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Synthesis and Anticancer Properties of Oxazepines Related to Azaisoerianin and IsoCoQuines

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Abstract: In this article, we report the synthesis and biological properties of a series of novel oxazepines related to *isoCA-4* having significant antitumor properties. Among them, three oxazepin-9-ol derivatives display a nanomolar or a (sub)nanomolar cytotoxicity level against five human cancer cell lines (HCT116, U87, A549, MCF7 and K562). It was demonstrated that the lead compound in this series inhibits tubulin assembly with an IC_{50} value of 1 μ M and totally arrested the cellular cycle in the G2/M phase at a low concentration of 5 nM in HCT116 and K562 cells. Molecular modelling studies perfectly corroborates these promising results.

Introduction

Combretastatin A-4 (CA-4, Figure 1), a natural (Z)-stilbene isolated from the African willow tree *Combretum cafrum* by G. Pettit^[1] in 1989, is a very potent antimitotic agent that binds to the colchicine site on β -tubulin causing inhibition of tubulin assembly.^[2] CA-4 also displayed a nanomolar level of cytotoxicity against a wide range of human cancer cells including multidrug resistant cells (MDRs).^[3] In addition to its highly antiproliferative properties and inhibitory activity on tubulin assembly, CA-4 is one of the few anti-microtubule agents reported to have a selective vascular targeting activity^[4,5] that induces an irreversible shutdown of blood flow to neoplastic cells.^[6] Its water-soluble phosphate prodrug (CA-4P, fosbretabulin, ZybrestatTM) has received in 2016 the orphan drug status in Europe and USA for the treatment of neuroendocrine tumors (NETs) and glioblastoma multiform (GBM).^[7] However, despite a marked efficacy in therapy, the (Z)-double bond of CA-4 and CA-4P isomerizes during storage, administration and metabolism to provide its 100-fold less active (E)-isomer.^[8,9] In an ongoing project aimed to develop stable CA-4 analogues having non-isomerizable linkers connecting the A- and B- rings,^[10] we discovered and synthesized *isoCA-4* in 2009.^[11,12] This 1,1-diarylethylene non-isomerizable isomer of CA-4 was found to be extremely stable and have the same biological activities as CA-4. Few years after, we demonstrated that the double bond of

isoCA-4 may be reduced or replaced by a *N*-Methyl linker to give respectively isoerianin^[13] and azaisoerianin^[14] which likewise displayed significant antitumor properties. In this medicinal chemistry program, we have next synthesized other highly bioactive analogues through chemical modifications on the B-ring of *isoCA-4*, and identified new stable and promising antiproliferative agents such as *isoNH₂CA-4* and *isoFCA-4*,^[15] or constrained *isoCA-4* derivatives of type naphthyls,^[16] chromenes,^[17] and benzoxepins.^[18] Recently, we successfully replaced for the first time, the classical 3,4,5-trimethoxyphenyl A-ring of *isoCA-4* derivatives by a quinazoline or a quinoline nucleus leading to *isoCombretaQuinazolines* (*isoCoQs*),^[19] *isoCombretaQuinolines* (*isoCoQuines*)^[20] and other bioactive analogues^[21,22] having comparable or better antiproliferative activities than those of *isoCA-4* and CA-4. Based on these results, Chen^[23] has combined into a single chemical structure azaisoerianin and *isoCoQuines* to give 4-anilinoquinolines as potent tubulin polymerization inhibitors which displayed nanomolar levels of cytotoxicity. Xie has also reported the synthesis of tetrahydro-1H-benzo[b]azepines^[24,25] as restricted analogues of AzixaTM^[26] which exhibited significant *in vitro* cytotoxic activities (GI_{50} ~ 20 nM) against various cancer cell lines. Due to the success of restricted *isoCA-4* analogues,^[16-18] these results encouraged us to synthesize and evaluate a novel series of benzo[b][1,4]oxazepines **1** which can be seen as constrained 4-anilinoquinoline derivatives (Figure 1) and also as structural analogues of benzo[b]azepines.

Results and Discussion

Chemistry

The target benzo[b][1,4]oxazepine **1a** having no substituent on C9 (R^1 = H) was achieved from the S_NAr reaction between 4-chloroquinoline and 2-amino-5-methoxyphenol **2a** (R^2 = H)^[27] in hot dioxane to give diarylamine **3a**. This compound was next dialkylated in the presence of 1,3-dibromopropane and NaH, leading to the desired benzo[b][1,4]oxazepine **1a**. Next, to prepare benzo[b][1,4]oxazepines **1b-e** having a benzyloxy group

Figure 1. Structures of CA-4, CA-4P, isoCA-4 and (restricted)-analogues, (aza)isoerianin, isoCoQ, isoCoQuine, 4-anilinoquinolines and targets benzo[b][1,4]oxazepine

on C9, we firstly synthesized amino derivative **6**^[28] via a Mitsunobu reaction (PPh₃, DEAD in THF for 12 h, 79%) between 2-(benzyloxy)-6-iodo-3-methoxyphenol **4**^[29] and *tert*-butyl (3-hydroxypropyl)carbamate, which was deprotected in CF₃CO₂H. Then we have fully studied the intramolecular cyclization of **6** using a variety of combinations of (i) palladium and copper catalysts (Pd(OAc)₂, Pd₂dba₃, Pd(Ph₃)₄, Cu(acac)₂), (ii) ligands (Xantphos, PPh₃, (±)BINAP, Xphos) and (iii) bases (Cs₂CO₃, K₃PO₄, NaOtBu) in hot toluene. In the majority of cases, the compound **6** was degraded or reduced upon hydrogenolysis of the C-I bond using, for example, a mixture of Pd(OAc)₂, Xantphos, Cs₂CO₃ in hot toluene for 28 h at 100 °C. Fortunately, by replacing Xantphos by (±) BINAP as ligand, we have isolated

after 12 h of reaction expected 9-(benzyloxy)-8-methoxy-2,3,4,5-tetra hydrobenzo [b][1,4]oxazepine **7** (yield 22%; calculated for carbamate **5** deprotection followed by the cyclization). Then, amine derivative **7** was coupled under Pd-catalysis (Pd₂dba₃ (5 mol%), Xantphos (10 mol%) with various 4-chloroquinolines in the presence of NaOtBu to provide benzo[b][1,4]oxazepines **1b-e** with modest yields (16 to 41%). These compounds were next O-debenzylated to provide benzoxepin-9-ols **1f-i** using AlCl₃ in DCM at rt. Following a recent work in Azixa's series, where Chen demonstrated the utility of a 2-chloroacetate function in place of the traditional OH substituent,^[23] we have also synthesized benzo[b][1,4]oxazepine **1j** by mixing **1f** with 2-chloroacetyl chloride in the presence of NEt₃.

Table 1. Cytotoxicity against HCT116 cells^[a] and ITP^[c] of benzo[b][1,4]oxazepines **1a-j**

Compounds	Cl ₅₀ [nM] ^[b]	ITP Cl ₅₀ [μM]	Compounds	Cl ₅₀ [nM]	ITP Cl ₅₀ [μM]	Compounds	Cl ₅₀ [nM]	ITP Cl ₅₀ [μM]
1a	28±1	10.4±2.5	1e	6414±1047	Na	1i	16±2.8	1.08±0.1
1b	3450±118	Na ^[d]	1f	8.0±0.1	4.16±0.6	1j	0.91±0.04	16±3.5
1c	1100±130	Na	1g	7.1±0.5	0.98±0.3	isoCA-4	2.3±0.2 ^[e]	1.0±0.1 ^[e]
1d	250±17	Na	1h	97±10	19.3±4.5			

[a] HCT116 human colon carcinoma [b] IC₅₀ is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug; for IC₅₀ determination, all products **1a-j** were tested at 10 different concentrations, each concentration being tested in triplicate. [c] ITP (Inhibition of Tubulin Polymerization) IC₅₀ is the concentration of compound required to inhibit 50% of the rate of

microtubule assembly (average of three experiments). [d] Not active. [e] The IC₅₀ values for *iso*CA-4 were determined in this study.

Scheme 1. Synthesis of target benzo[b][1,4]oxazepines **1a-j**.

Biology

(A) In vitro cell growth assay and inhibition of tubulin polymerization

In vitro cytotoxicity of synthesized benzo[b][1,4]oxazepines **1a-j** was evaluated against human colon carcinoma (HCT116) cell line (Table 1). A fluorimetry-based assay was used for determination of the drug concentration required to inhibit cell growth by 50% after incubation in the culture medium for 72 h. *iso*CA-4^[30] was included as reference compound for comparisons.

As it can be observed, all benzoxazepines **1b-e** having a benzyloxy-function on C9 display a modest micromolar level of cytotoxicity on HCT116 cells, except **1d** (IC₅₀ = 250 nM). These results are the consequence of a too large congestion on C9.^[31] This observation was confirmed by the cytotoxicity level of oxazepine **1a** having no substituent on C9, which display a promising level of cytotoxicity (**1a**, IC₅₀ = 28 nM). Comparison of antiproliferative properties of oxazepin-9-ol derivatives **1f** and **1g** revealed that a hydroxy-function on C9 increase the level of cytotoxicity and that -Me and -CN groups on C2 of the quinoline nucleus led to comparable cytotoxic agents (IC₅₀ values = 8 and 7 nM, respectively for **1f** and **1g**). We noticed that a chlorine atom or a nitro-group on the C6 position of the quinoline nucleus did not improve the cytotoxicity of oxazepin-9-ols **1h** and **1i**, which does not exclude that their chemical transformation could lead to highly cytotoxic compounds. Finally, esterification of the phenol function of **1f** by 2-chloroacetyl chloride improve the

cytotoxicity level, since ester **1j** (IC₅₀ = 0.9 nM) was found to be ~ 9-fold more cytotoxic against HCT116 cells than oxazepin-9-ol **1f**.

To investigate whether these derivatives were exerting their activity by interactions with microtubules, all benzo[b][1,4]oxazepines **1** were evaluated for their anti-tubulin activities. The inhibition of tubulin polymerization (ITP) results of benzo[b][1,4]oxazepines **1a-j** presented in Table 1 indicate that the most cytotoxic benzo[b][1,4]oxazepin-9-ols **1f,g** and **1i** were the more potent inhibitors of tubulin assembly with micromolar IC₅₀ values ranging from 1 to 10 μM.^[32] Without surprise, ester derivative **1j** (found as the more cytotoxic derivative against HCT116 cells), exhibited an IC₅₀ value against tubulin of 16 μM, substantially weaker than those of oxazepin-9-ols **1f** (IC₅₀ = 4 μM) and **1g** (IC₅₀ = 1 μM) because the anti-tubulin evaluation was achieved using a purified tubulin devoid of esterases.^[12] One also can note that the best inhibitor of tubulin assembly in this series is oxazepin-9-ol **1g** having a CN-function on C2. Oxazepin-9-ol **1g** was found a comparable inhibitor of tubulin polymerization to *iso*CA-4 and 4-times more potent than quinaldine **1f**. These preliminary biological results demonstrate that benzo[b][1,4]oxazepin-9-ols **1f,g** and ester derivative **1j** are promising candidates in this novel series of constrained derivatives.

We next investigated the effect on the proliferation of oxazepin-9-ols **1f**, **1g** and **1j** on other cell lines as human glioblastoma cells (U87), human lung epithelial cells (A549), human breast adenocarcinoma cell lines (MCF7) and chronic myeloid leukemia

cells in blast crisis (K562). The results of this study are depicted in Table 2 and reveal that selected oxazepin-9-ols **1f**, **1g** and **1j** are highly cytotoxic against all tested cancer cells.

Table 2. Cytotoxicity against human cancer cell lines, U87, A549, MCF7, K562 and HCT116					
Cytotoxicity IC ₅₀ , (nM) ^[a]					
Compound	U87 ^[b]	A549 ^[c]	MCF7 ^[d]	K562 ^[e]	HCT116 ^[f]
1f	1.27±0.40	9.64±0.65	4.5±0.3	3.10±0.21	8.00±0.01
1g	0.24±0.11	2.33±0.71	32±3	0.94±0.01	7.0±0.05
1j	2.82±0.49	3.17±0.10	30±2	3.25±0.02	0.91±0.04
<i>Iso</i> CA-4	7.8±0.06	8.2±0.7	3±0.2	1.0±0.1	2.3±0.2

[a] IC₅₀ is the concentration of oxazepines **1** needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug; For IC₅₀ determination, **1f**, **1g** and **1j** were tested at different concentrations, each concentration being tested in triplicate. [b] U87 Human glioblastoma cells. [c] A549, human lung epithelial cells. [d] MCF7 human breast adenocarcinoma cell line. [e] chronic myeloid leukemia cells in blast crisis. [f] HCT116 human colon carcinoma.

Except on HCT116 cells, which were used for the preliminary screening evaluation, oxazepin-9-ol **1f** and its ester derivative **1j** displayed a better level of cytotoxicity against U87 and MCF7 cancer cell lines. Oxazepin-9-ol **1g** was clearly the more cytotoxic derivative in this series against all tested cancer cells and particularly against U87 and MCF7 cells with IC₅₀ values of 0.24 and 0.43 nM, respectively. This study reveal that, when compared to *iso*CA-4, oxazepin-9-ol **1g** is ~ 32-fold more cytotoxic against U87 cells, ~ 3.5-fold more cytotoxic against A549 cells and ~ 19-fold more cytotoxic against MCF7 cells. These results suggests that oxazepin-9-ols **1f**, **1j** and especially **1g** are promising candidates in this novel series of 4-anilinoquinoline constrained derivatives.

(B) Cell cycle distribution

Figure 2. Effect of **1g** on cell cycle distribution in HCT116 (A) and K562 (B) cells determined by flow cytometry analysis. DNA content was assessed *via* propidium iodide staining.

Oxazepin-9-ol **1g**, which displayed against the five cancer cell lines the better cytotoxicity level ($0.24 \text{ nM} < \text{IC}_{50} < 7 \text{ nM}$), was next tested in dose-response experiments using HCT116 and K562 cells on cell cycle distribution.^[33] HCT116 and K562 cells were treated for 24 h with increasing concentrations (1 and 5 nM) of **1g** and DMSO was used as control. As can be seen in Fig. 2, oxazepin-9-ol **1g**, at a concentration of 5 nM, has arrested the entire population of HCT116 and K562 cells in the G2/M phase of the cellular cycle, similarly to those of *iso*CA-4.^[12] These observations consolidate our idea that β -tubulin is certainly one of the targets of **1g** and other benzoxazepins.

(C) Docking study

Figure 3. (A) Putative binding mode of oxazepin-9-ol **1g** (green color) within colchicine binding site of tubulin X-ray structure (accession code 6H9B) 1) with previously reported binding mode of reference compound *iso*CA-4 in overlay (magenta color). (B) Same figure (without *iso*CA-4 reference binding mode) showing expected hydrogen bonds between ligand atoms and cysteine β 241 and threonine α 179 protein residues and proximity between seven-membered ring hydrophobic part and side chains of lysine β 254 (represented in the front, starting from beta carbon as larger grey color atom) and leucine β 248.

Figure 3 presents the results of molecular docking calculations of the structure of oxazepin-9-ol **1g** within the colchicine binding site of tubulin β subunit (the structure of which was obtained from X-ray crystal structure with accession code 6H9B). The overall binding mode observed match that previously known for *iso*CA-4 (see overlay on subfigure A), with the quinoline ring system being accommodated by the lipophilic pocket ordinarily occupied by the 3,4,5-trimethoxyphenyl nucleus, as well as the substituted aromatic ring belonging the tetrahydrobenzo-oxazepin ring sitting at the same place as the B-ring of *iso*CA-4. Interactions that can be expected given this binding mode hypothesis include notably (see subfigure B) two potential

hydrogen bonds: one between the side-chain SH group of the cysteine β 241 residue and the endocyclic nitrogen atom of the quinoline moiety and the other between the hydroxy group belonging to the ligand and backbone carbonyl group of threonine α 179. The contribution of these point interactions to the overall binding are reinforced by the contact between the C2 carbon atom of the seven-membered ring and the hydrophobic side chain of leucine β 248, as well as between the C3 and C4 carbon atoms of the same ring and the hydrophobic part of the side chain of lysine β 254. The docking-derived superimposition of **1g** with *isoCA-4* show a binding pose comparable to the one observed with *isoCA-4*. As it was previously observed with *isoCoQs*, the nitrogen atom of the quinoline ring of **1g** is placed in proximity of Cys241 and the –OH group on C9 in proximity of Val181 to establish a hydrogen bond. All these biological results suggest that oxazepin-9-ol **1g** might be a lead compound for use as a vascular disrupting agent and a promising candidate for *in vivo* evaluation.

Conclusion

In summary, we have synthesized a series of benzoxazepines **1a-j** which were obtained by the Pd-coupling of 2,3,4,5-tetrahydrobenzo[b][1,4]oxazepine **7** with 4-chloroquinolines followed by a debenzoylation step. All oxazepines were evaluated for their antiproliferative properties and cytotoxicity results showed that oxazepines having a free OH on C9 (oxazepin-9-ols) displayed the best nanomolar cytotoxicities levels against human cancer cell lines and inhibited the tubulin assembly into microtubules. Among these oxazepines, oxazepin-9-ol **1g** having a CN-group on C2, a –OH on C9 and a –OMe on C8 appears to be the most promising agent in this series with sub-nanomolar IC₅₀ values on various human cancer cells. Oxazepin-9-ol **1g** is 32-fold more cytotoxic than *isoCA-4* against U87 cells and ~ 20-fold more cytotoxic against MCF7 cells. Moreover, we have also shown that, after 24 h of treatment using oxazepin-9-ol **1g**, K562 and HCT116 cells were totally arrested in the G2/M phase at a low concentration of 5 nM as it was observed with other *isoCA-4* derivatives.^[12,16]

Experimental Section

Chemistry

General considerations

The compounds were all identified by usual physical methods, *i.e.* ¹H NMR, ¹³C NMR, IR, HRMS. ¹H, ¹³C and spectra were measured in CDCl₃ with a Bruker AMX 200 (¹H, and ¹³C) or with a Bruker Avance 300 (¹H and ¹³C). ¹H chemical shifts are reported in ppm from an internal standard TMS or of residual chloroform (7.27 ppm). The following abbreviations are used: m (multiplet), s (singlet), d (doublet), t (triplet), quin (quintet). ¹³C chemical shifts are reported in ppm from the central peak of CDCl₃ (77.14). IR spectra were measured on a Bruker Vector 22 spectrophotometer (neat, cm⁻¹). Melting points (m.p.) were recorded on a Büchi B-450 apparatus and were uncorrected. Mass spectra were obtained with a LCT Micromass spectrometer. Analytical TLC was performed on Merck pre-coated silica gel 60F plates. Merck silica gel 60 (230-400 mesh) was used for column chromatography.

Procedure for the synthesis of new compounds

t-Butyl (3-(2-(benzyloxy)-6-iodo-3-methoxyphenoxy)propyl) carbamate **5** (79%)

2-(Benzyloxy)-6-iodo-3-methoxyphenol (3.2 g, 8.8 mmol) was mixed with *t*-butyl (3-hydroxypropyl)carbamate (1.4 g, 8 mmol) and PPh₃ (3.0 g, 11.2 mmol) in dry THF (16 mL). At 0 °C, DEAD (1.72 mL, 11.2 mmol) was added to the solution which was stirred for 12 h at 0 °C. Water was added to the mixture before extraction with EtOAc (3 x 10 mL). Organic phases were dried over sodium sulfate, filtered and evaporated. After concentration under reduced pressure, the crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (95:5) to give **5** (3.2 g, 79%) as a brown oil.

Brown oil (3.2 g, 79%). ¹H NMR (300 MHz, CDCl₃) δ 7.37 – 7.22 (m, 7H), 6.41 (d, *J* = 8.8 Hz, 1H), 4.90 (s, 2H), 3.96 (t, *J* = 5.8 Hz, 2H), 3.72 (s, 3H), 3.27 (t, *J* = 5.2 Hz, 2H), 1.80 (quin, *J* = 5.2 Hz, 2H), 1.33 (s, 9H). ¹³C NMR (75 MHz, CDCl₃) δ 156.0, 154.7, 152.7, 141.8, 137.2, 132.9, 128.4 (2), 128.3 (2), 128.2, 110.1, 81.7, 78.9, 75.6, 71.7, 56.2, 38.0, 30.2, 28.5 (3). IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3353, 2925, 1313, 1297. HRMS calcd for C₂₂H₂₉INO₅ [M+H]⁺: 514.1090., obsd 514.1071.

9-(Benzyloxy)-8-methoxy-2,3,4,5-tetrahydrobenzo [b] [1,4]oxazepine **7** (22% for the two steps)

To a solution of **5** (1.13 g, 2.20 mmol) in DCM (120 mL) was added slowly TFA (5 mL) at rt. After 30 min. of stirring, the mixture was neutralized with NaOH (1N) until pH = 7. After extraction with EtOAc (3 x 40 mL), drying on sodium sulfate, filtration and concentration, the crude product **6** was dissolved in toluene (30 mL). The solution was stirred at 130 °C for 12 h in a sealed tube with Pd₂dba₃-CHCl₃ (100 mg, 0.1 mmol), (±)-BINAP (120 mg, 0.2 mmol) and Cs₂CO₃ (1.44 g, 4.4 mmol). After cooling, the solution was filtered on a pad of celite and concentrated. The crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (95:5) to give **7** (137 mg, 22%) as a brown oil.

Brown oil (137 mg, 22%). ¹H NMR (300 MHz, CDCl₃) δ 7.40 – 7.31 (m, 5H), 6.50 (d, *J* = 7.2 Hz, 1H), 6.47 (d, *J* = 7.1 Hz, 1H), 5.07 (s, 2H), 4.00 (t, *J* = 5.6 Hz, 2H), 3.81 (s, 3H), 3.16 (t, *J* = 5.6 Hz, 2H), 2.00 (q, *J* = 5.6 Hz, 2H), NH not seen. ¹³C NMR (75 MHz, CDCl₃) δ 148.1, 145.9, 141.3, 138.3, 137.8, 128.5 (2), 128.3 (2), 127.8, 113.6, 107.4, 75.6, 72.2, 56.8, 46.7, 32.5. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3445, 2910, 1510, 1231. HRMS calcd for C₁₇H₂₀NO₃ [M+H]⁺: 286.1443., obsd 286.1446.

8-Methoxy-5-(2-methylquinolin-4-yl)-2,3,4,5-tetrahydro benzo[b][1,4]oxazepine **1a** (37% for the two steps)

In a sealed tube were successively added 4-chloroquinaldine (89 mg, 0.5 mmol), 2-amino-5-methoxyphenol (70 mg) and one drop of HCl (12N) in 1,4-dioxane (2 mL). The mixture was then stirred for 16 h at 140 °C. After cooling, the solution was neutralized with NaOH (5 N) until pH = 7 and extracted with EtOAc (3 x 10 mL). Organic phases were dried over sodium sulfate, filtered and evaporated. The crude product **3a** was dissolved at 0 °C in DMF (3 mL) containing NaH 60%, (96 mg, 2.5 mmol). 1,3-Dibromopropane (500 mg, 2.5 mmol) was slowly added to the mixture which was stirred at rt for 2 h. After concentration under reduced pressure, the crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (95:5) to give **1a** (59 mg, 37%) as a yellow solid.

Yellow solid (59 mg, 37%), m.p. = 89.7 - 92.1 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.97 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.53 (d, *J*

= 7.1 Hz, 1H), 7.20 (t, J = 7.7 Hz, 1H), 6.92 (s, 1H), 6.69 (d, J = 2.8 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.34 (dd, J = 8.8 Hz, J = 2.8 Hz, 1H), 4.35 (t, J = 5.9 Hz, 2H), 3.87 (t, J = 5.9 Hz, 2H), 3.77 (s, 3H), 2.72 (s, 3H), 2.13 (quin, J = 5.9 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 159.2, 157.7, 154.3, 154.0, 149.4, 133.6, 129.2, 128.7, 126.4, 124.8, 124.6, 122.0, 112.4, 109.4, 107.4, 70.3, 55.6, 51.5, 29.7, 25.5. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3050, 2979, 1562, 1454. HRMS calcd for $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$: 321.1603 cal., obsd 321.1610.

General procedure for the synthesis of **1b-e**

In a sealed tube were stirred at 130 °C for 12 h, $\text{Pd}_2\text{dba}_3\text{-CHCl}_3$ (13 mg, 0.01 mmol), Xantphos (15 mg, 0.02 mmol), NaOtBu (53 mg, 0.55 mmol), **7** (70 mg, 0.25 mmol) and the appropriate 4-chloroquinoline (0.28 mmol) in toluene (30 mL). After cooling, the solution was filtered on a pad of celite and concentrated. The crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (95:5 to 90:10) to give pure **1b-e**.

9-(Benzyloxy)-8-methoxy-5-(2-methylquinolin-4-yl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepine **1b** (41%)

Yellow solid (44 mg, 41%), m.p. = 158.7 – 159.2 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.02 (d, J = 8.3 Hz, 1H), 7.58 – 7.33 (m, 7H), 7.18 – 7.11 (m, 1H), 6.89 (s, 1H), 6.34 (d, J = 8.9 Hz, 1H), 6.24 (d, J = 8.9 Hz, 1H), 5.19 (s, 2H), 4.25 (t, J = 5.4 Hz, 2H), 3.80 (m, 5H), 2.74 (s, 3H), 2.05 (quin, J = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 158.5, 154.4, 151.5, 148.3, 147.4, 140.2, 137.6, 134.7, 129.3, 128.7 (2), 128.2 (2), 128.0, 127.8, 124.8, 124.62, 121.50, 119.91, 111.39, 106.54, 75.29, 70.25, 56.07, 51.13, 29.01, 24.91. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3068, 2718, 1544, 1374. HRMS calcd for $\text{C}_{27}\text{H}_{27}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 427.2022, obsd 427.2018.

4-(9-(Benzyloxy)-8-methoxy-3,4-dihydrobenzo[b][1,4]oxazepin-5(2H)-yl) quinoline-2-carbonitrile **1c** (19%)

Yellow solid (21 mg, 19%), m.p. = 137.5 – 137.9 °C. ^1H NMR (300 MHz, CDCl_3) δ 7.92 (d, J = 8.5 Hz, 1H), 7.52 (t, J = 8.2 Hz, 1H), 7.44 (d, J = 7.9 Hz, 2H), 7.36 – 7.28 (m, 3H), 7.19 – 7.11 (m, 3H), 6.27 (d, J = 8.9 Hz, 1H), 6.15 (d, J = 8.9 Hz, 1H), 5.10 (s, 2H), 4.12 (t, J = 5.4 Hz, 2H), 3.70 (m, 5H), 1.95 (quin, J = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 154.6, 152.2, 149.9, 140.3, 137.5, 134.0, 133.7, 130.2, 129.8, 128.8 (2), 128.3 (2), 128.1, 127.3, 124.9, 123.4, 120.2, 118.0, 112.5, 106.6, 75.3, 70.1, 56.1, 51.0, 28.5, one C not seen. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 2958, 2853, 1928, 1371, 1215. HRMS calcd for $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}]^+$: 438.1818, obsd 438.1814.

9-(Benzyloxy)-8-methoxy-5-(2-methyl-6-nitroquinolin-4-yl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepine **1d** (22%)

Yellow solid (26 mg, 22%), m.p. = 152.4 – 154.1 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.36 (s, 1H), 8.17 (d, J = 9.1 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.50 (d, J = 7.2 Hz, 2H), 7.32 (m, 3H), 6.83 (s, 1H), 6.32 (d, J = 8.9 Hz, 1H), 6.24 (d, J = 8.9 Hz, 1H), 5.11 (s, 2H), 4.21 (t, J = 5.3 Hz, 2H), 3.73 (m, 5H), 2.63 (s, 3H), 2.01 (quin, J = 5.3 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 163.2, 154.9, 152.6, 152.3, 148.1, 143.3, 141.4, 137.9, 133.8, 130.3, 128.5 (2), 128.3 (2), 127.9, 122.4, 122.3, 120.3, 120.3, 111.5, 107.1, 76.0, 70.3, 56.2, 50.9, 28.5, 25.9. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3122, 2923, 1614, 1239. HRMS calcd for $\text{C}_{27}\text{H}_{26}\text{N}_3\text{O}_5$ $[\text{M}+\text{H}]^+$: 472.1872, obsd 472.1868.

9-(Benzyloxy)-5-(6-chloro-2-methylquinolin-4-yl)-8-methoxy-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepine **1e** (16%)

Yellow solid (18 mg, 16%), m.p. = 128.4 – 131.7 °C. ^1H NMR (300 MHz, CDCl_3) δ 7.72 (d, J = 8.9 Hz, 1H), 7.44 – 7.18 (m, 7H), 6.76 (s,

1H), 6.23 (d, J = 8.9 Hz, 1H), 6.10 (d, J = 8.9 Hz, 1H), 5.01 (s, 2H), 4.08 (t, J = 5.7 Hz, 2H), 3.66 (m, 5H), 2.54 (s, 3H), 1.90 (quin, J = 5.3 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 159.5, 153.2, 151.5, 148.0, 147.9, 140.5, 137.7, 134.7, 130.4, 130.1, 129.7, 128.7 (2), 128.3 (2), 128.0, 123.7, 122.7, 119.9, 112.9, 106.7, 75.7, 70.4, 56.2, 51.2, 29.3, 25.5. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 2856, 2797, 1515, 1473, 1007. HRMS calcd for $[\text{M}+\text{H}]^+$ $\text{C}_{27}\text{H}_{26}\text{ClN}_2\text{O}_3$: 461.1632, obsd 461.1625.

General procedure for the synthesis of **1f-i**

Benzyl oxazepines **1b-e** were mixed with AlCl_3 (5 eq) in DCM for 12 h at rt. Then water (10 mL) was added to the solution which was extracted with DCM (3 x 20 mL). After concentration, the crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (90:10) to give pure compounds **1f-i**.

8-Methoxy-5-(2-methylquinolin-4-yl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-9-ol **1f** (92%)

Brown solid (48 mg, 92%), m.p. = 92.8 – 97.2 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.07 (d, J = 8.1 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.21 (t, J = 7.5 Hz, 1H), 6.95 (s, 1H), 6.36 (d, J = 8.9 Hz, 1H), 6.05 (d, J = 8.9 Hz, 1H), 4.40 (t, J = 5.4 Hz, 2H), 3.91 (t, J = 5.4 Hz, 2H), 3.84 (s, 3H), 2.77 (s, 3H), 2.18 (quin, J = 5.4 Hz, 2H), OH not seen. ^{13}C NMR (75 MHz, CDCl_3) δ 156.4, 156.3, 146.5, 143.6, 140.4, 138.7, 132.8, 131.3, 125.6, 125.2, 124.2, 120.2, 115.8, 109.1, 106.6, 70.5, 56.3, 51.6, 28.8, 22.3. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3254, 2857, 1404, 1266. HRMS calcd for $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 337.1552, obsd 337.1558.

4-(9-Hydroxy-8-methoxy-3,4-dihydrobenzo[b][1,4]oxazepin-5(2H)-yl)quinoline-2-carbonitrile **1g** (94%)

Brown solid (62 mg, 94%), m.p. = 93.7 – 95.4 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.04 (d, J = 8.4 Hz, 1H), 7.64 (t, J = 8.5 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.36 – 7.28 (m, 2H), 6.36 (d, J = 8.9 Hz, 1H), 6.04 (d, J = 8.9 Hz, 1H), 5.82 (s, 1H), 4.39 (t, J = 5.1 Hz, 2H), 3.86 (m, 5H), 2.19 (quin, J = 5.1 Hz, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 154.6, 149.9, 145.6, 140.7, 138.4, 134.1, 133.9, 130.3 (2), 127.5, 124.9, 123.8, 117.9, 115.4, 113.2, 106.5, 70.8, 56.3, 51.4, 29.6. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3397, 1649, 1563, 1342. HRMS calcd for $\text{C}_{20}\text{H}_{18}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}]^+$: 348.1348 cal., 348.1352 obsd.

8-Methoxy-5-(2-methyl-6-nitroquinolin-4-yl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-9-ol **1h** (69%)

Brown solid (52 mg, 69%), m.p. = 107.3 – 108.9 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.41 (s, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 9.2 Hz, 1H), 6.87 (s, 1H), 6.32 (d, J = 8.9 Hz, 1H), 6.04 (d, J = 8.8 Hz, 1H), 4.33 (t, J = 5.2 Hz, 2H), 3.85 (t, J = 5.2 Hz, 2H), 3.78 (s, 3H), 2.66 (s, 3H), 2.09 (quin, J = 5.2 Hz, 2H), OH not seen. ^{13}C NMR (75 MHz, CDCl_3) δ 162.8, 155.3, 146.0, 143.6, 141.2, 138.8, 137.1, 133.6, 129.9, 122.7, 122.2, 120.5, 115.4, 112.4, 106.9, 70.9, 57.4, 51.6, 29.5, 25.5. IR neat $\nu_{\text{max}}/\text{cm}^{-1}$: 3122, 2923, 1614, 1239. HRMS calcd for $\text{C}_{20}\text{H}_{20}\text{N}_3\text{O}_5$ $[\text{M}+\text{H}]^+$: 382.1403, obsd 382.1395.

5-(6-Chloro-2-methylquinolin-4-yl)-8-methoxy-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-9-ol **1i** (42%)

Brown solid (35 mg, 42%), m.p. = 92.3 – 94.1 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.26 (d, J = 9.0 Hz, 1H), 7.60 (dd, J = 8.7 Hz, J = 2.0 Hz, 1H), 7.48 (s, 1H), 6.99 (s, 1H), 6.52 (d, J = 8.9 Hz, 1H), 6.19 (d, J = 8.8 Hz, 1H), 4.46 (t, J = 5.1 Hz, 2H), 4.12 – 3.82 (m, 5H), 2.87 (s, 3H), 2.35 – 2.20 (quin, J = 5.1 Hz, 2H), OH not seen. ^{13}C NMR (75 MHz, CDCl_3) δ 158.5, 153.9, 145.6, 140.9, 138.6 (2C), 133.8, 130.7, 130.5, 128.9, 123.9, 122.3, 115.1, 112.3, 106.6, 70.9, 56.3, 51.7,

29.7, 24.4. IR neat $\nu_{\max}/\text{cm}^{-1}$: 2897, 2789, 1493, 1254. HRMS calcd for $\text{C}_{20}\text{H}_{20}\text{ClN}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 370.1084, obsd. 370.1080.

8-Methoxy-5-(2-methylquinolin-4-yl)-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-9-yl 2-chloroacetate **1j (45%)**

Oxazepine **1f** (20 mg) was added with NEt_3 (16.5 μL) in DCM (2 mL) at rt. To this solution was added dropwise 90 μL of a 2-chloroacetyl chloride solution (0.1 N). The resulting was stirred at rt for 12 h and then was hydrolyzed with 5 mL of water. After extraction with DCM (3 x 10 mL) and concentration, the crude mixture was purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate (90:10) to give pure oxazepine **1j** (45%).

Brown oil (13 mg, 45%). ^1H NMR (300 MHz, CDCl_3) δ 8.18 (d, J = 8.3 Hz, 1H), 7.60 (t, J = 8.3 Hz, 1H), 7.28 – 7.21 (m, 2H), 6.79 (s, 1H), 6.54 (d, J = 8.9 Hz, 1H), 6.46 (d, J = 9.0 Hz, 1H), 4.38 – 4.31 (m, 2H), 4.02 (s, 3H), 3.94 – 3.86 (m, 2H), 3.75 (s, 2H), 2.83 (s, 3H), 2.15 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 189.3, 158.1, 155.0, 152.0, 146.0, 140.2, 132.7, 130.9, 126.5, 125.7, 125.6, 124.4, 121.5, 119.0, 107.7, 107.1, 70.2, 56.3, 51.6, 40.5, 29.7, 20.9. IR neat $\nu_{\max}/\text{cm}^{-1}$: 3093, 2854, 1614, 1213. HRMS calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_4\text{Cl}$ $[\text{M}+\text{H}]^+$: 413.1268, obsd 413.1273.

Biology

Cell culture and proliferation assay

Cancer cell lines were obtained from the American type Culture Collection (Rockville, MD) and were cultured according to the supplier's instructions. Briefly, HCT116, U87, K562 and MCF7 cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. A549 lung carcinoma, cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine. All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 . The maximum number of passages for each cell line was eight (8). Cell viability was assessed using Promega CellTiter-Blue TM reagent according to the manufacturer's instructions. Cells were seeded in 96-well plates (5 x 103 cells/well) containing 50 μL growth medium. After 24 h of culture, the cells were supplemented with 50 μL of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 μL of resazurin was added for 2 h (the supplier's protocol recommends 1-4 h) before recording fluorescence (λ_{ex} = 560 nm, λ_{em} = 590 nm) using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA). The IC_{50} corresponds to the concentration of the tested compound that caused a decrease of 50% in fluorescence of drug treated cells compared with untreated cells. For IC_{50} determination, all products **1a-j** were tested at 10 different concentrations, each concentration being tested in triplicate.

Tubulin binding assay

Sheep brain tubulin was purified according to the method of Shelanski^[34] by two cycles of assembly-disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM MgCl_2 , 1 mM EGTA, and 1 mM GTP, pH 6.6 (the concentration of tubulin was about 2-3 mg/mL). Tubulin assembly was monitored by fluorescence according to reported procedure^[35] using DAPI as fluorescent molecule. Assays were realized on 96-well plates prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read at 37 °C on Wallac Victor fluorimeter from Perkin Elmer. The IC_{50} value of each compound was determined as the concentration which decreased the maximum assembly rate of tubulin by 50% compared to the rate in the absence of compound.

The IC_{50} values for all compounds were compared to the IC_{50} of isoCA-4 and measured the same day under the same conditions.

Cell cycle analysis

Exponentially growing cancer K562 and HCT116 cells were incubated with **1g** at different concentrations (1 and 5 nM) or in DMSO alone for 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously.^[36]

Molecular modeling

Atomic coordinates for tubulin α,β -dimer were retrieved from the Protein Data Bank (accession code 6H9B).^[21] Missing hydrogen atoms were added using the Dock Prep module from the UCSF Chimera v1.13 software package,^[37] and atoms from the ligand co-crystallized in the colchicine binding at the interface between chains C and D were deleted. Coordinates for a low-energy starting conformer of compound **1g** were obtained using the conformers function from MarvinSketch v19.12 software package^[38] with default parameters. Molecular docking was performed using AutoDock Vina v1.1.2 software package^[39] with default parameters and the binding site defined as the box circumscribed to all the protein residues in contact with the co-crystallized ligand. Analysis and depiction of poses were performed using UCSF Chimera v1.13 software package.^[37]

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Conflict of interest statement

The authors declare no conflict of interest.

Keywords: cancer • cytotoxicity • isoCA-4 • oxazepine • quinoline

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A series of novel benzo[*b*][1,4]oxazepines was synthesized and **1g** was found to be highly cytotoxic at a nanomolar level against 5 human cancer cell lines and inhibited tubulin assembly. Oxazepinol **1g** block the cellular cycle in the G2/M phase at a concentration of 5 nM. A Molecular docking study shows that **1g** perfectly overlays with isoCA-4 in β -tubulin at the colchicine binding site.

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