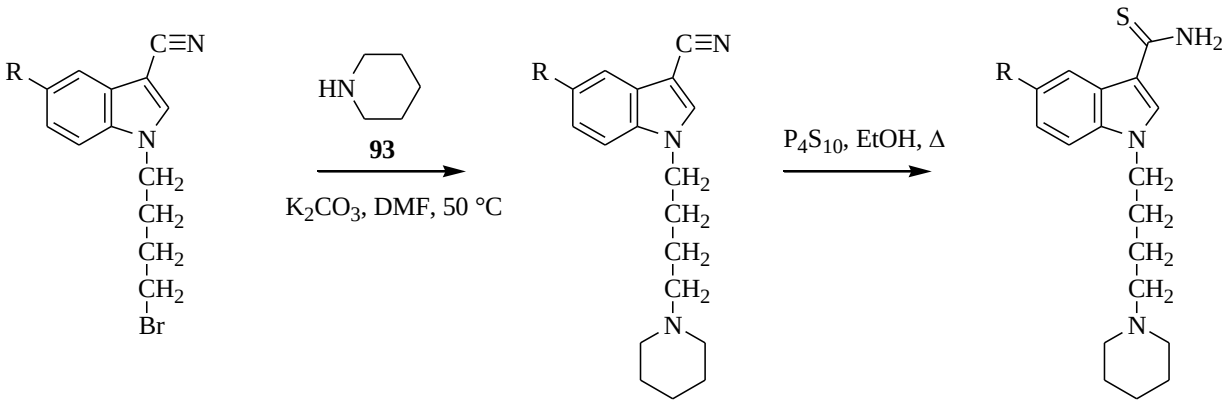
Table 13. 3-{2-[1-(4-Piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides **61a-h**.

Compd	R	R_1	Yield %
61a	H	H	**74**
61b	H	CH_3	**57**
61c	Br	H	**65**
61d	Br	CH_3	**83**
61e	F	H	**70**
61f	F	CH_3	**57**
61g	OCH_3	H	**74**
61h	OCH_3	CH_3	**76**

The key intermediates **95a-d** were synthesized from derivatives **86a-d**, whose synthesis has been previously reported in the scheme 11. In this case derivatives **86a-d** were reacted with piperidine **93** and led to the synthesis of derivatives **96a-d** in excellent yields (82-96%).

Treatment of these latter with P_4S_{10} in ethanol under reflux afforded the desired carbothioamides **95a-d** (56-75%) (Scheme 17).

Scheme 17.



86a-d
80-86%

96a-d
82-96%

95a-d
56-75%

RESULTS AND DISCUSSIONS:BIOLOGY

All synthesized thiazoles **55a-h**, **56a-h**, **57a-h**, **58a-h**, **59a-h**, **60a-h**, **61a-h** and **69a,b** (Tables 7-13) were submitted to the National Cancer Institute (NCI, Bethesda MD) in order to evaluate their antitumor activity.

Derivatives **55a**, **55e-h**, **57a,b**, **57e-g**, **58a**, **58f-h**, **69a,b** were selected, according to the NCI protocol, for the *in vitro* disease-oriented antitumor one dose screening (10^{-5} M) against a panel of about 60 human tumor cell lines derived from 9 human cancer cell types, grouped in disease sub-panel including leukemia, non-small cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cell lines (Tables 14-17).

Among thiazoles bearing the hydroxyethyl chain (**55a**, **55e-h**, Table 14), compounds **55f** and **55g** showed the best results with a mean growth percent value of 52.05 and 48.81 respectively. Both compounds were selective against the breast cancer subpanel with a mean growth percent value of 36.44 and 28.96 respectively, with significant antiproliferative effect against MDA-MB-468 (-16.49 for **55f** and 1.57 for **55g**) for which also derivatives **55a**, **55e** and **55h** showed the lowest growth percent values (4.37, -11.08 and -16.84 respectively). Moreover compound **55g** resulted selective against the renal cancer subpanel (mean growth percent value of 36.14) showing the best result against A498 cell line (mean growth percent value of -0.47)

Thiazoles bearing on the indole nitrogen the morpholine propyl chain (**57a,b,e-g**, Table 15), showed significant antiproliferative effect against the leukemia subpanel for which the best mean growth percent values were shown (16.39 for **57a**, 56.20 for **57b**, 19.85 for **57e**, 37.01 for **57f** and 5.27 for **57g**) if compared to those obtained for other subpanels. The most promising compound was derivative **57g** with a mean growth percent value of 23.90 and showing significant antiproliferative effect in a wide range of human tumor cell lines with different negative growth percent values (e.g. -54.15 for SK-MEL-28 of the melanoma subpanel and -38.46 for COLO-205 of colon cancer subpanel).

Also thiazoles bearing on the indole nitrogen the morpholine butyl chain (**58a**,**f-h**, Table 16) showed significant antiproliferative effect against the leukemia subpanel for which negative growth percent values were recorded for several cell lines. The mean growth percent values were -10.21 for compound **58a**, 16.6 for **58f**, -18.35 for compound **58g** and -8.95 for compound **58h**. Moreover they resulted active against melanoma subpanel with mean growth percent values of 19.44 for **58a**, 0.99

for **58f**, -12.15 for **58g** and -26.26 for **58h**. The most sensitive cell line of this subpanel was SK-MEL-28 in which the lower growth percent values were reached (-71.09, -87.10, -90.30, -93.55 for compounds **58a**, **58f**, **58g**, **58h** respectively). Compounds **58a**, **58g** and **58h** resulted also significant active against the colon cancer subpanel with mean growth percent values of 3.21, -29.07 and -14.55 respectively. The most active compound of this series was **58h** that also showed good results against the CNS cancer subpanel (mean growth percent values of 9.75).

Table 14. Mean Growth Percent of compounds **55a**, **55e-h**.

Cell line	**Growth Percent**				
	55a	**55e**	**55f**	**55g**	**55h**
Leukemia					
CCRF-CEM	84.83	55.96	44.39	41.27	43.33
HL-60(TB)	79.15	60.30	50.19	57.95	82.42
K-562	54.21	21.52	18.07	43.53	25.19
MOLT-4	71.22	54.54	46.70	54.99	58.24
RPMI-8226	57.82	53.59	62.58	39.90	50.54
SR	53.52	22.02	20.68	37.80	58.21
Non-Small Cell Lung Cancer					
A549/ATCC	77.52	62.98	49.82	66.94	79.82
EKVX	31.97	37.21	57.39	34.94	67.48
HOP-62	74.66	71.90	52.56	53.72	81.17
HOP-92	57.96	52.34	64.83	48.59	70.38
NCI-H226	58.02	48.45	76.00	36.54	70.08
NCI-H23	71.85	76.24	74.14	62.51	89.42
NCI-H322M	74.83	66.32	79.14	47.56	77.68
NCI-H460	78.63	65.73	27.05	46.34	74.87
NCI-H522	58.57	45.48	10.05	36.85	60.52
Colon Cancer					
COLO-205	85.90	66.44	46.03	56.94	81.17
HCC-2998	51.97	65.25	64.10	58.93	95.10
HCT-116	85.20	62.42	35.81	50.88	74.16
HCT-15	55.93	41.55	41.53	31.95	61.08
HT29	95.02	46.78	18.17	54.51	75.26
KM12	47.97	28.35	30.63	20.67	49.46
SW-620	91.50	57.93	29.76	63.07	93.58
CNS Cancer					
SF-268	84.12	77.02	77.60	66.57	91.11
SF-295	81.09	72.08	52.31	57.87	74.12
SF-539	88.45	81.46	69.94	49.99	75.02
SNB-19	88.11	79.45	69.62	82.36	90.18
SNB-75	55.18	72.48	46.39	31.73	91.34
U251	78.90	62.89	49.29	53.21	78.50
Melanoma					
LOX IMVI	77.72	65.50	44.78	42.77	59.29
MALME-3M	115.31	84.92	72.82	75.87	110.27
M14	97.75	63.84	35.74	59.33	75.10

MDA-MB-435	52.14	5.03	1.70	66.48	86.53
SK-MEL-2	98.57	66.20	52.17	48.68	92.99
SK-MEL-28	103.64	89.81	74.35	78.53	85.97
SK-MEL-5	77.50	63.04	62.89	58.43	74.18
UACC-257	93.05	87.81	77.37	91.02	92.45
UACC-62	69.91	63.27	52.39	58.44	80.45
Ovarian Cancer					
IGROV1	56.06	44.74	51.91	43.42	85.16
OVCAR-3	81.72	81.00	49.69	78.73	99.08
OVCAR-4	51.66	52.35	35.97	42.13	45.54
OVCAR-5	90.49	84.34	65.58	68.17	75.99
OVCAR-8	90.45	80.18	75.86	56.23	73.03
NCI/ADR-RES	94.60	82.57	45.45	76.04	90.04
SK-OV-3	70.16	70.10	73.03	30.01	89.77
Renal Cancer					
786-0	95.08	81.93	73.32	59.49	79.98
A498	34.69	22.98	45.35	-0.47	49.34
ACHN	69.30	65.80	69.49	26.66	72.10
CAKI-1	73.74	70.74	78.44	35.50	88.75
RXF 393	82.39	51.09	69.68	30.14	84.63
SN12C	80.02	80.34	73.19	63.51	86.11
TK-10	82.50	62.06	47.68	58.30	82.97
UO-31	37.83	39.36	70.72	16.06	67.69
Prostate Cancer					
PC-3	61.81	48.74	44.63	48.97	69.10
DU-145	93.48	83.68	95.38	56.48	90.94
Breast Cancer					
MCF7	32.92	48.11	34.86	20.06	12.04
MDA-MB-231/ATCC	66.42	64.47	57.93	51.51	56.96
HS 578T	71.29	70.93	79.85	33.51	80.94
BT-549	76.05	68.74	52.77	41.74	76.92
T-47D	37.25	35.17	9.75	23.37	10.12
MDA-MB-468	4.37	-11.08	-16.49	1.57	-16.84
MEAN	**71.57**	**59.61**	**52.05**	**48.81**	**72.12**

Table 15. Mean Growth Percent of compounds **57a,b,e-g**.

Cell line	Growth Percent				
	57a	**57b**	**57e**	**57f**	**57g**
Leukemia					
CCRF-CEM	17.37	61.99	21.82	36.37	13.08
HL-60(TB)	35.82	70.87	21.94	69.93	17.38
K-562	1.22	26.24	1.71	0.48	-5.12
MOLT-4	16.58	59.70	24.79	32.14	-1.04
RPMI-8226	24.42	59.46	36.64	34.76	0.66
SR	2.93	58.94	12.05	48.37	6.64
Non-Small Cell Lung Cancer					
A549/ATCC	54.36	79.29	42.57	66.12	42.02
EKVX	47.89	86.74	55.89	63.03	57.79
HOP-62	48.69	97.37	49.87	71.71	28.86
HOP-92	12.00	67.73	23.52	40.78	27.92
NCI-H226	61.48	75.71	59.29	51.90	45.41
NCI-H23	68.07	88.33	76.03	63.75	56.63
NCI-H322M	80.19	95.59	80.89	78.98	42.07
NCI-H460	45.44	88.52	37.80	41.00	25.70
NCI-H522	56.88	67.86	41.25	49.55	26.28
Colon Cancer					
COLO-205	31.23	73.92	18.08	51.26	-38.46
HCC-2998	58.63	92.88	74.26	65.42	65.52
HCT-116	29.79	84.38	40.44	72.31	25.28
HCT-15	13.98	55.53	24.47	27.08	13.73
HT29	7.69	32.87	12.87	1.97	-12.94
KM12	28.13	85.41	22.50	34.29	17.01
SW-620	39.48	94.62	24.95	61.03	21.22
CNS Cancer					
SF-268	52.34	91.69	50.78	66.61	43.86
SF-295	16.71	79.49	27.54	11.21	-34.44
SF-539	52.87	89.41	67.35	57.57	33.56
SNB-19	56.82	90.84	62.75	77.12	54.40
SNB-75	20.15	82.60	41.24	87.14	21.35
U251	32.94	64.25	30.35	51.05	16.32
Melanoma					
LOX IMVI	30.62	79.04	33.07	26.64	17.02
MALME-3M	61.14	107.68	59.21	87.38	5.55
M14	14.52	83.58	34.67	46.22	10.02
MDA-MB-435	6.18	91.58	-17.10	65.35	46.96
SK-MEL-2	82.63	91.75	60.49	86.29	11.35
SK-MEL-28	65.30	88.64	75.37	77.55	-54.15
SK-MEL-5	38.03	64.56	43.73	25.11	31.61
UACC-257	85.05	89.28	82.95	78.50	49.86
UACC-62	65.45	92.47	69.46	72.22	44.12
Ovarian Cancer					
IGROV1	35.27	98.56	38.63	76.25	22.87
OVCAR-3	64.40	96.32	59.80	77.57	44.02
OVCAR-4	45.12	97.79	56.22	56.59	41.53

OVCAR-5	62.12	84.72	82.28	72.99	68.08
OVCAR-8	62.43	82.60	59.72	47.59	25.31
NCI/ADR-RES	60.09	91.03	59.24	53.78	43.42
SK-OV-3	73.40	101.93	81.63	80.02	51.85
Renal Cancer					
786-0	28.39	74.44	57.29	57.43	-24.31
A498	53.86	55.20	50.99	36.14	27.80
ACHN	32.81	93.66	42.23	63.78	14.53
CAKI-1	47.46	92.03	56.15	78.99	28.83
RXF 393	24.87	65.74	39.51	36.07	-9.75
SN12C	64.49	87.16	73.90	78.48	52.65
TK-10	69.59	86.85	66.89	75.05	51.47
UO-31	23.03	70.98	31.54	50.24	11.87
Prostate Cancer					
PC-3	19.59	67.97	31.93	29.63	18.25
DU-145	82.03	97.89	79.79	80.30	41.48
Breast Cancer					
MCF7	28.62	85.65	22.00	34.09	13.30
MDA-MB-231/ATCC	27.10	76.52	36.04	25.09	9.85
HS 578T	28.47	87.98	38.31	46.61	2.12
BT-549	50.28	88.71	69.46	71.63	52.16
T-47D	27.08	62.10	38.14	41.15	31.70
MDA-MB-468	26.19	65.12	18.29	49.32	41.99
MEAN	**41.66**	**80.03**	**45.26**	**54.95**	**23.90**

Table 16. Mean Growth Percent of compounds **58a**,**f-h**.

Cell line	Growth Percent			
	58a	**58f**	**58g**	**58h**
Leukemia				
CCRF-CEM	10.12	-59.55	-8.39	2.06
HL-60(TB)	-58.24	76.21	7.66	24.65
K-562	-38.72	-49.74	-65.23	-41.02
MOLT-4	13.10	46.68	-21.00	-15.65
RPMI-8226	32.60	49.01	-5.60	-18.11
SR	-20.13	36.99	-17.53	-5.63
Non-Small Cell Lung Cancer				
A549/ATCC	56.90	75.11	49.70	48.31
EKVX	64.03	72.68	68.39	60.22
HOP-62	52.81	85.65	37.11	30.92
HOP-92	31.99	52.76	43.00	47.86
NCI-H226	72.43	60.21	70.25	70.13
NCI-H23	81.08	80.34	69.92	78.41
NCI-H322M	78.98	84.33	57.23	65.88
NCI-H460	31.44	93.47	4.29	-31.92
NCI-H522	49.07	57.18	39.91	3.68
Colon Cancer				
COLO-205	-77.55	65.94	-100.00	91.73
HCC-2998	56.87	92.42	26.72	7.68
HCT-116	40.32	83.00	32.53	11.37
HCT-15	5.17	61.67	-31.70	-42.12
HT29	-44.59	-64.03	-84.42	-87.82
KM12	24.13	52.48	16.39	-5.62
SW-620	18.09	19.68	-63.03	-77.06
CNS Cancer				
SF-268	49.73	63.04	52.37	52.79
SF-295	53.93	50.80	13.11	-48.46
SF-539	80.75	69.69	49.34	30.77
SNB-19	57.53	88.19	52.44	35.14
SNB-75	35.43	61.94	40.11	43.61
U251	20.05	67.27	4.84	-55.35
Melanoma				
LOX IMVI	20.76	67.61	-34.71	-78.84
MALME-3M	-24.96	-60.28	-66.68	-59.54
M14	-49.93	-53.87	-53.54	-66.87
MDA-MB-435	24.36	38.13	-59.02	-83.43
SK-MEL-2	87.77	78.72	68.98	64.35
SK-MEL-28	-71.09	-87.10	-90.30	-93.55
SK-MEL-5	51.25	48.10	25.97	14.46
UACC-257	67.84	-45.12	79.50	63.45
UACC-62	68.93	22.77	20.41	3.59
Ovarian Cancer				
IGROV1	34.89	65.37	36.07	67.80
OVCAR-3	59.85	89.99	58.26	50.81
OVCAR-4	74.82	84.55	50.78	60.17

OVCAR-5	72.49	79.47	58.31	56.86
OVCAR-8	60.59	81.26	55.65	48.51
NCI/ADR-RES	69.40	76.81	63.33	59.59
SK-OV-3	81.14	83.97	78.20	79.71
Renal Cancer				
786-0	53.95	63.16	-43.75	-71.67
A498	56.81	46.69	92.30	58.14
ACHN	32.35	86.29	17.11	32.00
CAKI-1	44.28	76.10	37.46	41.38
RXF 393	53.71	16.25	31.90	0.73
SN12C	66.97	72.16	61.90	67.98
TK-10	64.41	75.37	64.45	69.88
UO-31	25.40	44.41	22.75	33.97
Prostate Cancer				
PC-3	25.88	49.30	30.11	31.33
DU-145	70.03	100.06	48.85	46.77
Breast Cancer				
MCF7	25.14	78.36	18.08	15.10
MDA-MB-231/ATCC	32.80	35.20	23.36	5.75
HS 578T	54.86	41.06	44.77	18.76
BT-549	79.64	65.25	84.19	76.80
T-47D	36.13	76.87	47.97	64.50
MDA-MB-468	35.05	65.01	39.27	34.99
MEAN	**36.05**	**50.59**	**20.84**	**13.44**

Thiazoles **69a** and **69b**, bearing on the indole ring the ethyl carbamic chain, also showed inhibitory activity against several tumor cell lines (Table 17). In particular derivative **69a** showed the best mean growth percent value (33.31) with selectivity against leukemia, colon cancer and melanoma with mean growth percent values for these subpanels of 18.06, 24.98 and 21.92 respectively.

Table 17. Mean Growth Percent of compounds **69a** and **69b**.

Cell line	**Growth Percent**			**Growth Percent**	
	69a	**69b**		**69a**	**69b**
Leukemia			**Melanoma**		
CCRF-CEM	21.73	73.88	LOX IMVI	30.07	79.02
HL-60(TB)	6.14	86.87	MALME-3M	62.40	66.40
K-562	11.77	27.31	M14	11.83	71.12
MOLT-4	19.56	69.10	MDA-MB-435	-33.36	8.15
RPMI-8226	24.94	74.08	SK-MEL-2	2.38	84.85
SR	24.20	20.26	SK-MEL-28	61.66	92.51
Non-Small Cell Lung Cancer			SK-MEL-5	18.49	71.87
A549/ATCC	36.87	67.44	UACC-257	60.54	87.95
EKVX	42.11	68.74	UACC-62	32.91	72.20
HOP-62	39.17	74.46	**Ovarian Cancer**		
HOP-92	49.11	83.12	IGROV1	41.28	77.68
NCI-H226	53.00	86.41	OVCAR-3	27.93	94.94
NCI-H23	63.27	81.22	OVCAR-4	71.72	101.74
NCI-H322M	65.91	90.80	OVCAR-5	58.25	104.16
NCI-H460	17.82	82.36	OVCAR-8	41.57	89.35
NCI-H522	5.49	24.21	NCI/ADR-RES	38.11	82.90
Colon Cancer			SK-OV-3	41.64	95.18
COLO 205	4.32	77.56	**Renal Cancer**		
HCC-2998	71.67	90.44	786-0	42.87	87.36
HCT-116	30.79	86.50	A498	-11.71	19.02
HCT-15	28.04	63.95	ACHN	38.02	96.99
HT29	8.34	47.64	CAKI-1	34.47	88.24
KM12	23.03	67.04	RXF 393	46.30	91.38
SW-620	8.68	67.87	SN12C	49.10	88.32
CNS Cancer			TK-10	62.30	82.56
SF-268	50.00	95.79	UO-31	34.11	79.58
SF-295	25.51	86.25	**Prostate Cancer**		
SF-539	33.76	92.69	PC-3	33.22	63.56
SNB-19	42.23	78.64	DU-145	64.92	103.20
SNB-75	10.57	80.75	**Breast Cancer**		
U251	20.01	77.77	MCF7	18.26	58.18
			MDA-MB-231/ATCC	49.72	80.37
			HS 578T	41.41	92.58

	BT-549	32.84	79.46
	T-47D	44.70	45.80
	MDA-MB-468	12.45	16.23
MEAN	**69a: 33.31** **69b: 74.60**		

On the basis of the obtained results, compounds **57g**, **58a**, **58g**, **58h**, and **69a**, satisfying the criteria set by the NCI for activity, were selected for further screenings at 5 concentrations at 10-fold dilution (10^{-4}-10^{-8} M) on the full panel (Table 18). The antitumor activity of compounds was given by three parameters for each cell line: GI_{50} (GI_{50} is the molar concentration of the compound that inhibits 50% net cell growth), TGI (TGI is the molar concentration of the compound leading to total inhibition of net cell growth), and LC_{50} (LC_{50} is the molar concentration of the compound that induces 50% net cell death). The average values of mean graph midpoint (MG_MID) were calculated for each of these parameters.

Table 18. *In vitro* inhibition of cancer cell line growth by compounds **57g**, **58a**, **58g**, **58h**, **69a** (μM)[a].

Cell line	**GI_{50} (μM)**				
	57g	**58a**	**58g**	**58h**	**69a**
Leukemia					
CCRF-CEM	2.76	1.98	2.12	0.45	2.65
HL-60(TB)	2.11	1.96	1.93	1.67	2.12
K-562	2.08	2.02	1.95	0.24	1.55
MOLT-4	1.85	1.81	1.86	0.60	2.19
RPMI-8226	1.92	1.83	1.99	0.77	2.04
SR	2.30	1.91	2.01	0.27	1.08
Non-Small Cell Lung Cancer					
A549/ATCC	4.04	1.89	2.23	1.41	2.34
EKVX	3.01	2.07	2.66	1.50	1.30
HOP-62	2.24	1.70	1.91	1.72	1.07
HOP-92	2.42	1.43	1.67	0.52	0.67
NCI-H226	3.10	2.29	2.46	2.56	1.68
NCI-H23	2.68	2.04	2.15	2.02	2.02
NCI-H322M	1.96	1.79	1.82	1.63	1.95
NCI-H460	2.54	1.88	1.99	0.77	2.46
NCI-H522	2.07	1.95	2.34	1.64	1.49
Colon Cancer					
COLO-205	1.82	1.73	1.85	1.49	2.06
HCC-2998	2.04	1.74	1.91	1.47	1.99
HCT-116	1.85	1.65	1.78	0.28	1.72
HCT-15	2.32	1.81	1.96	0.24	1.92
HT29	2.22	2.10	2.18	0.31	1.77

KM12	2.41	1.72	1.93	1.09	1.44
SW-620	2.26	1.77	1.99	0.31	1.30
CNS Cancer					
SF-268	2.69	1.76	1.80	1.34	1.48
SF-295	1.91	1.92	1.93	1.53	2.29
SF-539	1.74	1.71	1.67	1.34	1.61
SNB-19	2.86	1.76	1.91	1.49	2.57
SNB-75	1.27	1.19	1.31	0.52	ND[b]
U251	2.25	1.81	1.73	0.81	1.91
Melanoma					
LOX IMVI	1.74	1.74	1.71	1.04	1.55
MALME-3M	1.39	1.32	1.55	1.59	ND[b]
M14	1.77	1.78	1.81	0.19	1.33
MDA-MB-435	1.75	1.72	1.70	0.76	ND[b]
SK-MEL-2	1.88	1.95	1.97	2.05	1.52
SK-MEL-28	1.95	1.81	1.85	1.32	2.05
SK-MEL-5	1.83	1.73	1.75	1.59	1.41
UACC-257	2.04	1.84	2.18	1.49	2.08
UACC-62	1.97	1.79	1.87	1.48	1.57
Ovarian Cancer					
IGROV1	2.09	1.43	2.48	3.43	0.14
OVCAR-3	2.57	1.64	1.87	1.46	1.27
OVCAR-4	2.14	2.06	2.21	1.48	1.78
OVCAR-5	2.60	1.82	1.99	2.03	2.21
NCI/ADR-RES	3.02	2.04	2.34	1.56	2.82
SK-OV-3	2.83	2.34	2.37	5.78	1.54
Renal Cancer					
786-0	1.74	1.85	1.78	0.42	2.38
A498	0.48	ND[b]	0.22	0.02	0.91
ACHN	2.50	1.79	1.79	1.50	2.05
CAKI-1	1.88	1.74	1.80	1.39	2.42
RXF 393	2.09	1.67	1.73	0.93	1.78
SN12C	3.16	2.98	1.91	6.05	2.46
TK-10	3.48	2.77	5.25	4.25	2.24
UO-31	1.37	1.15	1.43	1.11	0.62
Prostate Cancer					
PC-3	2.48	1.59	1.75	0.73	1.87
DU-145	3.15	2.12	3.07	1.44	3.07
Breast Cancer					
MCF7	1.43	1.56	1.56	1.65	0.99
MDA-MB-231/ATCC	1.86	1.58	1.70	0.52	1.10
HS 578T	2.08	1.77	2.05	0.77	1.07
BT-549	2.00	2.13	3.74	2.97	1.50
T-47D	2.07	1.33	2.12	12.00	ND[b]
MDA-MB-468	2.52	2.07	1.95	0.97	1.33

[a] Data obtained from NCI's in vitro disease-oriented tumor cells screen

[b] ND = Not Determined

An evaluation of the data reported in table 19 pointed out that all derivatives resulted active from micromolar to nanomolar concentration, against most of tested cell lines, as it has been confirmed by the range of GI_{50} value of 4.04-0.48, 2.98-1.15, 5.25-0.22, 12.00-0.02, 3.07-0.14 μM, respectively for compounds **57g**, **58a**, **58g**, **58h**, and **69a**.

Compound **58h**, resulted more active than thiazoles **57g**, **58a**, **58g**, and **69a** in terms either of GI_{50} (mean value 1.59, 2.21, 1.83, 2.01 and 1.82 μM, respectively) and percentage of sensitive cell lines out of the total number of cell lines investigated (100%, 100%, 98%, 100%, and 93% respectively).

It showed a remarkable inhibitory activity against a wide range of tumor cell lines. The leukemia subpanel resulted the most sensitive, showing a range of concentrations from micromolar to submicromolar (1.67-0.24 μM) followed by the colon cancer with a range from 1.49 to 0.24 μM.

Table 19. Overview of compounds **57g**, **58a**, **58g**, **58h**, **69a**.

Compd	**N° of cell line tested**	**N° of active cell lines**	**GI_{50} (μM)**	
			Range	**MG_MID**
57g	59	59	4.04-0.48	2.21
58a	59	58	2.98-1.15	1.83
58g	59	59	5.25-0.22	2.01
58h	59	59	12.00-0.02	1.59
69a	59	55	3.07-0.14	1.82

However studies on the potential cytotoxic activity of the other thiazoles synthesized **56a-h**, **59a-h**, **60a-h**, **61a-h** are in progress, as well as the evaluation of the selectivity of all the compounds **55a-h**, **56a-h**, **57a-h**, **58a-h**, **59a-h**, **60a-h**, **61a-h** against GSK-3β target.

DOCKING STUDIES

GSK-3β has the typical two-domain kinase fold[128-129] with a β-strand domain (residues 25-138, blue in Figure 12) at the N-terminal end and an α-helical domain at the C-terminal end (residues 139-343, magenta in Figure 12). The ATP-binding site is at the interface of the α-helical and β-strand domain and is bordered by the glycine-rich loop and the hinge. The activation loop (residues 200-226) runs along the surface of the substrate binding groove. The C-terminal 39 residues (residues 344-382) are outside the core kinase fold and form a small domain that packs against the α-helical domain.

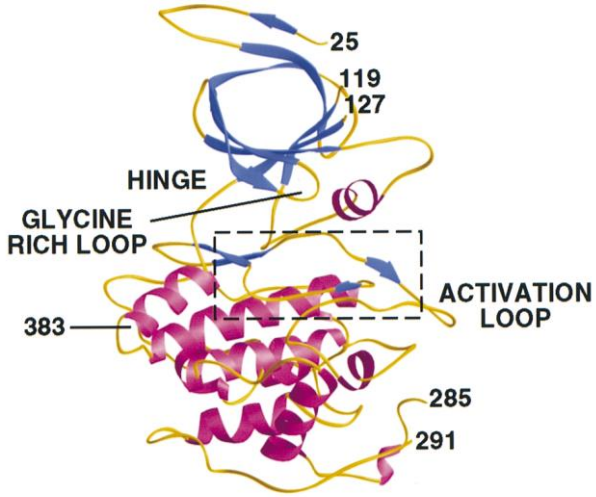


Figure 12. Structure of GSK-3β.

The β-strand domain consists of seven antiparallel β-strands: strands 2-6 form a β-barrel that is interrupted between strand 4 and 5 by a short helix (residue 96-102) that packs against the β-barrel. This helix is conserved in all kinases, and two of its residues play key roles in the catalytic activity of the enzyme. Arg 96 (R96, Figure 13) is involved in the alignment of the two domains. Glu 97 is positioned in the active site and forms a salt bridge with Lys 85, a key residue in catalysis. GSK-3β has two phosphorylation sites that influence the catalytic activity of the protein. Ser 9 is the phosphorylation site for Akt, and the phosphorylation of this residue inactivates GSK-3β. Tyr 216 (Y216, Figure 13), located on the activation loop, plays an important key role in the opening and closing of the substrate binding site.[130]

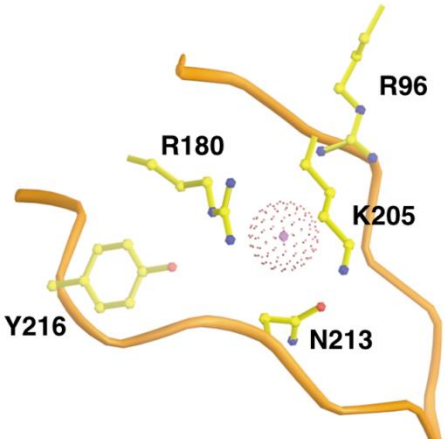
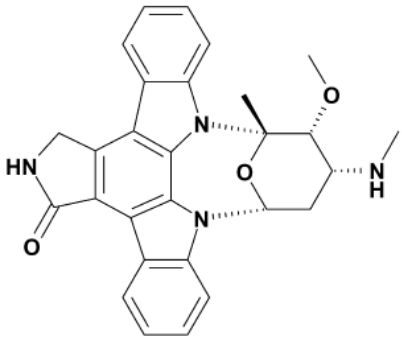


Figure 13. Activation loop of GSK-3β.

Different X-ray crystallographic structures of GSK-3β were published providing further information about its regulation mechanism, its kinase activity and its preference for pre-phosphorylated substrates.

In the crystal structure with pdb 1Q3D[131], GSK-3β is in complex with Staurosporine, a potent non-selective inhibitor for which is reported a IC_{50} value of 15 nM.[132]



Staurosporine

This structure reveals how the inhibitor binds the ATP-binding site. It utilizes a water network to bind the enzyme in a unique manner. Staurosporine infact, interacts through hydrogen bonds between its N1 and Asp 133 carbonyl oxygen of the hinge, and its O5 and the backbone nitrogen of Val 135. Moreover it establishes a water-polar-mediated interaction between the methylamino nitrogen (N4) of the glycosidic ring and the carbonyl oxygen of Gln 185. In the staurosporine complex, this water molecule is part of a hydrogen-bonding network that starts with O^{γ} of Thr 138, passes through four water molecules and ends with the carbonyl oxygen of Val 135 (Figure 14).

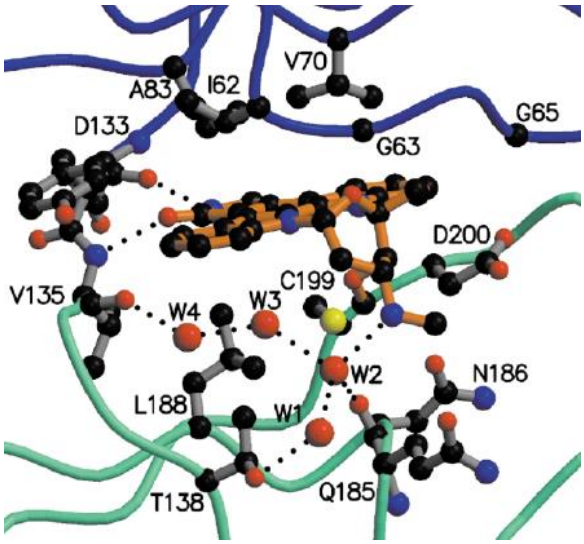


Figure 14. Staurosporine-GSK-3β complex in 1Q3D crystal structure.

In the crystal structure with pdb 1Q4I[131], indirubin-3'-monoxime, an extremely potent GSK-3β inhibitor (IC_{50} = 22 nM), is co-crystallized with GSK-3β.

The structure reveals a donor-acceptor-donor series of hydrogen bonds between the ligand and the hinge residues of GSK-3β. The N1 and O2 atoms form hydrogen bonds with the carbonyl oxygen of Asp 133 and the backbone nitrogen of Val 135, respectively (Figure 15).

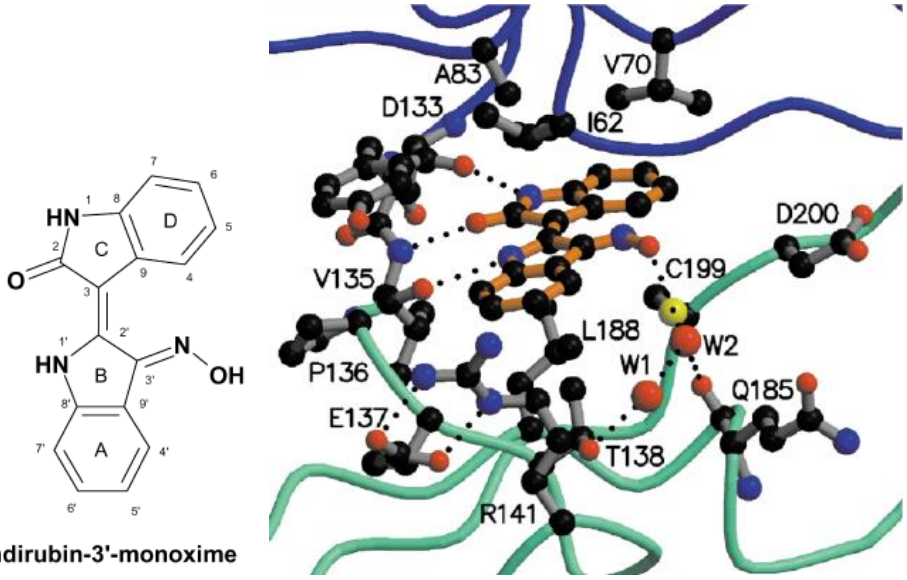


Figure 15. Indirubin-3'-monoxime-GSK3β complex in 1Q4I crystal structure.

The co-crystal structure 1Q3W[131], shows alsterpaullone bound in the GSK-3β active site (Figure 16). Alsterpaullone interact with the hinge area through two direct hydrogen bonds with Val 135 and one water mediated interaction with Asp 133. Moreover the N5 and carbonyl oxygen atoms of alsterpaullone make a pair of hydrogen bonds with the backbone nitrogen and carbonyl oxygen of Val 135, respectively, and the water molecule bridges between the carbonyl oxygen atoms of alsterpaullone and Asp 133. In addition to the hinge interactions, alsterpaullone also makes polar interactions between the nitro group in position 9 and the side-chain amino group of Lys 85. One feature that makes alsterpaullone unique is the positioning of its coupled ring systems.

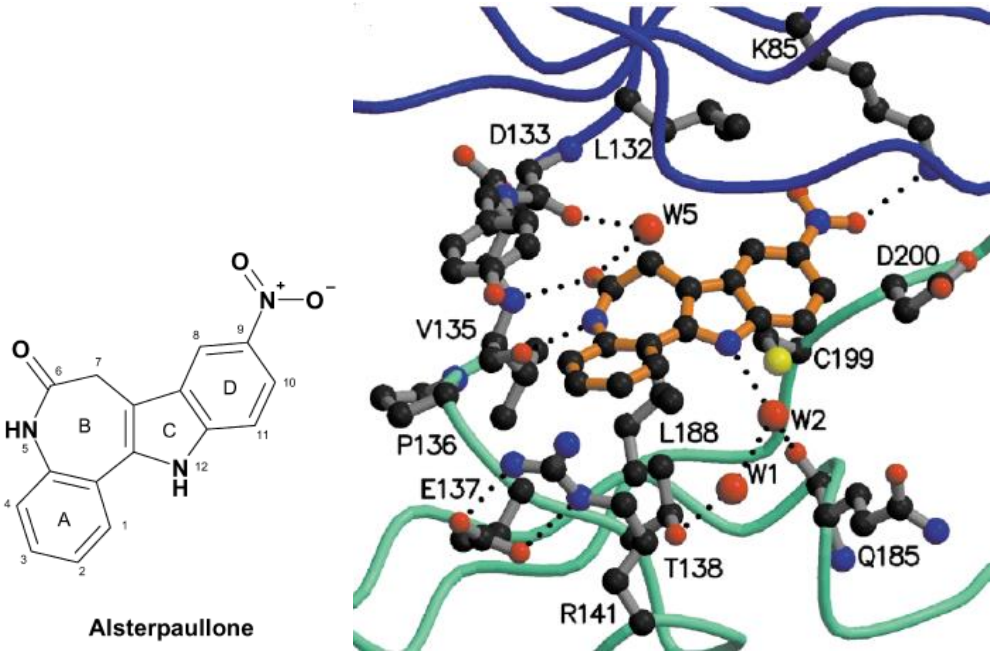


Figure 16. Alsterpaullone-GSK-3β complex in 1Q3W crystal structure.

In the complex with GSK-3β, the inhibitor's seven membered ring is situated slightly below the adenine-binding pocket and the pucker of ring B directs the attached ring. Ring A extends downward, following the path of the hinge towards the guanidine group of Arg 141. Rings C and D extend away from the hinge, passing through the ribose pocket and up towards the side-chain amino group of Lys 85. The shape of alsterpaullone also allows it to bury itself in the GSK-3β active site.

The X-ray crystal structure 2OW3[133], shows a bis-indolyl maleimidic derivative (**BIM**) complexed to GSK-3β in the ATP binding pocket (Figure 17). The maleimide portion makes key hydrogen bonding contacts with residues Asp 133 (amide CO) and Val 135 (Nα). The other maleimide CO hydrogen bonds with a water molecule that bridges to Asp 200 (Nα). **BIM** ligand makes also hydrophobic contacts with the side chains of Ile 62, Phe 67, Thr 138, Leu 188, and Cys 199.

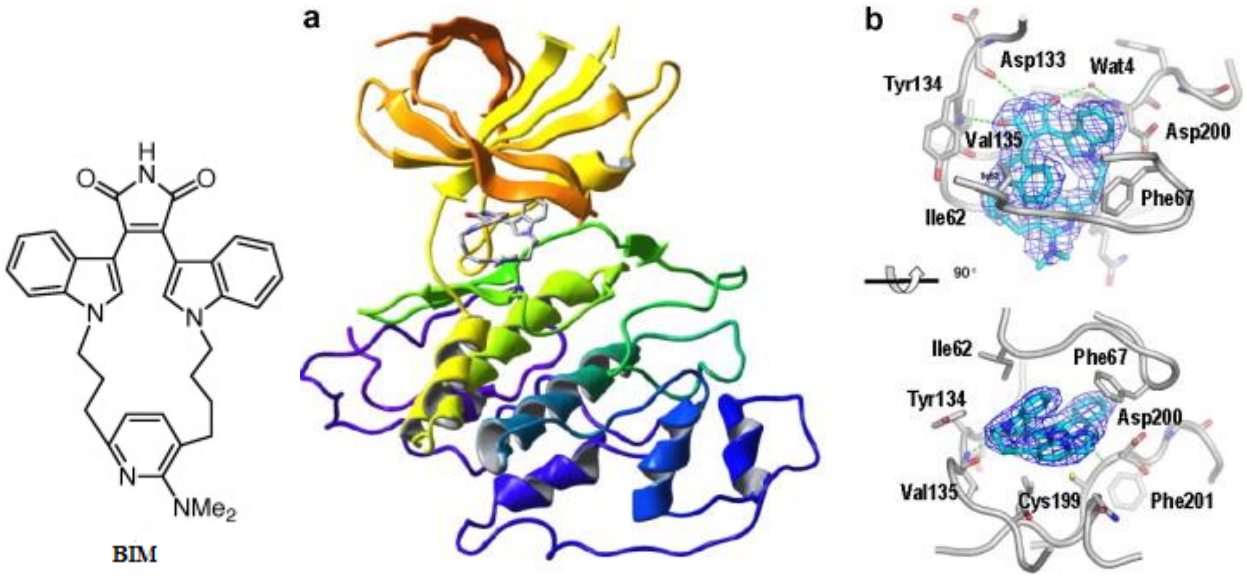


Figure 17. a) BIM-GSK-3β complex in 1Q3W crystal structure; b) two views of the complexed ligand, rotated 90° relative to each other.

The analysis of the interactions between GSK-3β structures and different inhibitors reveals how the enzyme can accommodate a number of diverse molecular scaffolds. The comparison of all these structures shows that the interaction of selective, and nonselective, inhibitors with GSK-3β usually involves key hydrogen bonding of the inhibitor ligand with Asp 133 and Val 135, which reside at the 'hinge region' of the ATP binding site.

In order to verify if the synthesized compounds are able to potentially interact with GSK-3β by forming the key hydrogen bonds observed for known inhibitors, docking studies were performed on this target.

For this purpose, different structures were preliminary used. All X-ray structures were downloaded from the Protein Data Bank (http://www.rcsb.org) and prepared using Schrodinger's Protein Preparation Wizard of Prime module[134] for protein structure refinement, through which the subunit B and its co-crystallized ligand were removed.

Ligands were prepared considering their protonated forms at physiological pH. Docking studies were performed using Extra Precision mode (XP) of Glide module of Schrodinger.[135] In order to evaluate the reliability of Glide protocol, the re-docking of the co-crystallized ligand was performed and its pose was compared to that of the crystallographic structure.

Considering the better results obtained in re-docking and docking procedures, the X-ray structure with pdb 1Q3D was selected for our studies.

All compounds bind GSK-3β similarly to Staurosporine. In table 20 are shown the docking score results and the key H-bond interactions for the synthesized compounds. The best results were obtained for derivatives bearing the free nitrogen on the azaindole moiety that is responsible for interaction with the key residues Asp 133 and Val 135 (Figure 18), as observed for the known inhibitors previously mentioned. The importance of the free nitrogen seems to be confirmed by the preliminary biological results obtained. Among the five most active compounds, selected for the five doses NCI screening, four compounds bear a free nitrogen in the azaindole portion. Moreover, all synthesized compounds bearing the unsubstituted azaindole moiety bind, through their chains, Asp 200 that seems to be a key residue for the new compounds (Figure 18, Table 20). No differences were observed on the basis of the inserted chains in terms of docking scores and binding poses probably due to the presence in all chains of an H-bond donor group that is able to bind Asp 200 residue.

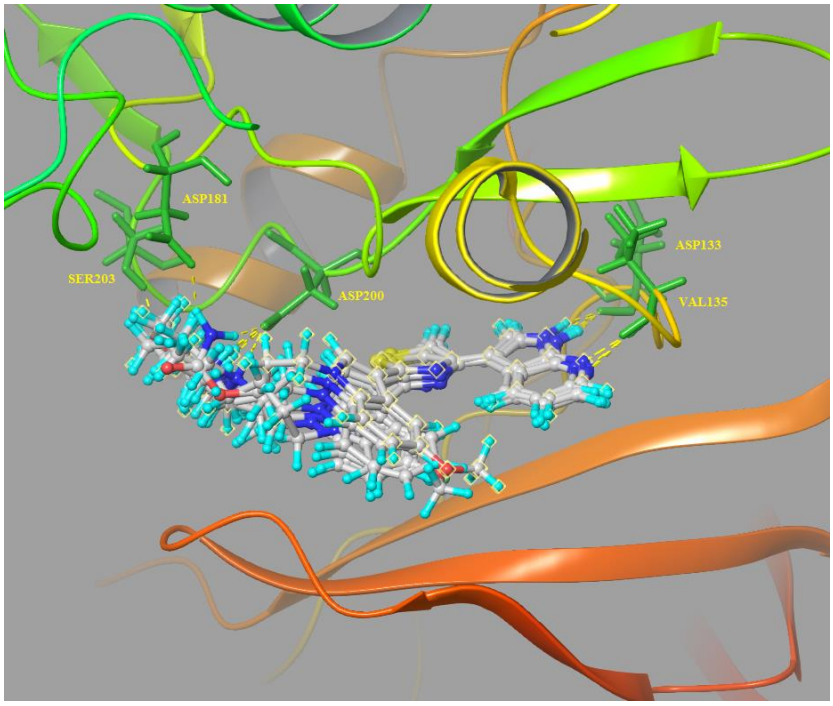


Figure 18. Docking poses of compounds **69a**, **55a**, **56g**, **57a**, **58a**, **59a**, **60e** and **61g**.

Table 20. Docking score results of compounds **69a,b**, **55a-h**, **56a-h**, **57a-h**, **58a-h**, **59a-h**, **60a-h**, **61a-h**.

Compound	D. Score	H-bond interaction residues	Compound	D. Score	H-bond interaction residues
69a	-9.323	ASP133, VAL135, ASP181, ASP200	**58d**	-6.680	LYS85
			58e	-9.665	ASP133, VAL135, ASP200
69b	-7.045	LYS85, HOH609	**58f**	-6.650	LYS85, HOH609
55a	-9.468	ASP133, VAL135, ASP200, LYS183	**58g**	-9.920	ASP133, VAL135, ASP200
55b	-6.817	LYS85	**58h**	-7.219	HOH589
55c	-8.411	ASP133, VAL135, LYS183	**59a**	-9.504	ASP133, VAL135, ASP200
55d	-7.044	PRO136, LYS85	**59b**	-6.719	ASP200
55e	-8.363	PRO136	**59c**	-9.225	ASP133, VAL135, ASP200
55f	-7.277	PRO136, LYS85			
			59d	-6.460	LYS85
55g	-9.056	ASP133, VAL135, ASP200, LYS183	**59e**	-9.015	ASP133, VAL135, ASP200
55h	-6.837	HOH589	**59f**	6.353	LYS85
56a	-7.426	ASP133, VAL135, ASP200, SER203	**59g**	-9.437	ASP133, VAL135, ASP200
56b	-4.270	LYS85	**59h**	-5.581	PRO136
56c	-6.734	ASP133, VAL135, ASP200, SER203	**60a**	-9.873	ASP133, VAL135, ASP200
56d	-4.720	LYS85	**60b**	-6.273	LYS85
56e	-7.348	ASP133, VAL135, ASP200, SER203	**60c**	-9.765	ASP133, VAL135, ASP200
56f	-4.977	PRO136, LYS85	**60d**	-6.072	PRO136
56g	-7.909	ASP133, VAL135, ASP200, SER203			
56h	-4.023	ASP200	**60e**	-10.174	ASP133, VAL135, ASP200
57a	-9.586	ASP133, VAL135, ASP200	**60f**	-6.589	ASP200
57a	-8.250	ASP133, VAL135	**60g**	-9.708	ASP133, VAL135, ASP200
57b	-6.805	ASP200			
57c	-9.289	ASP133, VAL135, ASP200	**60h**	-6.671	HOH589
57d	-6.097	ASP200	**61a**	-9.628	ASP133, VAL135, ASP200
57e	-8.941	ASP133, VAL135, ASP200	**61b**	-5.929	HOH589, ASP200
57f	-6.377	LYS85, HOH609	**61c**	-9.680	ASP133, VAL135, ASP200
57g	-8.958	ASP133, VAL135, ASP200	**61d**	-6.057	LYS85, PRO136
57h	-6.457	HOH589	**61e**	-9.623	ASP133, VAL135, ASP200
58a	-10.009	ASP133, VAL135, ASP200	**61f**	-5.895	LYS85, TYR134
58b	-6.086	LYS85, LYS60	**61g**	-9.913	ASP133, VAL135, ASP200
58c	-9.665	ASP133, VAL135, ASP200	**61h**	-7.029	HOH589, ASP200

EXPERIMENTAL SECTION

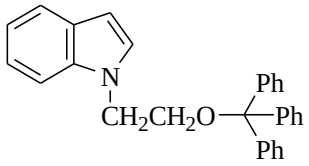
CHEMISTRY

General methods

All melting points were taken on a Buchi-Tottoly capillary apparatus and were uncorrected. IR spectra were determined in bromoform with a Shimadzu FT / IR 8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50 MHz, respectively, on DMSO-d_6 or $CDCl_3$ solution, using a Bruker Avance II series 200 MHz spectrometer. Chromatography column was performed with MERK silica gel 230-400 mesh ASTM or FLASH40i Biotage chromatography or with Buchi Sepacore chromatography module (prepacked cartridge reference). Elementar analyses (C, H, N) were within ± 0.4% of the theoretical values. Compounds **55e**, **55g**, **60h** were characterized only by ^{1}H NMR spectra, for their poor solubility the ^{13}C spectra were not performed.

General procedure for the synthesis of 1-(2-trityloxy-ethyl)-1*H*-indole (66a)

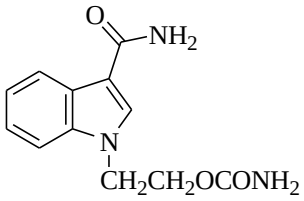
To a solution of indole **64a** (0.20 g, 1.71 mmol) in dry DMF (5 mL), cooled with an ice bath, was slowly added sodium hydride 60% dispersion in mineral oil (NaH) (0.06 g, 2.56 mmol). After 30 minutes stirring at 0-5 °C, the compound 1-bromo-2-trityloxy-ethane **65**[124] (0.75 g, 2.05 mmol) was added in one portion. The reaction mixture was then heated to 60 °C for 3 hours. After that, the mixture was poured into water and ice and the obtained precipitate was filtered off, dried, to give the desired derivative **66a**.



Yield: 98%, white solid; mp: 131-132 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.21 (2H, t, J = 4.8 Hz, CH_2), 4.40 (2H, t, J = 4.8 Hz, CH_2), 6.54 (1H, d, J = 3.0 Hz, H-3), 7.01-7.19 (15H, m, 3 × 5 H-Ph), 7.27-7.44 (3H, m, H-4, H-5 and H-6), 7.50 (1H, d, J = 3.0 Hz, H-2), 7.63 (1H, d, J = 7.1 Hz, H-7); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 45.7 (t), 62.8 (t), 86.0 (s), 100.5 (d), 110.1 (d), 118.9 (d), 120.3 (d), 120.9 (d), 126.9 (3 × d), 127.7 (6 × d), 128.1 (6 × d), 128.2 (s), 129.5 (d), 135.9 (s), 143.4 (3 × s); *Anal*. Calculated for $C_{29}H_{25}NO$ (MW: 403.51) : C, 86.32; H, 6.24; N, 3.47%. Found: C, 86.14; H, 6.07; N, 3.82%.

General procedure for the synthesis of carbamic acid 2-(3-carbamoyl-indol-1-yl)-ethyl ester (67a)

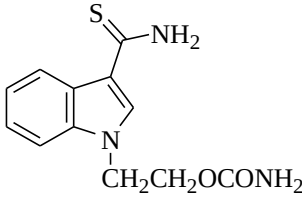
To a solution of the indole **66a** (0.30 g, 0.74 mmol), in anhydrous acetonitrile (2.5 mL) was added dropwise at 0 °C chlorosulfonyl isocyanate (CSI) (0.2 mL, 2.23 mmol). The reaction mixture was warmed to room temperature and stirred for 30 minutes. A solution of acetone (8 mL) and water (1 mL) was added and the mixture was basified using 10% aqueous solution of potassium hydroxide (KOH). The mixture was extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The obtained crude product was crystallized with dichloromethane to afford compound **67a**.



Yield: 70%, yellow solid; mp: 151-152 °C; IR: 3451, 3404 (NH_2), 1696 (CO) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 4.26 (2H, t, J = 4.5 Hz, CH_2), 4.42 (2H, t, J = 4.5 Hz, CH_2), 6.58 (2H, bs, NH_2), 6.91-7.37 (4H, m, NH_2, H-5 and H-6), 7.55 (1H, d, J = 8.2 Hz, H-7), 8.04 (1H, s, H-2), 8.17 (1H, dd, J = 7.1, 1.8 Hz, H-4); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 45.4 (t), 62.0 (t), 110.0 (s), 110.2 (d), 120.6 (d), 121.2 (d), 121.9 (d), 126.6 (s), 131.7 (d), 136.2 (s), 156.3 (s), 166.1 (s); *Anal*. Calculated for $C_{12}H_{13}N_3O_3$ (MW: 247.25) : C, 58.29; H, 5.30; N, 16.99%. Found: C, 58.10; H, 5.19; N, 17.29%.

General procedure for the synthesis of carbamic acid 2-(3-thiocarbamoyl-indol-1-yl)-ethyl ester (68a)

Lawesson's reagent (0.49 g, 1.21 mmol) was added to a suspension of derivative **67a** (0.30 g, 1.21 mmol) in anhydrous toluene (15 mL). The mixture was heated at reflux for 1 h. After cooling the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using dichloromethane:methanol (98:2) as eluent.

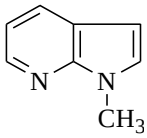


Yield: 45%, orange solid; mp: 149-150 °C; IR: 3404, 3309 (NH_2), 1684 (CO), 1646 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 4.26 (2H, t, J = 4.9 Hz, CH_2), 4.44 (2H, t, J = 4.7 Hz, CH_2), 6.59 (2H, bs, NH_2), 7.15-7.28 (2H, m, H-5 and H-6), 7.58 (1H, dd, J = 6.4, 1.7 Hz, H-7), 8.10 (1H, s, H-2), 8.59 (1H, dd, J = 7.1, 1.9 H-4), 8.81 (1H, s, SH), 9.04 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 45.5 (t), 61.9 (t), 110.4 (d), 115.8 (s), 121.1 (d), 121.9 (d), 122.2 (d), 126.1 (s), 131.5 (d), 136.7 (s), 156.2 (s), 193.1 (s); *Anal*. Calculated for $C_{12}H_{13}N_3O_2S$ (MW: 263.32) : C, 54.74; H, 4.98; N, 15.96%. Found: C, 54.87; H, 5.09; N, 15.72%.

Synthesis of 1-methyl-7-azaindole (74)[125]

Potassium *t*-butoxide (*t*-BuOK) (1.3g, 11.51 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) (1-2 drops) were added at 0 °C to a cold solution of derivative **73** (1.0 g, 8.46 mmol) in anhydrous toluene (85 mL). The reaction mixture was stirred at room temperature for 3 hours, and then methyl iodide (CH_3I) (0.7 mL, 8.46 mmol) was added at 0 °C. TLC analysis (dichloromethane:ethyl acetate 9:1) revealed that methylation was complete after 1 hour. The solvent was evaporated under reduced pressure. The residue was treated with water, extracted with dichloromethane (2 × 20 mL), dried (Na_2SO_4), evaporated and purified by column chromatography using dichloromethane:ethyl acetate (9:1) as eluent.

1-Methyl-1*H*-pyrrolo[2,3-*b*]pyridine (74)



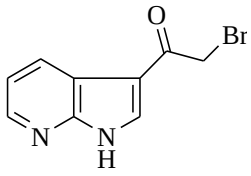
Yield: 96%, yellow oil; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.87 (3H, s, CH_3), 6.43 (1H, d, J = 3.4 Hz, H-3), 7.03 (1H, dd, J = 7.8, 4.8 Hz, H-5), 7.15 (1H, d, J = 3.4 Hz, H-2), 7.88 (1H, dd, J = 7.8, 1.5 Hz, H-4), 8.33 (1H, d, J = 4.8 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 31.1 (q), 99.1 (d), 115.3 (d), 120.4 (s), 128.6 (d), 128.9 (d + s), 142.6 (d); *Anal*. Calculated for $C_8H_8N_2$ (MW: 132.16) : C, 72.70; H, 6.10; N, 21.20%. Found: C, 72.60; H, 6.00; N, 21.40%.

Synthesis of 2-bromo-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-ethanones (63a,b)[125]

Anhydrous aluminum chloride ($AlCl_3$) (4.0 g, 29.99 mmol) was slowly added to a solution of the proper derivative **73**,**74** (8.46 mmol) in anhydrous dichloromethane (34 mL). The reaction mixture was heated under reflux and a solution of bromoacetyl bromide (0.7 mL, 8.46 mmol) in anhydrous dichlorometane (7 mL) was added dropwise. The resulting solution was allowed to stir under reflux for 40 minutes. After cooling, water and ice were slowly added and the obtained precipitate (for derivative **63a**) was filtered off or the oil residue (for derivative **63b**) was extracted with

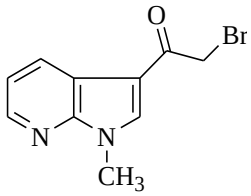
dichloromethane (3 × 20 mL), dried (Na_2SO_4), evaporated and purified by column chromatography using dichloromethane:ethyl acetate (9:1) as eluent.

2-Bromo-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-ethanone (63a)



Yield: 92%, white solid; mp: 280-281 °C; IR: 3556 (NH), 1678 (CO) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 4.71 (2H, s, CH_2), 7.30 (1H, dd, J = 7.8, 4.7 Hz, H-5), 8.37 (1H, d, J = 4.7 Hz, H-6), 8.47 (1H, d, J = 7.8 Hz, H-4), 8.65 (1H, s, H-2), 12.7 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 46.3 (t), 112.3 (s), 117.7 (s), 118.4 (d), 129.5 (d), 135.1 (d), 144.6 (d), 149.0 (s), 186.4 (s); *Anal*. Calculated for $C_9H_7BrN_2O$ (MW: 239.06): C, 45.22; H, 2.95; N, 11.72%. Found: C, 45.42; H, 2.85; N, 11.62%.

2-Bromo-1-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-ethanone (63b)

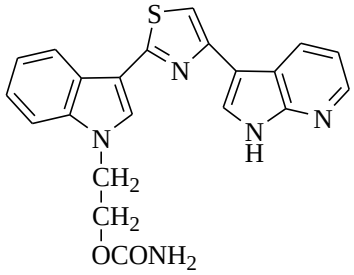


Yield: 86%, white solid; mp: 116-117 °C; IR: 1650 (CO) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.91 (3H, s, CH_3), 4.65 (2H, s, CH_2), 7.34 (1H, dd, J = 7.6, 4.7 Hz, H-5), 8.40-8.48 (2H, m, H-4 and H-6), 8.70 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.7 (q), 32.9 (t), 110.8 (s), 118.1 (d), 118.7 (s), 129.8 (d), 138.7 (d), 144.4 (d), 148.1 (s), 186.1 (s); *Anal*. Calculated for $C_{10}H_9BrN_2O$ (MW: 253.10) : C, 47.46; H, 3.58; N, 11.07%. Found: C, 47.56; H, 3.78; N, 10.77%.

General procedure for the synthesis of carbamic acid 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethyl esters (69a,b)

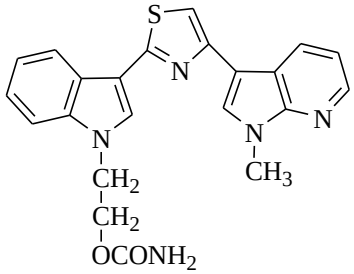
A suspension of the appropriate carbothioamide **68a**, (0.2 g, 0.67 mmol) and 2-bromo-1-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-ethanones **63a,b** (0.17 g, 0.67 mmol) in anhydrous ethanol (3 mL) was heated under reflux from 30 minutes to 1 hour. The precipitate, obtained after cooling, was filtered off, dried, and crystallized with ethanol to afford the desired final products.

Carbamic acid 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethyl ester (69a)



Conditions: 1 hour at reflux. Yield: 87%, orange solid; mp: 244-245 °C; IR: 3378, 3314 (NH_2), 3182 (NH), 1728 (CO), cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 4.34-4.36 (2H, m, CH_2), 4.50-4.53 (2H, m, CH_2), 6.58 (2H, bs, NH_2), 7.29-7.34 (2H, m, H-5' and H-6'), 7.40-7.47 (1H, m, H-5''), 7.64-7.68 (1H, m, H-7'), 7.84 (1H, s, H-2'), 8.23-8.35 (3H, m, H-2'', H-4' and H-5), 8.45 (1H, d, J = 5.0 Hz, H-6''), 8.92 (1H, d, J = 7.7 Hz, H-4''), 12.46 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 45.4 (t), 62.2 (t), 108.1 (d), 109.5 (s), 110.0 (s), 110.8 (d), 116.2 (d), 120.4 (d), 121.2 (d), 121.4 (s), 122.6 (d), 124.4 (s), 124.6 (s), 126.0 (d), 129.9 (d), 132.9 (d), 136.6 (s), 139.0 (d), 148.8 (s), 156.3 (s), 161.9 (s); *Anal*. Calculated for $C_{21}H_{17}N_5O_2S$ (MW: 403.46) : C, 62.52; H, 4.25; N, 17.36%. Found: C, 62.30; H, 4.12; N, 17.71%.

Carbamic acid 2-{3-[4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethyl ester (69b)



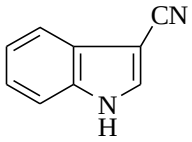
Conditions: 30 minutes at reflux. Yield: 88%, orange solid; mp: 234-235 °C; IR: 3346, 3255 (NH_2), 1710 (CO), cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.96 (3H, s, CH_3), 4.33-4.36 (2H, m, CH_2), 4.51-4.55 (2H, m, CH_2), 6.60 (2H, bs, NH_2), 7.29-7.38 (3H, m, H-5', H-5'' and H-6'), 7.66 (1H, d, J = 8.8 Hz, H-4'), 7.77 (1H, s, H-2'), 8.21 (1H, s, H-2''), 8.25 (1H, s, H-5), 8.34-8.44 (2H, m, H-6'' and H-7'), 8.74 (1H, d, J = 7.5 Hz, H-4''); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.8 (q), 45.4 (t), 62.2 (t), 107.8 (d), 109.5 (s), 109.9 (s), 110.7 (d), 116.1 (d), 119.0 (s), 120.5 (d), 121.2 (d), 122.6 (d), 124.6 (s), 129.7 (d), 129.9 (d), 131.2 (d), 136.6 (s), 140.3 (d), 148.3 (s), 148.4 (s), 156.3 (s),

162.0 (s); *Anal*. Calculated for $C_{22}H_{19}N_5O_2S$ (MW: 417.48) : C, 63.29; H, 4.59; N, 16.78%. Found: C, 63.13; H, 4.42; N, 17.11%.

General procedure for the synthesis of 1*H*-indol-3-carbonitriles (70a-d)

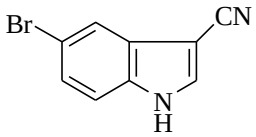
To a solution of the appropriate indole **64a-d** (1.0 g, 5.10 mmol) in anhydrous acetonitrile (4.5 mL) was added dropwise at 0 °C chlorosulfonyl isocyanate (CSI) (0.44 mL, 5.10 mmol). The reaction mixture was stirred at 0 °C for 2 hours. After that, it was added dropwise anhydrous dimethylformamide (DMF) (2.8 mL, 36.39 mmol) and the reaction mixture was stirred at 0 °C for 1 hour and 30 minutes. The mixture was poured into water and ice and the obtained precipitate was filtered off, dried, to give the desired derivatives **70a-d**.

1*H*-indole-3-carbonitrile (70a)



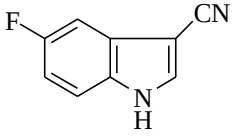
Yield: 90%, light orange solid; mp: 178-179 °C; IR: 3442 (NH), 2224 (CN) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 7.20-7.34 (2H, m, H-5 and H-6), 7.52-7.69 (2H, m, H-4 and H-7), 8.26 (1H, d, J = 3.0 Hz, H-2), 12.21 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 84.2 (s), 112.9 (d), 116.5 (s), 118.4 (d), 121.6 (d), 123.3 (d), 126.7 (s), 134.4 (d), 135.2 (s); *Anal*. Calculated for $C_9H_6N_2$ (MW: 142.16) : C, 76.04; H, 4.25; N, 19.71%. Found: C, 75.87; H, 4.03; N, 20.10%.

5-Bromo-1*H*-indole-3-carbonitrile (70b)



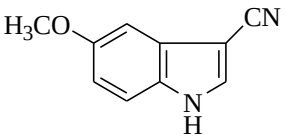
Yield: 98%, white solid; mp: 194-195 °C; IR: 3442 (NH), 2221 (CN) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 7.41 (1H, d, J = 8.4 Hz, H-6), 7.54 (1H, d, J = 8.4 Hz, H-7), 7.80 (1H, s, H-4), 8.32 (1H, s, H-2), 12.41 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 83.9 (s), 114.3 (s), 114.9 (d), 115.6 (s), 120.6 (d), 126.0 (d), 128.3 (s), 133.9 (s), 135.8 (d); *Anal*. Calculated for $C_9H_5BrN_2$ (MW: 221.05) : C, 48.90; H, 2.28; N, 12.67%. Found: C, 49.23; H, 2.15; N, 12.47%.

5-Fluoro-1*H*-indole-3-carbonitrile (70c)



Yield: 90%, light brown solid; mp: 176-177 °C; IR: 3443 (NH), 2231 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 7.15 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.42 (1H, dd, J = 9.2, 2.5 Hz, H-7), 7.54-7.61 (1H, m, H-4), 8.33 (1H, d, J = 3.1 Hz, H-2), 12.33 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 84.5 (d, J_{C7a-F} = 4.5 Hz), 103.6 (d, J_{C4-F} = 24.8 Hz), 111.8 (d, J_{C6-F} = 26.2 Hz), 114.3 (d, J_{C7-F} = 9.6 Hz), 115.9 (s), 127.2 (d, J_{C3a-F} = 10.9 Hz), 131.8 (s), 136.2 (d), 158.3 (d, J_{C5-F} = 236.1 Hz); *Anal*. Calculated for $C_9H_5FN_2$ (MW: 160.15) : C, 67.50; H, 3.15; N, 17.49%. Found: C, 67.60; H, 3.25; N, 17.29%.

5-Methoxy-1*H*-indole-3-carbonitrile (70d)

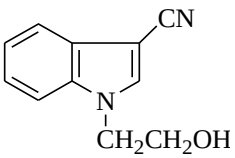


Yield: 90%, light brown solid; mp: 157-158 °C; IR: 3427 (NH), 2234 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.81 (3H, s, CH_3), 6.90 (1H, dd, J = 8.5, 2.4 Hz, H-6), 7.08 (1H, d, J = 2.4 Hz, H-4), 7.44 (1H, d, J = 8.5 Hz, H-7), 8.17 (1H, d, J = 3.1 Hz, H-2), 12.07 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.3 (q), 83.9 (s), 99.6 (d), 113.8 (2 × d), 116.6 (s), 127.5 (s), 129.9 (s), 134.3 (d), 155.2 (s); *Anal*. Calculated for $C_{10}H_8N_2O$ (MW: 172.18) : C, 69.76; H, 4.68; N, 16.27%. Found: C, 69.47; H, 4.57; N, 16.67%

General procedure for the synthesis of 1-(2-hydroxy-ethyl)-1*H*-indole-3-carbonitriles (72a-d)

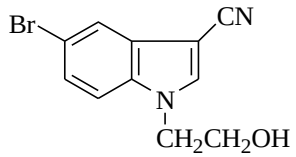
To a solution of the appropriate indole carbonitrile **70a-d** (0.5 g, 3.52 mmol) in dry DMF (10 mL), was slowly added at 0-5 °C, sodium hydride 60% dispersion in mineral oil (NaH) (0.13 g, 5.28 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. After that, it was added dropwise 2-bromoethanol **71** (0.50 mL, 7.04 mmol) and the reaction mixture was heated at 80 °C for 24 hours. After cooling, the mixture was poured into water and ice and extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4), and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using dichloromethane:ethyl acetate (9:1) as eluent.

1-(2-Hydroxy-ethyl)-1*H*-indole-3-carbonitrile (72a)



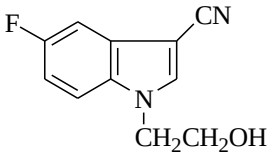
Yield: 65%, light orange solid; mp: 94-95 °C; IR: 3428 (OH), 2219 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.75 (2H, q, J = 5.3 Hz, CH_2), 4.32 (2H, t, J = 5.2 Hz, CH_2), 5.01 (1H, t, J = 5.3 Hz, OH), 7.24-7.38 (2H, m, H-5 and H-6), 7.64-7.71 (2H, m, H-4 and H-7), 8.26 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 49.0 (t), 59.6 (t), 83.1 (s), 111.7 (d), 116.1 (s), 118.6 (d), 121.8 (d), 123.1 (d), 127.1 (s), 135.3 (s), 137.5 (d); *Anal*. Calculated for $C_{11}H_{10}N_2O$ (MW: 186.21) : C, 70.95; H, 5.41; N, 15.04%. Found : C, 71.05; H, 5.51; N, 14.84%.

5-Bromo-1-(2-hydroxy-ethyl)-1*H*-indole-3-carbonitrile (72b)



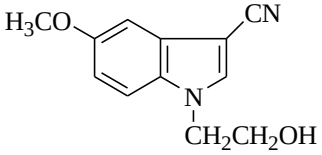
Yield: 70%, white solid; mp: 348-349 °C; IR: 3448 (OH), 2218 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.72 (2H, q, J = 5.3 Hz, CH_2), 4.32 (2H, t, J = 5.1 Hz, CH_2), 4.98 (1H, t, J = 5.3 Hz, OH), 7.46 (1H, dd, J = 8.8, 1.9 Hz, H-6), 7.70 (1H, d, J = 8.8 Hz, H-7), 7.81 (1H, d, J = 1.9 Hz, H-4), 8.31 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 49.3 (t), 59.6 (t), 82.9 (s), 114.0 (d), 114.6 (s), 115.4 (s), 120.8 (d), 125.9 (d), 128.7 (s), 134.3 (s), 138.7 (d); *Anal*. Calculated for $C_{11}H_9BrN_2O$ (MW: 265.11) : C, 49.84; H, 3.42; N, 10.57%. Found: C, 49.96; H, 3.55; N, 10.32%.

5-Fluoro-1-(2-hydroxy-ethyl)-1*H*-indole-3-carbonitrile (72c)



Yield: 75%, white solid; mp: 91-92 °C; IR: 3384 (OH), 2226 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.73 (2H, q, J = 5.3 Hz, CH_2), 4.32 (2H, t, J = 5.1 Hz, CH_2), 4.98 (1H, t, J = 5.3 Hz, OH), 7.20 (1H, td, J = 11.8, 9.3, 2.5 Hz, H-6), 7.43 (1H, dd, J = 9.3, 2.5 Hz, H-7), 7.71-7.77 (1H, m, H-4), 8.31 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 49.4 (t), 59.7 (t), 83.4 (d, $J_{C7a\text{-}F}$ = 4.6 Hz), 103.8 (d, $J_{C4\text{-}F}$ = 24.9 Hz), 111.6 (d, $J_{C6\text{-}F}$ = 26.1 Hz), 113.4 (d, $J_{C7\text{-}F}$ = 9.8 Hz), 115.7 (s), 127.6 (d, $J_{C3a\text{-}F}$ = 10.8 Hz), 132.2 (s), 139.0 (d), 158.4 (d, $J_{C5\text{-}F}$ = 239.5 Hz); *Anal*. Calculated for $C_{11}H_9FN_2O$ (MW: 204.20) : C, 64.70; H, 4.44; N, 13.72%. Found: C, 64.94; H, 4.59; N, 13.33%.

1-(2-Hydroxy-ethyl)-5-methoxy-1*H*-indole-3-carbonitrile (72d)

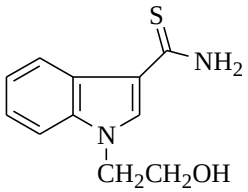


Yield: 62%, white solid; mp: 89-90 °C; IR: 3384 (OH), 2224 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.71 (2H, q, J = 5.3 Hz, CH_2), 3.82 (3H, s, CH_3), 4.27 (2H, t, J = 5.2 Hz, CH_2), 4.97 (1H, t, J = 5.2 Hz, OH), 6.94 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.08 (1H, d, J = 2.4 Hz, H-4), 7.59 (1H, d, J = 9.0 Hz, H-7), 8.16 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 44.3 (t), 50.5 (q), 55.7 (t), 79.6 (s), 95.5 (d), 106.2 (d), 109.2 (d), 111.0 (s), 123.5 (s), 125.1 (s), 130.4 (d), 150.6 (s); *Anal*. Calculated for $C_{12}H_{12}N_2O_2$ (MW: 216.24) : C, 66.65; H, 5.59; N, 12.96%. Found: C, 66.29; H, 5.73; N, 13.18%.

General procedure for the synthesis of 1-(2-hydroxy-ethyl)-1*H*-indole-3-carbothioamides (62a-d)

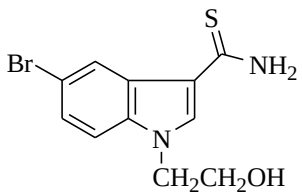
A solution of phosphorus pentasulfide (P_4S_{10}) (1.4 g, 4.22 mmol) in anhydrous ethanol (3 mL) was stirred at room temperature for 1 hour. The appropriate indole carbonitrile **72a-d** (0.7 g, 2.11 mmol) were added and the reaction mixture was stirred at room temperature for 2 hours. The precipitate obtained was filtered off, dried, and crystallized with ethanol to afford the desired products **62b-d**. In case of derivative **62a** the reaction mixture was concentrated under vacuum. The resulting crude was purified by column chromatography using dichloromethane:methanol (98:2) as eluent.

1-(2-Hydroxy-ethyl)-1*H*-indole-3-carbothioamide (62a)



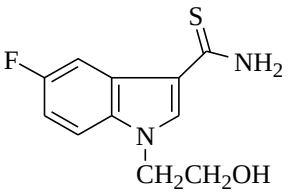
Yield: 80%, orange solid; mp: 130-131 °C; IR: 3384 (OH), 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.74 (2H, t, J = 5.0 Hz, CH_2), 4.26 (2H, t, J = 5.3 Hz, CH_2), 5.02 (1H, s, OH), 7.13-7.26 (2H, m, H-5 and H-6), 7.51-7.60 (1H, m, H-7), 8.14 (1H, s, H-2), 8.56-8.64 (1H, m, H-4), 8.81 (1H, s, SH), 8.98 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 48.7 (t), 59.6 (t), 110.6 (d), 115.2 (s), 120.9 (d), 121.8 (d), 121.9 (d), 126.1 (s), 132.3 (d), 136.8 (s), 193.0 (s); *Anal*. Calculated for $C_{11}H_{12}N_2OS$ (MW: 220.29) : C, 59.97; H, 5.49; N, 12.72%. Found: C, 59.66; H, 5.67; N, 12.85%.

5-Bromo-1-(2-hydroxy-ethyl)-1*H*-indole-3-carbothioamide (62b)



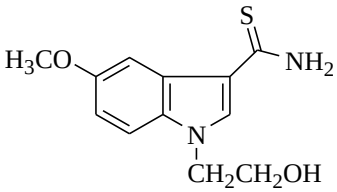
Yield: 70%, white solid; mp: 195-196 °C; IR: 3414 (OH), 1646 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.73 (2H, t, J = 5.2 Hz, CH_2), 4.25 (2H, t, J = 5.2 Hz, CH_2), 7.35 (1H, dd, J = 8.7, 2.0 Hz, H-6), 7.56 (1H, d, J = 8.7 Hz, H-7), 8.18 (1H, s, H-2), 8.89 (1H, d, J = 2.0 Hz, H-4), 8.92 (1H, s, SH), 9.04 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 49.0 (t), 59.6 (t), 112.8 (d), 113.9 (s), 114.4 (s), 124.1 (d), 124.4 (d), 128.1 (s), 132.6 (d), 135.7 (s), 192.5 (s); *Anal*. Calculated for $C_{11}H_{11}BrN_2OS$ (MW: 299.19) : C, 44.16; H, 3.71; N, 9.36%. Found: C, 43.93; H, 3.61; N, 9.69%.

5-Fluoro-1-(2-hydroxy-ethyl)-1*H*-indole-3-carbothioamide (62c)



Yield: 70%, light yellow solid; mp: 176-177 °C; IR: 3410 (OH), 1683 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.46 (2H, t, J = 5.1 Hz, CH_2), 4.26 (2H, t, J = 5.0 Hz, CH_2), 4.61 (1H, s, OH), 7.07 (1H, td, J = 11.1, 9.0, 2.6 Hz, H-6), 7.56-7.62 (1H, m, H-7), 8.21 (1H, s, H-2), 8.44 (1H, dd, J = 11.1, 2.5 Hz, H-4), 8.87 (1H, s, SH), 9.00 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 49.1 (t), 59.7 (t), 106.8 (d, $J_{C4\text{-}F}$ = 25.7 Hz), 110.1 (d, $J_{C6\text{-}F}$ = 26.3 Hz), 112.0 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 114.9 (d, $J_{C7a\text{-}F}$ = 4.6 Hz), 127.0 (d, $J_{C3a\text{-}F}$ = 11.0 Hz), 133.3 (d), 133.6 (s), 158.1 (d, $J_{C5\text{-}F}$ = 232.9 Hz), 192.6 (s); *Anal*. Calculated for $C_{11}H_{11}FN_2OS$ (MW: 238.28) : C, 55.45; H, 4.65; N, 11.76%. Found: C, 55.18; H, 4.52; N, 12.16%.

1-(2-Hydroxy-ethyl)-5-methoxy-1*H*-indole-3-carbothioamide (62d)

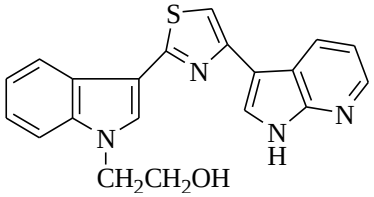


Yield: 75%, light orange solid; mp: 171-172 °C; IR: 3378 (OH), 1684 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.71 (2H, t, J = 5.1 Hz, CH_2), 3.78 (3H, s, CH_3), 4.21 (2H, t, J = 5.1 Hz, CH_2), 4.99 (1H, s, OH), 6.85 (1H, dd, J = 8.9, 2.4 Hz, H-6), 7.45 (1H, d, J = 8.9 Hz, H-7), 8.10 (1H, s, H-2), 8.19 (1H, d, J = 2.4 Hz, H-4), 8.75 (1H, s, SH), 8.91 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 48.9 (t), 55.2 (q), 59.7 (t), 103.8 (d), 111.4 (d), 111.6 (d), 114.7 (s), 126.8 (s), 131.8 (s), 132.6 (d), 154.8 (s), 192.8 (s); *Anal*. Calculated for $C_{12}H_{14}N_2O_2S$ (MW: 250.32) : C, 57.58; H, 5.64; N, 11.19%. Found: C, 57.76; H, 5.85; N, 10.80%.

General procedure for the synthesis of 2-{3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanols (55a-h)

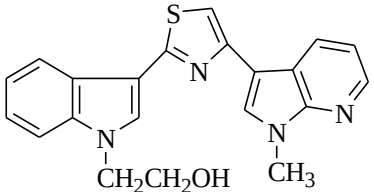
Compounds **55a-h** were prepared from **62a-d** using the same synthetic procedure described for compounds **69a,b**.

2-{3-[4-(1*H*-Pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55a)



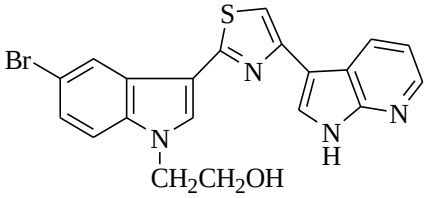
Conditions: 2 hours at reflux. Yield: 98%, yellow solid; mp: 243-244 °C; IR: 3365 (OH), 3136 (NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.80 (2H, t, J = 5.1 Hz, CH_2), 4.36 (2H, t, J = 5.2 Hz, CH_2), 7.26-7.34 (2H, m, H-5' and H-6'), 7.51-7.57 (1H, m, H-5''), 7.63-7.67 (1H, m, H-7'), 7.89 (1H, s, H-2'), 8.26-8.31 (3H, m, H-2'', H-4' and H-5), 8.51 (1H, d, J = 4.4 Hz, H-6''), 9.06 (1H, d, J = 7.3 Hz, H-4''), 12.74 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 48.6 (t), 59.9 (t), 108.3 (d), 109.3 (s), 111.0 (d), 111.2 (s), 116.2 (d), 120.2 (d), 121.0 (d), 122.3 (d), 124.3 (s), 124.6 (s), 126.4 (d), 130.4 (d), 132.2 (d), 136.6 (s), 138.4 (d), 142.5 (s), 147.8 (s), 162.2 (s); *Anal*. Calculated for $C_{20}H_{16}N_4OS$ (MW: 360.43) : C, 66.65; H, 4.47; N, 15.54%. Found: C, 66.31; H, 4.66; N, 15.69%.

2-{3-[4-(1-Methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55b)



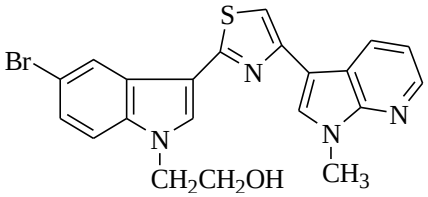
Conditions: 1 hour at reflux. Yield: 98%, yellow solid; mp: 257-258 °C; IR: 3222 (OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.80 (2H, t, J = 5.0 Hz, CH_2), 3.98 (3H, s, CH_3), 4.35 (2H, t, J = 4.9 Hz, CH_2), 7.25-7.34 (2H, m, H-5' and H-6'), 7.43-7.50 (1H, m, H-5''), 7.62-7.68 (1H, m,H-7'), 7.82 (1H, s, H-2'), 8.25-8.32 (3H, m, H-2'', H-4' and H-5), 8.51 (1H, d, J = 4.4 Hz, H-6''), 8.90 (1H, d, J = 7.1 Hz, H-4''); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 32.0 (q), 48.6 (t), 59.9 (t), 107.9 (d), 109.0 (s), 109.5 (s), 110.9 (d), 116.1 (d), 119.4 (s), 120.3 (d), 121.0 (d), 122.3 (d), 124.5 (s), 130.0 (d), 130.5 (d), 132.3 (d), 136.6 (s), 139.3 (d), 143.9 (s), 147.7 (s), 162.2 (s); *Anal*. Calculated for $C_{21}H_{18}N_4OS$ (MW: 374.46) : C, 67.36; H, 4.85; N, 14.96%. Found: C, 67.69; H, 4.71; N, 14.77%.

2-{5-Bromo-3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55c)



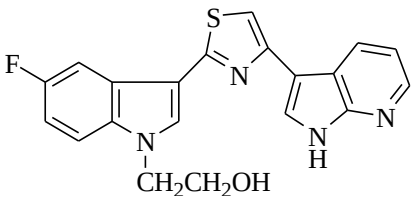
Conditions: 30 minutes at reflux. Yield: 90%, yellow solid; mp: 259-260 °C; IR: 3364 (OH), 3136 (NH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.77-3.81 (2H, m, CH_2), 4.33-4.37 (2H, m, CH_2), 7.40-7.44 (2H, m, H-5''and H-6'), 7.65 (1H, d, J = 8.7 Hz, H-7'), 7.83 (1H, s, H-2'), 8.20 (1H, s, H-2''), 8.27 (1H, s, H-5), 8.43-8.51 (2H, m, H-4' and H-6''), 8.88 (1H, d, J = 6.6 Hz, H-4''), 12.41 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 48.8 (t), 59.9 (t), 108.6 (d), 108.9 (s), 111.1 (s), 113.2 (d), 113.6 (s), 116.0 (d), 120.4 (s), 122.6 (d), 124.7 (d), 126.2 (s), 126.4 (d), 131.6 (d), 133.8 (d), 135.5 (s), 138.0 (d), 142.8 (s), 148.3 (s), 161.6 (s); *Anal*. Calculated for $C_{20}H_{15}BrN_4OS$ (MW: 439.33) : C, 54.68; H, 3.44; N, 12.75%. Found: C, 54.91; H, 3.55; N, 12.41%.

2-{5-Bromo-3-[4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55d)



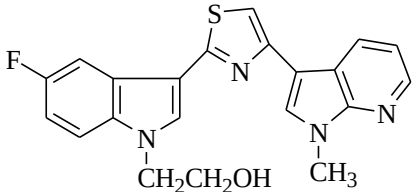
Conditions: 30 minutes at reflux. Yield: 90%, yellow solid; mp: 286-287 °C; IR: 3364 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.78 (2H, t, J = 5.0 Hz, CH_2), 3.96 (3H, s, CH_3), 4.34 (2H, t, J = 4.8 Hz, CH_2), 7.32-7.45 (2H, m, H-5'' and H-6'), 7.64 (1H, d, J = 8.8 Hz, H-7'), 7.77 (1H, s, H-2'), 8.22 (1H, s, H-2''), 8.26 (1H, s, H-5), 8.43-8.50 (2H, m, H-4' and H-6''), 8.77 (1H, d, J = 7.9 Hz, H-4''); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.5 (q), 48.8 (t), 60.0 (t), 107.1 (d), 107.6 (s), 109.0 (s), 109.2 (s), 113.2 (d), 113.5 (s), 116.0 (d), 118.4 (s), 122.6 (d), 124.8 (d), 126.2 (s), 129.1 (d), 130.3 (d), 131.5 (d), 135.5 (s), 141.2 (d), 148.9 (s), 161.4 (s); *Anal*. Calculated for $C_{21}H_{17}BrN_4OS$ (MW: 453.35) : C, 55.64; H, 3.78; N, 12.36%. Found: C, 55.47; H, 3.62; N, 12.69%.

2-{5-Fluoro-3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55e)



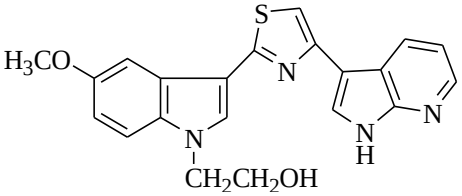
Conditions: 30 minutes at reflux. Yield: 98%, yellow solid; mp: 261-262 °C; IR: 3249 (OH), 3069 (NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.77-3.81 (2H, m, CH_2), 4.33-4.37 (2H, m, CH_2), 7.12-7.21 (1H, m, H-6'), 7.43-7.50 (1H, m, H-5''), 7.65-7.71 (1H, m, H-7'), 7.85 (1H, s, H-2'), 8.02 (1H, d, J = 9.3 Hz, H-4'), 8.28 (2H, d, J = 5.2 Hz, H-2'' and H-5), 8.47 (1H, d, J = 3.6 Hz, H-6''), 8.94 (1H, d, J = 7.6 Hz, H-4''), 12.59 (1H, bs, NH); *Anal*. Calculated for $C_{20}H_{15}FN_4OS$ (MW: 378.42) : C, 63.48; H, 4.00; N, 14.81%. Found: C, 63.83; H, 3.79; N, 14.67%.

2-{5-Fluoro-3-[4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55f)



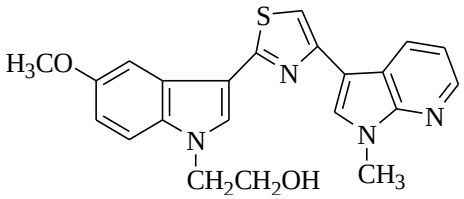
Conditions: 30 minutes at reflux. Yield: 98%, yellow solid; mp: 268-269 °C; IR: 3370 (OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.79 (2H, t, J = 4.6 Hz, CH_2), 3.97 (3H, s, CH_3), 4.35 (2H, t, J = 4.8 Hz, CH_2), 7.34-7.40 (2H, m, H-5'' and H-6'), 7.64-7.70 (1H, m, H-7'), 7.76 (1H, s, H-2'), 8.05 (1H, d, J = 9.5 Hz, H-4'), 8.27 (2H, s, H-2'' and H-5), 8.45 (1H, d, J = 4.5 Hz, H-6''), 8.75 (1H, d, J = 7.8 Hz, H-4''); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.5 (q), 48.9 (t), 60.0 (t), 105.3 (d, $J_{C4'-F}$ = 24.8 Hz), 107.4 (d), 109.3 (s), 109.5 (d, J_{C7a-F} = 4.6 Hz), 110.5 (d, $J_{C6'-F}$ = 25.9 Hz), 112.3 (d, $J_{C7'-F}$ = 10.2 Hz), 116.1 (d), 118.3 (s), 124.9 (d, J_{C3a-F} = 10.6 Hz), 129.6 (d), 130.6 (d), 131.9 (d), 133.4 (s), 140.9 (d), 145.8 (s), 148.7 (s), 158.1 (d, $J_{C5'-F}$ = 236.5 Hz), 161.7 (s); *Anal*. Calculated for $C_{21}H_{17}FN_4OS$ (MW: 392.45) : C, 64.27; H, 4.37; N, 14.28%. Found: C, 64.54; H, 4.48; N, 13.90%.

2-{5-Methoxy-3-[4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55g)



Conditions: 2 hours at reflux. Yield: 98%, yellow solid; mp: 253-254 °C; IR: 3371 (OH), 3176 (NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.78 (2H, t, J = 5.1 Hz, CH_2), 3.90 (3H, s, CH_3), 4.31 (2H, t, J = 5.0 Hz, CH_2), 6.93 (1H, dd, J = 8.9, 2.3 Hz, H-6'), 7.46-7.57 (2H, m, H-5''and H-7'), 7.84 (2H, s, H-2' and H-2''), 8.18 (1H, s, H-5), 8.29 (1H, s, H-4'), 8.49 (1H, d, J = 4.6 Hz, H-6''), 9.10 (1H, d, J = 7.5 Hz, H-4''), 12.69 (1H, bs, NH); *Anal*. Calculated for $C_{21}H_{18}N_4O_2S$ (MW: 390.46) : C, 64.60; H, 4.65; N, 14.35%. Found: C, 64.38; H, 4.55; N, 14.67%.

2-{5-Methoxy-3-[4-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-thiazol-2-yl]-indol-1-yl}-ethanol (55h)



Conditions: 30 minutes at reflux. Yield: 98%, yellow solid; mp: 258-259°C; IR: 3363 (OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.77 (2H, t, J = 5.0 Hz, CH_2), 3.90 (3H, s, CH_3), 3.97 (3H, s, CH_3), 4.30 (2H, t, J = 4.8 Hz, CH_2), 6.93 (1H, dd, J = 8.9, 2.2 Hz, H-6'), 7.40 (1H, dd, J = 7.9, 5.1 Hz, H-5''), 7.54 (1H, d, J = 8.9 Hz, H-7'), 7.75 (1H, s, H-2'), 7.81 (1H, d, J = 2.2 Hz, H-4'), 8.17 (1H, s, H-2''), 8.26 (1H, s, H-5), 8.48 (1H, d, J = 4.3 Hz, H-6''), 8.92 (1H, d, J = 7.3 Hz, H-4''); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.9 (q), 48.8 (t), 55.2 (q), 60.0 (t), 101.9 (d), 107.4 (d), 108.7 (s), 109.4 (s), 111.8 (d), 112.2 (d), 115.9 (d), 119.4 (s), 125.1 (s), 129.6 (d), 130.7 (d), 131.7 (s), 132.0 (d), 139.7 (d), 144.2 (s), 147.8 (s), 154.8 (s), 162.5 (s); *Anal*. Calculated for $C_{22}H_{20}N_4O_2S$ (MW: 404.48) : C, 65.33; H, 4.98; N, 13.85%. Found: C, 65.14; H, 4.86; N, 14.16%.

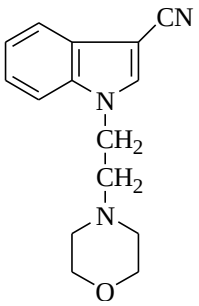
Synthesis of the 4-(2-chloroethyl)morpholine (77)

To a solution of 4-(2-chloroethyl)morpholine hydrochloride **76** (0.8 g, 4.30 mmol) in water (5 mL) was added 5% aqueous solution of sodium hydroxide (NaOH) up to pH = 11-12 and stirred at room temperature for 10 minutes. The resulting mixture was extracted with dichloromethane (2 × 10 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. Yield: 98%, yellow oil. Analytical and spectroscopic data were in accordance with those previously reported [126].

General procedure for the synthesis of 1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbonitriles (78a-d)

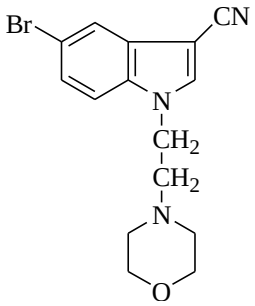
To a solution of the appropriate indole carbonitrile **70a-d** (0.3 g, 1.35 mmol) in dry DMF (2 mL), was slowly added at 0-5 °C, sodium hydride 60% dispersion in mineral oil (NaH) (0.05 g, 2.03 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. After that, it was added dropwise a solution of 4-(2-chloroethyl)morpholine **77** (0.8 g, 5.40 mmol) in dry DMF (2 mL), and the reaction mixture was stirred at 50 °C for 1-2 hours. After cooling, the mixture was poured into water and ice and the obtained precipitate was filtered off, dried, to give the desired products **78a-d**.

1-(2-Morpholin-4-yl-ethyl)-1*H*-indole-3-carbonitrile (78a)



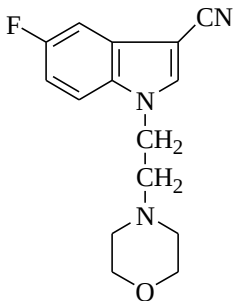
Conditions: heated for 2 hours at 50 °C. Yield: 94%, brown solid; mp: 89-90 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.42 (4H, t, J = 4.6 Hz, 2 × CH_2), 2.69 (2H, t, J = 6.2 Hz, CH_2), 3.53 (4H, t, J = 4.6 Hz, 2 × CH_2), 4.38 (2H, t, J = 6.2 Hz, CH_2), 7.23-7.38 (2H, m, H-5 and H-6), 7.63-7.73 (2H, m, H-4 and H-7), 8.31 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.3 (t), 53.1 (2 × t), 57.1 (t), 66.1 (2 × t), 83.2 (s), 111.5 (d), 116.1 (s), 118.6 (d), 121.8 (d), 123.2 (d), 127.0 (s), 135.2 (s), 137.3 (d); *Anal*. Calculated for $C_{15}H_{17}N_3O$ (MW: 255.31) : C, 70.56; H, 6.71; N, 16.46%. Found: C, 70.66; H, 6.91; N, 16.16%.

5-Bromo-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbonitrile (78b)



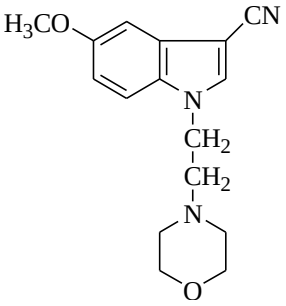
Conditions: heated for 1 hour at 50 °C. Yield: 95%, brown solid; mp: 128-129 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.41 (4H, t, J = 4.5 Hz, 2 × CH_2), 2.67 (2H, t, J = 6.1 Hz, CH_2), 3.51 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.38 (2H, t, J = 6.1 Hz, CH_2), 7.48 (1H, dd, J = 8.8, 1.8 Hz, H-6), 7.73 (1H, d, J = 8.8 Hz, H-7), 7.81 (1H, d, J = 1.8 Hz, H-4), 8.37 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.6 (t), 53.0 (2 × t), 57.1 (t), 66.1 (2 × t), 82.9 (s), 113.8 (d), 114.6 (s), 115.4 (s), 120.9 (d), 125.9 (d), 128.5 (s), 134.1 (s), 138.6 (d); *Anal*. Calculated for $C_{15}H_{16}BrN_3O$ (MW: 334.21) : C, 53.91; H, 4.83; N, 12.57%. Found: C, 54.28; H, 4.69; N, 12.34%.

5-Fluoro-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbonitrile (78c)



Conditions: heated for 1 hour at 50 °C. Yield: 95%, brown solid; mp: 86-87 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.41 (4H, t, J = 4.5 Hz, 2 × CH_2), 2.68 (2H, t, J = 6.1 Hz, CH_2), 3.52 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.38 (2H, t, J = 6.2 Hz, CH_2), 7.21 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.43 (1H, dd, J = 9.1, 2.4 Hz, H-7), 7.73-7.79 (1H, m, H-4), 8.37 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.6 (t), 53.1 (2 × t), 57.1 (t), 66.1 (2 × t), 83.4 (d, J_{C7a-F} = 4.4 Hz), 103.9 (d, J_{C4-F} = 24.9 Hz), 111.7 (d, J_{C6-F} = 26.3 Hz), 113.2 (d, J_{C7-F} = 9.6 Hz), 115.7 (s), 127.5 (d, J_{C3a-F} = 10.8 Hz), 132.0 (s), 138.9 (d), 158.4 (d, J_{C5-F} = 239.5 Hz); *Anal*. Calculated for $C_{15}H_{16}FN_3O$ (MW: 273.13) : C, 65.92; H, 5.90; N, 15.37%. Found: C, 66.20; H, 6.01; N, 14.98%.

5-Methoxy-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbonitrile (78d)

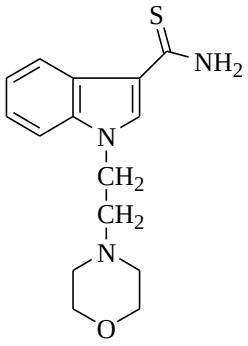


Conditions: heated for 1 hour at 50 °C. Yield: 88%, brown solid; mp: 78-79 °C; IR: 2217 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.41 (4H, t, J = 4.5 Hz, 2 × CH_2), 2.66 (2H, t, J = 6.2 Hz, CH_2), 3.52 (4H, t, J = 4.5 Hz, 2 × CH_2), 3.82 (3H, s, CH_3), 4.33 (2H, t, J = 6.2 Hz, CH_2), 6.93 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.09 (1H, d, J = 2.4 Hz, H-4), 7.61 (1H, d, J = 9.0 Hz, H-7), 8.22 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.5 (t), 53.1 (2 × t), 55.4 (q), 57.2 (t), 66.1 (2 × t), 82.8 (s), 100.0 (d), 112.5 (d), 113.5 (d), 116.3 (s), 127.8 (s), 130.1 (s), 137.1 (d), 155.4 (s); *Anal*. Calculated for $C_{16}H_{19}N_3O_2$ (MW: 285.15) : C, 67.35; H, 6.71; N, 14.73%. Found: C, 67.22; H, 6.97; N, 14.60%.

General procedure for the synthesis of 1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbothioamides (75a-d)

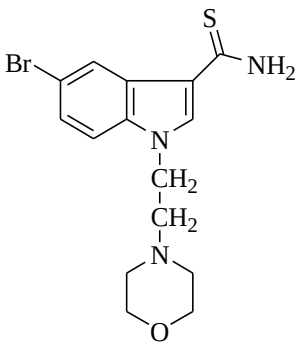
A solution of phosphorus pentasulfide (P_4S_{10}) (1.4 g, 3.17 mmol) in anhydrous ethanol (3 mL) was stirred at room temperature for 1 hour. The appropriate indole carbonitrile **78a-d**, (0.7 g, 2.11 mmol) were added and the reaction mixture was heated under reflux for 1 hour. The solvent was removed under reduced pressure and the residue was treated with 20% aqueous solution of sodium hydrogencarbonate ($NaHCO_3$) (22 mL) up to pH = 8-9 and stirred at room temperature for 24 hours. The resulting mixture was extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4), filtered and concentrated under vacuum. The product was purified by column chromatography using dichloromethane:methanol (98:2) as eluent.

1-(2-Morpholin-4-yl-ethyl)-1*H*-indole-3-carbothioamide (75a)



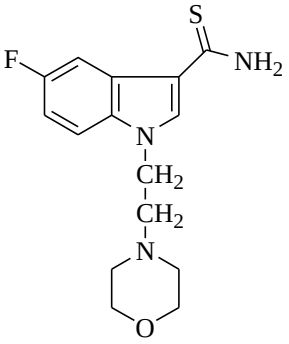
Yield: 73%, yellow solid; mp: 157-158 °C; IR: 1653 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.44 (4H, t, J = 4.5 Hz, 2 × CH_2), 2.69 (2H, t, J = 6.3 Hz, CH_2), 3.55 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.33 (2H, t, J = 6.3 Hz, CH_2), 7.13-7.27 (2H, m, H-5 and H-6), 7.56 (1H, dd, J = 6.1, 1.7 Hz, H-7), 8.16 (1H, s, H-2), 8.58 (1H, dd, J = 6.9, 1.0 Hz, H-4), 8.80 (1H, s, SH), 9.00 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.0 (t), 53.2 (2 × t), 57.3 (t), 66.1 (2 × t), 110.4 (d), 115.4 (s), 121.0 (d), 121.8 (d), 122.0 (d), 125.9 (s), 132.0 (d), 136.6 (s), 193.0 (s); *Anal*. Calculated for $C_{15}H_{19}N_3OS$ (MW: 289.40) : C, 62.25; H, 6.62; N, 14.52%. Found: C, 62.01; H, 6.49; N, 14.89%.

5-Bromo-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbothioamide (75b)



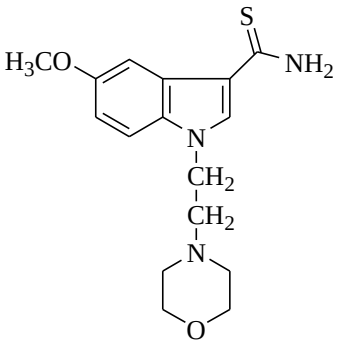
Yield: 81%, yellow solid; mp: 212-213 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.42 (4H, t, J = 4.4 Hz, 2 × CH_2), 2.67 (2H, t, J = 6.1 Hz, CH_2), 3.53 (4H, t, J = 4.4 Hz, 2 × CH_2), 4.33 (2H, t, J = 6.2 Hz, CH_2), 7.36 (1H, dd, J = 8.7, 2.0 Hz, H-6), 7.59 (1H, d, J = 8.7 Hz, H-7), 8.20 (1H, s, H-2), 8.88 (1H, d, J = 2.0 Hz, H-4), 8.92 (1H, s, SH), 9.07 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.6 (t), 53.1 (2 × t), 57.2 (t), 66.1 (2 × t), 112.7 (d), 114.0 (s), 114.6 (s), 124.1 (d), 124.5 (d), 127.9 (s), 132.4 (d), 135.5 (s), 192.4 (s); *Anal*. Calculated for $C_{15}H_{18}BrN_3OS$ (MW: 368.29) : C, 48.92; H, 4.93; N, 11.41%. Found: C, 48.63; H, 5.33; N, 11.30%.

5-Fluoro-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbothioamide (75c)



Yield: 76%, yellow solid; mp: 175-176 °C; IR: 1684 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.43 (4H, t, J = 4.6 Hz, 2 × CH_2), 2.68 (2H, t, J = 6.2 Hz, CH_2), 3.54 (4H, t, J = 4.4 Hz, 2 × CH_2), 4.33 (2H, t, J = 6.3 Hz, CH_2), 7.09 (1H, td, J = 11.0, 9.1, 2.6 Hz, H-6), 7.58-7.65 (1H, m, H-7), 8.23 (1H, s, H-2), 8.43 (1H, dd, J = 11.0, 2.6 Hz, H-4), 8.86 (1H, s, SH), 9.02 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.3 (t), 53.1 (2 × t), 57.3 (t), 66.1 (2 × t), 106.8 (d, $J_{C4\text{-}F}$ = 26.1 Hz), 110.1 (d, $J_{C6\text{-}F}$ = 26.1 Hz), 111.9 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 115.0 (d, $J_{C7a\text{-}F}$ = 4.6 Hz), 126.8 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 133.0 (d), 133.4 (s), 158.1 (d, $J_{C5\text{-}F}$ = 230.5 Hz), 192.6 (s); *Anal*. Calculated for $C_{15}H_{18}FN_3OS$ (MW: 307.39) : C, 58.61; H, 5.90; N, 13.67%. Found: C, 58.31; H, 6.08; N, 13.79%.

5-Methoxy-1-(2-morpholin-4-yl-ethyl)-1*H*-indole-3-carbothioamide (75d)

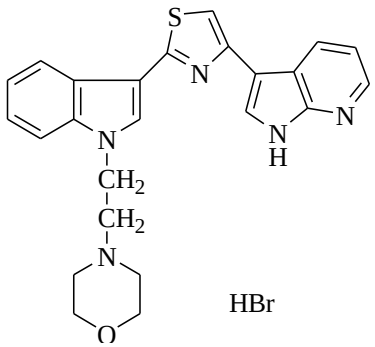


Yield: 77%, yellow solid; mp: 56-57 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.43 (4H, t, J = 4.5 Hz, 2 × CH_2), 2.67 (2H, t, J = 6.3 Hz, CH_2), 3.55 (4H, t, J = 4.4 Hz, 2 × CH_2), 3.78 (3H, s, CH_3), 4.29 (2H, t, J = 6.2 Hz, CH_2), 6.86 (1H, dd, J = 8.9, 2.5 Hz, H-6), 7.48 (1H, d, J = 8.9 Hz, H-7), 8.13 (1H, s, H-2), 8.18 (1H, d, J = 2.5 Hz, H-4), 8.74 (1H, s, SH), 8.92 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 43.2 (t), 53.2 (2 × t), 55.2 (q), 57.3 (t), 66.1 (2 × t), 103.8 (d), 111.2 (d), 111.7 (d), 114.9 (s), 126.7 (s), 131.7 (s), 132.3 (d), 154.8 (s), 192.8 (s); *Anal*. Calculated for $C_{16}H_{21}N_3O_2S$ (MW: 319.42) : C, 60.16; H, 6.63; N, 13.16%. Found: C, 60.03; H, 6.95; N, 12.97%.

General procedure for the synthesis of 3-{2-[1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (56a-h)

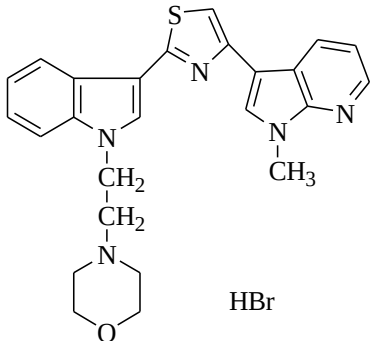
Compounds **56a-h** were prepared from **75a-d** using the same synthetic procedure described for compounds **69a,b**.

3-{2-[1-(2-Morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56a)



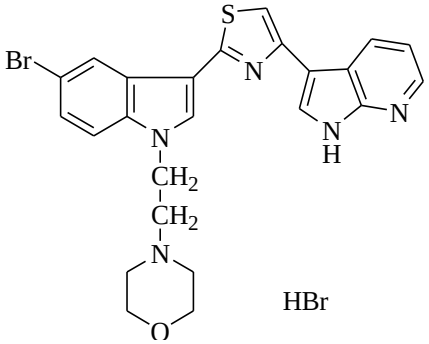
Conditions: 30 minutes at reflux. Yield: 70%, light brown solid; mp: 246-247 °C; IR: 3119 (NH), 2976 (NH^+) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.21-3.79 (8H, m, 4 × CH_2), 4.01 (2H, s, CH_2), 4.76 (2H, t, J = 6.7 Hz, CH_2), 7.25 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.32-7.42 (2H, m, H-5'and H-6'), 7.73-7.77 (2H, m, H-2' and H-7'), 8.12 (1H, d, J = 2.5 Hz, H-2''), 8.31-8.41 (3H, m, H-4', H-5 and H-6''), 8.65 (1H, dd, J = 8.0, 1.3 Hz, H-4''), 10.19 (1H, bs, NH^+), 12.00 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 40.1 (t), 51.5 (2 × t), 54.3 (t), 63.2 (2 × t), 107.2 (d), 109.9 (s), 110.7 (s), 110.8 (d), 116.1 (d), 117.5 (s), 120.7 (d), 121.5 (d), 122.9 (d), 124.8 (d + s), 128.6 (d), 129.4 (d), 136.3 (s), 142.9 (d), 148.6 (s), 149.9 (s), 161.3 (s); *Anal*. Calculated for $C_{24}H_{24}BrN_5OS$ (MW: 510.45) : C, 56.47; H, 4.74; N, 13.72%. Found: C, 56.25; H, 4.61; N, 14.07%.

1-Methyl-3-{2-[1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56b)



Conditions: 30 minutes at reflux. Yield: 80%, light yellow solid; mp: 240-241 °C; IR: 2951 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.20-3.80 (8H, m, 4 × CH_2), 3.94-4.08 (5H, m, CH_2 and CH_3), 4.76 (2H, s, CH_2), 7.24-7.43 (3H, m, H-5', H-5'' and H-6'), 7.74-7.78 (2H, m, H-2' and H-4'), 8.20 (1H, s, H-2''), 8.33 (1H, s, H-5), 8.36-8.44 (2H, m, H-6'' and H-7'), 8.63 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 10.16 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.0 (q), 40.7 (t), 51.5 (2 × t), 54.3 (t), 63.2 (2 × t), 107.1 (d), 108.9 (s), 110.7 (d), 110.8 (s), 116.2 (d), 117.3 (s), 120.9 (d), 121.5 (d), 122.9 (d), 124.8 (s), 128.7 (d), 128.8 (d), 129.4 (d), 136.3 (s), 142.9 (d), 147.6 (s), 149.5 (s), 161.4 (s); *Anal*. Calculated for $C_{25}H_{26}BrN_5OS$ (MW: 524.48) : C, 57.25; H, 5.00; N, 13.35%. Found: C, 57.61; H, 4.79; N, 13.20%.

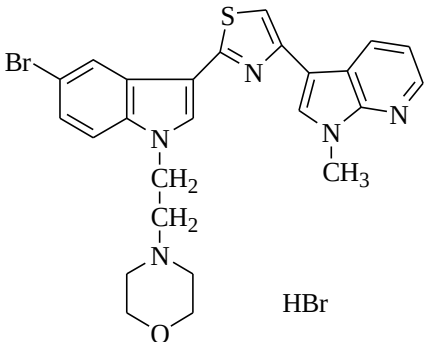
3-{2-[5-Bromo-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56c)



Conditions: 30 minutes at reflux. Yield: 81%, light brown solid; mp: 249-250 °C; IR: 3186 (NH), 2978 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.20-3.77 (8H, m, 4 × CH_2), 3.98-4.10 (2H, m, CH_2), 4.75 (2H, t, J = 6.7 Hz, CH_2), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.53 (1H, dd, J = 8.7, 1.8 Hz, H-6'), 7.77 (1H, d, J = 9.1 Hz, H-7'), 7.79 (1H, s, H-2'), 8.10 (1H, d, J = 2.5 Hz, H-2''), 8.34 (1H, dd, J = 4.7, 1.1 Hz, H-6''), 8.39 (1H, s, H-5), 8.60 (1H, d, J = 1.8 Hz, H-4'), 8.65 (1H, dd, J = 7.9, 1.1 Hz, H-4''), 10.18 (1H, bs, NH^+), 12.02 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 40.2 (t), 51.4 (2 × t), 54.2 (t), 63.1 (2 × t), 107.4 (d), 109.8 (s), 110.2 (s), 112.9 (d), 114.1 (s),

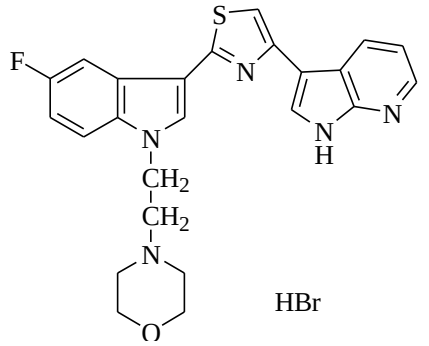
116.0 (d), 117.2 (s), 123.1 (d), 124.7 (d), 125.3 (d), 126.5 (s), 128.6 (d), 130.7 (d), 135.0 (s), 142.9 (d), 148.4 (s), 149.9 (s), 160.7 (s); *Anal*. Calculated for $C_{24}H_{23}Br_2N_5OS$ (MW: 589.35) : C, 48.91; H, 3.93; N, 11.88%. Found: C, 48.72; H, 4.27; N, 11.73%.

3-{2-[5-Bromo-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56d)



Conditions: 30 minutes at reflux. Yield: 98%, white solid; mp: 271-272 °C; IR: 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.20-3.84 (8H, m, 4 × CH_2), 3.94-4.07 (5H, m, CH_2 and CH_3), 4.72-4.78 (2H, m, CH_2), 7.27 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.54 (1H, dd, J = 8.7, 1.8 Hz, H-6'), 7.74-7.78 (2H, m, H-2' and H-7'), 8.16 (1H, s, H-2''), 8.37-8.40 (2H, m, H-5 and H-6''), 8.57 (1H, d, J = 1.8 Hz, H-4'), 8.64 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 10.0 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.0 (q), 40.2 (t), 51.4 (2 × t), 54.1 (t), 63.1 (2 × t), 107.4 (d), 108.6 (s), 110.2 (s), 113.0 (d), 114.1 (s), 116.1 (d), 117.3 (s), 123.0 (d), 125.4 (d), 126.4 (s), 128.5 (d), 128.6 (d), 130.8 (d), 135.1 (s), 142.9 (d), 147.5 (s), 149.6 (s), 160.8 (s); *Anal*. Calculated for $C_{25}H_{25}Br_2N_5OS$ (MW: 603.37) : C, 49.76; H, 4.18; N, 11.61%. Found: C, 49.55; H, 4.55; N, 11.45%.

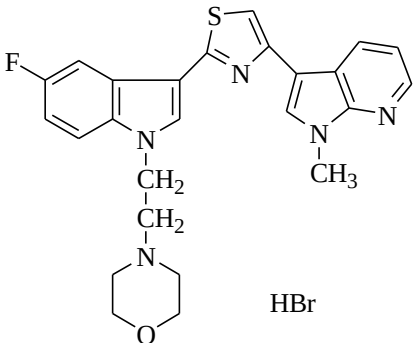
3-{2-[5-Fluoro-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56e)



Conditions: 30 minutes at reflux. Yield: 95%, light yellow solid; mp: 264-265 °C; IR: 3119 (NH), 2960 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.24-3.78 (8H, m, 4 × CH_2), 3.99-4.08 (2H, m, CH_2), 4.75 (2H, t, J = 5.5 Hz, CH_2), 7.21-7.32 (2H, m, H5'' and H-6'), 7.77-7.83 (2H, m, H-2' and

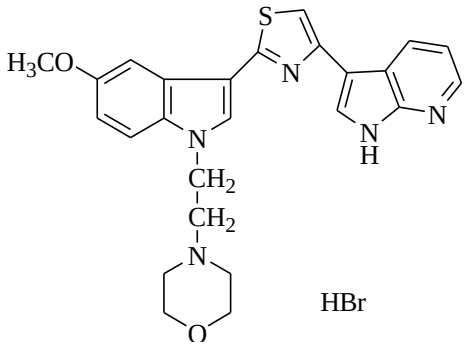
H-7'), 8.11 (1H, dd, J = 10.8, 2.5 Hz, H-4'), 8.14 (1H, d, J = 2.5 Hz, H-2''), 8.33 (1H, dd, J = 4.7, 1.4 Hz, H-6''), 8.40 (1H, s, H-5), 8.62 (1H, dd, J = 7.8, 1.4 Hz, H-4''), 10.17 (1H, bs, NH^+), 12.02 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 40.3 (t), 51.4 (2 × t), 54.2 (t), 63.2 (2 × t), 105.7 (d, $J_{C4'-F}$ = 25.7 Hz), 107.2 (d), 109.9 (s), 110.8 (d, J_{C7a-F} = 4.6 Hz), 111.1 (d, $J_{C6'-F}$ = 25.4 Hz), 112.2 (d, $J_{C7'-F}$ = 8.9 Hz), 116.1 (d), 117.1 (s), 124.9 (d), 125.2 (d, J_{C3a-F} = 10.7 Hz), 128.5 (d), 131.2 (d), 133.0 (s), 142.9 (d), 148.5 (s), 149.9 (s), 158.3 (d, $J_{C5'-F}$ = 234.6 Hz), 161.0 (s); *Anal*. Calculated for $C_{24}H_{23}BrFN_5OS$ (MW: 528.44) : C, 54.55; H, 4.39; N, 13.25%. Found: C, 54.43; H, 4.23; N, 13.53%.

3-{2-[5-Fluoro-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56f)



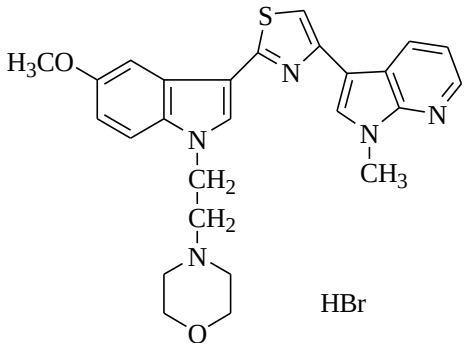
Conditions: 30 minutes at reflux. Yield: 98%, light yellow solid; mp: 281-282 °C; IR: 2975 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.20-3.86 (8H, m, 4 × CH_2), 3.94-4.07 (5H, m, CH_2 and CH_3), 4.72-4.78 (2H, m, CH_2), 7.24-7.32 (2H, m, H5'' and H-6'), 7.76-7.82 (2H, m, H-2' and H-7'), 8.13 (1H, dd, J = 9.8, 2.5 Hz, H-4'), 8.22 (1H, s, H-2''), 8.36-8.39 (2H, m, H-5 and H-6''), 8.59 (1H, dd, J = 8.0, 1.4 Hz, H-4''), 9.99 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 30.9 (q), 40.3 (t), 51.4 (2 × t), 54.2 (t), 63.1 (2 × t), 105.8 (d, $J_{C4'-F}$ = 24.4 Hz), 107.2 (d), 108.8 (s), 110.8 (d, J_{C7a-F} = 4.6 Hz), 111.1 (d, $J_{C6'-F}$ = 26.2 Hz), 112.2 (d, $J_{C7'-F}$ = 10.2 Hz), 116.2 (d), 117.3 (s), 125.2 (d, J_{C3a-F} = 10.7 Hz), 128.5 (d), 128.9 (d), 131.3 (d), 133.0 (s), 142.9 (d), 147.6 (s), 149.6 (s), 158.4 (d, $J_{C5'-F}$ = 234.1 Hz), 161.1 (s); *Anal*. Calculated for $C_{25}H_{25}BrFN_5OS$ (MW: 542.47) : C, 55.35; H, 4.65; N, 12.91%. Found: C, 55.45; H, 4.95; N, 12.51%.

3-{2-[5-Methoxy-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56g)



Conditions: 30 minutes at reflux. Yield: 92%, light brown solid; mp: 272-273 °C; IR: 3134 (NH), 2974 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.23-3.80 (8H, m, 4 × CH_2), 3.92-4.05 (5H, m, CH_2 and CH_3), 4.68-4.74 (2H, m, CH_2), 7.02 (1H, dd, J = 9.0, 2.4 Hz, H-6'), 7.21 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.66 (1H, d, J = 9.0 Hz, H-7'), 7.73 (1H, s, H-2'), 7.97 (1H, d, J = 2.4 Hz, H-4'), 8.11 (1H, d, J = 2.4 Hz, H-2''), 8.26 (1H, s, H-5), 8.32 (1H, dd, J = 4.7, 1.3 Hz, H-6''), 8.73 (1H, d, J = 7.9 Hz, H-4''), 10.20 (1H, bs, NH^+), 11.98 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 40.3 (t), 51.5 (2 × t), 54.3 (t), 55.2 (q), 63.2 (2 × t), 102.3 (d), 106.7 (d), 109.9 (s), 110.5 (s), 111.6 (d), 112.9 (d), 116.0 (d), 117.3 (s), 124.5 (d), 125.4 (s), 128.7 (d), 129.7 (d), 131.3 (s), 142.9 (d), 148.5 (s), 149.9 (s), 155.2 (s), 161.6 (s); *Anal*. Calculated for $C_{25}H_{26}BrN_5O_2S$ (MW: 540.48) : C, 55.56; H, 4.85; N, 12.96%. Found: C, 55.78; H, 4.52; N, 13.07%.

3-{2-[5-Methoxy-1-(2-morpholin-4-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (56h)



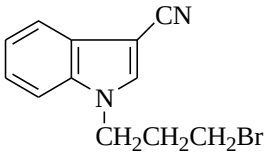
Conditions: 30 minutes at reflux. Yield: 93%, white solid; mp: 200-201 °C; IR: 2954 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 3.17-3.77 (8H, m, 4 × CH_2), 3.92-4.06 (8H, m, CH_2 and 2 × CH_3), 4.67-4.73 (2H, m, CH_2), 7.02 (1H, dd, J = 9.0, 2.4 Hz, H-6'), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.65 (1H, d, J = 9.0 Hz, H-7'), 7.71 (1H, s, H-2'), 7.95 (1H, d, J = 2.4 Hz, H-4'), 8.15 (1H, s,

H-2''), 8.25 (1H, s, H-5), 8.37 (1H, dd, J = 4.7, 1.5 Hz, H-6''), 8.72 (1H, dd, J = 7.9, 1.5 Hz, H-4''), 10.07 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.0 (q), 40.3 (t), 51.5 (2 × t), 54.3 (t), 55.3 (q), 63.2 (2 × t), 102.4 (d), 106.7 (d), 108.8 (s), 110.5 (s), 111.6 (d), 112.8 (d), 116.1 (d), 117.5 (s), 125.4 (s), 128.4 (d), 128.8 (d), 129.8 (d), 131.3 (s), 142.9 (d), 147.6 (s), 149.6 (s), 155.2 (s), 161.7 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5O_2S$ (MW: 554.50) : C, 56.32; H, 5.09; N, 12.63%. Found: C, 56.19; H, 4.86; N, 12.99%.

General procedure for the synthesis of 1-(3-bromo-propyl)-1*H*-indole-3-carbonitriles (81a-d)

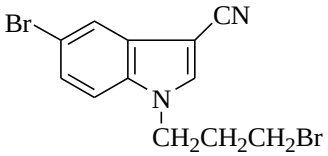
To a solution of the appropriate indole carbonitrile **70a-d** (0.4 g, 1.80 mmol) in dry DMF (2 mL), was slowly added at 0-5 °C, sodium hydride 60% dispersion in mineral oil (NaH) (0.06 g, 2.70 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. After that, it was added dropwise 1,3-dibromopropane **80** (0.91 mL, 9.00 mmol) and the reaction mixture was stirred at room temperature for 2-24 hours. Then, the mixture was poured into water and ice and extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether:ethyl acetate (7:3) as eluent.

1-(3-Bromo-propyl)-1*H*-indole-3-carbonitrile (81a)



Conditions: rt for 2 hours. Yield: 70%, white solid; mp: 52-53 °C; IR: 2220 (CN) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.27-2.40 (2H, m, CH_2), 3.47 (2H, t, J = 6.5 Hz, CH_2), 4.39 (2H, t, J = 6.8 Hz, CH_2), 7.24-7.40 (2H, m, H-5 and H-6), 7.64-7.73 (2H, m, H-4 and H-7), 8.32 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 31.1 (t), 32.3 (t), 44.7 (t), 83.7 (s), 111.4 (d), 115.9 (s), 118.8 (d), 122.0 (d), 123.5 (d), 127.1 (s), 135.0 (s), 136.8 (d); *Anal*. Calculated for $C_{12}H_{11}BrN_2$ (MW: 263.13) : C, 54.77; H, 4.21; N, 10.65%. Found: C, 54.53; H, 4.58; N, 10.52%.

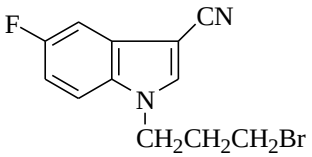
5-Bromo-1-(3-bromo-propyl)-1*H*-indole-3-carbonitrile (81b)



Conditions: rt for 24 hours. Yield: 80%, white solid; mp: 113-114°C; IR: 2220 (CN) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.25-2.39 (2H, m, CH_2), 3.47 (2H, t, J = 6.6 Hz, CH_2), 4.39 (2H, t, J = 6.8

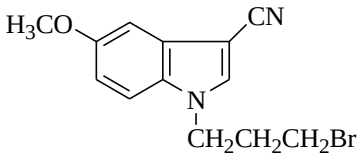
Hz, CH_2), 7.50 (1H, dd, J = 8.8, 1.9 Hz, H-6), 7.73 (1H, d, J = 8.8 Hz, H-7), 7.83 (1H, d, J = 1.9 Hz, H-4), 8.39 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ: 31.0 (t), 32.2 (t), 45.0 (t), 83.5 (s), 113.6 (d), 114.8 (s), 115.2 (s), 121.0 (d), 126.2 (d), 128.7 (s), 134.0 (s), 138.2 (d); *Anal*. Calculated for $C_{12}H_{10}Br_2N_2$ (MW: 342.03) : C, 42.14; H, 2.95; N, 8.19%. Found: C, 41.94; H, 3.29; N, 8.05%.

1-(3-Bromo-propyl)-5-fluoro-1*H*-indole-3-carbonitrile (81c)



Conditions: rt for 2 hours. Yield: 80%, white solid; mp: 108-109 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-*d*$_6$) δ: 2.29-2.36 (2H, m, CH_2), 3.47 (2H, t, J = 5.8 Hz, CH_2), 4.39 (2H, t, J = 6.1 Hz, CH_2), 7.18-7.27 (1H, m, H-6), 7.44 (1H, d, J = 8.5 Hz, H-7), 7.72-7.78 (1H, m, H-4), 8.38 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ: 31.0 (t), 32.2 (t), 45.1 (t), 84.0 (d, $J_{C7a\text{-}F}$ = 4.6 Hz), 104.1 (d, $J_{C4\text{-}F}$ = 24.9 Hz), 112.0 (d, $J_{C6\text{-}F}$ = 26.2 Hz), 113.0 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 115.5 (s), 127.6 (d, $J_{C3a\text{-}F}$ = 10.8 Hz), 131.8 (s), 138.4 (d), 158.5 (d, $J_{C5\text{-}F}$ = 236.9 Hz); *Anal*. Calculated for $C_{12}H_{10}BrFN_2$ (MW: 281.12) : C, 51.27; H, 3.59; N, 9.96%. Found: C, 51.49; H, 3.27; N, 10.06%.

1-(3-Bromo-propyl)-5-methoxy-1*H*-indole-3-carbonitrile (81d)

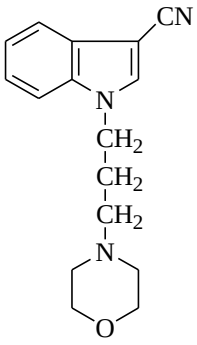


Conditions: rt for 2 hours. Yield: 70%, white solid; mp: 177-178 °C; IR: 2221 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-*d*$_6$) δ: 2.28-2.34 (2H, m, CH_2), 3.42-3.49 (2H, m, CH_2), 3.82 (3H, s, CH_3), 4.32-4.38 (2H, m, CH_2), 6.97 (1H, d, J = 8.4 Hz, H-6), 7.10 (1H, s, H-4), 7.61 (1H, d, J = 8.4 Hz, H-7), 8.23 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ: 31.1 (t), 32.3 (t), 44.9 (t), 55.4 (q), 83.3 (s), 100.1 (d), 112.4 (d), 113.8 (d), 116.1 (s), 127.9 (s), 130.0 (s), 136.6 (d), 155.5 (s); *Anal*. Calculated for $C_{13}H_{13}BrN_2O$ (MW: 293.16) : C, 53.26; H, 4.47; N, 9.56%. Found: C, 53.11; H, 4.35; N, 9.83%

General procedure for the synthesis of the derivatives 1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbonitriles (83a-d)

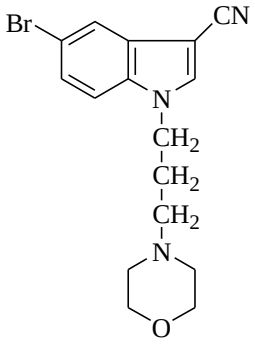
A mixture of appropriate indole carbonitrile **81a-d** (0.5 g, 1.46 mmol), morpholine **82** (1.27 mL, 14.69 mmol), and potassium carbonate (K_2CO_3) (0.35 g, 2.54 mmol), in dry DMF (5.5 mL) was stirred at 50 °C for 1-2 hours. After cooling, the mixture was poured into water and ice and the obtained precipitate was filtered off, dried, to give the desired products.

1-(3-Morpholin-4-yl-propyl)-1*H*-indole-3-carbonitrile (83a)



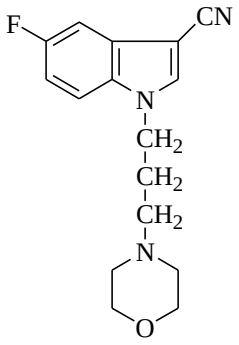
Conditions: heated for 1 hour at 50 °C. Yield: 90%, white solid; mp: 104-105 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.87-2.01 (2H, m, CH_2), 2.19 (2H, t, J = 6.4 Hz, CH_2), 2.27 (4H, t, J = 4.5 Hz, 2 × CH_2), 3.54 (4H, t, J = 4.6 Hz, 2 × CH_2), 4.31 (2H, t, J = 6.8 Hz, CH_2), 7.23-7.39 (2H, m, H-5 and H-6), 7.63-7.74 (2H, m, H-4 and H-7), 8.31 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.0 (t), 44.3 (t), 53.1 (2 × t), 54.6 (t), 66.0 (2 × t), 83.3 (s), 111.5 (d), 116.0 (s), 118.6 (d), 121.8 (d), 123.2 (d), 127.0 (s), 135.2 (s), 137.0 (d); *Anal*. Calculated for $C_{16}H_{19}N_3O$ (MW: 269.34) : C, 71.35; H, 7.11; N, 15.60%. Found: C, 70.95; H, 7.27; N, 15.84%.

5-Bromo-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbonitrile (83b)



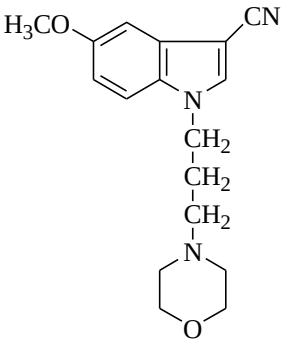
Conditions: heated for 2 hours at 50 °C. Yield: 98%, white solid; mp: 121-122 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.90-1.96 (2H, m, CH_2), 2.14-2.19 (2H, m, CH_2), 2.22-2.26 (4H, m, 2 × CH_2), 3.50-3.55 (4H, m, 2 × CH_2), 4.27-4.33 (2H, m, CH_2), 7.47 (1H, d, J = 8.8 Hz, H-6), 7.72 (1H, d, J = 8.8 Hz, H-7), 7.81 (1H, s, H-4), 8.38 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 25.9 (t), 44.5 (t), 53.0 (2 × t), 54.5 (t), 66.0 (2 × t), 83.1 (s), 113.7 (d), 114.6 (s), 115.3 (s), 120.9 (d), 126.0 (d), 128.6 (s), 134.1 (s), 138.3 (d); *Anal*. Calculated for $C_{16}H_{18}BrN_3O$ (MW: 348.24) : C, 55.18; H, 5.21; N, 12.07%. Found: C, 55.01; H, 5.05; N, 12.40%.

5-Fluoro-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbonitrile (83c)



Conditions: heated for 1 hour at 50 °C. Yield: 98%, white solid; mp: 76-77 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.87-2.00 (2H, m, CH_2), 2.19 (2H, t, J = 6.4 Hz, CH_2), 2.25 (4H, t, J = 4.2 Hz, 2 × CH_2), 3.53 (4H, t, J = 4.4 Hz, 2 × CH_2), 4.30 (2H, t, J = 6.7 Hz, CH_2), 7.21 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.43 (1H, dd, J = 9.2, 2.5 Hz, H-7), 7.73-7.80 (1H, m, H-4), 8.39 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.0 (t), 44.6 (t), 53.0 (2 × t), 54.6 (t), 66.0 (2 × t), 83.5 (d, $J_{C7a\text{-}F}$ = 4.5 Hz), 103.9 (d, $J_{C4\text{-}F}$ = 24.6 Hz), 111.7 (d, $J_{C6\text{-}F}$ = 26.3 Hz), 113.2 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 115.6 (s), 127.5 (d, $J_{C3a\text{-}F}$ = 11.0 Hz), 132.0 (s), 138.5 (d), 158.5 (d, $J_{C5\text{-}F}$ = 246.0 Hz); *Anal*. Calculated for $C_{16}H_{18}FN_3O$ (MW: 287.33) : C, 66.88; H, 6.31; N, 14.62%. Found: C, 67.02; H, 6.48; N, 14.31%.

5-Methoxy-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbonitrile (83d)



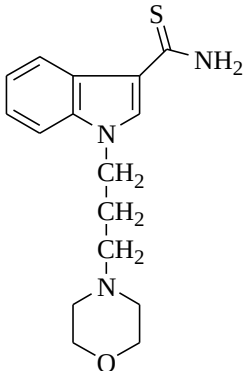
Conditions: heated for 1 hour at 50 °C; work-up: the oil residue was extracted with ethyl acetate (3 × 20 mL) and purified by column chromatography using dichloromethane:methanol (98:2) as eluent. Yield: 90%, yellow oil; IR: 2215 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.85-1.98 (2H, m, CH_2), 2.17 (2H, t, J = 6.4 Hz, CH_2), 2.25-2.29 (4H, m, 2 × CH_2), 3.55 (4H, t, J = 4.5 Hz, 2 × CH_2), 3.82 (3H, s, CH_3), 4.25 (2H, t, J = 6.7 Hz, CH_2), 6.94 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.08 (1H, d, J = 2.4 Hz, H-4), 7.60 (1H, d, J = 9.0 Hz, H-7), 8.22 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.1 (t), 44.3 (t), 53.1 (2 × t), 54.6 (t), 55.4 (q), 66.0 (2 × t), 82.9 (s), 99.9 (d), 112.5

(d), 113.6 (d), 116.2 (s), 127.9 (s), 130.1 (s), 136.7 (d), 155.4 (s); *Anal*. Calculated for $C_{17}H_{21}N_3O_2$ (MW: 299.37) : C, 68.20; H, 7.07; N, 14.04%. Found: C, 68.46; H, 6.68; N, 14.17%.

General procedure for the synthesis of 1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbothioamides (79a-d)

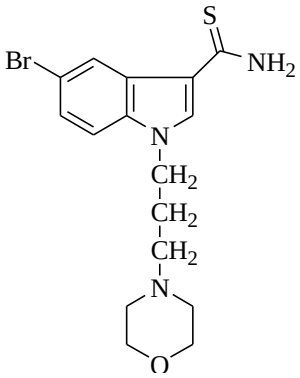
Compounds **79a-d** were prepared from **83a-d** using the same synthetic procedure described for compounds **75a-d**.

1-(3-Morpholin-4-yl-propyl)-1*H*-indole-3-carbothioamide (79a)



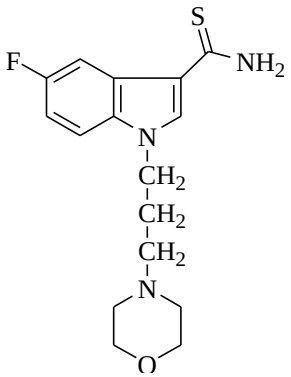
Conditions: 2 hours at reflux. Yield: 70%, yellow solid; mp: 168-169 °C; IR: 1653 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.87-2.00 (2H, m, CH_2), 2.21 (2H, t, J = 6.5 Hz, CH_2), 2.30 (4H, t, J = 4.3 Hz, 2 × CH_2), 3.58 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.26 (2H, t, J = 6.7 Hz, CH_2), 7.13-7.27 (2H, m, H-5 and H-6), 7.57 (1H, dd, J = 6.3, 1.7 Hz, H-7), 8.15 (1H, s, H-2), 8.56-8.61 (1H, m, H-4), 8.79 (1H, s, SH), 9.00 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.1 (t), 43.7 (t), 53.1 (2 × t), 54.7 (t), 66.1 (2 × t), 110.5 (d), 115.4 (s), 121.0 (d), 121.8 (d), 122.0 (d), 126.0 (s), 131.7 (d), 136.6 (s), 193.0 (s); *Anal*. Calculated for $C_{16}H_{21}N_3OS$ (MW: 303.42) : C, 63.33; H, 6.98; N, 13.85%. Found: C, 63.55; H, 7.09 ; N, 13.52%.

5-Bromo-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbothioamide (79b)



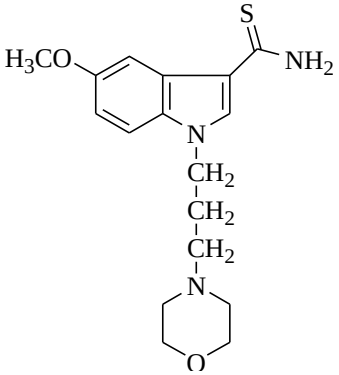
Conditions: 1 hour at reflux. Yield: 61%, yellow solid; mp: 99-100 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.85-1.99 (2H, m, CH_2), 2.19 (2H, t, J = 6.3 Hz, CH_2), 2.28 (4H, t, J = 4.2 Hz, 2 × CH_2), 3.56 (4H, t, J = 4.4 Hz, 2 × CH_2), 4.25 (2H, t, J = 6.6 Hz, CH_2), 7.35 (1H, dd, J = 8.7, 2.0 Hz, H-6), 7.58 (1H, d, J = 8.7 Hz, H-7), 8.19 (1H, s, H-2), 8.87 (1H, d, J = 2.0 Hz, H-4), 8.92 (1H, s, SH), 9.07 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.0 (t), 44.0 (t), 53.1 (2 × t), 54.6 (t), 66.0 (2 × t), 112.7 (d), 114.0 (s), 114.6 (s), 124.1 (d), 124.5 (d), 128.0 (s), 132.1 (d), 135.5 (s), 192.4 (s); *Anal*. Calculated for $C_{16}H_{20}BrN_3OS$ (MW: 382.32) : C, 50.26; H, 5.27; N, 10.99%. Found: C, 50.44; H, 5.40; N, 10.68%.

5-Fluoro-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbothioamide (79c)



Conditions: 2 hours at reflux. Yield: 60%, yellow solid; mp: 149-150 °C; IR: 1629 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.87-2.00 (2H, m, CH_2), 2.20 (2H, t, J = 6.4 Hz, CH_2), 2.28 (4H, t, J = 4.0 Hz, 2 × CH_2), 3.67 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.26 (2H, t, J = 6.6 Hz, CH_2), 7.08 (1H, td, J = 11.1, 9.0, 2.6 Hz, H-6), 7.58-7.64 (1H, m, H-7), 8.22 (1H, s, H-2), 8.43 (1H, dd, J = 11.1, 2.6 Hz, H-4), 8.85 (1H, s, SH), 9.02 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.0 (t), 44.1 (t), 53.1 (2 × t), 54.6 (t), 66.0 (2 × t), 106.9 (d, $J_{C4\text{-}F}$ = 25.6 Hz), 110.2 (d, $J_{C6\text{-}F}$ = 26.0 Hz), 111.9 (d, $J_{C7\text{-}F}$ = 10.0 Hz), 115.1 (d, $J_{C7a\text{-}F}$ = 4.9 Hz), 126.9 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 132.7 (d), 133.4 (s), 158.1 (d, $J_{C5\text{-}F}$ = 233.2 Hz), 192.6 (s); *Anal*. Calculated for $C_{16}H_{20}FN_3OS$ (MW: 321.41) : C, 59.79; H, 6.27; N, 13.07%. Found: C, 59.54; H, 6.14; N, 13.45%.

5-Methoxy-1-(3-morpholin-4-yl-propyl)-1*H*-indole-3-carbothioamide (79d)

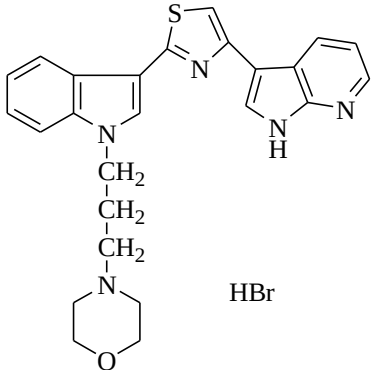


Conditions: 2 hours at reflux. Yield: 72%, yellow solid; mp: 157-158 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.85-1.98 (2H, m, CH_2), 2.19 (2H, t, J = 7.0 Hz, CH_2), 2.29 (4H, t, J = 4.4 Hz, 2 × CH_2), 3.58 (4H, t, J = 4.5 Hz, 2 × CH_2), 3.78 (3H, s, CH_3), 4.21 (2H, t, J = 6.6 Hz, CH_2), 6.86 (1H, dd, J = 8.9, 2.5 Hz, H-6), 7.47 (1H, d, J = 8.9 Hz, H-7), 8.11 (1H, s, H-2), 8.18 (1H, d, J = 2.5 Hz, H-4), 8.73 (1H, s, SH), 8.92 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 26.1 (t), 43.9 (t), 53.1 (2 × t), 54.6 (t), 55.2 (q), 66.1 (2 × t), 103.8 (d), 111.2 (d), 111.8 (d), 114.9 (s), 126.7 (s), 131.7 (s), 132.0 (d), 154.8 (s), 192.8 (s); *Anal*. Calculated for $C_{17}H_{23}N_3O_2S$ (MW: 333.45) : C, 61.23; H, 6.95; N, 12.60%. Found: C, 61.35; H, 7.10; N, 12.33%.

General procedure for the synthesis of 3-{2-[1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (57a-h)

Compounds **57a-h** were prepared from **79a-d** using the same synthetic procedure described for compounds **69a,b**.

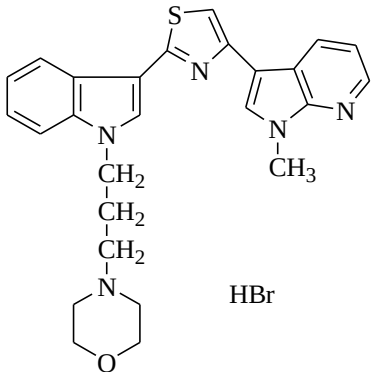
3-{2-[1-(3-Morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57a)



Conditions: 30 minutes at reflux. Yield: 80%, white solid; mp: 240-241 °C; IR: 3448 (NH), 2973 (NH^+) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.28-2.35 (2H, m, CH_2), 3.07-3.23 (4H, m, 2 × CH_2), 3.49-3.79 (4H, m, 2 × CH_2), 3.94-3.99 (2H, m, CH_2), 4.43 (2H, t, J = 6.3 Hz, CH_2), 7.21-7.35

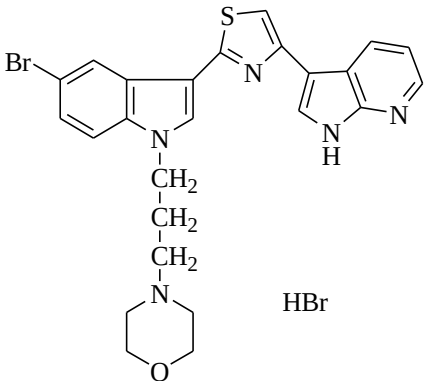
(3H, m, H-5', H-5'' and H-6'), 7.68-7.74 (2H, m, H-2' and H-7'), 8.12 (1H, d, J = 1.70 Hz, H-2''), 8.31-8.39 (3H, m, H-4', H-5 and H-6''), 8.64 (1H, d, J = 7.6 Hz, H-4''), 10.04 (1H, bs, NH^+), 11.99 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 43.2 (t), 51.1 (2 × t), 53.5 (t), 63.3 (2 × t), 106.9 (d), 110.0 (s), 110.3 (s), 110.7 (d), 116.1 (d), 117.1 (s), 120.7 (d), 121.2 (d), 122.6 (d), 124.8 (d + s), 128.5 (d), 129.3 (d), 136.3 (s), 143.0 (d), 148.7 (s), 149.8 (s), 161.5 (s); *Anal*. Calculated for $C_{25}H_{26}BrN_5OS$ (MW: 524.48) : C, 57.25; H, 5.00; N, 13.35%. Found: C, 57.15; H, 4.82; N, 13.63%.

1-Methyl-3-{2-[1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57b)



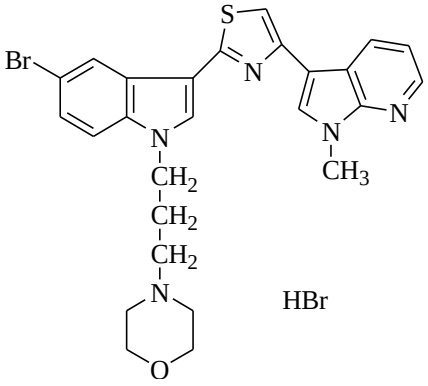
Conditions: 1 hour at reflux. Yield: 80%, brown solid; mp: 225-226 °C; IR: 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.24-2.34 (2H, m, CH_2), 3.07-3.20 (4H, m, 2 × CH_2), 3.50-3.78 (4H, m, 2 × CH_2), 3.94-3.99 (5H, m, CH_2 and CH_3), 4.43 (2H, t, J = 6.3 Hz, CH_2), 7.24-7.35 (3H, m, H-5', H-5'' and H-6'), 7.70 (1H, d, J = 8.7 Hz, H-4'), 7.73 (1H, s, H-2'), 8.20 (1H, s, H-2''), 8.30 (1H, s, H-5), 8.36-8.42 (2H, m, H-6'' and H-7'), 8.63 (1H, d, J = 7.5 Hz, H-4''), 9.99 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 30.9 (q), 43.1 (t), 51.1 (2 × t), 53.5 (t), 63.2 (2 × t), 106.8 (d), 108.9 (s), 110.2 (s), 110.6 (d), 113.6 (s), 116.2 (d), 117.2 (s), 120.8 (d), 121.2 (d), 122.6 (d), 124.7 (d), 128.6 (d), 129.4 (d), 136.3 (s), 142.9 (d), 147.6 (s), 149.4 (s), 161.6 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5OS$ (MW: 538.50) : C, 57.99; H, 5.24; N, 13.01%. Found: C, 58.09; H, 5.34; N, 12.81%.

3-{2-[5-Bromo-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridinehydrobromide (57c)



Conditions: 30 minutes at reflux. Yield: 87%, brown solid; mp: 252-253 °C; IR: 3450 (NH), 2975 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.20-2.39 (2H, m, CH_2), 3.06-3.16 (4H, m, 2 × CH_2), 3.50-3.77 (4H, m, 2 × CH_2), 3.94-4.00 (2H, m, CH_2), 4.42 (2H, t, J = 6.6 Hz, CH_2), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.48 (1H, dd, J = 8.7, 1.8 Hz, H-6'), 7.71 (1H, d, J = 8.7 Hz, H-7'), 7.77 (1H, s, H-2'), 8.10 (1H, d, J = 2.2 Hz, H-2''), 8.33 (1H, dd, J = 4.7, 1.2 Hz, H-6''), 8.37 (1H, s, H-5), 8.56 (1H, d, J = 1.8 Hz, H-4'), 8.65 (1H, d, J = 7.9 Hz, H-4''), 9.97 (1H, bs, NH^+), 12.01 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 43.3 (t), 51.1 (2 × t), 53.4 (t), 63.2 (2 × t), 107.1 (d), 109.7 (s), 109.8 (s), 112.9 (d), 113.8 (s), 116.0 (d), 117.2 (s), 123.0 (d), 124.7 (d), 125.1 (d), 126.4 (s), 128.5 (d), 130.7 (d), 135.1 (s), 142.9 (d), 148.5 (s), 149.8 (s), 161.0 (s); *Anal*. Calculated for $C_{25}H_{25}Br_2N_5OS$ (MW: 603.37) : C, 49.76; H, 4.18; N, 11.61%. Found: C, 49.49; H, 4.06; N, 12.00%.

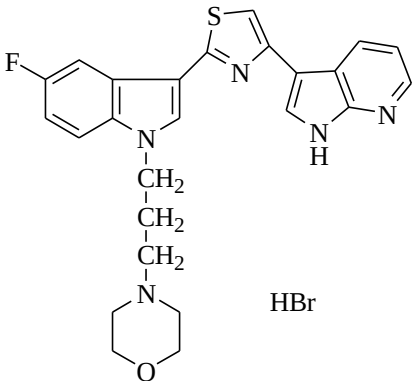
3-{2-[5-Bromo-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57d)



Conditions: 30 minutes at reflux. Yield: 90%, white solid; mp: 209-210 °C; IR: 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.18-2.37 (2H, m, CH_2), 3.05-3.16 (4H, m, 2 × CH_2), 3.49-3.73 (4H, m, 2 × CH_2), 3.94-4.00 (5H, m, CH_2 and CH_3), 4.42 (2H, t, J = 6.5 Hz, CH_2), 7.26 (1H, dd, J = 7.8, 4.7 Hz, H-5''), 7.49 (1H, d, J = 8.6 Hz, H-6'), 7.72 (1H, d, J = 8.6 Hz, H-7'), 7.74 (1H, s, H-

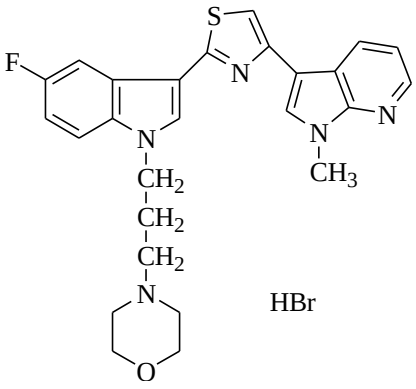
2’), 8.15 (1H, s, H-2’’), 8.35 (1H, s, H-5), 8.37 (1H, d, J = 4.7 Hz, H-6’’), 8.55 (1H, s, H-4’), 8.64 (1H, d, J = 7.8 Hz, H-4’’), 9.78 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 31.0 (q), 43.3 (t), 51.1 (2 × t), 53.4 (t), 63.3 (2 × t), 107.2 (d), 108.7 (s), 109.8 (s), 112.9 (d), 113.9 (s), 116.1 (d), 117.3 (s), 123.0 (d), 125.1 (d), 126.4 (s), 128.5 (d), 128.6 (d), 130.7 (d), 135.1 (s), 142.9 (d), 147.6 (s), 149.6 (s), 161.1 (s); *Anal*. Calculated for $C_{26}H_{27}Br_2N_5OS$ (MW: 617.40) : C, 50.58; H, 4.41; N, 11.34%. Found: C, 50.74; H, 4.12; N, 11.47%.

3-{2-[5-Fluoro-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57e)



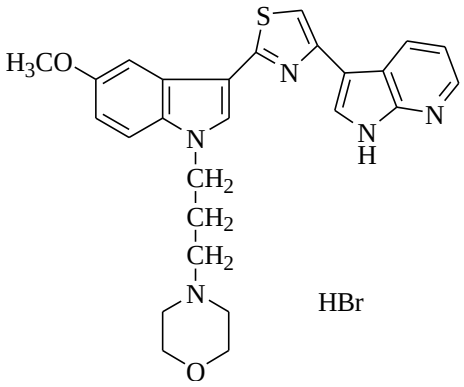
Conditions: 30 minutes at reflux. Yield: 85%, white solid; mp: 270-271 °C; IR: 3447 (NH), 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.23-2.37 (2H, m, CH_2), 3.06-3.17 (4H, m, 2 × CH_2), 3.50-3.78 (4H, m, 2 × CH_2), 3.94-4.00 (2H, m, CH_2), 4.43 (2H, t, J = 6.2 Hz, CH_2), 7.16-7.28 (2H, m, H5’’ and H-6’), 7.71-7.78 (2H, m, H-2’ and H-7’), 8.05-8.14 (2H, m, H-2’’ and H-4’), 8.32-8.38 (2H, m, H-5 and H-6’’), 8.62 (1H, d, J = 6.9 Hz, H-4’’), 10.06 (1H, bs, NH^+), 12.02 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 43.4 (t), 51.1 (2 × t), 53.4 (t), 63.2 (2 × t), 105.6 (d, $J_{C4'-F}$ = 24.9 Hz), 107.0 (d), 109.9 (s), 110.3 (d, J_{C7a-F} = 4.4 Hz), 110.8 (d, $J_{C6'-F}$ = 26.4 Hz), 112.1 (d, $J_{C7'-F}$ = 9.9 Hz), 116.1 (d), 117.2 (s), 124.9 (d), 125.1 (d, J_{C3a-F} = 10.5 Hz), 128.5 (d), 131.1 (d), 133.0 (s), 142.9 (d), 148.5 (s), 149.8 (s), 158.2 (d, $J_{C5'-F}$ = 234.7 Hz), 161.2 (s); *Anal*. Calculated for $C_{25}H_{25}BrFN_5OS$ (MW: 542.47) : C, 55.35; H, 4.65; N, 12.91%. Found: C, 55.17; H, 4.50; N, 13.24%.

3-{2-[5-Fluoro-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57f)



Conditions: 30 minutes at reflux. Yield: 98%, white solid; mp: 233-234 °C; IR: 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.22-2.36 (2H, m, CH_2), 3.07-3.18 (4H, m, 2 × CH_2), 3.50-3.77 (4H, m, 2 × CH_2), 3.94-4.00 (5H, m, CH_2 and CH_3), 4.42 (2H, t, J = 6.7 Hz, CH_2), 7.17-7.29 (2H, m, H5'' and H-6'), 7.71-7.78 (2H, m, H-2' and H-7'), 8.11 (1H, dd, J = 9.9, 2.4 Hz, H-4'), 8.22 (1H, s, H-2''), 8.28-8.37 (2H, m, H-5 and H-6''), 8.60 (1H, d, J = 6.8 Hz, H-4''), 9.96 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 30.9 (q), 43.4 (t), 51.1 (2 × t), 53.4 (t), 63.2 (2 × t), 105.7 (d, $J_{C4'-F}$ = 24.9 Hz), 106.9 (d), 108.8 (s), 110.3 (d, J_{C7a-F} = 4.4 Hz), 110.9 (d, $J_{C6'-F}$ = 26.1 Hz), 112.1 (d, $J_{C7'-F}$ = 10.1 Hz), 116.2 (d), 117.2 (s), 125.1 (d, J_{C3a-F} = 10.6 Hz), 128.5 (d), 128.8 (d), 131.2 (d), 133.1(s), 142.9 (d), 147.6 (s), 149.5 (s), 158.2 (d, $J_{C5'-F}$ = 234.0 Hz), 161.3 (s); *Anal*. Calculated for $C_{26}H_{27}BrFN_5OS$ (MW: 556.49) : C, 56.12; H, 4.89; N, 12.58%. Found: C, 56.48; H, 4.72; N, 12.39%.

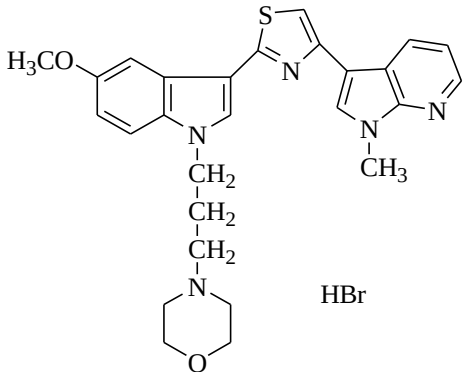
3-{2-[5-Methoxy-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57g)



Conditions: 30 minutes at reflux. Yield: 98%, white solid; mp: 256-257 °C; IR: 3450 (NH), 2974 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.24-2.35 (2H, m, CH_2), 3.06-3.16 (4H, m, 2 × CH_2), 3.39-3.55 (4H, m, 2 × CH_2), 3.73-3.79 (2H, m, CH_2), 3.91 (3H, s, CH_3), 4.37 (2H, t, J = 6.2 Hz, CH_2), 6.97 (1H, dd, J = 9.0, 2.3 Hz, H-6'), 7.20 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.60 (1H, d, J =

9.0 Hz, H-7'), 7.71 (1H, s, H-2'), 7.95 (1H, d, J = 2.3Hz, H-4'), 8.11 (1H, d, J = 2.1 Hz, H-2''), 8.23 (1H, s, H-5), 8.32 (1H, d, J = 4.7 Hz, H-6''), 8.73 (1H, d, J = 7.9Hz, H-4''), 10.05 (1H, bs, NH^+), 11.96 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 43.3 (t), 51.2 (2 × t), 53.5 (t), 55.2 (q), 63.3 (2 × t), 102.2 (d), 106.3 (d), 109.4 (s), 109.9 (s), 111.5 (d), 112.6 (d), 115.9 (d), 117.2 (s), 124.4 (d), 125.3 (s), 128.6 (d), 129.6 (d), 131.3 (s), 143.0 (d), 148.6 (s), 149.8 (s), 154.9 (s), 161.8 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5O_2S$ (MW: 554.50) : C, 56.32; H, 5.09; N, 12.63%. Found: C, 56.18; H, 4.88; N, 12.98%.

3-{2-[5-Methoxy-1-(3-morpholin-4-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (57h)

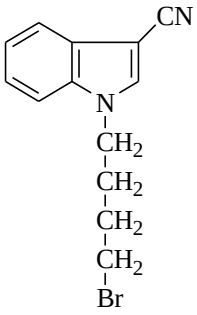


Conditions: 30 minutes at reflux. Yield: 98%, white solid; mp: 218-219 °C; IR: 2962 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 2.20-2.32 (2H, m, CH_2), 3.05-3.16 (4H, m, 2 × CH_2), 3.43-3.71 (4H, m, 2 × CH_2), 3.92 (3H, s, CH_3), 3.93 (3H, s, CH_3), 3.98-4.07 (2H, m, CH_2), 4.37 (2H, t, J = 6.2 Hz, CH_2), 6.99 (1H, dd, J = 8.9, 2.4 Hz, H-6'), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.59 (1H, d, J = 8.9 Hz, H-7'), 7.69 (1H, s, H-2'), 7.93 (1H, d, J = 2.4 Hz, H-4'), 8.15 (1H, s, H-2''), 8.22 (1H, s, H-5), 8.36 (1H, dd, J = 4.6, 1.4 Hz, H-6''), 8.71 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 9.74 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 31.0 (q), 43.3 (t), 51.1 (2 × t), 53.5 (t), 55.2 (q), 63.2 (2 × t), 102.3 (d), 106.4 (d), 108.9 (s), 109.9 (s), 111.5 (d), 112.6 (d), 116.0 (d), 117.4 (s), 125.3 (s), 128.3 (d), 128.8 (d), 129.6 (d), 131.3 (s), 142.9 (d), 147.5 (s), 149.5 (s), 154.9 (s), 161.9 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5O_2S$ (MW: 568.53) : C, 57.04; H, 5.32; N, 12.32%. Found: C, 57.37; H, 5.18; N, 12.13%.

General procedure for the synthesis of 1-(4-bromo-butyl)-1*H*-indole-3-carbonitriles (86a-d)

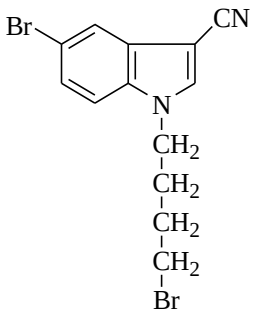
To a solution of the appropriate indole carbonitrile **70a-d** (0.5 g, 2.25 mmol) in dry DMF (2.6 mL), was slowly added at 0-5 °C, sodium hydride 60% dispersion in mineral oil (NaH) (0.08 g, 3.38 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. After that, it was added dropwise 1,4-dibromobutane **85** (1.34 mL, 11.25 mmol) and the reaction mixture was stirred at room temperature for 1-2 hours. Then, the mixture was poured into water and ice and extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether:ethyl acetate (8:2) as eluent.

1-(4-Bromo-butyl)-1*H*-indole-3-carbonitrile (86a)



Conditions: 1 hour at rt. Yield: 86%, yellow oil; IR: 2219 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.72-1.99 (4H, m, 2 × CH_2), 3.55 (2H, t, J = 6.5 Hz, CH_2), 4.32 (2H, t, J = 6.6 Hz, CH_2), 7.24-7.40 (2H, m, H-5 and H-6), 7.64-8.32 (2H, m, H-4 and H-7), 8.34 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 28.0 (t), 29.3 (t), 34.3 (t), 45.4 (t), 83.4 (s), 111.5 (d), 116.0 (s), 118.7 (d), 121.9 (d), 123.4 (d), 127.1 (s), 135.1 (s), 136.8 (d); *Anal*. Calculated for $C_{13}H_{13}BrN_2$ (MW: 277.16) :C, 56.34; H, 4.73; N, 10.11%. Found: C, 56.08; H, 4.62; N, 10.48%.

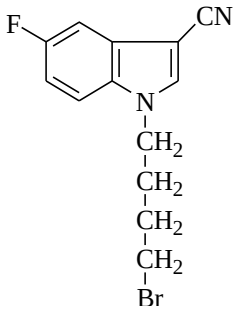
5-Bromo-1-(4-bromo-butyl)-1*H*-indole-3-carbonitrile (86b)



Conditions: 2 hours at rt. Yield: 80%, white solid; mp: 112-113 °C; IR: 2222 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.70-1.94 (4H, m, 2 × CH_2), 3.55 (2H, t, J = 6.5 Hz, CH_2), 4.32 (2H, t, J = 6.6 Hz, CH_2), 7.49 (1H, dd, J = 8.8, 1.9 Hz, H-6), 7.74 (1H, d, J = 8.8 Hz, H-7), 7.82 (1H, d, J = 1.9

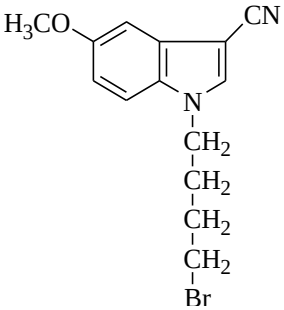
Hz, H-4), 8.40 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 28.0 (t), 29.2 (t), 34.3 (t), 45.6 (t), 83.2 (s), 113.7 (d), 114.8 (s), 115.2 (s), 121.0 (d), 126.1 (d), 128.6 (s), 134.0 (s), 138.1 (d); *Anal*. Calculated for $C_{13}H_{12}Br_2N_2$ (MW: 356.06) : C, 43.85; H, 3.40; N, 7.87%. Found: C, 43.63; H, 3.26; N, 8.23%.

1-(4-Bromo-butyl)-5-fluoro-1*H*-indole-3-carbonitrile (86c)



Conditions: 2 hours at rt. Yield: 80%, yellow solid; mp: 66-67 °C; IR: 2221 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.67-1.99 (4H, m, 2 × CH_2), 3.55 (2H, t, J = 6.5 Hz, CH_2), 4.32 (2H, t, J = 6.6 Hz, CH_2), 7.22 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.45 (1H, dd, J = 9.1, 2.4 Hz, H-7), 7.75-7.81 (1H, m, H-4), 8.41 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 28.0 (t), 29.2 (t), 34.3 (t), 45.7 (t), 83.7 (d, $J_{C7a\text{-}F}$ = 4.5 Hz), 104.0 (d, $J_{C4\text{-}F}$ = 24.8 Hz), 111.9 (d, $J_{C6\text{-}F}$ = 26.2 Hz), 113.2 (d, $J_{C7\text{-}F}$ = 9.7 Hz), 115.5 (s), 127.6 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 131.9 (s), 138.4 (d), 158.5 (d, $J_{C5\text{-}F}$ = 236.4 Hz); *Anal*. Calculated for $C_{13}H_{12}BrFN_2$ (MW: 295.15) : C, 52.90; H, 4.10; N, 9.49%. Found: C, 52.67; H, 4.47; N, 9.35%.

1-(4-Bromo-butyl)-5-methoxy-1*H*-indole-3-carbonitrile (86d)



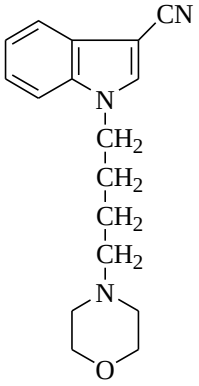
Conditions: 1 hour at rt. Yield: 80%, white solid; mp: 69-70 °C; IR: 2217 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.67-1.96 (4H, m, 2 × CH_2), 3.54 (2H, t, J = 6.5 Hz, CH_2), 3.82 (3H, s, CH_3), 4.27 (2H, t, J = 6.5 Hz, CH_2), 6.96 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.10 (1H, d, J = 2.4 Hz, H-4), 7.62 (1H, d, J = 9.0 Hz, H-7), 8.25 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 28.1 (t), 29.3 (t), 34.3 (t), 45.5 (t), 55.4 (q), 83.0 (s), 100.0 (d), 112.5 (d), 113.7 (d), 116.2 (s), 127.9 (s), 130.0 (s), 136.5

(d), 155.4 (s); *Anal*. Calculated for $C_{14}H_{15}BrN_2O$ (MW: 307.19) : C, 54.74; H, 4.92; N, 9.12%. Found: C, 54.37; H, 5.07; N, 9.34%.

General procedure for the synthesis of 1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbonitriles (87a-d)

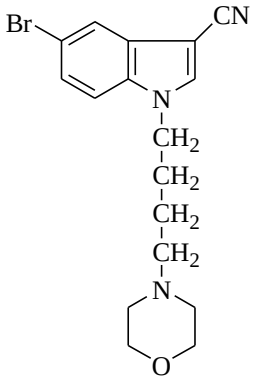
Compounds **87a-d** were prepared from **86a-d** using the same synthetic procedure described for compounds **83a-d**.

1-(4-Morpholin-4-yl-butyl)-1*H*-indole-3-carbonitrile (87a)



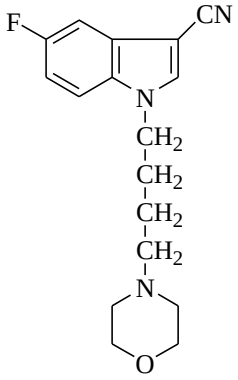
Conditions: heated for 1 hour at 50 °C. Yield: 90%, white solid; mp: 79-80 °C; IR: 2257 (CN), cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.30-1.45 (2H, m, CH_2), 1.73-1.87 (2H, m, CH_2), 2.20-2.28 (6H, m, 3 × CH_2), 3.52 (4H, t, J = 4.6 Hz, 2 × CH_2), 4.28 (2H, t, J = 7.0 Hz, CH_2), 7.23-7.39 (2H, m, H-5 and H-6), 7.63-7.73 (2H, m, H-4 and H-7), 8.33 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.0 (t), 46.2 (t), 53.1 (2 × t), 57.2 (t), 66.1 (2 × t), 83.2 (s), 111.6 (d), 116.0 (s), 118.7 (d), 121.8 (d), 123.3 (d), 127.1 (s), 135.1 (s), 136.8 (d); *Anal*. Calculated for $C_{17}H_{21}N_3O$ (MW: 283.37) : C, 72.06; H, 7.47; N, 14.83%. Found: C, 72.16; H, 7.64; N, 14.56%.

5-Bromo-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbonitrile (87b)



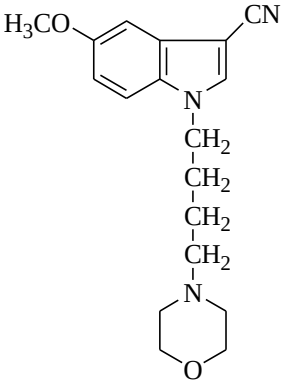
Conditions: heated for 1 hour at 50 °C. Yield: 92%, white solid; mp: 110-111 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.30-1.43 (2H, m, CH_2), 1.71-1.85 (2H, m, CH_2), 2.20-2.26 (6H, m, 3 × CH_2), 3.52 (4H, s, 2 × CH_2),4.27 (2H, t, J = 6.6 Hz, CH_2), 7.48 (1H, d, J = 8.9 Hz, H-6), 7.72 (1H, d, J = 8.9 Hz, H-7), 7.81 (1H, s, H-4), 8.39 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.0 (t), 46.4 (t), 53.1 (2 × t), 57.2 (t), 66.1 (2 × t), 83.0 (s), 113.7 (d), 114.7 (s), 115.3 (s), 121.0 (d), 126.0 (d), 128.7 (s), 134.0 (s), 138.1 (d); *Anal*. Calculated for $C_{17}H_{20}BrN_3O$ (MW: 362.26) : C, 56.36; H, 5.56; N, 11.60%. Found: C, 56.20; H, 5.42; N, 11.90%.

5-Fluoro-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbonitrile (87c)



Conditions: heated for 2 hours at 50 °C. Yield: 90%, white solid; mp: 89-90 °C; IR: 2221 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.30-1.45 (2H, m, CH_2), 1.72-1.87 (2H, m, CH_2), 2.20-2.29 (6H, m, 3 × CH_2), 3.52 (4H, t, J = 4.6 Hz, 2 × CH_2), 4.27 (2H, t, J = 7.0 Hz, CH_2), 7.21 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.44 (1H, dd, J = 9.2, 2.5 Hz, H-7), 7.73-7.79 (1H, m, H-4), 8.40 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.0 (t), 46.5 (t), 53.1 (2 × t), 57.2 (t), 66.1 (2 × t), 83.5 (d, J_{C7a-F} = 4.6 Hz), 104.0 (d, J_{C4-F} = 24.7 Hz), 111.8 (d, J_{C6-F} = 26.2 Hz), 113.2 (d, J_{C7-F} = 9.9 Hz), 115.6 (s), 127.6 (d, J_{C3a-F} = 10.9 Hz), 131.9 (s), 138.4 (d), 158.5 (d, J_{C5-F} = 233.0 Hz); *Anal*. Calculated for $C_{17}H_{20}FN_3O$ (MW: 301.36) : C, 67.75; H, 6.69; N, 13.94%. Found: C, 67.38; H, 6.91; N, 14.09%.

5-Methoxy-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbonitrile (87d)

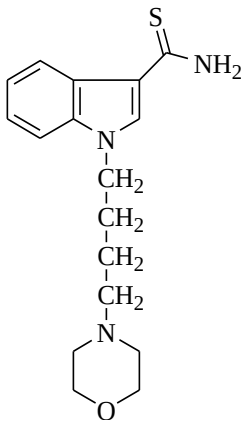


Conditions: heated for 1 hour at 50 °C. Yield: 90%, white solid; mp: 86-87 °C; IR: 2217 (CN), cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28-1.43 (2H, m, CH_2), 1.70-1.85 (2H, m, CH_2), 2.19-2.26 (6H, m, 3 × CH_2), 3.52 (4H, t, J = 4.5 Hz, 2 × CH_2), 3.82 (3H, s, CH_3), 4.23 (2H, t, J = 6.9 Hz, CH_2), 6.95 (1H, dd, J = 9.0, 2.3 Hz, H-6), 7.09 (1H, d, J = 2.3 Hz, H-4), 7.61 (1H, d, J = 9.0 Hz, H-7), 8.23 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.1 (t), 46.3 (t), 53.1 (2 × t), 55.4 (q), 57.2 (t), 66.1 (2 × t), 82 8 (s), 100.0 (d), 112.5 (d), 113.6 (d), 116.2 (s), 127.9 (s), 130.0 (s), 136.6 (d), 155.4 (s); *Anal*. Calculated for $C_{18}H_{23}N_3O_2$ (MW: 313.39) : C, 68.98; H, 7.40; N, 13.41%. Found: C, 68.69; H, 7.30; N, 13.80%.

General procedure for the synthesis of 1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbothioamides (84a-d)

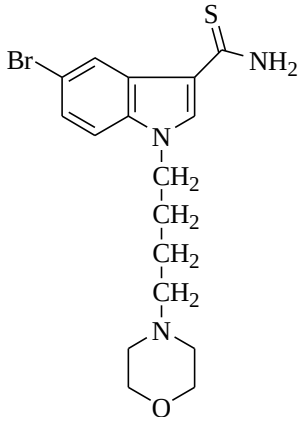
Compounds **84a-d** were prepared from **87a-d** using the same synthetic procedure described for compounds **75a-d**.

1-(4-Morpholin-4-yl-butyl)-1*H*-indole-3-carbothioamide (84a)



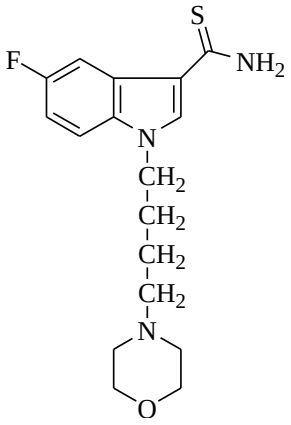
Conditions: 3 hours at reflux. Yield: 90%, yellow solid; mp: 85-86 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.49 (2H, m, CH_2), 1.74-1.88 (2H, m, CH_2), 2.23-2.30 (6H, m, 3 × CH_2), 3.54 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.23 (2H, t, J = 6.9 Hz, CH_2), 7.14-7.27 (2H, m, H-5 and H-6), 7.58 (1H, dd, J = 6.3, 1.7 Hz, H-7), 8.14 (1H, s, H-2), 8.59 (1H, dd, J = 6.1, 1.8 Hz, H-4), 8.81 (1H, s, SH), 9.00 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.9 (t), 27.1 (t), 45.7 (t), 53.1 (2 × t), 57.3 (t), 66.1 (2 × t), 110.5 (d), 115.4 (s), 121.0 (d), 121.9 (d), 122.0 (d), 126.0 (s), 131.3 (d), 136.5 (s), 193.0 (s); *Anal*. Calculated for $C_{17}H_{23}N_3OS$ (MW: 317.45) : C, 64.32; H, 7.30; N, 13.24%. Found: C, 64.15; H, 7.16; N, 13.55%.

5-Bromo-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbothioamide (84b)



Conditions: 2 hours at reflux. Yield: 84%, yellow solid; mp: 175-176 °C; IR: 1653 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.36-1.43 (2H, m, CH_2), 1.72-1.83 (2H, m CH_2), 2.22-2.29 (6H, m, 3 × CH_2), 3.53 (4H, t, J = 4.5 Hz, 2 × CH_2), 4.23 (2H, t, J = 6.8 Hz, CH_2), 7.37 (1H, dd, J = 8.7, 2.0 Hz, H-6), 7.59 (1H, d, J = 8.7 Hz, H-7), 8.19 (1H, s, H-2), 8.89 (1H, d, J = 2.0Hz, H-4), 8.93 (1H, s, SH), 9.08 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.9 (t), 27 1 (t), 45.9 (t), 53.1 (2 × t), 57.3 (t), 66.1 (2 × t), 112.7 (d), 114.0 (s), 114.5 (s), 124.2 (d), 124.6 (d), 128.1 (s), 131.7 (d), 135.4 (s), 192.4 (s); *Anal*. Calculated for $C_{17}H_{22}BrN_3OS$ (MW: 396.35) : C, 51.52; H, 5.59; N, 10.60%. Found: C, 51.16; H, 5.77; N, 10.78%.

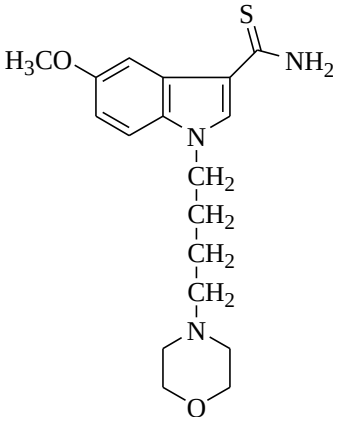
5-Fluoro-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbothioamide (84c)



Conditions: 1 hour at reflux. Yield: 89%, yellow solid; mp: 122-123 °C; IR: 1683 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.54 (2H, m, CH_2), 1.78-1.93 (2H, m CH_2), 2.29-2.34 (6H, m, 3 × CH_2), 3.59 (4H, t, J = 4.4, 2 × CH_2), 4.28 (2H, t, J = 7.0 Hz, CH_2), 7.15 (1H, td, J = 11.1, 9.1, 2.6 Hz, H-6), 7.63-7.70 (1H, m, H-7), 8.27 (1H, s, H-2), 8.48 (1H, dd, J = 11.1, 2.6 Hz, H-4), 8.91 (1H, s, SH), 9.07 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.1 (t), 46.0 (t), 53.1 (2 × t), 57.3 (t), 66.0 (2 × t), 106.9 (d, $J_{C4\text{-}F}$ = 25.8 Hz), 110.2 (d, $J_{C6\text{-}F}$ = 26.1 Hz), 111.9 (d, $J_{C7\text{-}F}$ = 9.1 Hz), 115.0 (d, $J_{C7a\text{-}F}$ = 4.7 Hz), 127.0 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 132.4 (d), 133.3 (s), 158.1 (d, $J_{C5\text{-}F}$ =

233.3 Hz), 192.6 (s); *Anal*. Calculated for $C_{17}H_{22}FN_3OS$ (MW: 335.44) : C, 60.87; H, 6.61; N, 12.53%. Found: C, 60.53; H, 6.84; N, 12.64%.

5-Methoxy-1-(4-morpholin-4-yl-butyl)-1*H*-indole-3-carbothioamide (84d)

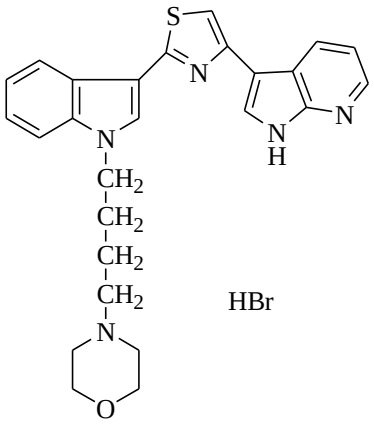


Conditions: 1 hour at reflux. Yield: 91%, yellow solid; mp: 120-121 °C; IR: 1623 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.47 (2H, m, CH_2), 1.72-1.86 (2H, m, CH_2), 2.22-2.29 (6H, m, 3 × CH_2), 3.53 (4H, t, J = 4.5 Hz, 3 × CH_2), 3.78 (3H, s, CH_3), 4.18 (2H, t, J = 7.0 Hz, CH_2), 6.86 (1H, dd, J = 8.9, 2.5 Hz, H-6), 7.48 (1H, d, J = 8.9 Hz, H-7), 8.11 (1H, s, H-2), 8.19 (1H, d, J = 2.4 Hz, H-4), 8.75 (1H, s, SH), 8.92 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.8 (t), 27.1 (t), 45.9 (t), 53.1 (2 × t), 55.2 (q), 57.3 (t), 66.0 (2 × t), 103.9 (d), 111.3 (d), 111.8 (d), 114 8 (s), 116.1 (s), 126.8 (s), 131.6 (d), 154.8 (s), 192.8 (s); *Anal*. Calculated for $C_{18}H_{25}N_3O_2S$ (MW: 347.48) : C, 62.22; H, 7.25; N, 12.09%. Found: C, 61.91; H, 7.37; N, 12.28%.

General procedure for the synthesis of 3-{2-[1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (58a-h)

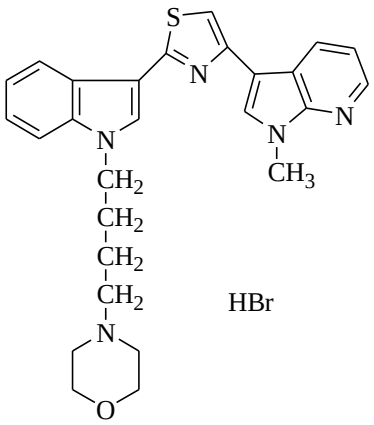
Compounds **58a-h** were prepared from **84a-d** using the same synthetic procedure described for compounds **69a,b**.

3-{2-[1-(4-Morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58a)



Conditions: rt for 24 hours. Yield: 82%, white solid; mp: 243-244 °C; IR: 3450 (NH), 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.66-1.68 (2H, m, CH_2), 1.86-1.90 (2H, m, CH_2), 3.06-3.26 (4H, m, 2 × CH_2), 3.43-3.69 (4H, m, 2 × CH_2), 3.93-3.98 (2H, m, CH_2), 4.37 (2H, t, J = 6.1 Hz, CH_2), 7.20-7.34 (3H, m, H-5', H-5'' and H-6'), 7.66-7.73 (2H, m, H-2' and H-7'), 8.11 (1H, d, J = 2.5 Hz, H-2''), 8.28-8.38 (3H, m, H-4', H-5 and H-6''), 8.63 (1H, d, J = 6.9 Hz, H-4''), 9.59 (1H, bs, NH^+), 11.98 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.2 (t), 26.6 (t), 45.2 (t), 51.0 (2 × t), 55.4 (t), 63.2 (2 × t), 106.9 (d), 109.9 (s), 110.0 (s), 110.7 (d), 116.1 (d), 117.3 (s), 120.6 (d), 121.0 (d), 122.4 (d), 124.7 (d), 124.8 (s), 128.8 (d), 129.5 (d), 136.3 (s), 142.6 (d), 148.3 (s), 149.7 (s), 161.6 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5OS$ (MW: 538.50) : C, 57.99; H, 5.24; N, 13.01%. Found: C, 58.09; H, 5.34; N, 12.81%.

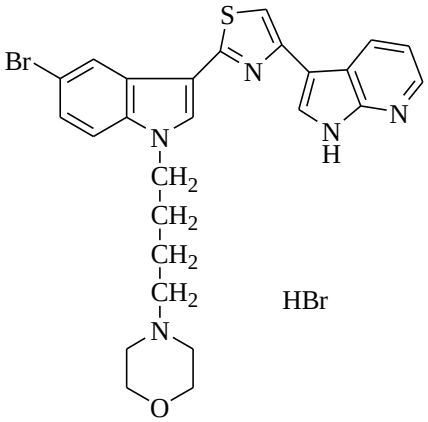
1-Methyl-3-{2-[1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58b)



Conditions: rt for 24 hours. Yield: 72%, white solid; mp: 195-196 °C; IR: 2970 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.66-1.68 (2H, m, CH_2), 1.86-1.90 (2H, m, CH_2), 3.01-3.16 (4H, m, 2 × CH_2), 3.43-3.68 (4H, m, 2 × CH_2), 3.94-3.99 (5H, m, CH_2 and CH_3), 4.37 (2H, t, J = 6.2 Hz,

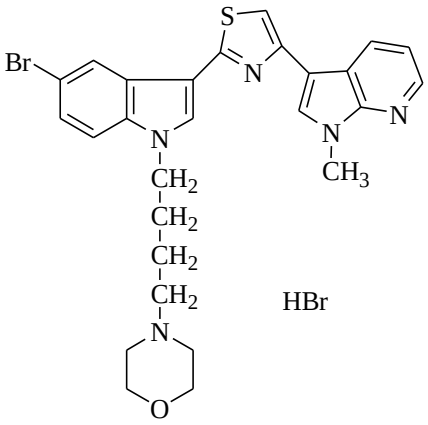
CH_2), 7.24-7.37 (3H, m, H-5', H-5'' and H-6'), 7.66-7.72 (2H, m, H-2' and H-4'), 8.20 (1H, s, H-2''), 8.28 (1H, s, H-5), 8.36-8.41 (2H, m, H-6'' and H-7'), 8.62 (1H, d, J = 7.9 Hz, H-4''), 9.52 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.3 (t), 26.6 (t), 30.9 (q), 45.2 (t), 51.0 (2 × t), 55.4 (t), 63.2 (2 × t), 106.7 (d), 108.9 (s), 109.9 (s), 110.7 (d), 116.2 (d), 117.2 (s), 120.7 (d), 121.0 (d), 122.5 (d), 124.7 (s), 128.6 (d), 128.7 (d), 129.6 (d), 136.3 (s), 142.9 (d), 147.6 (s), 149.4 (s), 161.6 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5OS$ (MW: 552.53) : C, 58.69; H, 5.47; N, 12.68%. Found: C, 58.29; H, 5.72; N, 12.83%.

3-{2-[5-Bromo-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58c)



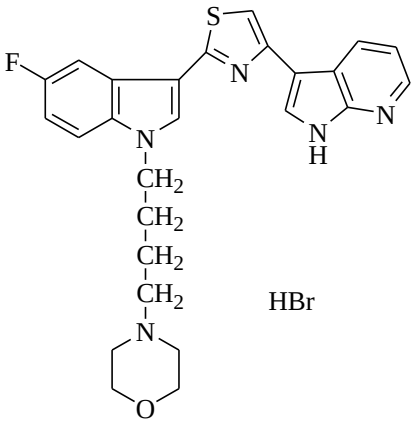
Conditions: rt for 24 hours. Yield: 70%, brown solid; mp: 169-170 °C; IR: 3450 (NH), 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.57-1.66 (2H, m, CH_2), 1.84-1.92 (2H, m, CH_2), 2.84-3.12 (4H, m, 2 × CH_2), 3.42-3.71 (4H, m, 2 × CH_2), 3.93-3.98 (2H, m, CH_2), 4.36 (2H, s, CH_2), 7.23 (1H, dd, J = 7.4, 4.8 Hz, H-5''), 7.46 (1H, d, J = 8.5 Hz, H-6'), 7.70 (1H, d, J = 8.6 Hz, H-7'), 7.75 (1H, s, H-2'), 8.09 (1H, s, H-2''), 8.32-8.35 (2H, m, H-4' and H-5), 8.57-8.66 (2H, m, H-4'' and H-6''), 9.87 (1H, bs, NH^+), 12.00 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.2 (t), 26.7 (t), 45.4 (t), 51.0 (2 × t), 55.4 (t), 63.2 (2 × t), 107.0 (d), 109.5 (s), 109.8 (s), 112.9 (d), 113.7 (s), 116.0 (d), 117.0 (s), 123.0 (d), 124.6 (d), 125.0 (d), 126.3 (s), 128.4 (d), 130.8 (d), 135.1 (s), 143.1 (d), 148.7 (s), 149.9 (s), 161.0 (s); *Anal*. Calculated for $C_{26}H_{27}Br_2N_5OS$ (MW: 617.40) : C, 50.58; H, 4.41; N, 11.34%. Found: C, 50.32; H, 4.79; N, 11.22%.

3-{2-[5-Bromo-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58d)



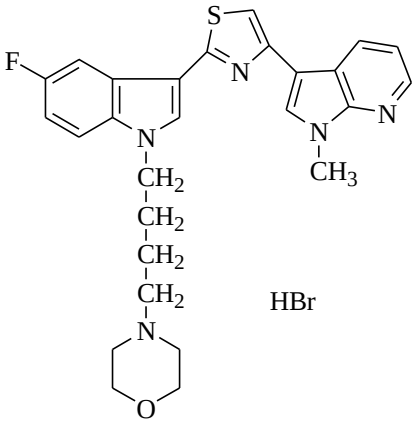
Conditions: rt for 24 hours. Yield: 74%, white solid; mp: 187-188 °C; IR: 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.57-1.69 (2H, m, CH_2), 1.84-1.92 (2H, m, CH_2), 3.01-3.26 (4H, m, 2 × CH_2), 3.45-3.67 (4H, m, 2 × CH_2), 3.94-3.99 (5H, m, CH_2 and CH_3), 4.36 (2H, t, J = 6.2 Hz, CH_2), 7.26 (1H, dd, J = 8.0, 4.6 Hz, H-5''), 7.47 (1H, dd, J = 8.8, 1.9 Hz, H-6'), 7.70 (1H, d, J = 8.8 Hz, H-7'), 7.74 (1H, s, H-2'), 8.15 (1H, s, H-2''), 8.34 (1H, s, H-5), 8.38 (1H, dd, J = 4.6, 1.4 Hz, H-6''), 8.55 (1H, d, J = 1.9 Hz, H-4'), 8.64 (1H, dd, J = 8.0, 1.4 Hz, H-4''), 9.50 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.3 (t), 26.7 (t), 30.9 (q), 45.4 (t), 51.2 (2 × t), 55.5 (t), 63.4 (2 × t), 107.1 (d), 108.7 (s), 109.5 (s), 112.9 (d), 113.7 (s), 116.1 (d), 117.3 (s), 122.9 (d), 125.0 (d), 126.3 (s), 128.4 (d), 128.6 (d), 130.9 (d), 135.1 (s), 143.0 (d), 147.6 (s), 149.6 (s), 161.1 (s); *Anal*. Calculated for $C_{27}H_{29}Br_2N_5OS$ (MW: 631.43) : C, 51.36; H, 4.63; N, 11.09%. Found: C, 50.99; H, 4.85; N, 11.24%.

3-{2-[5-Fluoro-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58e)



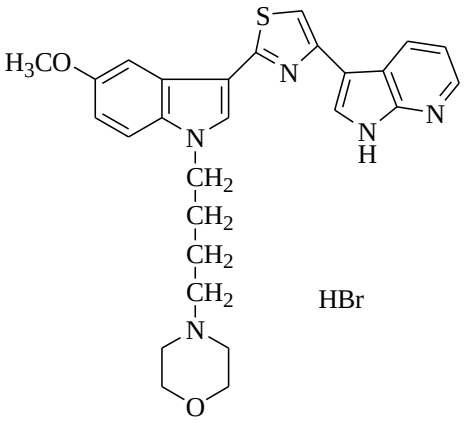
Conditions: rt for 24 hours. Yield: 92%, yellow solid; mp: 226-227 °C; IR: 3449 (NH), 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.64-1.66 (2H, m, CH_2), 1.78-1.95 (2H, m, CH_2), 2.92-3.24 (4H, m, 2 × CH_2), 3.53-3.79 (4H, m, 2 × CH_2), 3.85-4.07 (2H, m, CH_2), 4.36 (2H, s, CH_2), 7.08-7.24 (2H, m, H5'' and H-6'), 7.60-7.75 (2H, m, H-2' and H-7'), 7.88-8.11 (2H, m, H-2'' and H-4'), 8.31-8.35 (2H, m, H-5 and H-6''), 8.60 (1H, d, J = 7.7 Hz, H-4''), 9.62 (1H, bs, NH^+), 11.98 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.3 (t), 26.7 (t), 45.5 (t), 51.1 (2 × t), 55.5 (t), 63.3 (2 × t), 105.5 (d, $J_{C4'-F}$ = 23.5 Hz), 106.8 (d), 109.9 (s), 110.0 (d, J_{C7a-F} = 4.7 Hz), 110.7 (d, $J_{C6'-F}$ = 26.6 Hz), 112.2 (d, $J_{C7'-F}$ = 9.9 Hz), 116.1 (d), 117.1 (s), 124.8 (d), 125.0 (d, J_{C3a-F} = 10.7 Hz), 128.3 (d), 131.2 (d), 133.1 (s), 143.1 (d), 148.8 (s), 149.9 (s), 158.1 (d, $J_{C5'-F}$ = 234.2 Hz), 161.3 (s); *Anal*. Calculated for $C_{26}H_{27}BrFN_5OS$ (MW: 556.49) : C, 56.12; H, 4.89; N, 12.58%. Found: C, 56.22; H, 5.09; N, 12.28%.

3-{2-[5-Fluoro-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58f)



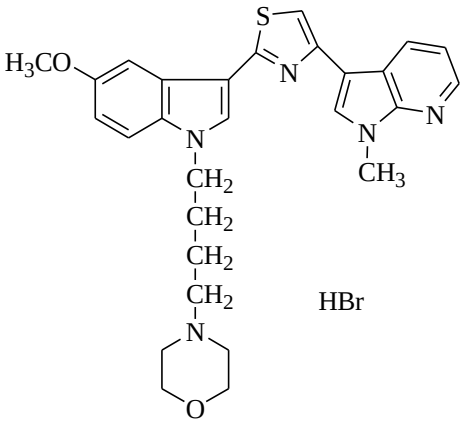
Conditions: rt for 24 hours. Yield: 95%, white solid; mp: 245-246 °C; IR: 2969 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.64-1.67 (2H, m, CH_2), 1.85-1.92 (2H, m, CH_2), 3.01-3.16 (4H, m, 2 × CH_2), 3.43-3.69 (4H, m, 2 × CH_2), 3.94-3.99 (5H, m, CH_2 and CH_3), 4.37 (2H, t, J = 6.3 Hz, CH_2), 7.15-7.30 (2H, m, H5'' and H-6'), 7.69-7.76 (2H, m, H-2' and H-7'), 8.10 (1H, dd, J = 9.9, 2.5 Hz, H-4'), 8.21 (1H, s, H-2''), 8.35 (1H, s, H-5), 8.37 (1H, dd, J = 4.6, 1.4 Hz, H-6''), 8.56 (1H, dd, J = 8.0, 1.4 Hz, H-4''), 9.52 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.3 (t), 26.6 (t), 30.9 (q), 45.4 (t), 51.0 (2 × t), 55.4 (t), 63.2 (2 × t), 105.6 (d, $J_{C4'-F}$ = 24.6 Hz), 106.8 (d), 108.8 (s), 110.0 (d, J_{C7a-F} = 4.5 Hz), 110.8 (d, $J_{C6'-F}$ = 26.6 Hz), 112.2 (d, $J_{C7'-F}$ = 9.6 Hz), 116.2 (d), 117.2 (s), 125.0 (d, J_{C3a-F} = 10.8 Hz), 128.5 (d), 128.8 (d), 131.3 (d), 133.1 (s), 142.9 (d), 147.7 (s), 149.5 (s), 158.2 (d, $J_{C5'-F}$ = 233.9 Hz), 161.4 (s); *Anal*. Calculated for $C_{27}H_{29}BrFN_5OS$ (MW: 570.52) : C, 56.84; H, 5.12; N, 12.28%. Found : C, 56.68; H, 5.02; N, 12.54%.

3-{2-[5-Methoxy-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58g)



Conditions: rt for 24 hours. Yield: 76%, brown solid; mp: 233-234 °C; IR: 3451 (NH), 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.64-1.67 (2H, m, CH_2), 1.84-1.87 (2H, m, CH_2), 3.00-3.13 (4H, m, 2 × CH_2), 3.47-3.69 (4H, m, 2 × CH_2), 3.91-3.99 (5H, m, CH_2 and CH_3), 4.32 (2H, s, CH_2), 6.96 (1H, d, J = 8.9 Hz, H-6'), 7.21 (1H, dd, J = 7.7, 4.9 Hz, H-5''), 7.59 (1H, d, J = 8.9 Hz, H-7'), 7.70 (1H, s, H-2'), 7.94 (1H, s, H-4'), 8.11 (1H, d, J = 2.0 Hz, H-2''), 8.21 (1H, s, H-5), 8.32 (1H, d, J = 4.9 Hz, H-6''), 8.74 (1H, d, J = 7.7 Hz, H-4''), 9.62 (1H, bs, NH^+), 11.99 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.2 (t), 26.7 (t), 45.3 (t), 51.0 (2 × t), 55.1 (q), 55.4 (t), 63.2 (2 × t), 102.1 (d), 106.4 (d), 109.6 (s), 110.0 (s), 111.6 (d), 112.6 (d), 115.9 (d), 117.4 (s), 124.5 (d), 125.3 (s), 128.9 (d), 129.8 (d), 131.3 (s), 142.7 (d), 148.2 (s), 149.7 (s), 154.8 (s), 161.9 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5O_2S$ (MW: 567.13) : C, 57.04; H, 5.32; N, 12.32%. Found: C, 56.71; H, 5.52; N, 12.45%.

3-{2-[5-Methoxy-1-(4-morpholin-4-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (58h)



Conditions: rt for 24 hours. Yield: 88%, white solid; mp: 216-217 °C; IR: 2951 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.64-1.67 (2H, m, CH_2), 1.84-1.92 (2H, m, CH_2), 3.01-3.26 (4H, m, 2 × CH_2), 3.43-3.68 (4H, m, 2 × CH_2), 3.92 (3H, s, CH_3), 3.93 (3H, s, CH_3), 3.93-3.99 (2H, m, CH_2), 4.32 (2H, t, J = 6.3 Hz, CH_2), 6.97 (1H, dd, J = 8.9, 2.5 Hz, H-6'), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.59 (1H, d, J = 8.9 Hz, H-7'), 7.68 (1H, s, H-2'), 7.92 (1H, d, J = 2.5 Hz, H-4'), 8.15 (1H, s, H-2''), 8.21 (1H, s, H-5), 8.37 (1H, dd, J = 4.7, 1.4 Hz, H-6''), 8.71 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 9.52 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.3 (t), 26.7 (t), 31.0 (q), 45.3 (t), 51.0 (2 × t), 55.2 (q), 55.4 (t), 63.2 (2 × t), 102.2 (d), 106.3 (d), 108.9 (s), 109.6 (s), 111.6 (d), 112.5 (d), 116.0 (d), 117.4 (s), 125.3 (s), 128.2 (d), 128.8 (d), 129.8 (d), 131.3 (s), 142.9 (d), 147.5 (s), 149.5 (s), 154.9 (s), 161.9 (s); *Anal*. Calculated for $C_{28}H_{32}BrN_5O_2S$ (MW: 582.55) : C, 57.73; H, 5.54; N, 12.02%. Found: C, 57.43; H, 5.66; N, 12.20%.

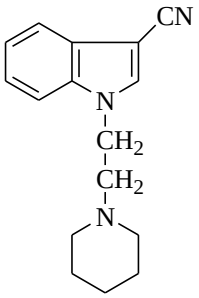
Synthesis of the 1-(2-chloroethyl)piperidine hydrochloride (90)

To a solution of 1-(2-chloroethyl)piperidine hydrochloride **89** (0.5 g, 2.72 mmol) in water (3 mL), was added 5% aqueous solution of sodium hydroxide (NaOH) up to pH = 11-12 and stirred at room temperature for 10 minutes. The resulting mixture was extracted with dichloromethane (2 × 10 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. Yield: 87%, yellow oil. Analytical and spectroscopic data were in accordance with those previously reported [127].

General procedure for the synthesis of 1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbonitriles (91a-d)

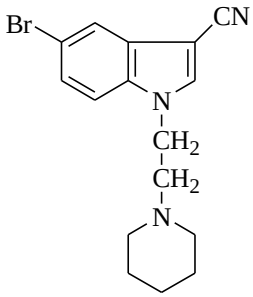
To a solution of the appropriate indole carbonitrile **70a-d** (0.5 g, 2.90 mmol) in dry DMF (6.5 mL), was slowly added at 0-5 °C, sodium hydride 60% dispersion in mineral oil (NaH) (0.10 g, 4.35 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. After that, it was added dropwise a solution of 1-(2-chloroethyl)piperidine hydrochloride **90** (1.7 g, 11.60 mmol) in dry DMF (2 mL), and the reaction mixture was stirred at room temperature for 12 hours. Then, the mixture was poured into water and ice and extracted with ethyl acetate (3 × 20 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether:ethyl acetate (6:4) as eluent.

1-(2-Piperidin-1-yl-ethyl)-1*H*-indole-3-carbonitrile (91a)



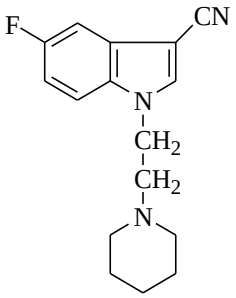
Yield: 98%, yellow solid; mp: 92-93 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.44 (6H, m, 3 × CH_2), 2.35-2.38 (4H, m, 2 × CH_2), 2.63 (2H, t, J = 6.3 Hz, CH_2), 4.36 (2H, t, J = 6.4 Hz, CH_2), 7.23-7.38 (2H, m, H-5 and H-6), 7.62-7.72 (2H, m, H-4 and H-7), 8.28 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 43.8 (t), 53.9 (2 × t), 57.5 (t), 83.1 (s), 111.5 (d), 116.1 (s), 118.6 (d), 121.8 (d), 123.2 (d), 127.0 (s), 135.2 (s), 137.3 (d); *Anal*. Calculated for $C_{16}H_{19}N_3$ (MW: 253.34) : C, 75.85; H, 7.56; N, 16.59%. Found: C, 75.56; H, 7.68; N, 16.76%.

5-Bromo-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbonitrile (91b)



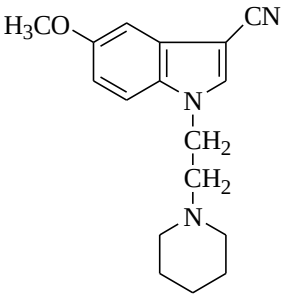
Yield: 90%, white solid; mp: 100-101 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.41 (6H, m, 3 × CH_2), 2.35 (4H, s, 2 × CH_2), 2.61 (2H, t, J = 6.2 Hz, CH_2), 4.35 (2H, t, J = 6.2 Hz, CH_2), 7.47 (1H, dd, J = 8.8, 1.8 Hz, H-6), 7.70 (1H, d, J = 8.8 Hz, H-7), 7.79 (1H, d, J = 1.8 Hz, H-4), 8.34 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 44.1 (t), 53.9 (2 × t), 57.5 (t), 82.8 (s), 113.8 (d), 114.6 (s), 115.4 (s), 120.8 (d), 125.9 (d), 128.5 (s), 134.2 (s), 138.6 (d); *Anal*. Calculated for $C_{16}H_{18}BrN_3$ (MW: 332.24) : C, 57.84; H, 5.46; N, 12.65%. Found: C, 57.95; H, 5.57; N, 12.43%.

5-Fluoro-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbonitrile (91c)



Yield: 98%, white solid; mp: 56-57 °C; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.46 (6H, m, 3 × CH_2), 2.34-2.37 (4H, m, 2 × CH_2), 2.62 (2H, t, J = 6.2 Hz, CH_2), 4.36 (2H, t, J = 6.3 Hz, CH_2), 7.20 (1H, td, J = 11.8, 9.3, 2.5 Hz, H-6), 7.43 (1H, dd, J = 9.1, 2.4 Hz, H-7), 7.72-7.78 (1H, m, H-4), 8.34 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 44.1 (t), 53.9 (2 × t), 57.5 (t), 83.4 (d, J_{C7a-F} = 4.5 Hz), 103.9 (d, J_{C4-F} = 24.8 Hz), 111.7 (d, J_{C6-F} = 26.2 Hz), 113.2 (d, J_{C7-F} = 9.8 Hz), 115.7 (s), 127.5 (d, J_{C3a-F} = 10.9 Hz), 132.0 (s), 138.8 (d), 158.4 (d, J_{C5-F} = 236.6 Hz); *Anal*. Calculated for $C_{16}H_{18}FN_3$ (MW: 271.33) : C, 70.82; H, 6.69; N, 15.49%. Found: C, 71.04; H, 6.79; N, 15.17%.

5-Methoxy-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbonitrile (91d)

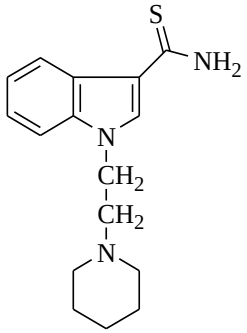


Yield: 94%, yellow oil; IR: 2217 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.46 (6H, m, 3 × CH_2), 2.34-2.36 (4H, m, 2 × CH_2), 2.61 (2H, t, J = 6.3 Hz, CH_2), 3.82 (3H, s, CH_3), 4.30 (2H, t, J = 6.4 Hz, CH_2), 6.94 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.08 (1H, d, J = 2.4 Hz, H-4), 7.59 (1H, d, J = 9.0 Hz, H-7), 8.19 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 44.0 (t), 53.9 (2 × t), 55.3 (q), 57.6 (t), 82.7 (s), 99.9 (d), 112.5 (d), 113.5 (d), 116.3 (s), 127.8 (s), 130.1 (s), 137.0 (d), 155.3 (s); *Anal*. Calculated for $C_{17}H_{21}N_3O$ (MW: 283.37) : C, 72.06; H, 7.47; N, 14.83%. Found: C, 71.90; H, 7.34; N, 15.12%.

General procedure for the synthesis of 1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbothioamides (88a-d)

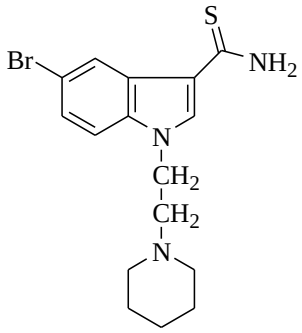
Compounds **88a-d** were prepared from **91a-d** using the same synthetic procedure described for compounds **75a-d**.

1-(2-Piperidin-1-yl-ethyl)-1*H*-indole-3-carbothioamide (88a)



Yield: 72%, yellow solid; mp: 126-127 °C; IR: 1654 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.47 (6H, m, 3 × CH_2), 2.38-2.40 (4H, m, 2 × CH_2), 2.64 (2H, t, J = 6.4 Hz, CH_2), 4.31 (2H, t, J = 6.5 Hz, CH_2), 7.13-7.26 (2H, m, H-5 and H-6), 7.56 (1H, dd, J = 6.2, 1.6 Hz, H-7), 8.15 (1H, s, H-2), 8.57 (1H, dd, J = 6.9, 2.8 Hz, H-4), 8.90 (1H, s, SH), 8.98 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.9 (t), 25.5 (2 × t), 43.5 (t), 54.0 (2 × t), 57.7 (t), 110.4 (d), 115.4 (s), 120.9 (d), 121.8 (d), 122.0 (d), 125.9 (s), 132.0 (d), 136.6 (s), 193.0 (s); *Anal*. Calculated for $C_{16}H_{21}N_3S$ (MW: 287.42) : C, 66.86; H, 7.36; N, 14.62%. Found: C, 67.03; H, 7.51; N, 14.30%.

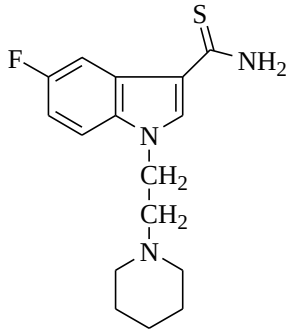
5-Bromo-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbothioamide (88b)



Yield: 71%, yellow solid; mp: 177-178 °C; IR: 1607 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.35-1.45 (6H, m, 3 × CH_2), 2.37-2.38 (4H, m, 2 × CH_2), 2.62 (2H, t, J = 5.9 Hz, CH_2), 4.30 (2H, t, J = 5.3 Hz, CH_2), 7.35 (1H, d, J = 8.7 Hz, H-6), 7.57 (1H, d, J = 8.7 Hz, H-7), 8.18 (1H, s, H-2), 8.86 (1H, s, H-4), 8.91 (1H, s, SH), 9.06 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 43.8 (t), 54.0 (2 × t), 57.7 (t), 112.7 (d), 113.9 (s), 114.5 (s), 124.0 (d), 124.5 (d), 127.9

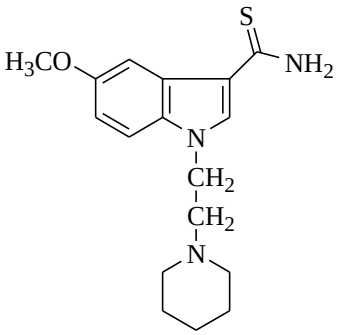
(s), 132.4 (d), 135.5 (s), 192.4 (s); *Anal*. Calculated for $C_{16}H_{20}BrN_3S$ (MW: 366.32) : C, 52.46; H, 5.50; N, 11.47%. Found: C, 52.80; H, 5.40; N, 11.23%.

5-Fluoro-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbothioamide (88c)



Yield: 67%, yellow solid; mp: 144-145 °C; IR: 1623 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.36-1.46 (6H, m, 3 × CH_2), 2.36-2.39 (4H, m, 2 × CH_2), 2.63 (2H, t, J = 6.3 Hz, CH_2), 4.31 (2H, t, J = 6.3 Hz, CH_2), 7.08 (1H, td, J = 11.0, 9.0, 2.6 Hz, H-6), 7.57-7.64 (1H, m, H-7), 8.22 (1H, s, H-2), 8.42 (1H, dd, J = 11.0, 2.6 Hz, H-4), 8.86 (1H, s, SH), 9.03 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 43.8 (t), 54.0 (2 × t), 57.7 (t), 106.8 (d, $J_{C4\text{-}F}$ = 25.9 Hz), 110.1 (d, $J_{C6\text{-}F}$ = 26.5 Hz), 111.9 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 115.0 (d, $J_{C7a\text{-}F}$ = 4.7 Hz), 126.8 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 133.1 (d), 133.5 (s), 158.1 (d, $J_{C5\text{-}F}$ = 232.9 Hz), 192.6 (s); *Anal*. Calculated for $C_{16}H_{20}FN_3S$ (MW: 305.41) : C, 62.92; H, 6.60; N, 13.76%. Found: C, 62.70; H, 6.93; N, 13.65%.

5-Methoxy-1-(2-piperidin-1-yl-ethyl)-1*H*-indole-3-carbothioamide (88d)



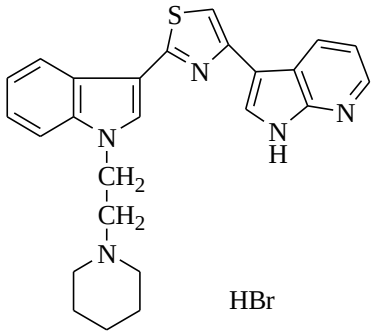
Yield: 70%, yellow solid; mp: 152-153 °C; IR: 1654 (CS) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.42-1.46 (6H, m, 3 × CH_2), 2.36-2.39 (4H, m, 2 × CH_2), 2.62 (2H, t, J = 6.3 Hz, CH_2), 3.78 (3H, s, CH_3), 4.26 (2H, t, J = 6.4 Hz, CH_2), 6.85 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.46 (1H, d, J = 9.0 Hz, H-7), 8.11 (1H, s, H-2), 8.17 (1H, d, J = 2.4 Hz, H-4), 8.73 (1H, s, SH), 8.90 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.5 (2 × t), 43.7 (t), 54.0 (2 × t), 55.2 (q), 57.8 (t), 103.8 (d), 111.2 (d), 111.7 (d), 114.8 (s), 126.6 (s), 131.7 (s), 132.3 (d), 154.8 (s), 192.8 (s); *Anal*. Calculated

for $C_{17}H_{23}N_3OS$ (MW: 317.45) : C, 64.32; H, 7.30; N, 13.24%. Found: C, 64.20; H, 7.65; N, 13.01%.

General procedure for the synthesis of 3-{2-[1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (59a-h)

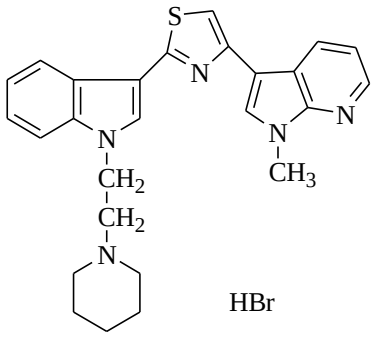
Compounds **59a-h** were prepared from **88a-d** using the same synthetic procedure described for compounds **69a,b**.

3-{2-[1-(2-Piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59a)



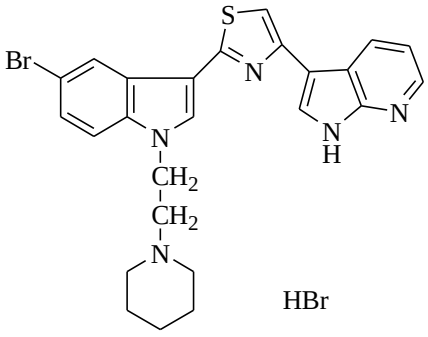
Conditions: 30 minutes at reflux. Yield: 86%, light brown solid; mp: 258-259 °C; IR: 3106 (NH), 2979 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.83 (6H, m, 3 × CH_2), 2.85-3.05 (2H, m, CH_2), 3.57-3.61 (4H, m, 2 × CH_2), 4.76 (2H, t, J = 6.6 Hz, CH_2), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.32-7.42 (2H, m, H-5'and H-6'), 7.74-7.78 (2H, m, H-2' and H-7'), 8.12 (1H, d, J = 2.6 Hz, H-2''), 8.31-8.41 (3H, m, H-4', H-5 and H-6''), 8.64 (1H, dd, J = 7.9, 1.1 Hz, H-4''), 9.62 (1H, bs, NH^+), 11.99 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 40.3 (t), 52.5 (2 × t), 54.0 (t), 107.1(d), 109.9 (s), 110.7 (d), 110.8 (s), 116.1 (d), 117.1 (s), 120.7 (d), 121.5 (d), 122.9 (d), 124.8 (d + s), 128.5 (d), 129.4 (d), 136.3 (s), 143.1 (d), 148.7 (s), 149.9 (s), 161.2 (s); *Anal*. Calculated for $C_{25}H_{26}BrN_5S$ (MW: 508.48) : C, 59.05; H, 5.15; N, 13.77%. Found: C, 58.90; H, 4.97; N, 14.10%.

1-Methyl-3-{2-[1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59b)



Conditions: 30 minutes at reflux. Yield: 70%, light pink solid; mp: 209-210 °C; IR: 2954 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.42-1.83 (6H, m, 3 × CH_2), 2.89-3.04 (2H, m, CH_2), 3.57-3.61 (4H, m, 2 × CH_2), 3.94 (3H, s, CH_3), 4.75 (2H, s, CH_2), 7.24-7.42 (3H, m, H-5', H-5'' and H-6'), 7.74-7.78 (2H, m, H-2' and H-4'), 8.07 (1H, s, H-2''), 8.21 (1H, s, H-5), 8.32-8.43 (2H, m, H-6'' and H-7'), 8.63 (1H, d, J = 6.7 Hz, H-4''), 9.57 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 30.9 (q), 40.3 (t), 52.5 (2 × t), 54.1 (t), 107.0 (d), 108.7 (s), 110.6 (s), 110.7 (d), 116.0 (d), 117.1 (s), 120.8 (d), 121.3 (d), 122.8 (d), 124.8 (s), 128.4 (d), 128.6 (d), 129.2 (d), 136.3 (s), 142.9 (d), 147.5 (s), 149.6 (s), 161.4 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5S$ (MW: 522.50) : C, 59.77; H, 5.40; N, 13.40%. Found: C, 59.60; H, 5.76; N, 13.21%.

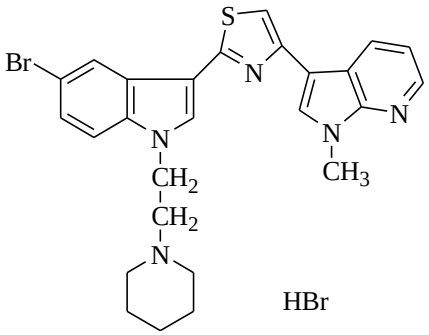
3-{2-[5-Bromo-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59c)



Conditions: 30 minutes at reflux. Yield: 71%, yellow solid; mp: 277-278 °C; IR: 3124 (NH), 2972 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.83 (6H, m, 3 × CH_2), 2.87-3.03 (2H, m, CH_2), 3.54-3.59 (4H, m, 2 × CH_2), 4.75 (2H, t, J = 6.6 Hz, CH_2), 7.23 (1H, dd, J = 7.8, 4.7 Hz, H-5''), 7.53 (1H, d, J = 8.4 Hz, H-6'), 7.77 (1H, d, J = 6.8 Hz, H-7'), 7.78 (1H, s, H-2'), 8.10 (1H, d, J = 1.9 Hz, H-2''), 8.33 (1H, s, H-4'), 8.38 (1H, s, H-5), 8.61 (1H, d, J = 4.7 Hz, H-6''), 8.64 (1H, d, J = 7.8 Hz, H-4''), 9.59 (1H, bs, NH^+), 12.01 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 40.5 (t), 52.5 (2 × t), 54.0 (t), 107.3 (d), 109.8 (s), 110.3 (s), 112.9 (d), 114.1 (s),

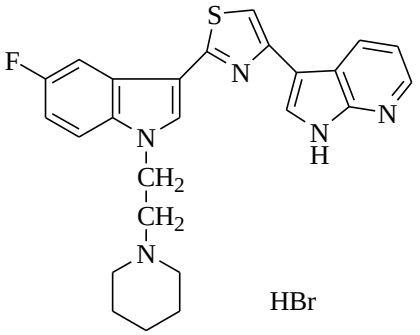
116.0 (d), 117.1 (s), 123.1 (d), 124.7 (d), 125.3 (d), 126.4 (s), 128.4 (d), 130.7 (d), 135.1 (s), 143.0 (d), 148.6 (s), 149.9 (s), 160.7 (s); *Anal*. Calculated for $C_{25}H_{25}Br_2N_5S$ (MW: 587.37) : C, 51.12; H, 4.29; N, 11.92%. Found: C, 51.50; H, 4.16; N, 11.67%.

3-{2-[5-Bromo-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59d)



Conditions: 30 minutes at reflux. Yield: 78%, white solid; mp: 251-252 °C; IR: 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.90 (6H, m, 3 × CH_2), 2.98-3.09 (2H, m, CH_2), 3.54-3.60 (4H, m, 2 × CH_2), 3.94 (3H, s, CH_3), 4.50 (2H, t, J = 6.3 Hz, CH_2), 7.26 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.53 (1H, dd, J = 8.8, 1.8 Hz, H-6'), 7.74-7.79 (2H, m, H-2' and H-7'), 8.16 (1H, s, H-2''), 8.38-8.39 (2H, m, H-5 and H-6''), 8.57 (1H, d, J = 1.8 Hz, H-4'), 8.63 (1H, dd, J = 7.9, 1,4 Hz, H-4''), 9.52 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 31.0 (q), 40.5 (t), 52.5 (2 × t), 53.9 (t), 107.4 (d), 108.6 (s), 110.2 (s), 112.9 (d), 114.1 (s), 116.1 (d), 117.3 (s), 123.0 (d), 125.4 (d), 126.4 (s), 128.5 (d), 128.6 (d), 130.8 (d), 135.1 (s), 143.0 (d), 147.6 (s), 149.7 (s), 160.8 (s); *Anal*. Calculated for $C_{26}H_{27}Br_2N_5S$ (MW: 601.40) : C, 51.93; H, 4.53; N, 11.65%. Found: C, 51.79; H, 4.90; N, 11.42%.

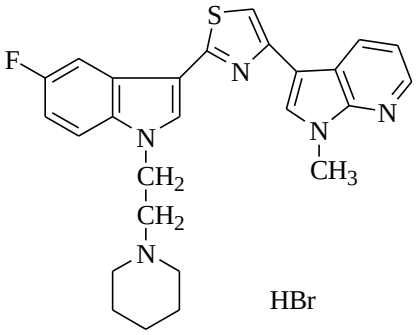
3-{2-[5-Fluoro-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59e)



Conditions: 30 minutes at reflux. Yield: 86%, light yellow solid; mp: 263-264 °C; IR: 3110 (NH), 2979 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.90 (6H, m, 3 × CH_2), 2.98-3.08 (2H, m, CH_2), 3.55-3.61 (4H, m, 2 × CH_2), 4.75 (2H, t, J = 7.3 Hz, CH_2), 7.20-7.32 (2H, m, H5'' and H-6'),

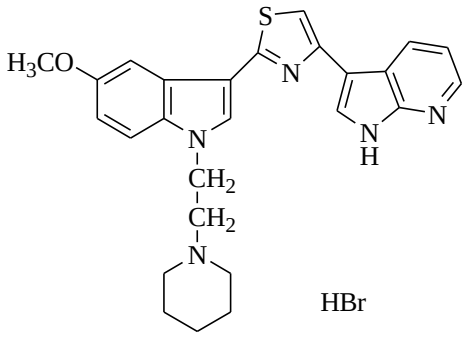
7.77-7.83 (2H, m, H-2' and H-7'), 8.08 (1H, dd, J = 10.4, 2.5 Hz, H-4'), 8.13 (1H, d, J = 2.5 Hz, H-2''), 8.32 (1H, dd, J = 4.7, 1.4 Hz, H-6''), 8.40 (1H, s, H-5), 8.61 (1H, dd, J = 8.2, 1.4 Hz, H-4''), 9.60 (1H, bs, NH^+), 11.99 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 40.6 (t), 52.5 (2 × t), 54.0 (t), 105.7 (d, $J_{C4'-F}$ = 23.6 Hz), 107.2 (d), 109.8 (s), 110.8 (d, J_{C7a-F} = 4.6 Hz), 111.1 (d, $J_{C6'-F}$ = 26.2 Hz), 112.2 (d, $J_{C7'-F}$ = 9.9 Hz), 116.1 (d), 117.0 (s), 124.9 (d), 125.1 (d, J_{C3a-F} = 10.6 Hz), 128.3 (d), 131.1 (d), 133.0 (s), 143.1 (d), 148.7 (s), 149.9 (s), 158.3 (d, $J_{C5'-F}$ = 235.5 Hz), 161.0 (s); *Anal*. Calculated for $C_{25}H_{25}BrFN_5S$ (MW: 526.47) : C, 57.03; H, 4.79; N, 13.30%. Found: C, 57.40; H, 4.65; N, 13.07%.

3-{2-[5-Fluoro-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobrimide (59f)



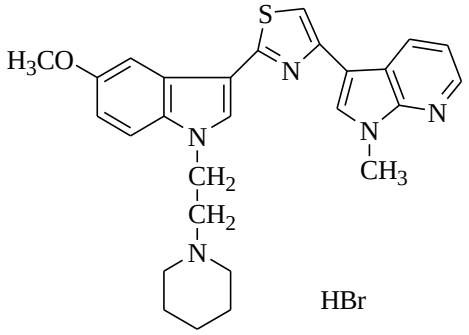
Conditions: 30 minutes at reflux. Yield: 91%, light pink solid; mp: 244-245 °C; IR: 2954 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.90 (6H, m, 3 × CH_2), 2.85-3.08 (2H, m, CH_2), 3.55-3.61 (4H, m, 2 × CH_2), 3.94 (3H, s, CH_3), 4.75 (2H, t, J = 7.0 Hz, CH_2), 7.22-7.32 (2H, m, H5'' and H-6'), 7.76-7.83 (2H, m, H-2' and H-7'), 8.13 (1H, dd, J = 9.8, 2.5 Hz, H-4'), 8.22 (1H, s, H-2''), 8.36-8.40 (2H, m, H-5 and H-6''), 8.59 (1H, dd, J = 8.0, 1.4 Hz, H-4''), 9.54 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 30.9 (q), 40.6 (t), 52.5 (2 × t), 54.0 (t), 105.8 (d, $J_{C4'-F}$ = 24.0 Hz), 107.1 (d), 108.8 (s), 110.8 (d, J_{C7a-F} = 4.6 Hz), 111.1 (d, $J_{C6'-F}$ = 25.4 Hz), 112.2 (d, $J_{C7'-F}$ = 9.6 Hz), 116.2 (d), 117.2 (s), 125.1 (d, J_{C3a-F} = 10.7 Hz), 128.5 (d), 128.8 (d), 131.2 (d), 133.0 (s), 142.9 (d), 147.6 (s), 149.6 (s), 158.3 (d, $J_{C5'-F}$ = 235.1 Hz), 161.1 (s); *Anal*. Calculated for $C_{26}H_{27}BrFN_5S$ (MW: 540.49) : C, 57.78; H, 5.04; N, 12.96%. Found: C, 57.61; H, 5.38; N, 12.79%.

3-{2-[5-Methoxy-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59g)



Conditions: 30 minutes at reflux. Yield: 80%, light yellow solid; mp: 290-291 °C; IR: 3072 (NH), 2944 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.42-1.82 (6H, m, 3 × CH_2), 2.86-3.02 (2H, m, CH_2), 3.53-3.58 (4H, m, 2 × CH_2), 3.92 (3H, s, CH_3), 4.68-4.75 (2H, m, CH_2), 7.02 (1H, d, J = 8.9 Hz, H-6'), 7.21 (1H, dd, J = 7.5, 4.7 Hz, H-5''), 7.67 (1H, d, J = 8.9 Hz, H-7'), 7.73 (1H, s, H-2'), 7.96 (1H, s, H-4'), 8.11 (1H, s, H-2''), 8.26 (1H, s, H-5), 8.31 (1H, d, J = 4.7 Hz, H-6''), 8.72 (1H, d, J = 7.5 Hz, H-4''), 9.66 (1H, bs, NH^+), 11.98 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 40.4 (t), 52.5 (2 × t), 54.1 (t), 55.2 (q), 102.3 (d), 106.6 (d), 109.9 (s), 110.5 (s), 111.5 (d), 112.9 (d), 115.9 (d), 117.2 (s), 124.5 (d), 125.4 (s), 128.6 (d), 129.6 (d), 131.2 (s), 142.9 (d), 148.5 (s), 149.9 (s), 155.1 (s), 161.5 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5OS$ (MW: 538.50) : C, 57.99; H, 5.24; N, 13.01%. Found: C, 58.09; H, 5.44; N, 12.71%.

3-{2-[5-Methoxy-1-(2-piperidin-1-yl-ethyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (59h)



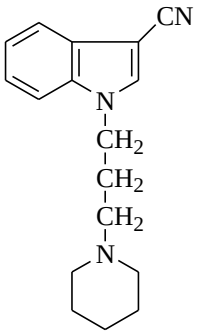
Conditions: 30 minutes at reflux. Yield: 91%, light orange solid; mp: 147-148 °C; IR: 2954 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.43-1.89 (6H, m, 3 × CH_2), 2.84-3.07 (2H, m, CH_2), 3.54-3.59 (4H, m, 2 × CH_2), 3.92 (3H, s, CH_3), 3.93 (3H, s, CH_3), 4.70 (2H, t, J = 6.8 Hz, CH_2), 7.02 (1H, dd, J = 9.0, 2.4 Hz, H-6'), 7.24 (1H, dd, J = 7.9, 4.6 Hz, H-5''), 7.67 (1H, d, J = 9.0 Hz, H-7'), 7.71 (1H, s, H-2'), 7.94 (1H, d, J = 2.4 Hz, H-4'), 8.16 (1H, s, H-2''), 8.26 (1H, s, H-5), 8.37 (1H, dd, J = 4.6, 1.4 Hz, H-6''), 8.72 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 9.56 (1H, bs, NH^+); ^{13}C NMR (50

MHz, DMSO-d_6) δ: 21.1 (t), 22.3 (2 × t), 31.0 (q), 40.5 (t), 52.5 (2 × t), 54.1 (t), 55.3 (q), 102.4 (d), 106.7 (d), 108.8 (s), 110.4 (s), 111.6 (d), 112.8 (d), 116.0 (d), 117.4 (s), 125.3 (s), 128.3 (d), 128.7 (d), 129.7 (d), 131.2 (s), 142.9 (d), 147.5 (s), 149.6 (s), 155.1 (s), 161.6 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5OS$ (MW: 552.53) : C, 58.69; H, 5.47; N, 12.68%. Found: C, 58.85; H, 5.12; N, 12.87%.

General procedure for the synthesis of 1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbonitriles (94a-d)

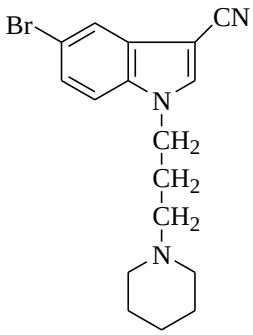
A mixture of appropriate indole carbonitrile **81a-d** (0.4 g, 1.43 mmol), piperidine **93** (1.45 mL, 14.68 mmol), and potassium carbonate (K_2CO_3) (0.34 g, 2.49 mmol), in dry DMF (5.4 mL) was stirred at 50 °C for 1 hour. After cooling, the mixture was poured into water and ice and extracted with dichloromethane (3 × 20 mL), dried (Na_2SO_4), filtered and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using dichloromethane:methanol (98:2) as eluent.

1-(3-Piperidin-1-yl-propyl)-1*H*-indole-3-carbonitrile (94a)



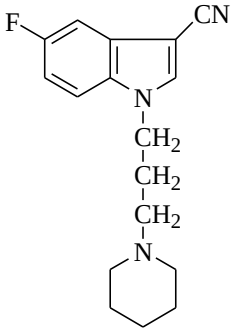
Yield: 93%, yellow oil; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.35-1.47 (6H, m, 3 × CH_2), 1.86-1.99 (2H, m, CH_2), 2.15 (2H, t, J = 7.4 Hz, CH_2), 2.21-2.26 (4H, m, 2 × CH_2), 4.29 (2H, t, J = 6.7 Hz, CH_2), 7.23-7.38 (2H, m, H-5 and H-6), 7.63-7.72 (2H, m, H-4 and H-7), 8.29 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 25.4 (2 × t), 26.5 (t), 44.3 (t), 53.8 (2 × t), 54.9 (t), 83.2 (s), 111.5 (d), 116.0 (s), 118.6 (d), 121.8 (d), 123.2 (d), 127.0 (s), 135.2 (s), 136.9 (d); *Anal*. Calculated for $C_{17}H_{21}N_3$ (MW: 267.37) : C, 76.37; H, 7.92; N, 15.72%. Found: C, 76.51; H, 8.05; N, 15.45%.

5-Bromo-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbonitrile (94b)



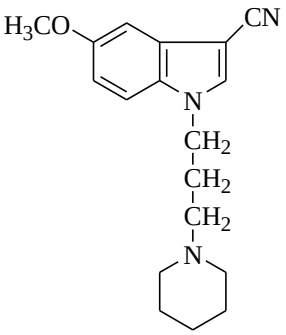
Yield: 81%, yellow oil; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.45 (6H, m, 3 × CH_2), 1.85-1.98 (2H, m, CH_2), 2.12 (2H, t, J = 7.4 Hz, CH_2), 2.18-2.24 (4H, m, 2 × CH_2), 4.28 (2H, t, J = 6.7 Hz, CH_2), 7.47 (1H, dd, J = 8.8, 1.9 Hz, H-6), 7.71 (1H, d, J = 8.8 Hz, H-7), 7.81 (1H, d, J = 1.8 Hz, H-4), 8.36 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 25.4 (2 × t), 26.4 (t), 44.6 (t), 53.8 (2 × t), 54.8 (t), 83.0 (s), 113.7 (d), 114.6 (s), 115.3 (s), 120.9 (d), 125.9 (d), 128.6 (s), 134.2 (s), 138.3 (d); *Anal*. Calculated for $C_{17}H_{20}BrN_3$ (MW: 346.26) : C, 58.97; H, 5.82; N, 12.14%. Found: C, 59.23; H, 5.96; N, 11.74%.

5-Fluoro-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbonitrile (94c)



Yield: 70%, yellow oil; IR: 2220 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.34-1.48 (6H, m, 3 × CH_2), 1.85-1.98 (2H, m, CH_2), 2.13 (2H, t, J = 7.4 Hz, CH_2), 2.19-2.24 (4H, m, 2 × CH_2), 4.28 (2H, t, J = 6.7 Hz, CH_2), 7.20 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.43 (1H, dd, J = 9.2, 2.4 Hz, H-7), 7.71-7.78 (1H, m, H-4), 8.36 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 25.4 (2 × t), 26.4 (t), 44.6 (t), 53.8 (2 × t), 54.8 (t), 83.5 (d, $J_{C7a\text{-}F}$ = 4.5 Hz), 103.9 (d, $J_{C4\text{-}F}$ = 24.7 Hz), 111.7 (d, $J_{C6\text{-}F}$ = 26.1 Hz), 113.2 (d, $J_{C7\text{-}F}$ = 9.8 Hz), 115.6 (s), 127.5 (d, $J_{C3a\text{-}F}$ = 10.9 Hz), 132.0 (s), 138.5 (d), 158.4 (d, $J_{C5\text{-}F}$ = 236.7 Hz); *Anal*. Calculated for $C_{17}H_{20}FN_3$ (MW: 285.36) : C, 71.55; H, 7.06; N, 14.73%. Found: C, 71.22; H, 7.18; N, 14.94%.

5-Methoxy-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbonitrile (94d)

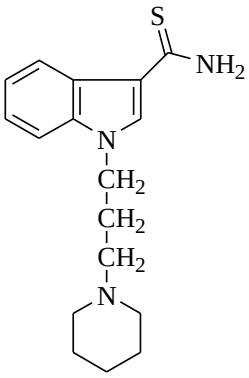


Yield: 82%, yellow oil; IR : 2216 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.35-1.50 (6H, m, 3 × CH_2), 1.84-1.97 (2H, m, CH_2), 2.13 (2H, t, J = 7.2 Hz, CH_2), 2.21-2.25 (4H, m, 2 × CH_2), 3.82 (3H, s, CH_3), 4.24 (2H, t, J = 6.7 Hz, CH_2), 6.95 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.09 (1H, d, J = 2.4 Hz, H-4), 7.60 (1H, d, J = 9.0 Hz, H-7), 8.21 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 25.4 (2 × t), 26.5 (t), 44.4 (t), 53.8 (2 × t), 54.9 (t), 55.4 (q), 82.8 (s), 100.0 (d), 112.5 (d), 113.5 (d), 116.2 (s), 127.8 (s), 130.1 (s), 136.7 (d), 155.4 (s); *Anal*. Calculated for $C_{18}H_{23}N_3O$ (MW: 297.39) : C, 72.70; H, 7.80; N, 14.13%. Found: C, 72.33; H, 7.94; N, 14.36%.

General procedure for the synthesis of 1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbothioamides (92a-d)

Compounds **92a-d** were prepared from **94a-d** using the same synthetic procedure described for compounds **75a-d**.

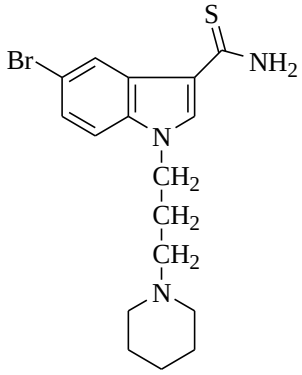
1-(3-Piperidin-1-yl-propyl)-1*H*-indole-3-carbothioamide (92a)



Yield: 80%, yellow solid; mp: 112-113 °C; IR: 1684 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.53 (6H, m, 3 × CH_2), 1.92-1.99 (2H, m, CH_2), 2.26-2.35 (6H, m, 3 × CH_2), 4.24 (2H, t, J = 6.6 Hz, CH_2), 7.14-7.26 (2H, m, H-5 and H-6), 7.56 (1H, d, J = 7.1 Hz, H-7), 8.15 (1H, s, H-2), 8.59 (1H, d, J = 8.4 Hz, H-4), 8.80 (1H, s, SH), 8.99 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.8 (t), 25.2 (2 × t), 26.3 (t), 43.7 (t), 53.7 (2 × t), 54.7 (t), 110.5 (d), 115.2 (s), 120.8 (d), 121.9 (d),

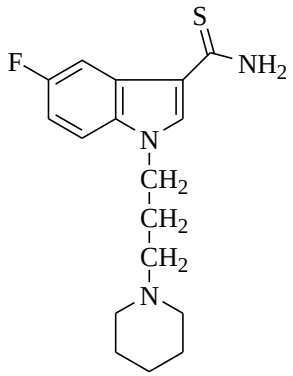
122.1 (d), 126.1 (s), 131.3 (d), 136.9 (s), 193.3 (s); *Anal*. Calculated for $C_{17}H_{23}N_3S$ (MW: 301.45) : C, 67.73; H, 7.69; N, 13.94%. Found: C, 67.48; H, 7.58; N, 14.30%.

5-Bromo-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbothioamide (92b)



Yield: 82%, yellow solid; mp: 197-198 °C; IR: 1636 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.40-1.52 (6H, m, 3 × CH_2), 1.91-1.98 (2H, m, CH_2), 2.27-2.37 (6H, m, 3 × CH_2), 4.24 (2H, t, J = 6.5 Hz, CH_2), 7.36 (1H, dd, J = 8.8, 1.8 Hz, H-6), 7.57 (1H, d, J = 8.8 Hz, H-7), 8.19 (1H, s, H-2), 8.88 (1H, d, J = 1.8 Hz, H-4), 8.92 (1H, s, SH), 9.07 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.5 (t), 25.0 (2 × t), 26.1 (t), 43.9 (t), 53.6 (2 × t), 54.6 (t), 112.6 (d), 114.0 (s), 114.6 (s), 124.1 (d), 124.5 (d), 128.0 (s), 132.0 (d), 135.5 (s), 192.4 (s); *Anal*. Calculated for $C_{17}H_{22}BrN_3S$ (MW: 380.35) : C, 53.68; H, 5.83; N, 11.05%. Found: C, 53.87; H, 5.95; N, 10.74%.

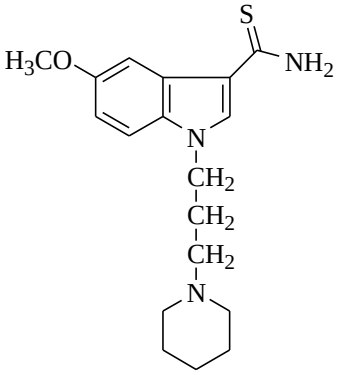
5-Fluoro-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbothioamide (92c)



Yield: 86%, yellow solid; mp: 77-78 °C; IR: 1623 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.42-1.56 (6H, m, 3 × CH_2), 1.94-2.04 (2H, m, CH_2), 2.22 (2H, t, J = 7.2 Hz, CH_2), 2.32 (4H, s, 2 × CH_2), 4.29 (2H, t, J = 6.7 Hz, CH_2), 7.14 (1H, td, J = 11.0, 9.1, 2.6 Hz, H-6), 7.62-7.69 (1H, m, H-7), 8.27 (1H, s, H-2), 8.48 (1H, dd, J = 11.0, 2.6 Hz, H-4), 8.91 (1H, s, SH), 9.07 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 24.0 (t), 25.5 (2 × t), 26.5 (t), 44.1 (t), 53.8 (2 × t), 54.9 (t), 106.9 (d, $J_{C4\text{-}F}$ = 26.0 Hz), 110.2 (d, $J_{C6\text{-}F}$ = 26.3 Hz), 111.8 (d, $J_{C7\text{-}F}$ = 9.7 Hz), 115.0 (d, $J_{C7a\text{-}F}$ = 4.6 Hz), 126.9 (d, $J_{C3a\text{-}F}$ = 11.1 Hz), 132.6 (d), 133.5 (s), 158.1 (d, $J_{C5\text{-}F}$ = 233.0 Hz), 192.6 (s); *Anal*.

Calculated for $C_{17}H_{22}FN_3S$ (MW: 319.44) : C, 63.92; H, 6.94; N, 13.15%. Found: C, 63.74; H, 6.74; N, 13.53%.

5-Methoxy-1-(3-piperidin-1-yl-propyl)-1*H*-indole-3-carbothioamide (92d)

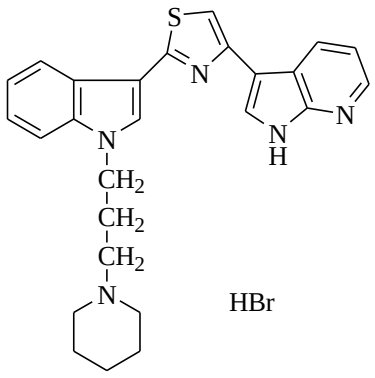


Yield: 88%, yellow solid; mp: 74-75 °C; IR: 1623 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.42-1.56 (6H, m, 3 × CH_2), 1.95-2.01 (2H, m, CH_2), 2.28-2.47 (6H, m, 3 × CH_2), 3.79 (3H, s, CH_3), 4.21 (2H, t, J = 6.5 Hz, CH_2), 6.86 (1H, dd, J = 8.9, 2.4 Hz, H-6), 7.47 (1H, d, J = 8.9 Hz, H-7), 8.12 (1H, s, H-2), 8.17 (1H, d, J = 2.4 Hz, H-4), 8.74 (1H, s, SH), 8.92 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.4 (t), 24.7 (2 × t), 26.0 (t), 43.8 (t), 53.4 (2 × t), 54.5 (t), 55.2 (q), 103.9 (d), 111.2 (d), 111.8 (d), 115.0 (s), 126.7 (s), 131.7 (s), 131.8 (d), 154.8 (s), 192.9 (s); *Anal*. Calculated for $C_{18}H_{25}N_3OS$ (MW: 331.48) : C, 65.22; H, 7.60; N, 12.68%. Found: C, 65.46; H, 7.75; N, 12.29%.

General procedure for the synthesis of 3-{2-[1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (60a-h)

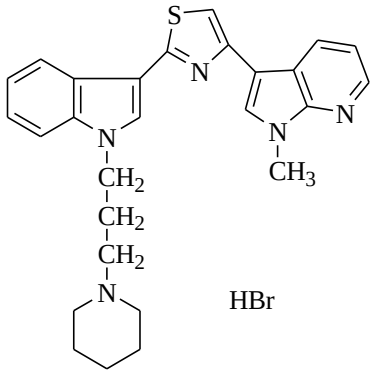
Compounds **60a-h** were prepared from **92a-d** using the same synthetic procedure described for compounds **69a,b**.

3-{2-[1-(3-Piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60a)



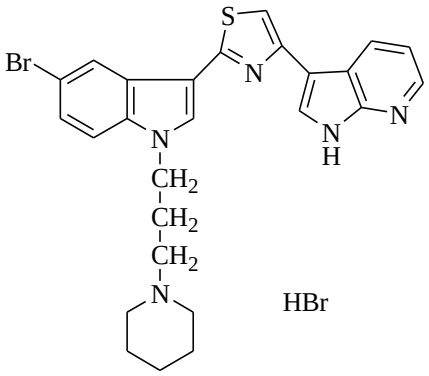
Conditions: 30 minutes at reflux. Yield: 64%, light brown solid; mp: 228-229 °C; IR: 3113 (NH), 2953 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.71-2.29 (6H, m, 3 × CH_2), 2.62-2.78 (2H, m, CH_2), 3.21-3.61 (4H, m, 2 × CH_2), 3.88-3.90 (2H, m, CH_2), 4.83 (2H, t, J = 6.4 Hz, CH_2), 7.63-7.78 (3H, m, H-5', H-5'' and H-6'), 8.09-8.17 (2H, m, H-2' and H-7'), 8.54 (1H, d, J = 2.5 Hz, H-2''), 8.71-8.81 (3H, m, H-4', H-5 and H-6''), 9.06 (1H, d, J = 7.4 Hz, H-4''), 9.56(1H, bs, NH^+), 12.42 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (t), 22.5 (2 × t), 24.3 (t), 43.2 (t), 52.1 (2 × t), 53.4 (t), 106.9 (d), 110.0 (s), 110.3 (s), 110.7 (d), 116.1 (d), 117.1 (s), 120.7 (d), 121.2 (d), 122.6 (d), 124.8 (d + s), 128.5 (d), 129.3 (d), 136.3 (s), 143.0 (d), 148.7 (s), 149.8 (s), 161.5 (s); *Anal*. Calculated for $C_{26}H_{28}BrN_5S$ (MW: 522.50) : C, 59.77; H, 5.40; N, 13.40%. Found: C, 59.44; H, 5.56; N, 13.57%.

1-Methyl-3-{2-[1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60b)



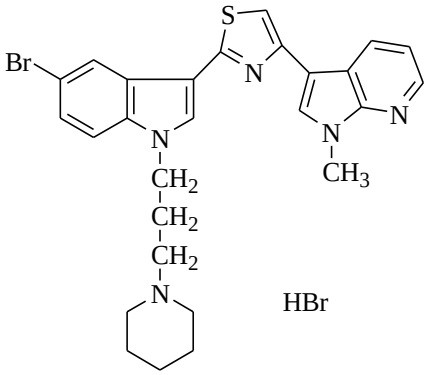
Conditions: 30 minutes at reflux. Yield: 56%, light brown solid; mp: 219-220 °C; IR: 2954 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.24-1.84 (6H, m, 3 × CH_2), 2.15-2.35 (2H, m, CH_2), 2.80-3.17 (4H, m, 2 × CH_2), 3.42-3.46 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.40 (2H, t, J = 6.0 Hz, CH_2), 7.24-7.39 (3H, m, H-5', H-5'' and H-6'), 7.67-7.73 (2H, m, H-2' and H-4'), 8.20 (1H, s, H-2''), 8.28 (1H, s, H-5), 8.37-8.42 (2H, m, H-6'' and H-7'), 8.62 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 9.09 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (t), 22.5 (2 × t), 24.3 (t), 31.0 (q), 43.2 (t), 52.1 (2 × t), 53.4 (t), 106.9 (d), 108.9 (s), 110.3 (s), 110.7 (d), 116.2 (d), 117.3 (s), 120.8 (d), 121.2 (d), 122.6 (d), 124.8 (s), 128.7 (2 × d), 129.4 (d), 136.3 (s), 142.9 (d), 147.6 (s), 149.4 (s), 161.6 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5S$ (MW: 536.53) : C, 60.44; H, 5.64; N, 13.05%. Found: C, 60.16; H, 5.52; N, 13.45%.

3-{2-[5-Bromo-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60c)



Conditions: 30 minutes at reflux. Yield: 66%, yellow solid; mp: 259-260 °C; IR: 3182 (NH), 2959 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.29-1.85 (6H, m, 3 × CH_2), 2.22-2.32 (2H, m, CH_2), 2.79-3.13 (4H, m, 2 × CH_2), 3.39-3.50 (2H, m, CH_2), 4.40 (2H, t, J = 6.6 Hz, CH_2), 7.25 (1H, dd, J = 7.9, 4.6 Hz, H-5''), 7.49 (1H, dd, J = 8.6, 1.4 Hz, H-6'), 7.71 (1H, d, J = 8.6 Hz, H-7'), 7.77 (1H, s, H-2'), 8.11 (1H, d, J = 2.0 Hz, H-2''), 8.34-8.35 (2H, m, H-5 and H-6''), 8.58 (1H, d, J = 1.4 Hz, H-4'), 8.67 (1H, dd, J = 7.8, 1.0 Hz, H-4''), 9.20 (1H, bs, NH^+), 12.05 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (t), 22.5 (2 × t), 24.2 (t), 43.5 (t), 52.1 (2 × t), 53.3 (t), 107.4 (d), 109.8 (s), 110.0 (s), 112.9 (d), 113.9 (s), 116.1 (d), 117.6 (s), 123.0 (d), 124.9 (d), 125.1 (d), 126.4 (s), 129.3 (d), 130.7 (d), 135.1 (s), 142.3 (d), 147.8 (s), 149.7 (s), 161.1 (s); *Anal*. Calculated for $C_{26}H_{27}Br_2N_5S$ (MW: 601.40) : C, 51.93; H, 4.53; N, 11.65%. Found: C, 52.03; H, 4.63; N, 11.45%.

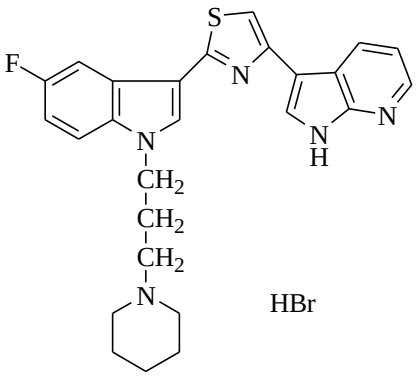
3-{2-[5-Bromo-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60d)



Conditions: 30 minutes at reflux. Yield: 68%, light brown solid; mp: 285-286 °C; IR: 2952 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.27-1.84 (6H, m, 3 × CH_2), 2.15-2.35 (2H, m, CH_2), 2.75-3.16 (4H, m, 2 × CH_2), 3.40-3.47 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.37-4.43 (2H, m, CH_2), 7.26 (1H, dd, J = 7.8, 4.7 Hz, H-5''), 7.49 (1H, dd, J = 8.9, 1.8 Hz, H-6'), 7.69-7.75 (2H, m, H-2' and H-7'), 8.16 (1H, s, H-2''), 8.35-8.39 (2H, m, H-5 and H-6''), 8.56 (1H, d, J = 1.8 Hz, H-4'), 8.63 (1H,

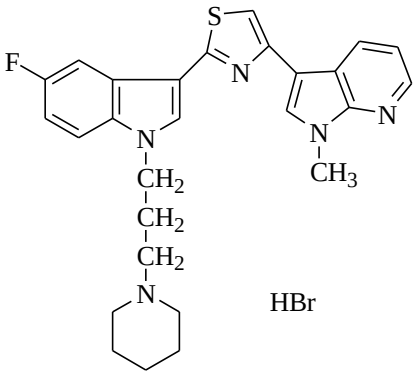
dd, J = 7.8, 1.0 Hz, H-4''), 9.09 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.5 (2 × t), 24.2 (t), 31.1 (q), 43.4 (t), 52.1 (2 × t), 53.3 (t), 107.4 (d), 108.9 (s), 109.8 (s), 112.9 (d), 113.9 (s), 116.1 (d), 117.7 (s), 123.0 (d), 125.2 (d), 126.4 (s), 128.7 (d), 129.2 (d), 130.8 (d), 135.1 (s), 142.5 (d), 147.2 (s), 149.5 (s), 161.2 (s); *Anal*. Calculated for $C_{27}H_{29}Br_2N_5S$ (MW: 615.43) : C, 52.69; H, 4.75; N, 11.38%. Found: C, 52.30; H, 4.98; N, 11.54%.

3-{2-[5-Fluoro-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60e)



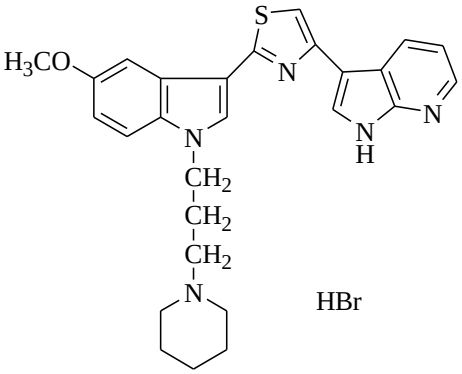
Conditions: 30 minutes at reflux. Yield: 87%, yellow solid; mp: 247-248 °C; IR: 3177 (NH), 2952 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.24-1.80 (6H, m, 3 × CH_2), 2.16-2.36 (2H, m, CH_2), 2.79-3.10 (4H, m, 2 × CH_2), 3.43-3.51 (2H, m, CH_2), 4.36-4.43 (2H, m, CH_2), 7.17-7.26 (2H, m, H-5'' and H-6'), 7.70-7.74 (2H, m, H-2' and H-7'), 8.05-8.11 (2H, m, H-2'' and H-4'), 8.28-8.42 (2H, m, H-5 and H-6''), 8.61 (1H, d, J = 7.9 Hz, H-4''), 9.12(1H, bs, NH^+), 11.98 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (t), 22.6 (2 × t), 24.3 (t), 43.5 (t), 52.1 (2 × t), 53.4 (t), 105.6 (d, $J_{C4'-F}$ = 23.9 Hz), 106.9 (d), 109.9 (s), 110.3 (d, J_{C7a-F} = 4.4 Hz), 110.8 (d, $J_{C6'-F}$ = 25.9 Hz), 112.1 (d, $J_{C7'-F}$ = 9.9 Hz), 116.1 (d), 117.1 (s), 124.8 (d), 125.0 (d, J_{C3a-F} = 10.3 Hz), 128.3 (d), 131.1 (d), 133.1 (s), 143.1 (d), 148.7 (s), 149.9 (s), 158.2 (d, $J_{C5'-F}$ = 234.9 Hz), 161.2 (s); *Anal*. Calculated for $C_{26}H_{27}BrFN_5S$ (MW: 540.49) : C, 57.78; H, 5.04; N, 12.96%. Found: C, 57.90; H, 5.29; N, 12.59%.

3-{2-[5-Fluoro-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60f)



Conditions: 30 minutes at reflux. Yield: 98%, yellow solid; mp: 217-218 °C; IR: 2965 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.25-1.75 (6H, m, 3 × CH_2), 2.25-2.36 (2H, m, CH_2), 2.80-3.17 (4H, m, 2 × CH_2), 3.40-3.47 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.41 (2H, t, J = 6.3 Hz, CH_2), 7.17-7.30 (2H, m, H-5'' and H-6'), 7.71-7.78 (2H, m, H-2' and H-7'), 8.11 (1H, dd, J = 9.9, 2.4 Hz, H-4'), 8.22 (1H, s, H-2''), 8.36-8.39 (2H, m, H-5 and H-6''), 8.61 (1H, dd, J = 8.0, 1.3 Hz, H-4''), 9.38 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.1 (t), 22.5 (2 × t), 24.2 (t), 31.0 (q), 43.5 (t), 52.0 (2 × t), 53.3 (t), 105.7 (d, $J_{C4'-F}$ = 23.8 Hz), 107.0 (d), 108.8 (s), 110.2 (d, J_{C7a-F} = 4.5 Hz), 110.9 (d, $J_{C6'-F}$ = 26.6 Hz), 112.1 (d, $J_{C7'-F}$ = 9.1 Hz), 116.2 (d), 117.3 (s), 125.0 (d, J_{C3a-F} = 10.8 Hz), 128.6 (d), 128.8 (d), 131.1 (d), 133.1 (s), 142.8 (d), 147.5 (s), 149.5 (s), 158.2 (d, $J_{C5'-F}$ = 233.9 Hz), 161.3 (s); *Anal*. Calculated for $C_{27}H_{29}BrFN_5S$ (MW: 554.52) : C, 58.48; H, 5.27; N, 12.63%. Found: C, 58.34; H, 5.06; N, 12.98%.

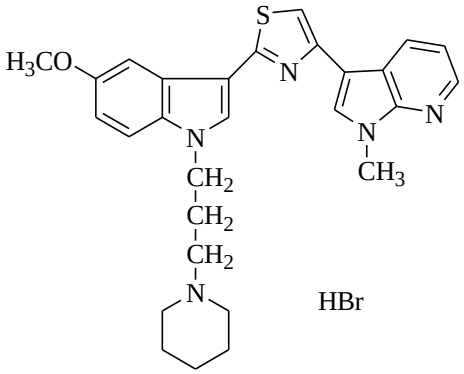
3-{2-[5-Methoxy-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60g)



Conditions: 30 minutes at reflux. Yield: 82%, yellow solid; mp: 261-262 °C; IR: 3062 (NH), 2953 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.28-1.82 (6H, m, 3 × CH_2), 2.16-2.34 (2H, m, CH_2), 2.76-3.12 (4H, m, 2 × CH_2), 3.39-3.50 (2H, m, CH_2), 3.91 (3H, s, CH_3), 4.36 (2H, t, J = 6.6 Hz, CH_2), 7.00 (1H, dd, J = 9.0, 2.5 Hz, H-6'), 7.23 (1H, dd, J = 7.9, 4.8 Hz, H-5''), 7.60 (1H, d, J =

9.0 Hz, H-7'), 7.72 (1H, s, H-2'), 7.95 (1H, d, J = 2.5 Hz, H-4'), 8.12 (1H, d, J = 2.5 Hz, H-2''), 8.22 (1H, s, H-5), 8.33 (1H, dd, J = 4.8, 1.4 Hz, H-6''), 8.76 (1H, dd, J = 7.9, 1.4 Hz, H-4''), 9.31 (1H, bs, NH^+), 12.03 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2 (t), 22.5 (2 × t), 24.3 (t), 43.4 (t), 52.1 (2 × t), 53.4 (t), 55.2 (q), 102.2 (d), 106.8 (d), 109.9 (s), 110.2 (s), 111.6 (d), 112.7 (d), 116.0 (d), 117.9 (s), 124.8 (d), 125.3 (s), 129.6 (d), 129.7 (d), 131.4 (s), 142.0 (d), 147.5 (s), 149.6 (s), 154.9 (s), 161.9 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5OS$ (MW: 552.53) : C, 58.69; H, 5.47; N, 12.68%. Found: C, 58.50; H, 5.35; N, 12.99%.

3-{2-[5-Methoxy-1-(3-piperidin-1-yl-propyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (60h)

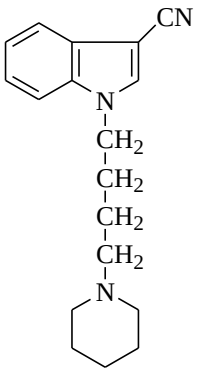


Conditions: 30 minutes at reflux. Yield: 65%, yellow solid; mp: 262-263 °C; IR: 2934 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.23-1.83 (6H, m, 3 × CH_2), 2.19-2.35 (2H, m, CH_2), 2.78-3.14 (4H, m, 2 × CH_2), 3.40-3.46 (2H, m, CH_2), 3.91 (3H, s, CH_3), 3.94 (3H, s, CH_3), 4.36 (2H, t, J = 6.5 Hz, CH_2), 6.98 (1H, dd, J = 8.9, 2.4 Hz, H-6'), 7.29 (1H, dd, J = 7.9, 4.8 Hz, H-5''), 7.61 (1H, d, J = 8.9 Hz, H-7'), 7.71 (1H, s, H-2'), 7.92 (1H, d, J = 2.4 Hz, H-4'), 8.18 (1H, s, H-2''), 8.23 (1H, s, H-5), 8.41 (1H, dd, J = 4.8, 1.3 Hz, H-6''), 8.79 (1H, dd, J = 7.9, 1.3 Hz, H-4''), 9.31 (1H, bs, NH^+); *Anal*. Calculated for $C_{28}H_{32}BrN_5OS$ (MW: 566.56) : C, 59.36; H, 5.69; N, 12.36%. Found: C, 59.57; H, 5.82; N, 12.02%.

General procedure for the synthesis of 1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbonitriles (96a-d)

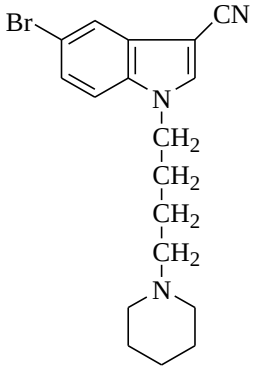
Compounds **96a-d** were prepared from **86a-d** using the same synthetic procedure described for compounds **94a-d**.

1-(4-Piperidin-1-yl-butyl)-1*H*-indole-3-carbonitrile (96a)



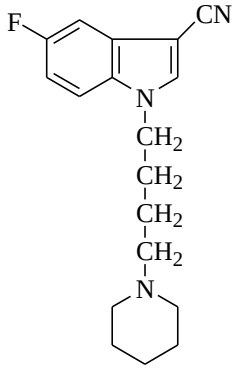
Yield: 95%, orange solid; mp: 46-47 °C; IR: 2219 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.31-1.50 (8H, m, 4 × CH_2), 1.72-1.87 (2H, m, CH_2), 2.17-2.24 (6H, m, 3 × CH_2), 4.28 (2H, t, J = 7.0 Hz, CH_2), 7.24-7.39 (2H, m, H-5 and H-6), 7.63-7.73 (2H, m, H-4 and H-7), 8.32 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.2 (t), 24.0 (t), 25.6 (2 × t), 27.3 (t), 46.2 (t), 54.0 (2 × t), 57.7 (t), 83.3 (s), 111.5 (d), 115.9 (s), 118.6 (d), 121.8 (d), 123.2 (d), 127.1 (s), 125.0 (s), 136.7 (d); *Anal*. Calculated for $C_{18}H_{23}N_3$ (MW: 281.40) : C, 76.83; H, 8.24; N, 14.93%. Found: C, 76.52; H, 8.38; N, 15.10%.

5-Bromo-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbonitrile (96b)



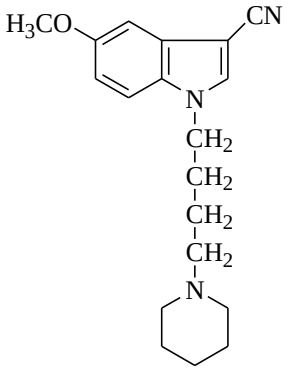
Yield: 96%, white solid; mp: 85-86 °C; IR: 2222 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28-1.43 (8H, m, 4 × CH_2), 1.69-1.83 (2H, m, CH_2), 2.15-2.22 (6H, m, 3 × CH_2), 4.27 (2H, t, J = 7.0 Hz, CH_2), 7.47 (1H, d, J = 8.8 Hz, H-6), 7.72 (1H, d, J = 8.8 Hz, H-7), 7.81 (1H, d, J = 1.8 Hz, H-4), 8.39 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.3 (t), 24.0 (t), 25.2 (2 × t), 27.2 (t), 46.5 (t), 54.0 (2 × t), 57.7 (t), 82.8 (s), 113.9 (d), 114.8 (s), 115.2 (s), 120.8 (d), 125.9 (d), 128.6 (s), 133.9 (s), 138.1 (d); *Anal*. Calculated for $C_{18}H_{22}BrN_3$ (MW: 360.29) : C, 60.00; H, 6.15; N, 11.66%. Found: C, 60.22; H, 5.83; N, 11.76%.

5-Fluoro-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbonitrile (96c)



Yield: 82%, yellow oil; IR: 2221 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.50 (8H, m, 4 × CH_2), 1.71-1.86 (2H, m, CH_2), 2.21-2.40 (6H, m, 3 × CH_2), 4.27 (2H, t, J = 7.0 Hz, CH_2), 7.22 (1H, td, J = 11.7, 9.3, 2.5 Hz, H-6), 7.45 (1H, dd, J = 9.1, 2.5 Hz, H-7), 7.74-7.7.81 (1H, m, H-4), 8.41 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.7 (t), 24.4 (t), 25.0 (2 × t), 27.0 (t), 46.4 (t), 53.4 (2 × t), 57.6 (t), 83.5 (d, J_{C7a-F} = 4.6 Hz), 104.0 (d, J_{C4-F} = 24.9 Hz), 111.8 (d, J_{C6-F} = 26.1 Hz), 113.2 (d, J_{C7-F} = 9.9 Hz), 115.6 (s), 127.6 (d, J_{C3a-F} = 10.8 Hz), 131.8 (s), 138.4 (d), 158.5 (d, J_{C5-F} = 233.0 Hz); *Anal*. Calculated for $C_{18}H_{22}FN_3$ (MW: 299.39) : C, 72.21; H, 7.41; N, 14.04%. Found: C, 72.08; H, 7.23; N, 14.35%.

5-Methoxy-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbonitrile (96d)

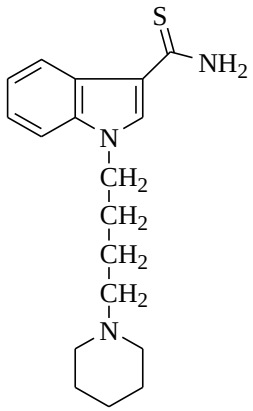


Yield: 96%, yellow oil; IR: 2216 (CN) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.28-1.47 (8H, m, 4 × CH_2), 1.69-1.83 (2H, m, CH_2), 2.17-2.24 (6H, m, 3 × CH_2), 3.82 (3H, s, CH_3), 4.22 (2H, t, J = 7.0 Hz, CH_2), 6.95 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.09 (1H, d, J = 2.4 Hz, H-4), 7.61 (1H, d, J = 9.0 Hz, H-7), 8.24 (1H, s, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.2 (t), 24.0 (t), 25.4 (2 × t), 27.3 (t), 46.4 (t), 54.0 (2 × t), 55.4 (q), 57.6 (t), 82.7 (s), 100.1 (d), 112.6 (d), 113.7 (d), 116.2 (s), 127.8 (s), 129.9 (s), 136.6 (d), 155.4 (s); *Anal*. Calculated for $C_{19}H_{25}N_3O$ (MW: 311.42) : C, 73.28; H, 8.09; N, 13.49%. Found: C, 73.52; H, 8.21; N, 13.13%.

General procedure for the synthesis of 1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbothioamides (95a-d)

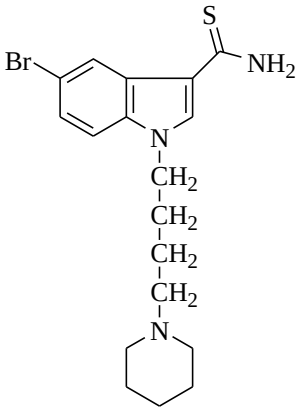
Compounds **95a-d** were prepared from **96a-d** using the same synthetic procedure described for compounds **75a-d**.

1-(4-Piperidin-1-yl-butyl)-1*H*-indole-3-carbothioamide (95a)



Yield: 70%, yellow solid; mp: 100-101°C; IR: 1522 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.45 (8H, m, 4 × CH_2), 1.71-1.82 (2H, m, CH_2), 2.24-2.30 (6H, m, 3 × CH_2), 4.22 (2H, t, J = 6.9 Hz, CH_2), 7.14-7.26 (2H, m, H-5 and H-6), 7.57 (1H, dd, J = 6.9, 1.8 Hz, H-7), 8.15 (1H, s, H-2), 8.61 (1H, dd, J = 6.9, 1.8 Hz, H-4), 8.82 (1H, s, SH), 9.00 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.3 (t), 24.0 (t), 25.3 (2 × t), 27.3 (t), 45.8 (t), 53.8 (2 × t), 57.6 (t), 110.5 (d), 115.4 (s), 121.0 (d), 121.9 (d), 122.1 (d), 126.1 (s), 131.4 (d), 136.6 (s), 193.0 (s); *Anal*. Calculated for $C_{18}H_{25}N_3S$ (MW: 315.48) : C, 68.53; H, 7.99; N, 13.32%. Found: C, 68.36; H, 7.84; N, 13.64%.

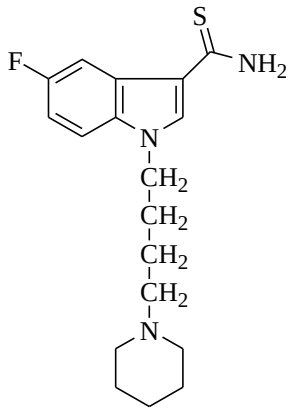
5-Bromo-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbothioamide (95b)



Yield: 74%, yellow oil; IR: 1521 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.37-1.47 (8H, m, 4 × CH_2), 1.70-1.84 (2H, m, CH_2), 2.29-2.31 (6H, m, 3 × CH_2), 4.21 (2H, t, J = 6.8 Hz, CH_2), 7.36

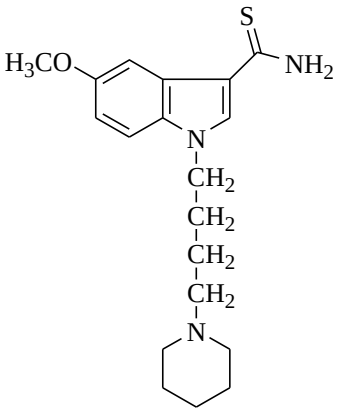
(1H, dd, J = 8.8, 1.9 Hz, H-6), 7.58 (1H, d, J = 8.8 Hz, H-7), 8.21 (1H, s, H-2), 8.90 (1H, d, J = 1.9 Hz, H-4), 8.96 (1H, s, SH), 9.08 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ: 23.1 (t), 23.9 (t), 25.3 (2 × t), 27.2 (t), 46.0 (t), 53.7 (2 × t), 57.5 (t), 112.7 (d), 114.1 (s), 114.6 (s), 124.2 (d), 124.6 (d), 128.2 (s), 131.8 (d), 135.4 (s), 192.4 (s); *Anal.* Calculated for $C_{18}H_{24}BrN_3S$ (MW: 394.37) : C, 54.82; H, 6.13; N, 10.65%. Found: C, 54.68; H, 5.94; N, 10.98%.

5-Fluoro-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbothioamide (95c)



Yield: 75%, yellow oil; IR: 1521 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-*d*$_6$) δ: 1.39-1.47 (8H, m, 4 × CH_2), 1.75-1.82 (2H, m, CH_2), 2.25-2.34 (6H, m, 3 × CH_2), 4.22 (2H, t, J = 6.8 Hz, CH_2), 7.08 (1H, td, J = 11.1, 9.0, 2.6 Hz, H-6), 7.57-7.64 (1H, m, H-7), 8.26 (1H, s, H-2), 8.46 (1H, dd, J = 11.1, 2.6 Hz, H-4), 8.91 (1H, s, SH), 9.01 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ: 23.0 (t), 23.7 (t), 25.1 (2 × t), 27.1 (t), 46.0 (t), 53.6 (2 × t), 57.3 (t), 106.9 (d, $J_{C4\text{-}F}$ = 25.6 Hz), 110.2 (d, $J_{C6\text{-}F}$ = 26.3 Hz), 111.8 (d, $J_{C7\text{-}F}$ = 9.9 Hz), 115.0 (d, $J_{C7a\text{-}F}$ = 4.7 Hz), 127.1 (d, $J_{C3a\text{-}F}$ = 11.1 Hz), 132.4 (d), 133.3 (s), 158.1 (d, $J_{C5\text{-}F}$ = 233.3 Hz), 192.6 (s); *Anal*. Calculated for $C_{18}H_{24}FN_3S$ (MW: 333.47) : C, 64.83; H, 7.25; N, 12.60%. Found: C, 64.57; H, 7.14; N, 12.97%.

5-Methoxy-1-(4-piperidin-1-yl-butyl)-1*H*-indole-3-carbothioamide (95d)

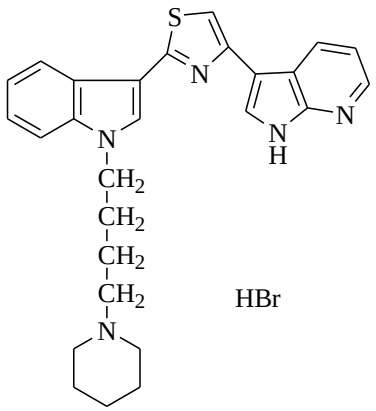


Yield: 56%, yellow oil; IR: 1520 (CS) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.48 (8H, m, 4 × CH_2), 1.69-1.83 (2H, m, CH_2), 2.29-2.32 (6H, m, 3 × CH_2), 3.79 (3H, s, CH_3), 4.17 (2H, t, J = 6.8 Hz, CH_2), 6.86 (1H, dd, J = 8.9, 2.4 Hz, H-6), 7.47 (1H, d, J = 8.9 Hz, H-7), 8.13 (1H, s, H-2), 8.21 (1H, d, J = 2.4 Hz, H-4), 8.78 (1H, s, SH), 8.93 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 23.1 (t), 23.9 (t), 25.2 (2 × t), 27.3 (t), 45.9 (t), 53.7 (2 × t), 55.3 (q), 57.5 (t), 103.9 (d), 111.3 (d), 111.8 (d), 114.8 (s), 126.9 (s), 131.7 (d), 154.9 (s × 2), 192.8 (s); *Anal*. Calculated for $C_{19}H_{27}N_3OS$ (MW: 345.50) : C, 66.05; H, 7.88; N, 12.16%. Found: C, 65.84; H, 8.28; N, 11.97%.

General procedure for the synthesis 3-{2-[1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromides (61a-h)

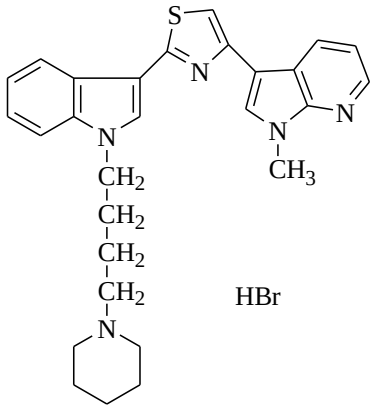
Compounds **61a-h** were prepared from **95a-d** using the same synthetic procedure described for compounds **69a,b**.

3-{2-[1-(4-Piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61a)



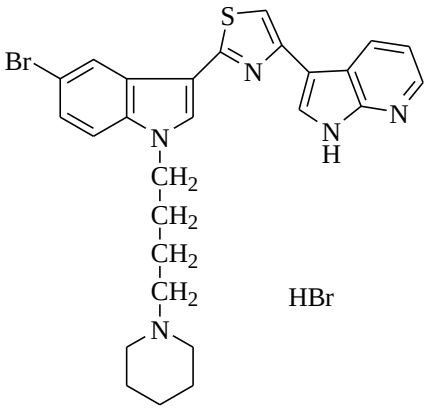
Conditions: 30 minutes at reflux. Yield: 74%, light yellow solid; mp: 275-276 °C; IR: 3132 (NH), 2971 (NH^+) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.88 (10H, m, 5 × CH_2), 2.76-3.12 (4H, m, 2 × CH_2), 3.35-3.40 (2H, m, CH_2), 4.36 (2H, t, J = 6.1 Hz, CH_2), 7.21-7.34 (3H, m, H-5', H-5'' and H-6'), 7.65-7.74 (2H, m, H-2' and H-7'), 8.12 (1H, d, J = 2.5 Hz, H-2''), 8.30-8.38 (3H, m, H-4', H-5 and H-6''), 8.65 (1H, d, J = 6.9 Hz, H-4''), 9.25 (1H, bs, NH^+), 12.00 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.6 (t), 21.2 (t), 22.4 (2 × t), 26.8 (t), 45.2 (t), 52.0 (2 × t), 55.2 (t), 106.8 (d), 110.0 (2 × s), 110.8 (d), 116.1 (d), 117.1 (s), 120.6 (d), 121.1 (d), 122.5 (d), 124.7 (d + s), 128.5 (d), 129.6 (d), 136.3 (s), 143.0 (d), 148.7 (s), 149.8 (s), 161.6 (s); *Anal*. Calculated for $C_{27}H_{30}BrN_5S$ (MW: 536.53) : C, 60.44; H, 5.64; N, 13.05%. Found: C, 60.10; H, 5.79; N, 13.24%.

1-Methyl-3-{2-[1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61b)



Conditions: 30 minutes at reflux. Yield: 57%, yellow solid; mp: 179 °C; IR: 2951 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.88 (10H, m, 5 × CH_2), 2.81-3.08 (4H, m, 2 × CH_2), 3.63-3.66 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.36 (2H, t, J = 5.9 Hz, CH_2), 7.24-7.37 (3H, m, H-5', H-5'' and H-6'), 7.66-7.73 (2H, m, H-2' and H-4'), 8.21 (1H, s, H-2''), 8.30 (1H, s, H-5), 8.37-8.41 (2H, m, H-6'' and H-7'), 8.64 (1H, d, J = 6.8 Hz, H-4''), 9.22 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.6 (t), 21.2 (t), 22.4 (2 × t), 26.8 (t), 31.0 (q), 45.2 (t), 52.0 (2 × t), 55.2 (t), 106. 8 (d), 108.9 (s), 109.9 (s), 110.8 (d), 116.2 (d), 117.4 (s), 120.7 (d), 121.1 (d), 122.5 (d), 124.7 (s), 128.8 (2 × d), 129.6 (d), 136.3 (s), 142.8 (d), 147.5 (s), 149.4 (s), 161.7 (s); *Anal*. Calculated for $C_{28}H_{32}BrN_5S$ (MW: 550.56) : C, 61.08; H, 5.86; N, 12.72%. Found: C, 61.35; H, 5.72; N, 12.59%.

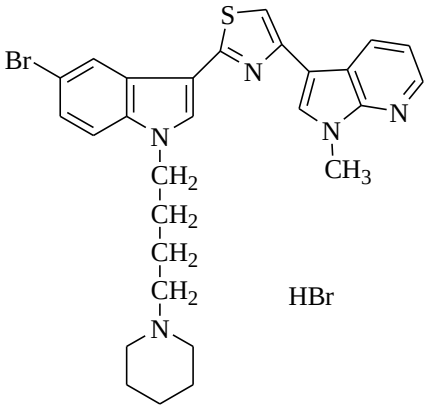
3-{2-[5-Bromo-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61c)



Conditions: 30 minutes at reflux. Yield: 65%, light yellow solid; mp: 259-260 °C; IR: 3194 (NH), 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.38-1.86 (10H, m, 5 × CH_2), 2.76-3.06 (4H, m, 2 × CH_2), 3.35-3.41 (2H, m, CH_2), 4.35 (2H, t, J = 6.0 Hz, CH_2), 7.24 (1H, dd, J = 7.9, 4.7 Hz,

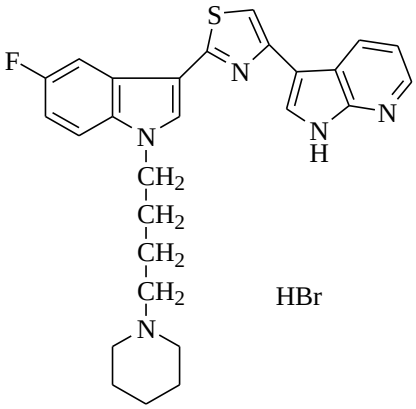
H-5’’), 7.45 (1H, dd, J = 8.8, 1.8 Hz, H-6’), 7.70 (1H, d, J = 8.8 Hz, H-7’), 7.76 (1H, s, H-2’), 8.11 (1H, d, J = 2.4 Hz, H-2’’), 8.34 (1H, dd, J = 4.7, 1.8 Hz, H-6’’), 8.37 (1H, s, H-5), 8.57 (1H, d, J = 1.8 Hz, H-4’), 8.66 (1H, d, J = 7.9 Hz, H-4’’), 9.28 (1H, bs, NH^+), 12.03 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.5 (t), 21.2 (t), 22.4 (2 × t), 26.8 (t), 45.4 (t), 52.0 (2 × t), 55.2 (t), 107.1 (d), 109.5 (s), 109.9 (s), 113.0 (d), 113.7 (s), 116.1 (d), 117.2 (s), 123.0 (d), 124 7 (d), 125.0 (d), 126.4 (s), 128.7 (d), 130.9 (d), 135.1 (s), 142.9 (d), 148.4 (s), 149.8 (s), 161.1 (s); *Anal*. Calculated for $C_{27}H_{29}Br_2N_5S$ (MW: 615.43) : C, 52.69; H, 4.75; N, 11.38%. Found: C, 52.53; H, 5.02; N, 11.27%.

3-{2-[5-Bromo-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61d)



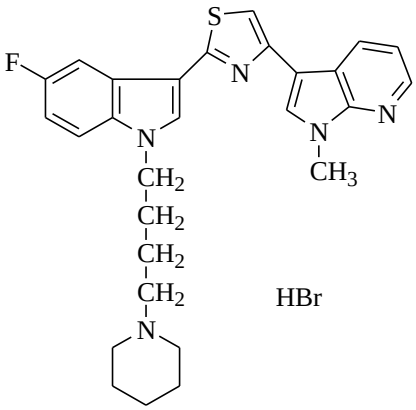
Conditions: 30 minutes at reflux. Yield: 83%, yellow solid; mp: 254-255 °C; IR: 2957 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.29-1.87 (10H, m, 5 × CH_2), 2.76-3.19 (4H, m, 2 × CH_2), 3.38-3.41 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.36 (2H, t, J = 6.5 Hz, CH_2), 7.26 (1H, dd, J = 7.9, 4.7 Hz, H-5’’), 7.47 (1H, dd, J = 8.7, 1.9 Hz, H-6’), 7.68-7.74 (2H, m, H-2’ and H-7’), 8.15 (1H, s, H-2’’), 8.34-8.39 (2H, m, H-5 and H-6’’), 8.55 (1H, d, J = 1.9 Hz, H-4’), 8.64 (1H, dd, J = 7.9, 1,4 Hz, H-4’’), 9.02 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.5 (t), 21.2 (t), 22.4 (2 × t), 26.8 (t), 31.0 (q), 45.4 (t), 52.0 (2 × t), 55.2 (t), 107.2 (d), 108.8 (s), 109.5 (s), 113.0 (d), 113.8 (s), 116.1 (d), 117.4 (s), 122.9 (d), 125.0 (d), 126.3 (s), 128.5 (d), 128.7 (d), 130.9 (d), 135.1 (s), 142.9 (d), 147.5 (s), 149.6 (s), 161.2 (s); *Anal*. Calculated for $C_{28}H_{31}Br_2N_5S$ (MW: 629.45) : C, 53.43; H, 4.96; N, 11.13%. Found: C, 53.73; H, 4.86; N, 10.93%.

3-{2-[5-Fluoro-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61e)



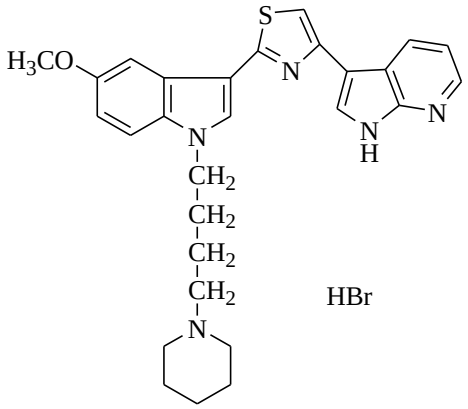
Conditions: 30 minutes at reflux. Yield: 70%, yellow solid; mp: 240-241 °C; IR: 3132 (NH), 2970 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.87 (10H, m, 5 × CH_2), 2.80-3.07 (4H, m, 2 × CH_2), 3.35-3.41 (2H, m, CH_2), 4.35 (2H, t, J = 6.3 Hz, CH_2), 7.14-7.27 (2H, m, H5'' and H-6'), 7.69-7.74 (2H, m, H-2' and H-7'), 8.07 (1H, dd, J = 9.9, 2.4 Hz, H-4'),8.14 (1H, d, J = 2.3 Hz, H-2''), 8.32-8.38 (2H, m, H-5 and H-6''), 8.63 (1H, d, J = 7.1 Hz, H-4''), 9.37 (1H, bs, NH^+), 12.03 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.5 (t), 21.3 (t), 22.3 (2 × t), 26.8 (t), 45.5 (t), 51.9 (2 × t), 55.2 (t), 105.5 (d, $J_{C4'-F}$ = 24.4 Hz), 106.9 (d), 109.9 (s), 110.0 (d, J_{C7a-F} = 4.5 Hz), 110.7 (d, $J_{C6'-F}$ = 26.1 Hz), 112.2 (d, $J_{C7'-F}$ = 9.9 Hz), 116.1 (d), 117.2 (s), 124.9 (d), 125.0 (d, J_{C3a-F} = 10.5 Hz), 128.6 (d), 131.3 (d), 133.0 (s), 142.9 (d), 148.5 (s), 149.8 (s), 158.1 (d, $J_{C5'-F}$ = 234.2 Hz), 161.3 (s); *Anal*. Calculated for $C_{27}H_{29}BrFN_5S$ (MW: 554.52) : C, 58.48; H, 5.27; N, 12.63%. Found: C, 58.21; H, 5.39; N, 12.78%.

3-{2-[5-Fluoro-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61f)



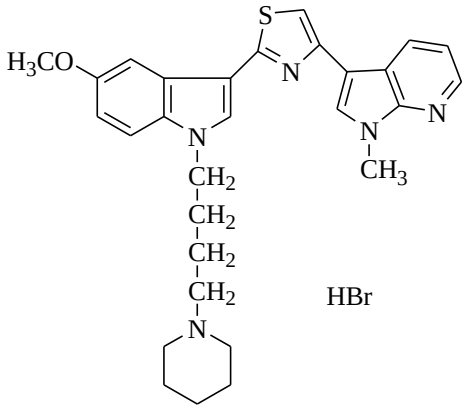
Conditions: 30 minutes at reflux. Yield: 57%, yellow solid; mp: 194-195 °C; IR: 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.39-1.87 (10H, m, 5 × CH_2), 2.80-3.07 (4H, m, 2 × CH_2), 3.49-3.52 (2H, m, CH_2), 3.94 (3H, s, CH_3), 4.35 (2H, t, J = 6.5 Hz, CH_2), 7.14-7.30 (2H, m, H5'' and H-6'), 7.69-7.75 (2H, m, H-2' and H-7'), 8.09 (1H, dd, J = 9.8, 2.5 Hz, H-4'), 8.21 (1H, s, H-2''), 8.36-8.38 (2H, m, H-5 and H-6''), 8.61 (1H, d, J = 7.9 Hz, H-4''), 9.30 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.5 (t), 21.3 (t), 22.3 (2 × t), 26.8 (t), 31.1 (q), 45.5 (t), 51.9 (2 × t), 55.2 (t), 105.6 (d, $J_{C4'-F}$ = 24.7 Hz), 107.0 (d), 108.9 (s), 109.9 (d, J_{C7a-F} = 4.4 Hz), 110.7 (d, $J_{C6'-F}$ = 26.1 Hz), 112.2 (d, $J_{C7'-F}$ = 9.6 Hz), 116.2 (d), 117.4 (s), 125.0 (d, J_{C3a-F} = 11.0 Hz), 128.8 (d), 128.9 (d), 131.4 (d), 133.0 (s), 142.6 (d), 147.4 (s), 149.4 (s), 158.2 (d, $J_{C5'-F}$ = 234.6 Hz), 161.5 (s); *Anal*. Calculated for $C_{28}H_{31}BrFN_5S$ (MW: 568.55) : C, 59.15; H, 5.50; N, 12.32%. Found: C, 59.50; H, 5.28; N, 12.19%.

3-{2-[5-Methoxy-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61g)



Conditions: 30 minutes at reflux. Yield: 74%, yellow solid; mp: 247-248 °C; IR: 3137 (NH), 2971 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.86 (10H, m, 5 × CH_2), 2.76-3.07 (4H, m, 2 × CH_2), 3.35-3.39 (2H, m, CH_2), 3.91 (3H, s, CH_3), 4.31 (2H, t, J = 6.0 Hz, CH_2), 6.95 (1H, dd, J = 8.9, 2.4 Hz, H-6'), 7.26 (1H, dd, J = 7.9, 4.8 Hz, H-5''), 7.58 (1H, d, J = 8.9 Hz, H-7'), 7.73 (1H, s, H-2'), 7.93 (1H, d, J = 2.4 Hz, H-4'), 8.15 (1H, d, J = 2.2 Hz, H-2''), 8.24 (1H, s, H-5), 8.35 (1H, dd, J = 4.8, 1.1 Hz, H-6''), 8.80 (1H, d, J = 7.9 Hz, H-4''), 9.34 (1H, bs, NH^+), 12.11 (1H, bs, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.5 (t), 21.3 (t), 22.3 (2 × t), 26.8 (t), 45.4 (t), 51.9 (2 × t), 55.2 (t + q), 102.1 (d), 106.6 (d), 109.6 (s), 110.2 (s), 111.7 (d), 112.6 (d), 116.0 (d), 117.8 (s), 124.8 (d), 125.3 (s), 129.6 (d), 129.9 (d), 131.4 (s), 142.2 (d), 147.7 (s), 149.6 (s), 154.9 (s), 162.0 (s); *Anal*. Calculated for $C_{28}H_{32}BrN_5OS$ (MW: 566.56) : C, 59.36; H, 5.69; N, 12.36%. Found: C, 59.12; H, 5.56; N, 12.73%.

3-{2-[5-Methoxy-1-(4-piperidin-1-yl-butyl)-1*H*-indol-3-yl]-thiazol-4-yl}-1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine hydrobromide (61h)



Conditions: 30 minutes at reflux. Yield: 76%, yellow solid; mp: 253-254 °C; IR: 2953 (NH^+) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ: 1.32-1.86 (10H, m, 5 × CH_2), 2.75-3.12 (4H, m, 2 × CH_2), 3.43-3.46 (2H, m, CH_2), 3.92 (3H, s, CH_3), 3.93 (3H, s, CH_3), 4.32 (2H, t, J = 6.6 Hz, CH_2), 6.97 (1H, dd, J = 9.0, 2.5 Hz, H-6'), 7.24 (1H, dd, J = 7.9, 4.7 Hz, H-5''), 7.59 (1H, d, J = 9.0 Hz, H-7'), 7.68 (1H, s, H-2'), 7.92 (1H, d, J = 2.5 Hz, H-4'), 8.15 (1H, s, H-2''), 8.21 (1H, s, H-5), 8.37 (1H, dd, J = 4.7, 1.4 Hz, H-6''), 8.72 (1H, dd, J = 7.9, 1.5 Hz, H-4''), 9.05 (1H, bs, NH^+); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 20.6 (t), 21.2 (t), 22.4 (2 × t), 26.8 (t), 31.0 (q), 45.4 (t), 52.0 (2 × t), 55.2 (t), 55.3 (q), 102.3 (d), 106.4 (d), 108.9 (s), 109.6 (s), 111.7 (d), 112.5 (d), 116.0 (d), 117.5 (s), 125.3 (s), 128.3 (d), 129.0 (d), 129.9 (d), 131.4 (s), 142.8 (d), 147.5 (s), 149.5 (s), 154.9 (s), 162.0 (s); *Anal*. Calculated for $C_{29}H_{34}BrN_5OS$ (MW: 580.58) : C, 59.99; H, 5.90; N, 12.06%. Found: C, 59.67; H, 6.06; N, 12.22%.

DOCKING STUDIES

Protein Preparation

The Uniprot database (http://www.uniprot.org) was used to collect available GSK-3β structures, PDB files were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org). Schrödinger's Protein Preparation Wizard of the Prime module was used for protein structure refinements (Prime, 4.1 version, Schrödinger, LLC, New York, NY, 2015). In the preprocess phase, hydrogen atoms were added and bond orders were assigned, missing loops were filled (if needed) according to the amino acid sequence. In the refinement phase the hydrogen bond (H-bond) network was restored using the H-bond assignment option, which was followed by a restrained minimization (converged heavy atoms to RMSD: 0.3 Å) with OPLS_2005 force field. Schrödinger's Maestro was used to visualize interactions between binding site and inhibitors (Maestro, 10.3 version, Schrödinger, LLC, New York, NY, 2015).

Ligand preparation

Structures were drawn manually, 2D structures were optimized with Schrödinger's LigPrep module with standard settings using the OPLS_2005 forcefield (LigPrep, 3.5 version, Schrödinger, LLC, New York, NY, 2015). For compounds **56a-h**, **57a-h**, **58a-h**, **59a-h**, **60a-h**, **61a-h** only their protonated forms were used.

Docking studies

All docking studies were performed with Schrödinger's Glide software package (Glide, 6.8 version, Schrödinger, LLC, New York, NY, 2015). In the grid generation phase, the position of the grid box was determined by the co-crystallized ligand: the center of the docking box was fixed on the center of the inhibitor. The size of the boxes was 20x20x20 Å (the inner box was 10x10x10 Å). Standard settings (Van der Waals Radius and Charge Scaling) were used. Flexible docking on XP (extra precision) level was used, which in terms of glide means that the enzyme was rigid during the conformation of the ligands evaluation. The complementarity of ligand-receptor interactions was examined using the empirical ChemScore function. The active comformer ('pose') of each ligand with the highest score was selected as a result. In case of the re-dockings, scores were also calculated for the co-crystallized poses (score-in-place-protocol).

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