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Benzopyridooxathiazepine derivatives as novel potent antimitotic agents

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ABSTRACT

Herein, we describe the structure–activity relationship study of a new 1-(arylalkyl)-11*H*-benzo[*f*]-1,2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide series of antimitotic agents. The pharmacological results obtained from previous works allowed us to identify compound **1** as a new cytotoxic agent inhibiting tubulin polymerization. We have undertaken the synthesis of its non-methylated analogue **7** and have extended our investigations to a novel, structurally related benzopyridooxathiazepine dioxide series. Among all analogues synthesized in this study, compound **10b** was the most promising, being 12-fold more potent than compound **1**. Its activity over a panel of five tumoral cell lines was in the nanomolar range for all of the histological types tested and flow cytometric studies performed on L1210 cells showed an accumulation of the cells in the G2/M phases of the cell cycle with a significant percentage of tetraploid cells (8N DNA content). This interesting pharmacological profile, resulting from inhibition of tubulin polymerization, encouraged us to perform preliminary in vivo studies.

1. Introduction

In our continuing search for antitumor agents, we synthesized a large series of benzopyridothiadiazepine derivatives and found that many of them were cytotoxics agent inhibiting tubulin polymerization.[1,2] Pharmacomodulations performed within this class of compounds led to the discovery of the potent compound **1**, exhibiting inhibitory activity against tubulin polymerization comparable to that of the potent antimitotic sulfonamides **E7010** and **ER-67865**[3–10] (Fig. 1). The structure–activity relationship (SAR) we had established showed that the best activities were obtained when the benzopyridothiadiazepine tricyclic moiety is substituted on the pyridine core by a 4-methoxyphenylethyl chain and bears no substitution on the benzenic part of the tricycle. Compound **1** exhibited good in vitro cytotoxicity with IC_{50} values in the submicromolar range toward the L1210 leukemia cell line, but was devoided of significant in vivo activity in a P388 murine leukemia model (iv/ip) probably for ADME problems.

Encouraged by these promising results, we have undertaken the synthesis of the non-methylated analogue **7** in order to mimic more closely the potent antimitotic sulfonamides **E7010** and **ER-67865**. Our investigations of the benzopyridothiadiazepine dioxide series were then extended to a novel, structurally related benzopyridooxathiazepine dioxide[11] in which the sulfonamide moiety is replaced by a sulfonate group. Preliminary studies showed that introduction of the arylalkyl chain at the *N*-11 position do not result in significantly cytotoxic compounds, while incorporation of the arylalkyl chain at the *N*-1 position gave active agents at least as potent as their corresponding benzopyridothiadiazepines analogues. Compound **14b,** which is the direct analogue of compound **1** in the benzopyridooxathiazepine series, was about 12 times more cytotoxic than compound **1** (IC_{50} of 9.5 nM vs 110 nM) toward the L1210 leukemia cell line (Table 1). This compound exhibits also cytotoxic effects on other cell lines (Table 2). Based on these initial results, a systematic investigation of substituted benzopyridooxathiazepine dioxide derivatives bearing various arylalkyl substituents, was undertaken, and is reported here.

2. Chemistry

Non-methylated thiadiazepine dioxide **7** was synthesized according to the general method described in Scheme 1. Tricyclic thiadiazepine **5**, bearing a methoxyethoxymethyl substituent, as protecting group, at the nitrogen sulfonamide position, was prepared according to a modification of the previously described procedure.[1] Alkylation of **5** with 4-methoxyphenylethyl methanesulfonate proceeded either on the nitrogen in position 1 or on the nitrogen in position 11 affording predominantly compound **6b** and to its less extended regioisomer **6a.** The structures of these compounds, that were separated by preparative HPLC, with respectively 40% and 15% yield, were determined by NMR spectra, including steady state NOE measurements. In a last step, compound **7** was obtained in 78% yield by refluxing compound **6b** in 6 N HCl .

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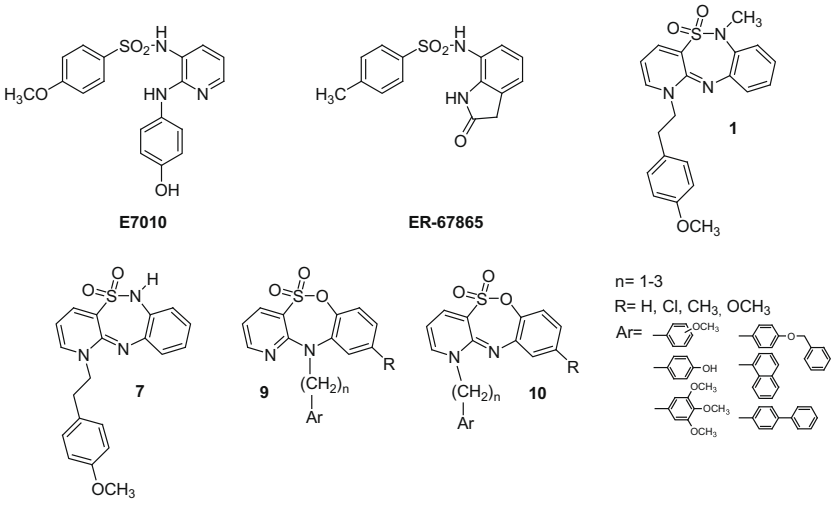


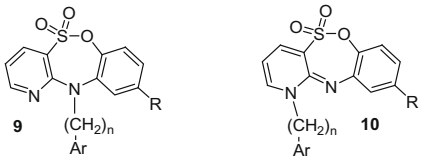
Figure 1. References and synthesized compounds.

The synthesis of the oxathiazepinic compounds is reported in Scheme 2. Reaction of the already described tricyclic derivatives[11] **8a–d** with 4-methoxybenzyl chloride or arylalkyl methanesulfonates[1,18,19] afford the two regioisomers **9** and **10** as already described in Scheme 1.

3. Results and discussion

The substituted azepine derivatives **7, 9a–g,i,k–m**, and **10a–m** were evaluated for their antiproliferative activity against the L1210 leukemia cell line. The results expressed as IC_{50}, are reported in Table 1. In a previous study, we demonstrated that introduction of bulky substituent (compared to methyl) on the sulfonamide nitrogen resulted in inactive compounds (IC_{50} on L1210 > 100 μM) compared to the N-methylated thiadiazepine **1** (IC_{50} = 0.11 μM). In order to mimic more closely the potent antimitotic sulfonamides **E7010** and **ER-67865**, which were all non N-substituted on the sulfonamide moiety, the unsubstituted compound **7** was prepared and evaluated. Surprisingly, **7** was slightly less cytotoxic than **1** (IC_{50} of 0.3 μM vs 0.11 μM for **1**) whose substitution with a methyl group seemed to be most tolerant of the steric bulk of substituents. As some papers[12,13] report that it is possible, and sometimes favorable to replace the sulfonamide moiety of antimitotic compounds by a sulfonate function, we have prepared and evaluated the oxathiazepine analogues (compounds **9** and **10**) of our previously published thiadiazepine derivatives. In most of the cases, compounds substituted on the pyridine moiety (**10**) were clearly more cytotoxic than their counterpart (**9**) substituted on the oxathiazepine moiety. Four compounds, **10b**, **10d**, **10e**, and **10f** were found markedly cytotoxic with IC_{50} on L1210 cell line in the nanomolar range (respectively 9.5, 27, 37, and 43 nM). The perturbations of the cell cycle induced by these four active compounds were studied on the L1210 cell line by flow cytometry (Table 2). When exposed to the cells for two doubling times (21 h), all these compounds induced a marked accumulation of tetraploid cells (8N).

Table 1
In vitro antiproliferative activities of compounds **7, 9a–g,i,k–m**, and **10a–m** on L1210 cell line



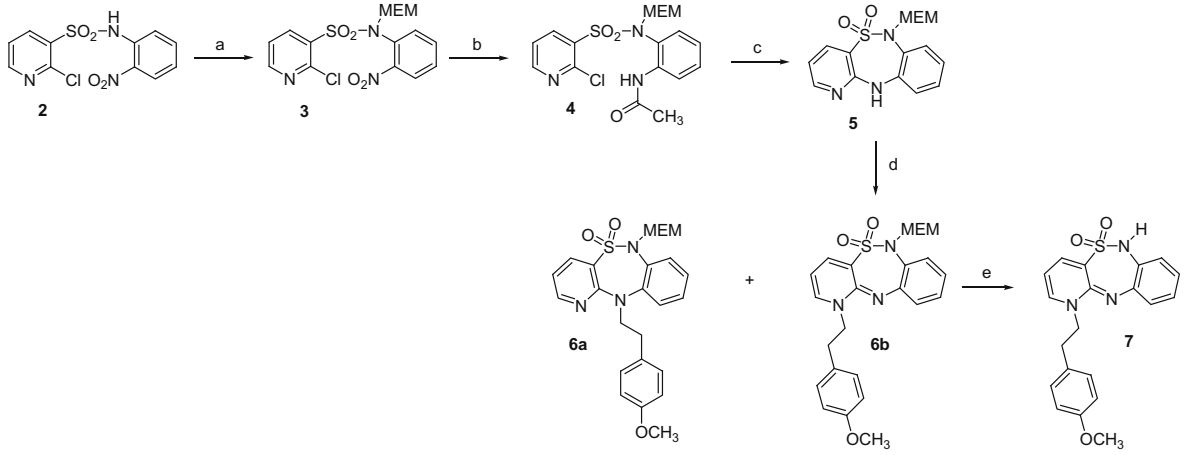
N°	*n*	R	Ar	IC_{50}[a] (μM)	N°	*n*	R	Ar	IC_{50} (μM)
					1				0.11
					7				0.3
9a	1	H	4-Methoxyphenyl	0.77	**10a**	1	H	4-Methoxyphenyl	3.2
9b	2	H	4-Methoxyphenyl	26.5	**10b**	2	H	4-Methoxyphenyl	0.0095
9c	3	H	4-Methoxyphenyl	15.4	**10c**	3	H	4-Methoxyphenyl	8.6
9d	2	9-Cl	4-Methoxyphenyl	n.t.[b]	**10d**	2	9-Cl	4-Methoxyphenyl	0.027
9e	2	9-CH_3	4-Methoxyphenyl	22.5	**10e**	2	9-CH_3	4-Methoxyphenyl	0.037
9f	2	9-OCH_3	4-Methoxyphenyl	11.2	**10f**	2	9-OCH_3	4-Methoxyphenyl	0.043
9g	2	H	2-Methoxyphenyl	>10	**10g**	2	H	2-Methoxyphenyl	14.8
					10h	2	H	3-Methoxyphenyl	0.8
9i	2	H	4-Benzyloxyphenyl	n.t.	**10i**	2	H	4-Benzyloxyphenyl	>100
					10j	2	H	4-Hydroxyphenyl	0.6
9k	2	H	3,4,5-Trimethoxyphenyl	36.8	**10k**	2	H	3,4,5-Trimethoxyphenyl	27.7
9l	2	H	1-Naphthyl	>100	**10l**	2	H	1-Naphthyl	9.6
9m	2	H	4-Biphenyl	n.t.	**10m**	2	H	4-Biphenyl	5

[a] Concentration inhibiting L1210 cell proliferation by 50% relative to untreated controls after 48 h of drug exposure.
[b] Not tested.

Table 2
Antiproliferative activity of **10b,d,e,f** on selected tumoral cell lines (nM) and effects on L1210 cell cycle

In vitro antiproliferative activities (nM)						% of L1210 Cells in each phase of the cell cycle[b]				
Cell type	Prostate	Colon	Lung	Skin	Skin	G1	S	G2 + M	8N	Concentration (nM)
Cell line	DU145	HT29	A549	KB 3-1	KB-A1[a]					
10b	17	10	11.5	16	13.2	4	3	25	60	25
10d	42.3	27.1	n.t.[c]	n.t.	n.t.	3	3	29	65	50
10e	n.t.	50.2	n.t.	n.t.	n.t.	4	3	17	75	100
10f	116	n.t.	99.1	n.t.	n.t.	8	11	22	59	200

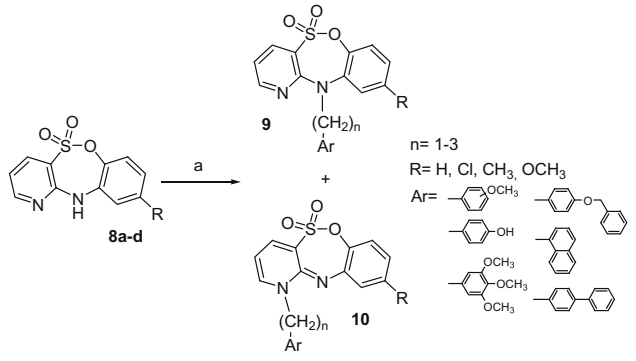
[a] KB-A1, adriamycine resistant.
[b] Distribution of control cells in the cell cycle: 45% (G1), 31% G2, 23% (G2 + M), 1% (8N).
[c] Not tested.



Scheme 1. Reagents and conditions: (a) NaH, MEMCl, DMF, rt, 78%; (b) (i) H_2, Ni-R, EtOH; (ii) Ac_2O, rt, 95%; (c) K_2CO_3, DMF, reflux, 45%; (d) NaH, DMF, 4-methoxyphenylethyl methanesulfonate, 60 °C, 15–40%; (e) EtOH 95%, 6 N HCl, reflux, 78%.

This accumulation, is generally observed for numerous tubulin interacting drugs.

As for the thiadiazepine derivatives, the best results were obtained when the benzopyridooxathiazepine is substituted on the pyridine moiety by a 4-methoxyphenylethyl chain (**10b**). Modification of the length of this substituent (4-methoxybenzyl **10a**, or 4-methoxypropyl **10c**) resulted in a clear decrease of the cytotoxicity. The conclusion was the same when the 4-methoxyphenylethyl side chain was replaced by a 2-methoxy (**10g**), 3-methoxy (**10h**), 4-hydroxy (**10j**), and 3,4,5-trimethoxy (**10k**) phenylethyl group. Some paper have shown that it was possible to replace the polyoxygenated rings of combretastatin A-4 with naphthalene, quinoline or quinoxaline moieties without significant loss of cytotoxicity and inhibition of tubulin polymerization potency.[14–17] Transposed to our series, the replacement of the 4-methoxyphenyl side chain with (1-naphthyl)ethyl (**10l**) or (4-biphenyl)ethyl (**10m**) resulted in clearly less active compounds (IC_{50} of respectively 9.6 and 5.0 μM). These results suggest that aromatic hydrophobic rings are not good surrogates for the 4-methoxyphenyl ring and that the interaction with tubulin is not only hydrophobic in nature but needs some polar interactions. In a second step, we investigated the influence of some substituents on the benzenic part of the tricycle (no substituent **10b**, 9-Cl **10d**, 9-Me **10e**, and 9-MeO **10f**). As for the benzopyridothiadiazepine series, compounds substituted on the phenyl moiety of the oxathiazepine were slightly less active than their unsubstituted derivative **10b**.



Scheme 2. Reagents and conditions: (a) NaH, DMF, 4-methoxybenzyl chloride or arylalkyl methanesulfonates, 60 °C, 3–39%.

The most active compound within this series (**10b**, IC_{50}(L1210) = 9.5 nM) was about 12 times more cytotoxic than its corresponding sulfonamide direct counterpart **1** (IC_{50}(L1210) = 110 nM). Compound **10b** was evaluated on five other cancer cell lines (DU145, HT29, A549, KB 3-1, KB-A-1) and showed potent nanomolar cytotoxic effects with IC_{50} of respectively 17, 10, 11.5, 16, and 13.2 nM (Table 2). As compounds **10b** induced a marked accumulation of tetraploid cells (8N), this compound was evaluated for its ability to inhibit the polymerization of tubulin and was found to be potent inhibitor of tubulin polymerization with IC_{50} of 2.2 μM compared to 2.4 μM for desoxypodophyllotoxin used as a reference compound. No effect was observed on tubulin depolymerization. In a last step, compound **10b** was evaluated in vivo on a P388 murine leukemia model. On day 0, 106 leukemic cells were grafted in the peritoneal cavity of B6D2F1 mice as described.[23] Administered iv or ip on day 1, **10b** proved to be moderately but significantly active, inducing an increase in survival of 41% (T/C = 141%) for a 100 mg/kg iv dose and 61% (T/C = 161%) for 50–100 mg/kg ip doses. The relatively low in vivo antitumor activity of compound **10b** considering its high in vitro antiprolifer-

ative activity toward cancer cell lines, could be explained by its weak metabolic bioavailability on mice and human liver microsomes (<12%) while its intestinal permeability on Caco2 cells is nearly full.

4. Conclusion

We have synthesized sulfonate analogues of the thiadiazepine compound **1,** and have found many of these compounds to be potent in vitro inhibitors of the proliferation of the murine L1210 leukemia cell line. Compound **10b**, which is the strict analogue in the benzopyridooxathiazepine series of the sulfonamide **1** is a nanomolar cytotoxic agent clearly (12 times) more potent than **1**. This compound is a potent inhibitor of tubulin polymerization with 100% of inhibition at 10 μM. Surprisingly within the benzopyridothiadiazepine series, compound **7**, which is not methylated on the sulfonamide moiety, and therefore mimic more closely than compound **1** does the potent antimitotic sulfonamides **E7010** and **ER-67865**, was found slightly less active on L1210 cell line (IC_{50} of 300 nM vs 110 nM) than **1**. Our lead compound **10b** was found moderately but significantly active in vivo on a P388 murine leukemia model. Its relatively low in vivo antitumor activity, considering its high in vitro antiproliferative activity toward cancer cell lines, could be explained by poor metabolic stability. The design of new compounds with improved in vivo antitumor activity is currently under investigation.

5. Experimental

5.1. Chemistry

Melting points were determined on a BÜCHI B-540 apparatus and are uncorrected. Infrared spectra were recorded as thin films on potassium bromide disks on a Beckman Acculab IV spectrophotometer. Mass spectra were performed on a Finnigan MAT SSQ 710 Advantage spectrometer. ^{1}H NMR spectra (LARMN, Université de Lille 2) were recorded on a Bruker AC 300 P and 2D NMR spectra on a Bruker DPX 300, using tetramethylsilane as internal standard. Elemental analyses were performed by C.N.R.S—Vernaison, and were in agreement with the calculated values within ±0.4%.

5.1.1. 2-Chloro-*N*-(methoxyethoxymethyl)-*N*-(2-nitro-phenyl)-3-pyridinesulfonamide (3)

2-Chloro-*N*-(2-nitrophenyl)-3-pyridinesulfonamide 2 (10 mmol) dissolved in DMF (25 mL) is added dropwise to a suspension of sodium hydride (15 mmol) in DMF (5 mL) and stirred for 1 h at room temperature. Methoxyethoxymethyl chloride (12 mmol) in DMF (5 mL) is added dropwise to the previous solution and stirred for 12 h at room temperature. The resulting solution is concentrated under reduced pressure, hydrolyzed, extracted twice with DCM, dried over sodium sulfate, filtered and concentrated under vacuo. The resulting residue is then recrystallized from diisopropylic ether. Yellow powder; mp 72–74 °C (diisopropyl ether) (yield 78%). IR (KBr) cm^{-1}: 1160, 1350, 1520. ^{1}H NMR ($CDCl_3$): δ 3.40 (s, 3H), 3.62 (t, 2H), 3.95 (m, 2H), 5.50 (m, 2H), 7.29 (dd, 1H), 7.50–7.65 (m, 3H), 7.71 (dd, 1H), 8.04 (dd, 1H), 8.58 (dd, 1H).

5.1.2. 2-Chloro-*N*-(methoxyethoxymethyl)-*N*-(2-acetamido-phenyl)-3-pyridinesulfonamide (4)

A solution of **3** (10 mmol) in ethanol was stirred in the presence of 0.5 g of Raney Nickel under an atmosphere of hydrogen at room temperature until reaction was completed as checked by TLC. The solution was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was dissolved in acetic anhydride and stirred for 12 h. The solution was diluted with water, extracted with DCM, dried over sodium sulfate, filtered and concentrated under vacuo. The resulting oil was used for next step without further purification. Brown oil, yield 99%.

5.1.3. 6,11-Dihydro-6-methoxyethoxymethylbenzo[*c*] pyrido-[2,3,*f*][1,2,5] thiadiazepines 5,5-dioxide (5)

A solution of **4** (10 mmol) in DMF (40 mL) with potassium carbonate (20 mmol) is refluxed for 8 h. The reaction mixture is evaporated under reduced pressure. The residue is poured in water, extracted with DCM, dried, filtered, concentrated under reduced pressure, and purified by silica gel chromatography with petroleum ether/ethyl acetate (7:3). White solid; mp 119–121 °C (yield 45%). IR (KBr) cm^{-1}: 1180, 1360, 3280. ^{1}H NMR ($CDCl_3$): δ 3.34 (s, 3H), 3.49 (t, 2H), 3.78 (m, 2H), 4.93 (s, 2H), 6.90 (dd, 1H), 6.99 (dd, 1H), 7.13 (dd, 1H), 7.33 (dd, 1H), 7.38 (dd, 1H), 7.56 (s, 1H), 8.15 (dd, 1H), 8.33 (dd, 1H).

5.1.4. Synthesis of (6a) and (6b)

A solution of **5** (10 mmol) in DMF (20 mL) is added dropwise to a suspension of sodium hydride (20 mmol) in DMF (10 mL) and stirred for 3 h at 60 °C. A solution of 4-methoxyphenylethyl methanesulfonate[1] (25 mmol) in DMF (15 mL) is then added dropwise to the previous solution and stirred for 12 h at 60 °C. The resulting solution is concentrated under reduced pressure, the residue is diluted in DCM, washed with water, dried, concentrated and the two regioisomers separated by preparative HPLC (petroleum ether/ethyl acetate 6:4).

5.1.5. 6,11-Dihydro-11-(4-methoxyphenylethyl)-6-methoxyethoxymethylbenzo[*c*]pyrido[2,3-*f*][1,2,5] thiadiazepine 5, 5-dioxide (6a)

Beige solid; mp 80–83 °C (yield 15%). IR (KBr) cm^{-1}: 1170, 1340. ^{1}H NMR ($CDCl_3$): δ 2.92 (t, 2H), 3.42 (s, 3H), 3.63 (t, 2H), 3.78 (s, 3H), 3.93 (t, 2H), 4.36 (t, 2H), 4.78 (s, 2H), 6.83 (d, 2H), 6.99–7.07 (m, 3H), 7.28–7.43 (m, 3H), 7.51 (dd, 1H), 8.12 (dd, 1H), 8.47 (dd, 1H).

5.1.6. 1-(4-Methoxyphenylethyl)-6-methoxyethoxymethyl-benzo[*c*]-1,2-dihydropyrido[2,3-*f*][1,2,5] thiadiazepine 5, 5-dioxide (6b)

Yellow oil (yield 40%). IR (KBr) cm^{-1}: 1170, 1340. ^{1}H NMR ($CDCl_3$): δ 3.10 (t, 2H), 3.34 (s, 3H), 3.52 (t, 2H), 3.81 (s, 3H), 3.95 (t, 2H), 4.30 (t, 2H), 4.90 (t, 2H), 5.81 (dd, 1H), 6.86 (d, 2H), 7.0–7.15 (m, 4H), 7.20–7.35 (m, 3H), 7.85 (dd, 1H).

5.1.7. 1-(4-Methoxyphenylethyl)-benzo[*c*]-1,2-dihydro-pyrido [2,3-*f*][1,2,5] thiadiazepine 5,5-dioxide (7)

A solution of **6b** (1 mmol) in ethanol 95% (10 mL) and 6 N hydrochloric acid (10 mL) is refluxed for 1 h 30 min. The reaction mixture is concentrated and the residue poured into water. Saturated aqueous sodium bicarbonate is added until pH 5 and the solution is extracted with ethyl acetate, washed with water, dried, filtered and concentrated under reduced pressure. The resulting powder is recrystallized from diisopropylic ether. Yellow solid; mp 174–175 °C (yield 78%). IR (KBr) cm^{-1}: 1170, 1315, 3210. ^{1}H NMR ($CDCl_3$): δ 3.10 (t, 2H), 3.81 (s, 3H), 4.26 (t, 2H), 5.76 (dd, 1H), 6.26 (s, 1H), 6.86 (d, 2H), 6.95–7.25 (m, 7H), 7.77 (d, 1H). MS (EI) *m/z* 381 (M+). Anal. Calcd for $C_{20}H_{19}N_3O_3S$: C, 62.97; H, 5.02; N, 11.02. Found: C, 62.84; H, 5.11; N, 11.09.

5.1.8. General procedure for the synthesis of substituted 11-(arylalkyl)-11*H*-benzo[*f*]pyrido[3,2,*c*][1,2,5]oxa-thiazepine 5,5-dioxide (9a–m) and 1-(arylalkyl)-11*H*-benzo[*f*]-1,2-dihydropyrido [3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10a–m)

A solution of substituted 11*H*-benzo[*f*]pyrido[3,2-*c*][1,2,5]oxa-thiazepine 5,5-dioxide **8a–d** (4 mmol) in DMF (10 mL) is added

dropwise to a suspension of sodium hydride (8 mmol) in DMF (5 mL) and stirred for 3 h at 60 °C. A solution of commercially available 4-methoxybenzyl chloride or synthesized arylalkyl methanesulfonate[1,18,19] (12 mmol) in DMF (10 mL) is then added dropwise to the previous solution and stirred for 24 h at 60 °C. The resulting solution is concentrated under reduced pressure, the residue is diluted in DCM, washed with water, dried, concentrated, and the two regioisomers are separated by preparative HPLC.

5.1.9. 11-(4-Methoxybenzyl)-11*H*-benzo[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9a)

Beige solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 126–127 °C (ethanol) (yield 26%). IR (KBr) cm^{-1}: 1180, 1370. ^1H NMR ($CDCl_3$): δ 3.76 (s, 3H), 5.38 (s, 2H), 6.81 (d, 2H), 6.98 (dd, 1H), 7.20–7.40 (m, 6H), 8.13 (dd, 1H), 8.43 (dd, 1H). MS (EI) *m/z* 368 (M+). Anal. Calcd for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.34; N, 7.60. Found: C, 61.97; H, 4.37; N, 7.67.

5.1.10. 11-(4-Methoxyphenylethyl)-11*H*-benzo[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9b)

White solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 93–94 °C (ethanol) (yield 12%). IR (KBr) cm^{-1}: 1180, 1370. ^1H NMR ($CDCl_3$): δ 2.95 (t, 2H), 3.79 (s, 3H), 4.44 (t, 2H), 6.83 (d, 2H), 6.99 (dd, 1H), 7.12 (d, 2H), 7.25–7.50 (m, 4H), 8.11 (dd, 1H), 8.50 (dd, 1H). MS (EI) *m/z* 382 (M+). MS (EI); Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.32. Found: C, 62.91; H, 4.68; N, 7.46.

5.1.11. 11-[3-(4-Methoxyphenyl)propyl]-11*H*-benzo[*f*]pyrido-[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9c)

White solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 86–87 °C (ethanol) (yield 31%). IR (KBr) cm^{-1}: 1170, 1370. ^1H NMR ($CDCl_3$): δ 1.90 (m, 2H), 2.62 (t, 2H), 3.77 (s, 3H), 4.23 (t, 2H), 6.77 (d, 2H), 6.90–7.60 (m, 7H), 8.11 (d, 1H), 8.50 (d, 1H). MS (EI) *m/z* 396 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07. Found: C, 63.47; H, 5.17; N, 7.19.

5.1.12. 9-Chloro-11-(4-methoxyphenylethyl)-11*H*-benzo-[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9d)

White solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 133–135 °C (ethanol 95%) (yield 3%). IR (KBr) cm^{-1}: 1170, 1360. ^1H NMR ($CDCl_3$): δ 2.96 (t, 2H), 3.79 (s, 3H), 4.41 (t, 2H), 6.83 (d, 2H), 7.03 (dd, 1H), 7.11 (d, 2H), 7.20–7.45 (m, 4H), 8.12 (dd, 1H), 8.52 (dd, 1H). MS (EI) *m/z* 416 (M+). Anal. Calcd for $C_{20}H_{17}ClN_2O_4S$: C, 57.62; H, 4.11; N, 6.72. Found: C, 57.54; H, 4.03; N, 6.98.

5.1.13. 11-(4-Methoxyphenylethyl)-9-methyl-11*H*-benzo-[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9e)

White solid; purified by chromatography with petroleum ether/ethyl acetate (8:2); mp 137–138 °C (ethanol 95%) (yield 25%). IR (KBr) cm^{-1}: 1170, 1350. ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.95 (t, 2H), 3.79 (s, 3H), 4.41 (t, 2H), 6.83 (d, 2H), 6.97 (dd, 1H), 7.05–7.19 (m, 3H), 7.13–7.32 (m, 2H), 8.10 (dd, 1H), 8.50 (dd, 1H). MS (EI) *m/z* 396 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07. Found: C, 63.58; H, 5.09; N, 7.13.

5.1.14. 9-Methoxy-11-(4-methoxyphenylethyl)-11*H*-benzo-[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9f)

White solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 110–114 °C (ethanol 95%) (yield 13%). IR (KBr) cm^{-1}: 1170, 1360. ^1H NMR ($CDCl_3$): δ 3.01 (t, 2H), 3.80 (s, 6H), 4.39 (t, 2H), 6.80–6.90 (m, 3H), 6.99 (dd, 1H), 7.12–7.20 (m, 3H), 7.31 (d, 1H), 8.10 (dd, 1H), 8.49 (dd, 1H). MS (EI) *m/z* 412 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_5S$: C, 61.15; H, 4.89; N, 6.79. Found: C, 61.05; H, 5.01; N, 6.46.

5.1.15. 11-(2-Methoxyphenylethyl)-11*H*-benzo[*f*]pyrido-[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9g)

White solid; purified by chromatography with petroleum ether/ethyl acetate (9:1); mp 132–133 °C (ethanol) (yield 11%). IR (KBr) cm^{-1}: 1180, 1350. ^1H NMR ($CDCl_3$): δ 3.04 (t, 2H), 3.77 (s, 3H), 4.44 (t, 2H), 6.81 (d, 2H), 6.87 (dd, 1H), 6.96 (dd, 1H), 7.10–7.40 (m, 5H), 7.50 (d, 1H), 8.10 (dd, 1H), 8.49 (dd, 1H). MS (EI) *m/z* 382 (M+). Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.32. Found: C, 62.92; H, 4.70; N, 7.32.

5.1.16. 11-[2-(4-Benzyloxyphenyl)ethyl]-11*H*-benzo[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9i)

White solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 92–94 °C (methanol) (yield 14%). IR (KBr) cm^{-1}: 1190, 1360. ^1H NMR ($CDCl_3$): δ 2.95 (t, 2H), 4.33 (t, 2H), 5.11 (s, 2H), 6.99 (dd, 1H), 6.80–7.55 (m, 13H), 8.11 (dd, 1H), 8.49 (dd, 1H). MS (EI) *m/z* 458 (M+). Anal. Calcd for $C_{26}H_{22}N_2O_4S$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.21; H, 4.76; N, 6.14.

5.1.17. 11-[2-(3,4,5-Trimethoxyphenyl)ethyl]-11*H*-benzo-[*f*]pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9k)

White solid; purified by chromatography with petroleum ether/ethyl acetate (6:4); mp 108–110°C (ethanol 95%) (yield 18%). IR (KBr) cm^{-1}: 1180, 1360. ^1H NMR ($CDCl_3$): δ 2.94 (t, 2H), 3.81 (s, 9H), 4.51 (t, 2H), 6.37 (s, 2H), 7.00 (dd, 1H), 7.20–7.50 (m, 4H), 8.11 (dd, 1H), 8.51 (dd, 1H). MS (EI) *m/z* 442 (M+). Anal. Calcd for $C_{22}H_{22}N_2O_6S$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.97; H, 5.02; N, 6.46.

5.1.18. 11-[2-(1-Naphthyl)ethyl]-11*H*-benzo[*f*]pyrido-[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9l)

White solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 59–61 °C (ethanol 95%) (yield 10%). IR (KBr) cm^{-1}: 1180, 1360. ^1H NMR ($CDCl_3$): δ 3.52 (t, 2H), 4.57 (t, 2H), 7.03 (dd, 1H), 7.25–7.45 (m, 6H), 7.45–7.60 (m, 2H), 7.75 (d, 1H), 7.87 (dd, 1H), 8.15 (dd, 1H), 8.26 (d, 1H), 8.57 (dd, 1H). MS (EI) *m/z* 402 (M+). Anal. Calcd for $C_{23}H_{18}N_2O_3S$: C, 68.64; H, 4.51; N, 6.96. Found: C, 68.41; H, 4.23; N, 7.17.

5.1.19. 11-[2-(4-Biphenyl)ethyl]-11*H*-benzo[*f*]pyrido-[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (9m)

White solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 55–58 °C (propanol) (yield 6%). IR (KBr) cm^{-1}: 1180, 1360. ^1H NMR ($CDCl_3$): δ 3.06 (t, 2H), 4.52 (t, 2H), 6.83 (d, 2H), 7.00 (dd, 1H), 7.25–7.45 (m, 9H), 7.50–7.60 (m, 4H), 8.12 (dd, 1H), 8.51 (dd, 1H). MS (EI) *m/z* 428 (M+). Anal. Calcd for $C_{25}H_{20}N_2O_3S$: C, 70.07; H, 4.70; N, 6.54. Found: C, 70.12; H, 4.59; N, 6.38.

5.1.20. 1-(4-Methoxybenzyl)-11*H*-benzo[*f*]-1,2-dihydropyrido-[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10a)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 162–163 °C (ethanol) (yield 29%). IR (KBr) cm^{-1}: 1180, 1360. ^1H NMR ($CDCl_3$): δ 3.82 (s, 3H), 5.28 (s, 2H), 5.98 (dd, 1H), 6.91 (d, 2H), 7.00–7.30 (m, 4H), 7.33 (d, 2H), 7.50 (dd, 1H), 7.90 (dd, 1H). MS (EI) *m/z* 368 (M+). Anal. Calcd for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.34; N, 7.60. Found: C, 61.92; H, 4.38; N, 7.62.

5.1.21. 1-(4-Methoxyphenylethyl)-11*H*-benzo[*f*]-1,2-dihydro-pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10b)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 115–116 °C (ethanol) (yield

15%). IR (KBr) cm^{-1}: 1180, 1370. ^1H NMR ($CDCl_3$): δ 3.11 (t, 2H), 3.81 (s, 3H), 4.43 (t, 2H), 5.84 (dd, 1H), 6.87 (d, 2H), 7.00–7.35 (m, 7H), 7.89 (dd, 1H). MS (EI) *m/z* 382 (M+). Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.32. Found: C, 62.62; H, 4.74; N, 7.39.

5.1.22. 1-[3-(4-Methoxyphenyl)propyl]-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10c)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 99–100 °C (ethanol) (yield 38%). IR (KBr) cm^{-1}: 1170, 1370. ^1H NMR ($CDCl_3$): δ 2.15 (m, 2H), 2.70 (t, 2H), 3.82 (s, 3H), 4.12 (t, 2H), 5.96 (dd, 1H), 6.87 (d, 2H), 6.95–7.25 (m, 7H), 7.39 (dd, 1H), 7.90 (dd, 1H). MS (EI) *m/z* 396 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07. Found: C, 63.39; H, 5.23; N, 7.26.

5.1.23. 9-Chloro-1-(4-methoxyphenylethyl)-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10d)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 126–127 °C (ethanol 95%) (yield 18%). IR (KBr) cm^{-1}: 1190, 1360. ^1H NMR ($CDCl_3$): δ 3.10 (t, 2H), 3.81 (s, 3H), 4.33 (t, 2H), 5.89 (dd, 1H), 6.86 (d, 2H), 7.00–7.35 (m, 7H), 7.92 (dd, 1H). MS (EI) *m/z* 416 (M+). Anal. Calcd for $C_{20}H_{17}ClN_2O_4S$: C, 57.62; H, 4.11; N, 6.72. Found: C, 57.94; H, 4.16; N, 6.81.

5.1.24. 1-(4-Methoxyphenylethyl)-9-methyl-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10e)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (8:2); mp 114–115 °C (ethanol 95%) (yield 31%). IR (KBr) cm^{-1}: 1170, 1350. ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H), 3.12 (t, 2H), 3.81 (s, 3H), 4.31 (t, 2H), 5.80 (dd, 1H), 6.80–6.91 (m, 3H), 7.01–7.16 (m, 5H), 7.87 (dd, 1H). MS (EI) *m/z* 396 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07. Found: C, 63.68; H, 5.13; N, 7.01.

5.1.25. 9-Methoxy-1-(4-methoxyphenylethyl)-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10f)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 115–116 °C (ethanol 95%) (yield 21%). IR (KBr) cm^{-1}: 1150, 1360. ^1H NMR ($CDCl_3$): δ 3.11 (t, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 4.32 (t, 2H), 5.84 (dd, 1H), 6.60 (dd, 1H), 6.80 (d, 1H), 6.86 (d, 2H), 7.05–7.15 (m, 4H), 7.89 (dd, 1H). MS (EI) *m/z* 412 (M+). Anal. Calcd for $C_{21}H_{20}N_2O_5S$: C, 61.15; H, 4.89; N, 6.79. Found: C, 61.40; H, 5.08; N, 6.51.

5.1.26. 1-(2-Methoxyphenylethyl)-11*H*-benzo[*f*]-1,2-dihydro-pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10g)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (9:1); mp 138–140 °C (propan-2-ol/water 9:1) (yield 27%). IR (KBr) cm^{-1}: 1180, 1370. ^1H NMR ($CDCl_3$): δ 3.20 (t, 2H), 3.81 (t, 3H), 4.37 (t, 2H), 5.78 (dd, 1H), 6.80–6.90 (m, 2H), 7.00–7.35 (m, 7H), 7.86 (dd, 1H). MS (EI) *m/z* 382 (M+). Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.32. Found: C, 62.57; H, 4.69; N, 7.25.

5.1.27. 1-(3-Methoxyphenylethyl)-11*H*-benzo[*f*]-1,2-dihydro-pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10h)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 133–134 °C (ethanol 95%) (yield 18%). IR (KBr) cm^{-1}: 1180, 1370. ^1H NMR ($CDCl_3$): δ 3.15 (t, 2H), 3.81 (s, 3H), 4.36 (t, 2H), 5.85 (dd, 1H), 6.72–6.83 (m, 3H), 7.05 (td, 1H), 7.12 (dd, 1H), 7.18 (dd, 1H), 7.21–7.28 (m, 3H), 7.32 (dd, 1H), 7.89 (dd, 1H). MS (EI) *m/z* 382 (M+). Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.32. Found: C, 62.64; H, 4.78; N, 7.36.

5.1.28. 1-(4-Benzyloxyphenylethyl)-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10i)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 142–143 °C (ethanol) (yield 23%). IR (KBr) cm^{-1}: 1190, 1360. ^1H NMR ($CDCl_3$): δ 3.11 (t, 2H), 4.31 (t, 2H), 5.07 (s, 2H), 5.82 (dd, 1H), 6.85–7.55 (m, 14H), 7.89 (dd, 1H). MS (EI) *m/z* 458 (M+). Anal. Calcd for $C_{26}H_{22}N_2O_4S$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.08; H, 5.04; N, 5.72.

5.1.29. 1-(4-Hydroxyphenylethyl)-11*H*-benzo[*f*]-1,2-dihydro-pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10j)

A solution of **14i** (4 mmol) in acetic acid (6 mL) and 33% hydrobromic acid (4 mL) is heated at 35 °C for five days. The reaction mixture is hydrolyzed with ice, extracted with ethyl acetate, washed with aqueous saturated sodium bicarbonate solution. The organic layer is dried, filtered, evaporated and recrystallized in ethanol 95%. Red solid; mp 58–61 °C (ethanol 95%) (yield 53%). IR (KBr) cm^{-1}: 1180, 1350, 3400. ^1H NMR ($CDCl_3$): δ 3.11 (t, 2H), 4.32 (t, 2H), 5.20 (m, 1H), 5.84 (dd, 1H), 6.79 (d, 2H), 7.00–7.35 (m, 7H), 7.89 (dd, 1H). MS (EI) *m/z* 368 (M+). Anal. Calcd for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.38; N, 7.60. Found: C, 62.03; H, 4.35; N, 7.54.

5.1.30. 1-(3,4,5-Trimethoxyphenylethyl)-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10k)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (6:4); mp 165–166 °C (ethanol 95%) (yield 39%). IR (KBr) cm^{-1}: 1170, 1380. ^1H NMR ($CDCl_3$): δ 3.12 (t, 2H), 3.82 (s, 9H), 4.36 (t, 2H), 5.84 (dd, 1H), 6.37 (s, 2H), 6.95–7.40 (m, 5H), 7.90 (dd, 1H). MS (EI) *m/z* 442 (M+). Anal. Calcd for $C_{22}H_{22}N_2O_6S$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.67; H, 4.99; N, 6.41.

5.1.31. 1-[2-(1-Naphthyl)ethyl]-11*H*-benzo[*f*]-1,2-dihydro-pyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10l)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (7:3); mp 201–203 °C (ethanol 95%) (yield 16%;). IR (KBr) cm^{-1}: 1160, 1350. ^1H NMR ($CDCl_3$): δ 3.63 (t, 2H), 4.50 (t, 2H), 5.76 (dd, 1H), 7.01 (dd, 1H), 7.09 (dd, 1H), 7.23 (dd, 1H), 7.25–7.35 (m, 2H), 7.40–7.45 (m, 2H), 7.50–7.60 (m, 2H), 7.79 (d, 1H), 7.80–7.90 (m, 2H), 8.24 (d, 1H). MS (EI) *m/z* 402 (M+). Anal. Calcd for $C_{23}H_{18}N_2O_3S$: C, 68.64; H, 4.51; N, 6.96. Found: C, 68.91; H, 4.48; N, 7.01.

5.1.32. 1-[2-(4-Biphenyl)ethyl]-11*H*-benzo[*f*]-1, 2-dihydropyrido[3,2,*c*][1,2,5]oxathiazepine 5,5-dioxide (10m)

Yellow solid; purified by chromatography with petroleum ether/ethyl acetate (75:25); mp 163–165 °C (ethanol) (yield 23%;). IR (KBr) cm^{-1}: 1190, 1360. ^1H NMR ($CDCl_3$): δ 3.22 (t, 2H), 4.39 (t, 2H), 5.86 (dd, 1H), 7.06 (dd, 1H), 7.15–7.20 (m, 2H), 7.20–7.30 (m, 3H), 7.30–7.40 (m, 2H), 7.46 (dd, 2H), 7.55–7.65 (m, 4H), 7.91 (dd, 1H). MS (EI) *m/z* 428 (M+). Anal. Calcd for $C_{25}H_{20}N_2O_3S$: C, 70.07; H, 4.70; N, 6.54. Found: C, 70.27; H, 4.73; N, 6.40.

5.2. Cell culture and cytotoxicity assays

Cells were cultivated in RPMI 1640 medium (*Invitrogen Inc.*) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay (MTA). Adherent cells (DU145, HT29, A549, KB 3-1 and KB-A1)were seeded in 96 well microplates and incubated for 2 days. Tested compounds were then added and plates were incubated for 96 h (about 4 doubling times).

The murine L1210 cells were exposed for 48 h to graded concentrations of drug. At the end of this period, 15 μL of 5 mg/mL of 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT, *Sigma*) were added to each well and the plates were incubated for 4 h at 37 °C. The medium was aspirated and the formazan solubilized by 100 μL of DMSO. The IC_{50}, concentration reducing by 50% the optical density at 540 nm, was calculated by a linear regression performed on the linear zone of the dose–response curve. All the measurements were performed in triplicate.

5.3. Cell cycle analysis[20,21]

L1210 cells (2.5×10^5 cells/mL) were incubated for 21 h with various concentrations of the compounds. Cells were then fixed in 70% ethanol (v/v), washed and incubated in Dulbecco's phosphate buffered saline (D-PBS) containing 100 mg/mL RNAse and 25 mg/mL propidium iodide for 30 min. at 20 °C. For each sample, 10^4 cells were analyzed on a Epics XL/MCL flow cytometer (Beckman Coulter, France).

5.4. Tubulin test

Effects of the compounds on tubulin polymerization were evaluated as previously reported.[22]

5.5. In vivo antitumor activity[23]

The in vivo antitumor activity of compound **14b** was evaluated on the P388 leukemia murine model. P388 cells (NCI, Frederik) were inoculated ip (10^6 cells/mouse) into B6D2F1 mice (Iffa credo) on day 0. Compound 14b was administered ip or iv on day one. The results are expressed in term of percent T/C (median survival time of treated animals divided by median survival time of control, X 100).

5.6. Determination of metabolic bioavailability[24]

Metabolic bioavailability predictions were based on in vitro metabolic stability measurements with hepatic microsomes assuming total absorption.

5.7. Determination of intestinal absorption[25]

Assuming no possible limitation due to solubility, absorbed fractions (Abs%) were estimated using permeability measurements through Caco2 cell monolayers.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.12.039.

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