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Recurrence of the oxazole motif in tubulin colchicine site inhibitors with anti-tumor activity

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ABSTRACT

Because of its wide spectrum of targets and biological activities, the oxazole ring is a valuable heterocyclic scaffold in the design of new therapeutic agents with anticancer, antiviral, antibacterial, anti-inflammatory, neuroprotective, antidiabetic and antidepressant properties. The presence of two heteroatoms, oxygen and nitrogen, offers possible interactions (hydrogen, hydrophobic, van der Waals or dipoles bonds) with a broad range of receptors and enzymes. Furthermore, the oxazole core conjugates low cytotoxicity with improved compound solubility and is well suited to structural modifications such as substitution with different groups and condensation to aromatic, heteroaromatic or non-aromatic rings, offering diversity when introduced into scaffolds. These features make it a very attractive nucleus in medicinal chemistry.

Herein we present a diverse array of oxazole derivatives with potential therapeutic use in multiple tumor models. The emphasis has been addressed to compounds with anti-tubulin activity reported in literature in the last decade, describing their structural features, efficiency and future perspectives.

1. Introduction

Nitrogen heterocycles represent the core structure of many drug candidates with a broad spectrum of pharmaceutical applications and therapeutic perspectives [1–6]. In this context, particular relevance has been attributed to oxazoles, five-membered heterocycles containing an oxygen and a nitrogen atom. Oxazole-based compounds, including [1,2]oxazoles and [1,3]oxazoles, attract considerable attention in medicinal chemistry because of the recurrence of the oxazole core as pharmacophore moiety in analgesic, anticancer, antimicrobial, antiviral, anticonvulsant, antidepressant, antituberculosis and immunosuppressant agents. Several drugs, belonging to diverse pharmacological classes, have reached the market (Table 1).

Due to the straightforward synthetic access, the oxazole ring is used as scaffold for the development of new drugs and represents a common feature in numerous anticancer agents. Variations in the oxazole core are associated with a variety of mechanism of actions responsible of tumor suppression, involving heat shock proteins, caspases, kinases and microtubules as main targets (Fig. 1).

Isoxazoles **1**–**3** (Fig. 1) have been reported as heat shock protein inhibitors with interesting clinical potential. Compound **1**, Luminespib (AUY-922; NVP-AUY-922; VER-52296) (Kd of 1.7 ± 0.5 nmol/L) potently inhibited proliferation with GI_{50} values ranging from 2.3 to 49.6 nM in a wide variety of human tumor xenografts models [7] and it was used in clinical trials both as single agent or in combination regimens for the treatment of multiple myeloma, lymphoma, HER2-positive breast cancer, advanced non-small cell lung cancer (NSCLC), pancreatic cancer and other solid tumors [8–10]. Substitution of the isopropyl moiety with the chlorine atom produced compound **2**, VER-50589, with improved potency compared to the parent pyrazole analogue (GI_{50} 78 ± 15 nM vs 685 ± 119 nM) [11]. The 4-methoxy-5-ethyl [1,2]oxazole derivative **3**, KRIBB3 (Fig. 1) showed binding affinity to Hsp27, blocking migration and invasion of a triple negative breast cancer (TNBC) cell line (MDA-MB-231, IC_{50} = 150 nM) [12]. Bis-oxazole derivatives, among which compound **4** bearing arylalkylamino substituents, showed low micromolar cell growth inhibitory activity in cancer cells due to high selectivity for the Hsp90A over Hsp90B quadruplexes [13].

The development of new indole-containing diarylisoxazoles led to the identification of pro-apoptotic antitumor agents targeting caspases 3 and

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List of abbreviations

CA-4	Combretastatin A-4
CDK1	Cyclin-dependent kinase 1
DMPM	Diffuse Malignant Peritoneal Mesothelioma
EBI	*N,N′*-ethylene-bis(iodoacetamide)
HDAC	Histone deacetylases
HER2	Human epidermal growth factor receptor 2
HSP	Heat shock protein
MTs	Microtubules
NCI	National Cancer Institute
NSCLC	Non-small cell lung cancer
PARP	Poly (ADP-ribose) polymerase
PDGFR	Platelet-derivative growth factor receptor
ROS	Reactive oxygen species
RTKs	Receptor tyrosine kinases
SAR	Structure activity relationship
VEGFR	Vascular endothelial growth receptor

7. Strong activity was observed for compounds **5** and **6** (Fig. 1) against colon adenocarcinoma (COLO 320, IC_{50} = 13.5 and 9.0 μM respectively) and lung adenocarcinoma (Calu-3, IC_{50} = 22.5 and 13.3 μM respectively) cell lines [14].

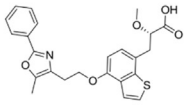
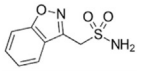
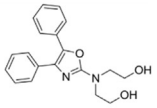
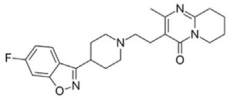
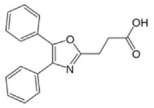
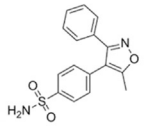
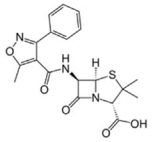
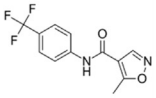
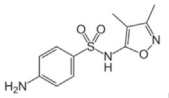
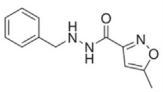
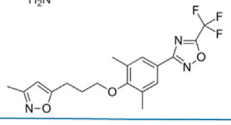
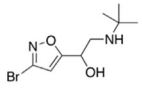
Several condensed oxazole derivatives have also been reported as kinase inhibitors. Within the benzo-condensed series, 3-amino-benzo [*d*] isoxazoles of type **7** were evaluated as c-Met or receptor tyrosine kinases (RTKs) inhibitors, exhibiting IC_{50} values lower than 10 nM both at enzymatic and cellular levels [15]. Extensive structure-activity relationship (SAR) studies demonstrated that the presence of a *N,N′*-diphenyl urea residue (**8**) potently inhibits both vascular endothelial growth receptor (VEGFR) and platelet-derivative growth factor receptor (PDGFR) families of RTKs, displaying high oral bioavailability and excellent *in vivo* efficacy [16]. Condensation to the imidazo system, as in imidazo [2,1-*b*]oxazole-based compounds **9**, led to strong inhibitory effects against V600E-B-RAF and RAF-1 with IC_{50} values ranging from 34 to 930 nM [17].

Another attractive pharmacological target for the development of new anticancer drugs are microtubules (MTs) [3] because even minor alteration of their dynamics can engage the spindle assembly, inhibiting chromosome segregation during mitosis, arresting cell cycle progression and ultimately leading to apoptotic cell death. Despite literature search highlights the oxazole ring as a valuable pharmacophore for the design of new anticancer agents accounting more than five thousand results, only a very limited number (about 80 hits) is related to tubulin polymerization inhibitors. Considering that the latter class of pharmacological agents still represents a gold standard treatment for tumor malignancies, we decided to present a comprehensive overview on oxazole-based compounds with anti-tubulin activity in different cancer models to highlight the drug-discovery process over the past 10 years, to inspire new perspectives.

2. Oxazoles as anti-tubulin agents

Microtubules are highly dynamic, cytoskeletal protein-polymer involved in many fundamental cell functions such as cellular architecture maintenance, motility, intracellular trafficking, cell division and signalling [18]. They are formed by the polymerization of two globular proteins, α- and β-tubulin, both with a molecular weight of 50 kDa [19]. Anti-tubulin agents interfere with the mitotic apparatus by targeting microtubules. Among them, novel compounds with promising activity are based on different oxazole scaffolds. Few examples of compounds containing the [1,3]oxazole substituent were reported before the decade of our interest, among which emerged a family of oxazole and imidazole

Table 1
Marketed drugs containing the oxazole moiety.

Drug	Structure	Pharmacological class	Drug	Structure	Pharmacological class
Aleglitazar		Antidiabetic	Zonisamide		Anticonvulsant Antiobesity
Ditazole		Anti-inflammatory	Risperidone		Antipsycotic
Oxaprozin		Anti-inflammatory	Valdecoxib		COX-2 inhibitor
Oxacillin		Antibacterial	Leflunomide		Antirheumatic
Sulfisoxazole		Antibacterial	Isocarboxazid		Antidepressant
Pleconaril		Antiviral	Broxaterol		Bronchodilatory agent

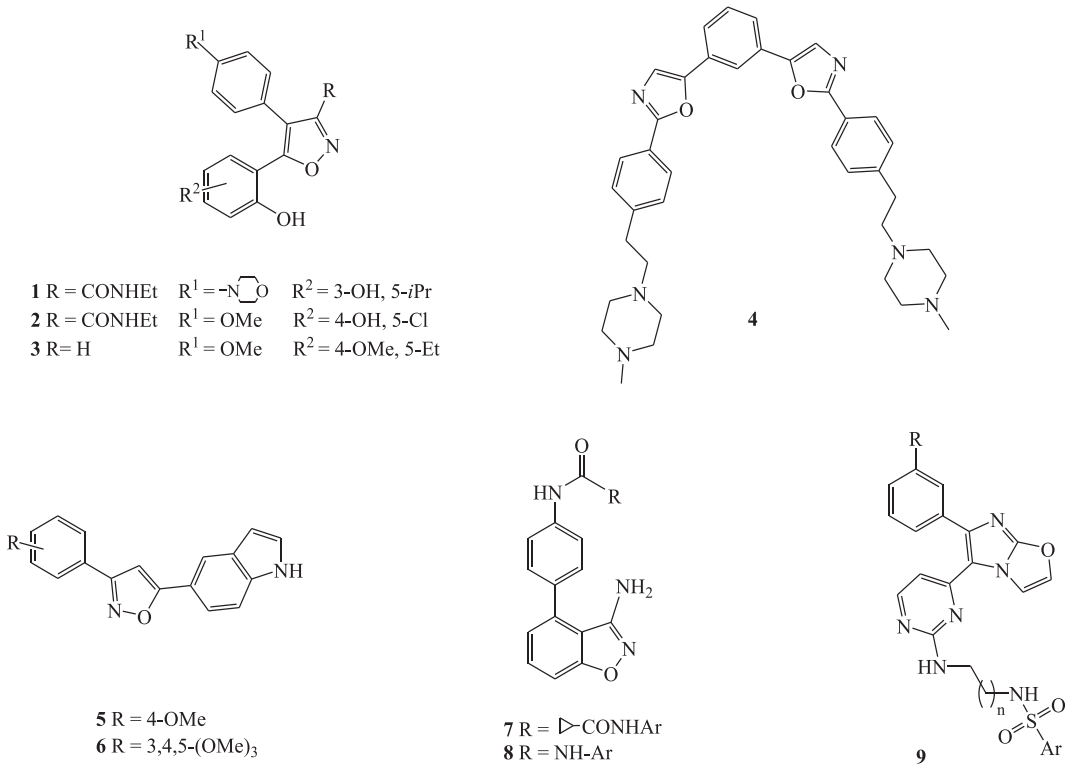


Fig. 1. Anticancer drugs containing the oxazole-moiety.

derivatives with nanomolar activity against cell lines derived from colon adenocarcinoma (HT-29), breast cancer (MCF7) and testicular germ tumor cells resistant to the treatment with combretastatin A-4 (CA-4) or cisplatin (1411HP). The diamino substituted oxazole was one of the most active compound among oxazole and imidazole-bridged combretastatin derivatives with halo- or amino-substituted rings against cell lines derived from melanoma (518A2), leukemia (HL-60), colon adenocarcinoma (HT-29), cervix cancer (KB–V1/Vbl), and breast cancer (MCF-7/Topo) [20]. To identify novel tubulin inhibitors with strong inhibition of tubulin polymerization, a wide SAR analysis of small molecules based on the benzophenone scaffold incorporating different heterocycles were investigated. The oxazole core was evaluated as substitution at C5 of the so-called B-ring in conjunction of a trimethoxyphenyl moiety to probe the effect of substitutions in the framework. In this case, compounds with nanomolar potency against the leukemia HL-60 cell line were generated, even though this result was not paralleled by appreciable *in vivo* antitumor activity compared to their thiazole analogues [21].

Since the discover of CA-4 as potent anti-tubulin agent and representative ligand of the colchicine site [22,23], medicinal chemists have focused on the development of new analogues with enhanced activity. Promising compounds were obtained replacing the double bond with heterocyclic moieties, such as indole, indazole, triazole, imidazole, pyrazoline, furazan, oxazole, cyclopentenone and thiadiazole [24–28] or incorporating the heterocyclic motif in a more rigid polycondensed scaffold [29]. Among the five-membered rings, oxazoles showed higher

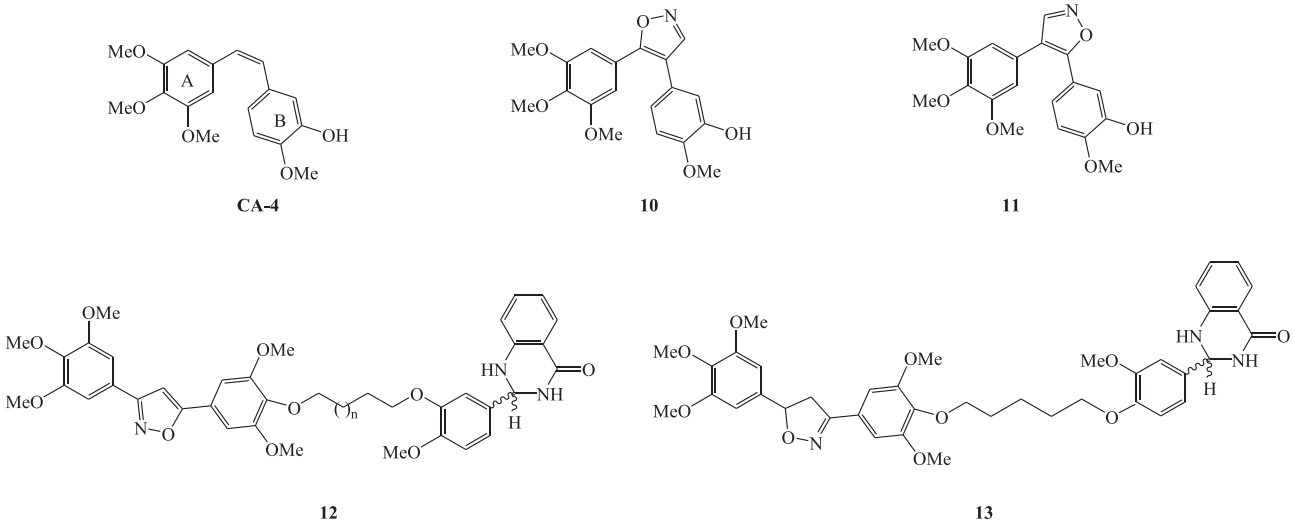


Fig. 2. CA-4 and [1,2]oxazole-bridged derivatives.

cytotoxicity and affinity to tubulin towards several tumor cells without affecting normal tissues as described in the following paragraphs.

2.1. [1,2]Oxazole-bridged derivatives

The [1,2]oxazole ring is suitable to replace the ethylene bridge of CA-4 (Fig. 2) because it maintains the two aromatic rings in the active *cis* conformation, preventing *cis-trans* isomerization. In the first decade of 2000s, anti-tubulin CA-4 analogues bearing the [1,2]oxazole moiety as linker of rings A and B were identified by different research groups. Both 3,4-diarylisoxazoles and 4,5-diarylisoxazoles, maintaining the trimethoxyphenyl (ring A) and 4-methoxyphenyl groups (ring B), exhibited interesting activity as depolymerizing agent and showed high cytotoxicity against different tumor cell lines (leukemia, esophageal carcinoma, NSCLC, hepatocellular carcinoma and prostate cancer) [30–32]. More recently, 4,5-diarylisoxazoles **10** and **11** (Fig. 2) have been evaluated for their antimitotic tubulin-binding activity using the phenotypic sea urchin embryo assay. Effects of **10** and **11** were higher than CA-4 (Fig. 2), inducing embryo spinning and formation of tuberculate arrested eggs typical of microtubule destabilizing agents [33].

3,5-Diarylisoxazolines/isoxazoles were linked to 2,3-dihydroquinazolinones using different alkane spacers, since quinazolinone-based anticancer agents are associated with the inhibition of tubulin polymerization [34]. Among the [1,2]oxazole derivatives, compound **12** (Fig. 2) showed promising anticancer activity against cell lines derived from lung adenocarcinoma (A549, GI_{50} = 0.18 μM), ovarian cancer (A2780, GI_{50} = 2 μM)) and prostate cancer (PC3, GI_{50} = 2 μM) [35]. However, 3,5-diaryl isoxazoline analogue **13** (Fig. 2) exhibited a more significant efficacy with GI_{50} values lower than 1 μM against 18 cancer cell lines (especially leukemia and melanoma models) and the ability to induce microtubule disruption as well as fragmentation of nuclei at concentration similar to that of CA-4. Compound **13** further caused inhibition of cyclin B1 and CDK1 and increased level of cleaved PARP (Poly (ADP-ribose) polymerase) in a breast cancer cell line (MCF-7). Although maintaining the same functionalizations of compound **12**, the replacement of the isoxazole with an isoxazoline unit and the variations in position and length of spacer in compound **13** led to improved activity as anti-tubulin agent.

The 3,4,5-trimethoxyphenyl group close to the oxygen atom of the oxazole ring proved to be important to get more potent compounds, whilst the replacement of any methoxy group in the structures displayed a decrease in the activity [35].

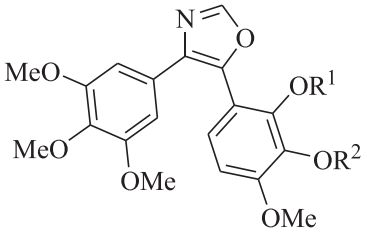
2.2. [1,3]Oxazole-bridged derivatives

2.2.1. Insight on 4,5- and 2,5-disubstituted [1,3]oxazoles

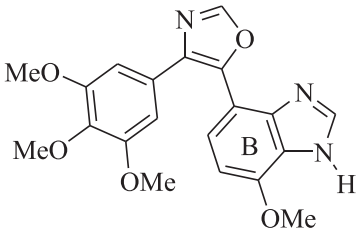
CA-1H and its prodrug CA-1HP are analogues of combretastatin A-4 (CA-4) bearing a [1,3]oxazole ring between the two aryl moieties (Fig. 3).

CA-1H displayed the ability to inhibit tubulin polymerization both in a cell-free system and in Human Umbilical Vein Endothelial Cells (HUVEC) through the binding to colchicine site, showing a moderately stronger activity than CA-4. CA-1H was able to cause disruption in the cytoskeleton organization leading to inhibition of the proliferation of tumor vasculatures with high selectivity. Thus, it was evaluated the *in vivo* disruptive effect of prodrug CA-1HP considering that vascular disrupting agents generally induce concentration dependent necrosis. In *vivo* models of lung adenocarcinoma (NCI–H1975) and mouse hepatocellular carcinoma (H22), CA-1HP displayed the capability to inhibit tumor growth at not toxic concentration and its efficacy could be enhanced by combination with other antitumor agents [36].

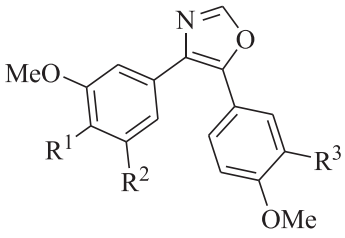
A new class of [1,3]oxazole-bridged compounds, in which the B ring of CA-4 was replaced by a 4-methoxy-1*H*-benzo [*d*]-imidazole (Fig. 3), showed antiproliferative activity up to nanomolar level against five cell lines from different tumor types (MCF-7, A549, HT29, HepG2 and BxPC3). Among the synthetized derivatives, emerged the *N*-unsubstituted benzoimidazole compound **14** (Fig. 3), which displayed highly potent cytotoxicity against breast cancer (MCF-7), colon adenocarcinoma (HT29) and pancreatic ductal adenocarcinoma (BxPC-3) cell lines (IC_{50} 25 nM, 8 nM and 31 nM, respectively). Further insight in the mechanism



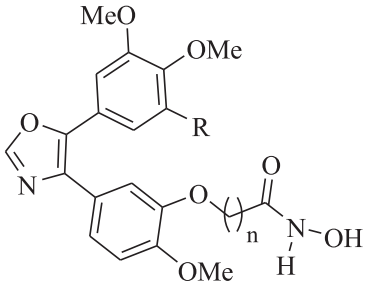
CA-1H $R^1 = R^2 = H$
CA-1HP $R^1 = R^2 = PO(ONa)_2$



14



15a R^1= SMe, R^2 = OMe, R^3 = OH
15b R^1= OMe, R^2 = Br, R^3 = SMe



(n = 3-5)
16a-c R = Cl
16d-f R = Br
16g-i R = OMe

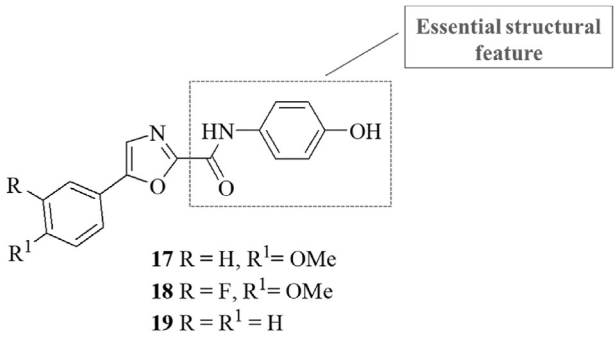
Figs. 3. 4,5-Disubstituted [1,3]oxazoles.

of action demonstrated that [1,3]oxazole **14** was able to inhibit tubulin polymerization activity with IC_{50} value of 0.39 μM, resulting more active than CA-4 (IC_{50} 2.7 μM), causing cell cycle arrest at G2/M phase in a concentration dependent manner. This compound can be regarded as a promising example of the class, as *in vivo* study with a murine hepatocellular carcinoma xenograft (H22) model revealed a significant antitumor activity with a tumor grown inhibition up to 66% [37].

A series of thio analogues of CA-4 (Fig. 3) belonging to the [1,3] oxazole-bridged derivatives were evaluated against six different cancer cell lines (A431, HeLa, MCF7, MDA-MB-231, A549 and SKOV) and two non-neoplastic cell lines (HaCaT and CCD39Lu), showing IC_{50} values in the low nanomolar-micromolar range (0.009–17.87 μM). The activity was strongly influenced by the number and the position of the substituents on the B ring. In fact, 3,4-disubstituted derivatives were more active than both the 2-thiomethyl decorated compound and 3,4,5-trisubstituted derivatives. Replacement of one the methoxy group of the phenyl A-ring with a bromine atom led to derivatives with comparable activity. Overall, the most active compounds were **15a** (IC_{50} 0.009–0.71 μM) and **15b** (IC_{50} 0.43–2.78 μM) (Fig. 3). The same compounds tested against the non-neoplastic HaCaT and CCD39Lu cell lines produced in some cases cytotoxicity against HaCaT cells. All [1,3]oxazole compounds were evaluated for their ability to inhibit tubulin polymerization and derivatives **15a** and **15*b*** well inhibited tubulin polymerization (IC_{50} values of 1.05, and 0.85 μM, respectively) inducing cell cycle arrest in G2/M phase in cell line-dependent manner and cell death by apoptosis [38].

Considering that tubulin-binding antitumor drugs showed synergistic effects when administrated with HDAC inhibitors, a new class of 4,5-diaryloxazole derivatives bearing a hydroxamate group were designed as dual target and evaluated for their anticancer activity. The synthetized compounds were screened for cytotoxic activity against a panel of six cancer cell lines (518A2, HT-29, DLD-1, HCT-116, KB-V1^{Vbl}, MCF-7^{Topo}) as well as against the human endothelial hybrid cell line Ea. Hy926 and the non-malignant human dermal fibroblasts HDFa. Within this class of compounds, those bearing the hydroxamate side chain showed the best activity, in the nanomolar-micromolar range. In particular, the antitumor activity against Ea. Hy926 increased as the length of the side chain decreased, and with the replacement of trimethoxy group (**16g-i**, R = OMe, n = 3–5) with chlorine (**16a-c,** R = Cl, n = 3–5) or bromine (**16d-f,** R = Br, n = 3–5) atoms. Among the bromo derivatives, a different behaviour was highlighted. In fact, compounds **16d** and **16e** were able to disrupt microtubule organization with IC_{50} values of 0.5 and 1.5 μM respectively with cell cycle arrest in G2/M phase, whereas derivative **16f** did not interfere with microtubule even at 4 μM but showed the highest activity in the inhibition of HDAC1 and HDAC6 (IC_{50} 0.49 and 0.32 μM) with cell cycle arrest in G1 phase. Moreover derivative **16d** was well tolerated in mice after administration of high doses (1 × 100 mg/kg i. p., 1 × 200 mg/kg orally) [39].

Acyl-5-phenyloxazoles were evaluated against cell lines derived from lung adenocarcinoma (A549), cervical cancer (HeLa), and hepatocellular carcinoma (HepG2). Among the screened compounds, promising results were obtained with derivative **17** (Fig. 4) which showed IC_{50} values of 1.44, 1.93, and 2.76 μM, respectively. The introduction of a fluorine atom in ortho position of the methoxy group led to compound **18**, which maintained antiproliferative activity in the low micromolar range (IC_{50} 1.12, 1.20, and 1.81 μM, respectively). The removal of methoxy group yielded derivative **19** which showed the best activity in sub micromolar - low micromolar range (IC_{50} 0.78, 1.08, and 1.83 μM, respectively). Several structural modification in the acyl moiety, such as the change of position of hydroxyl group or its substitution with methoxy, methyl, *N*,*N*-dimethyl, *N*,*N*-diethyl, and chlorine groups or substitution of B ring with an arylpiperazine produced loss of activity. The most active compound **19** demonstrated the ability to inhibit tubulin polymerization activity in a concentration dependent manner with an IC_{50} value of 16.7 μM and induced a marked cell cycle arrest in G2/M phase [40].



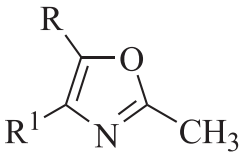
Figs. 4. 2,5-Disubstituted [1,3]oxazoles.

2.2.2. Insight on 2,4,5-trisubstituted [1,3]oxazoles

Among *cis*-constrained CA-4 analogues, novel 2-methyl-4,5-disubstituted oxazole derivatives of type **20** and **21** were designed and synthesized by Romagnoli and coll. In 2017 (Fig. 5) [41].

The two different regioisomeric series of derivatives of type **20** and **21** were designed maintaining, at C-4 and C-5 respectively, the 3′,4′,5′-trimethoxyphenyl moiety corresponding to the A-ring of CA-4 and replacing its B ring with different aryl moieties either substituted with electron-withdrawing or electron-releasing groups. Antiproliferative activity was evaluated against a panel of seven human tumor cell lines (HeLa, A549, HT-29, MCF-7, Jurkat, RS4; 11, SEM) in comparison with the reference drug CA-4 (IC_{50} = 0.8–3100 nM). The most active compounds proved to be **20d** and **20e** with IC_{50} values of 0.35–4.6 nM and 0.5–20.2 nM, respectively. Moreover, derivatives **20a**, **20c**, **20f** and **21a**, which can be regarded as positional isomer of **20e**, showed cell growth inhibitory effects against most of the investigated cancer cell lines at micromolar concentrations (IC_{50} = 0.4–179.3 nM). SAR studies indicated the 3′,4′,5′-trimethoxyphenyl moiety at C-4 as a crucial structural requirements of this class of compounds, since the corresponding 5-(3′,4′,5′-trimethoxyphenyl)oxazoles **21** were generally less active. Electron-releasing groups at the *para*-position of the phenyl ring, increased the activity, whilst moving them in *meta* position was detrimental for the activity.

The most active derivatives were also tested for their antitubulin activity, but there were some discrepancies between *in vitro* antiproliferative activity and inhibition of tubulin assembly. In fact, in the



20a-f, 21a

20a R = naphth-2-yl, $R^1 = (OMe)_3Ph$
20b R = 4'-Me-C_6H_4, $R^1 = (OMe)_3Ph$
20c R = 4'-OMe-C_6H_4, $R^1 = (OMe)_3Ph$
20d R=4'-OMe, 3'-F-C_6H_3, $R^1 = (OMe)_3Ph$
20e R=4'-OEt-C_6H_4, $R^1 = (OMe)_3Ph$
20f R=4'-OEt, 3'-Cl-C_6H_3, $R^1 = (OMe)_3Ph$
21a R=$(OMe)_3Ph$, R^1 = 4'-OEt-C_6H_4

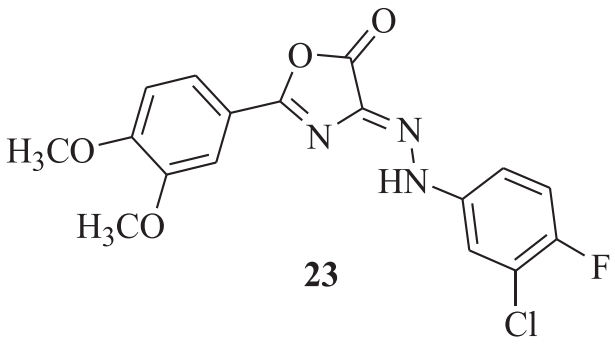
Fig. 5. 2-Methyl-4,5-disubstituted oxazoles.

assembly assay, the best tubulin polymerization inhibitors proved to be derivatives **20e** and **20b** (IC_{50} values of 0.56 and 0.66 μM, respectively) which were even more potent than CA-4 (IC_{50} = 1.3 μM), while **20d** showed comparable antitubulin activity with CA-4. Compound **20e**, caused a dose-dependent increase in the percentage of mitotic cells associated with alteration of the expression of cdc2/cyclin B1 and increased phosphorylation of γH2A.X, thus suggesting DNA damage. The mitochondrial pathway, associated to increased ROS production, PARP cleavage and down regulation of anti-apoptotic proteins Mcl-1 and XIAP was involved in the apoptotic death. *In vivo* studies performed on compound **20e** displayed its ability to produce a reduction in tumor mass of 34.9%, and 52.5% at 3.0 and 7.5 mg/kg respectively, without showing any toxicity or decrease in animal body weight.

No vascular disrupting activity was detected in this class of compounds, differently from 2-methyl-4-phenyloxazol-5-yl) (4-phenylpiperazin-1-yl)methanone derivative **22a** (Fig. 6) synthesized by Choi and coll [42]. Considering that combretastatin A-4 phosphate (CA-4P) is a well-known vascular-disrupting agent [43], a class of aryloxazoles was designed and synthesized as dual agents, able to produce both mitotic arrest and tumor vasculature disruption. From a screening of an *in-house* library of compounds, derivative **22a** emerged for its cytotoxicity against leukemia (HL-60) and colon carcinoma (HCT-116) cell lines (IC_{50} values 36 and 4.5 nM, respectively) as well as potent tubulin polymerization inhibitor (IC_{50} = 4.32 μM). Molecular modeling studies pointed out the ability of derivative **22a** to occupy the colchicine binding site, partially overlapping with docked colchicine in the hydrophobic binding pocket.

Structural modification were therefore designed in order to improve the interaction with the target and a series of aryl [1,3]oxazoles emerged among [1,2]oxazole and thiazole derivatives synthesized by the same authors [42]. Antiproliferative activity against human leukemia cells (HL-60) was evaluated, using also the vascular disrupting agent CYT99718 as reference drug. From a SAR point of view concerning the piperazine moiety, which was selected among other heterocycloalkyl moieties (piperidines, homopiperazines), 3-aryl substituted derivatives **22b**, **22c**, and **22d** showed cytotoxic effects at nanomolar level (IC_{50} values 38.4–45 nM) regardless the electronegativity of the substituent, whereas bulkier substituent at position 3 or 4 led to a reduction of potency.

Introduction of an additional 5-methoxy group as in compound **22e**, a 3,5-dimethoxy-substituted derivative, produced the highest antiproliferative activity with IC_{50} value of 19.2 nM. Any decoration of the phenyl ring at position 4 with a hydroxy group led to less active compounds, in comparison with the corresponding unsubstituted derivatives



23

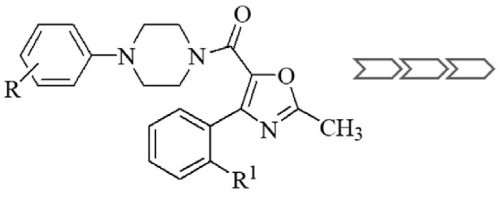
Fig. 7. 3-Chloro 4-fluoro aryl hydrazono oxazolone.

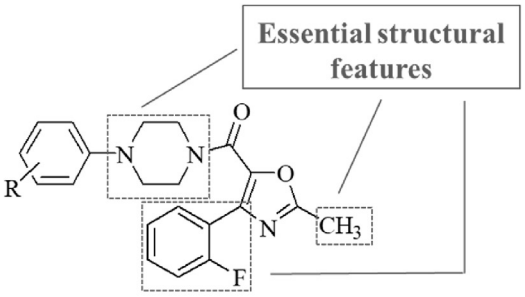
thus proving the importance of van der Waals interaction with the active site of the receptor. Once fixed the 3,5-dimethoxy motif, various fluorine substitutions were evaluated leading to define 2-fluoro substituent as important for the activity. The best compounds were fluoro-substituted derivatives decorated with a dimethoxy group on the phenylpiperazine moiety which were endowed with good cytotoxic activity with IC_{50} value from 10.3 to 38.5 nM, indicating that hydrogen-bond interactions may play an important role. The most potent compounds **22f** and **22g** were chosen for further *in vivo* evaluation of antitumor activity.

Interestingly **22f** was able to reduce tumor growth of 42.3% in size at 100 mg/kg, while compound **22g** did not affect tumor growth, probably due to its poor metabolic stability.

In a study involving several azole derivatives (oxazolones, imidazolones, hydrazono-oxazolones and 1,2,4-triazoles) screened against HepG2 cell line, 3-chloro, 4-fluoro aryl hydrazono substituted derivative **23** (IC_{50} = 2.91 μM) (Fig. 7) was the most significative compound emerged among the oxazole class, even if it was less active than imidazolones and triazoles analogues investigated by the authors [44].

In this case, the decoration at C-2 with trimethoxyphenyl moiety led to a decrement of the cytotoxic activity compared to dimethoxyphenyl substituted derivatives. Investigation of the mechanism of action revealed that compound **23** caused inhibition of β-tubulin polymerization (79.27%), accumulation of the cells at G2/M phase and apoptosis mediated by upregulation of p53, increased expression of Fas-L and of BAX/BCL-2 ratio.





22a R = 3-Cl, R^1 = H
22b R = 3-OMe, R^1 = H
22c R = 3-CN, R^1 = H
22d R = 3-F, R^1 = H
22e R = 3,5-$(OMe)_2$, R^1 = H
22f R = 3,5-$(Cl)_2$, R^1 = F
22g R = 3,5-$(OMe)_2$, R^1 = F

22f,g

Fig. 6. 2-Methyl-4-phenyloxazol-5-yl-(phenylpiperazin-1-yl)methanone derivatives.

2.3. Benzocondensed oxazoles

Interesting scaffolds have been highlighted along the years in this field of research. Typically, in these compounds, which can be generalized as in Fig. 8, the benzocondensed oxazole moiety represents one of the pharmacophore unit connected to the recurrent trimethoxyphenyl moiety through spacers with different chemical features. The smallest spacer is represented by a pyridine ring in bridged compounds. This chemical feature emerged from previous studies leading to potent tubulin inhibitors binding at the colchicine site as analogues of CA-4 [23]. Interestingly, the most promising anticancer activity and tubulin polymerization inhibition was achieved for trimethoxyphenyl substituted pyridines bearing in α position a 6-methoxy benzo [*d*]oxazole group **24**, which showed comparable activity to CA-4 against lung adenocarcinoma (A549) (GI_{50} = 0.82 μM) and astrocytoma (U251) (GI_{50} = 0.17 μM) cells, strong tubulin polymerization inhibitory activity (IC_{50} = 2.1 μM) at the colchicine binding site, in which it adopted a similar conformation to that of CA-4, and producing cell cycle arrest in the G2/M phase by disrupting the microtubules network [45].

Supported by virtual screening insight of a library of more than one thousand tubulin inhibitors, one lead candidate bearing a larger spacer, based on four carbon atoms and an aryl substituted ring, between the benzoxazole and the trimethoxyphenyl moieties was identified. From further SAR analysis, covering the chemical space around the parent structure highlighted a potent compound which showed improved activity at micromolar level against multiple cancer cell lines (MDA-MB-231, HeLa, A549, HepG2, CNE2, and HCT116) with IC_{50} values of 5.45, 8.61, 7.47, 2.29, 2.91, and 4.10 μM, respectively, confirming the cytotoxicity against resistant tumor cell lines. Tubulin polymerization inhibition, by immunofluorescence analysis, indicated that **25** induced cell cycle arrest in the G2/M phase, inhibited cell migration of TNBC cells (MDA-MB-231) and demonstrated to be an effective inhibitor by binding to the colchicine site of tubulin [46].

Introduction of heteroatoms (N,S) into the spacer led to 2-(benzo[*d*]oxazol-2-ylthio)-*N*-(4-methoxybenzyl)-*N*-(3,4,5)-trimethoxyphenyl)acetamide **26** which was able to interfere with polymerization of tubulin by binding to colchicine site, assessed by immunofluorescence studies on a cancer cell line (MCG-803, believed to be derived from gastric mucinous adenocarcinoma, but more likely a hybrid cell line derivative of the cervix cancer HeLa cell line) [47] and the *N, N′*-ethylene-bis(iodoacetamide) (EBI) competition assay, at low micromolar level (IC_{50} = 3.35 μM). The compound showed potent antiproliferative activity against MGC-803 cells with an IC_{50} value of 0.45 μM by induction of G2/M phase arrest and cell apoptosis [48].

2.4. Tricyclic [1,2]oxazoles

In the past 10 years, many efforts have been devoted to the synthesis of new polycondensed heterocyclic systems incorporating the [1,2]oxazole ring. In the attempt to improve the modest antiproliferative activity of pyrrolo [3,4-*g*]indazoles [49], two classes of pyrazole- and pyrrole-fused systems bearing the [1,2]oxazole unit were investigated. The [1,2]oxazolo [5,4-*e*]indazoles of type **27** and [1,2]oxazolo [4,5-*g*] indoles of type **28** showed potent antiproliferative activity against the NCI human tumor cell lines panel with GI_{50} values in the nanomolar – low micromolar range (Fig. 9) [50,51]. In search of novel tricyclic [1,2] oxazole-based antimitotic agents [1,2],oxazolo [5,4-*e*]isoindoles **29** as positional isomers of compounds **28** were further reported (Fig. 9). The annelation of the [1,2]oxazole ring into the isoindole moiety strongly improved the antitumor properties, reducing *in vitro* and *in vivo* cell growth of different tumor models, including NCI 60 human tumor cell line panel and diffuse malignant peritoneal mesothelioma (DMPM). The antiproliferative activity of compounds **29a,b** was found to rely on microtubule impairment during mitosis, in a vinca alkaloid-like manner, with consequent cell cycle arrest at the G2/M phase and caspase-dependent apoptosis [52,53]. Since anti-tubulin agents are included in the commonest chemotherapy regimens for lymphomas [1–3],oxazolo [5,4-*e*]isoindoles **29a,b** were further evaluated in cell lines derived from different lymphoma subtypes, providing promising results (IC_{50} values in the low micromolar to nanomolar range) [54].

From the investigation of optimal structural requirements for the design of anti-tubulin tricyclic [1,2]oxazoles, the pyrrolo [2′,3':3,4] cyclohepta [1,2-*d*] [1,2]oxazoles **30** have recently emerged as promising cyclohepta analogues of compounds **28** (Fig. 9). Their inhibition of tubulin polymerization (IC_{50} values of 1.9–8.2 μM) through binding to the colchicine site, induced growth reduction against multiple malignant cell types [55]. Expansion of the cyclohexyl central ring by one member, in analogy with the cyclohepta rings in the structure of colchicine, enhanced the activity of the parent analogue **28**, albeit not as much as compounds **29**.

SAR studies suggested that the isoindole scaffold provide the best antimitotic effect, whilst the indole and indazole moieties induce a progressive reduction of potency. The presence of a benzyl or substituted benzyl groups at the pyrrole nitrogen is crucial in conferring good activity to all tricyclic [1,2]oxazolo derivatives. In particular, methoxy groups in position 3,4 and/or 5 are relevant. Removal of a methoxy group from position 5 of the benzyl portion led to a substantial decrease in activity. Within series **28** and **30**, the ethoxycarbonyl substituent in the position 2 of the pyrrole is a main feature to obtain active compounds. The most active compounds belonging to tricyclic [1,2]oxazoles are depicted in Fig. 9.

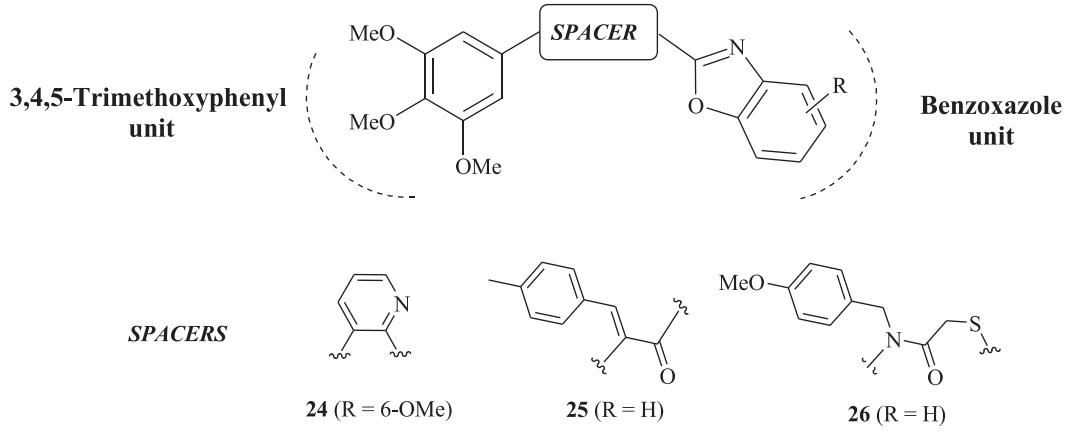


Fig. 8. Benzoxazole derivatives.

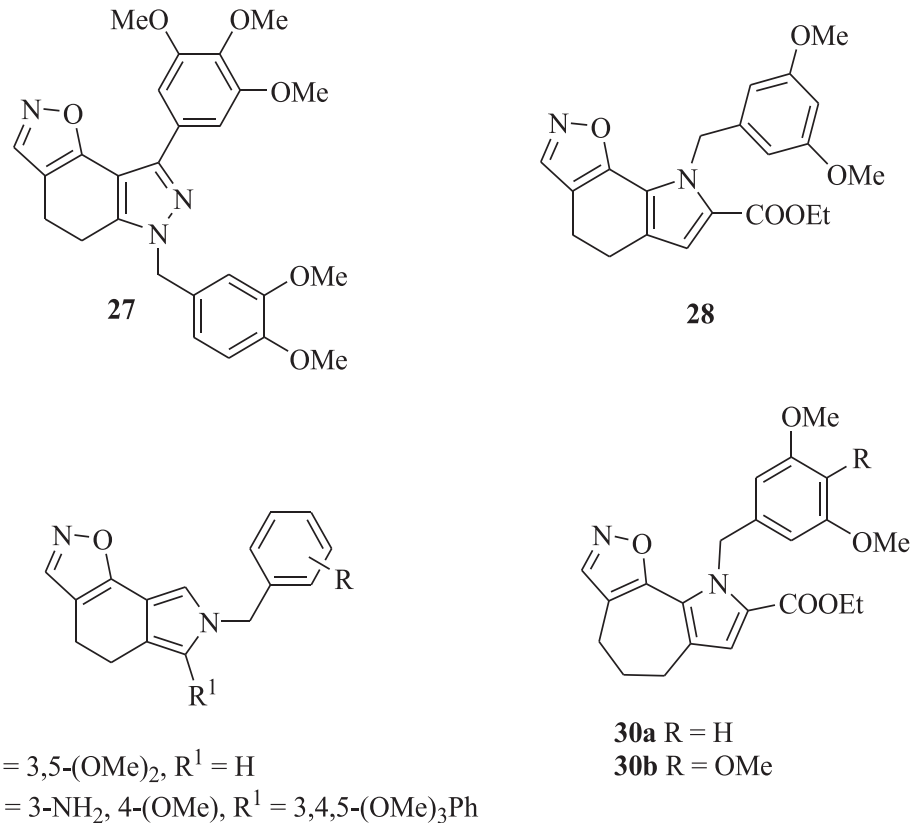


Fig. 9. Tricyclic [1,2]oxazoles.

3. Conclusions

The notion that tubulin inhibitors, such as the FDA approved paclitaxel and vincristine, have a well-established clinical activity that, however, is associated with toxicity and development of drug resistance has boosted several medicinal chemistry efforts in the discovery of members of this class of anti-cancer agents. Small molecules targeting the colchicine binding site attract much attention as potential drug candidates. Starting from the *cis* stilbene CA-4, numerous derivatives endowed with high anti-tubulin activity were obtained by constraining the optimal configuration through the insertion of the oxazole ring. Its straightforward synthetic access, coupled to its favourable pharmacokinetics properties represent a successful strategy to achieve derivatives endowed with high anti-proliferative activity.

Among the classes of compounds which were presented in our overview, recurrent structural features proved to be at least a 3,4,5-trimethoxyphenyl-moiety or a 3,5-dimethoxyphenyl group at the A-ring of CA-4. A slightly higher variability is tolerated at B-ring, even if the presence of a halogen atom and a methoxy group is a generally recurring decoration.

The exploration of the chemical space around the oxazole core provided a deeper insight into the structural requirements that allowed an optimal interaction with the colchicine binding site, paving the way to promising advances in the discovery of potent tubulin inhibitors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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