Direct metallation of thienopyrimidines using a mixed lithium–cadmium base and antitumor activity of functionalized derivatives†

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A series of thieno[2,3-*d*]- and thieno[3,2-*d*]pyrimidines have been easily synthesized using as key step a deproto-cadmiation–trapping sequence. Some of the compounds thus synthesized were screened for anticancer (cytotoxic) activities, and (*S*)-2-(6-iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)-3-phenylpropanoic acid proved to have a significant activity towards liver, human breast and cervix carcinoma cell lines.

Introduction

Thienopyrimidine derivatives, which are structural analogues of purines, have been drawn attention due to their large range of pharmacological activities.[1] Numerous thieno[2,3-*d*]pyrimidines have been proved to be phosphodiesterase inhibitors and various receptor antagonists; compounds were designed to act as immunomodulators or to be used in case of cerebral ischemia, malaria, tuberculosis, Alzheimer's and Parkinson's diseases.[1] Many thieno[2,3-*d*]- and thieno[3,2-*d*]pyrimidines exhibit antitumor and radioprotective activities.[1]

Lithium bases have been largely employed for the deproto-metallation of aromatic rings.[2] Nevertheless, for aromatics bearing reactive functions (*e.g.*, ester or cyano groups) or sensitive π-deficient heterocycles, either extremely low reaction temperatures or *in situ* electrophilic trapping are required due to the high reactivity of the corresponding (hetero)aryllithiums.

The use of metal additives in order to get more efficient or more chemoselective bases is a challenging field, and various R_nMLi type compounds already prepared behave as superbases since such species exhibit behaviours that cannot be reproduced by the monometallic compounds on their own. Among them, the R_nMLi mixtures of organolithiums and M alkali metal alkoxides described by Schlosser,[3] Lochmann,[4] and Caubère[5] are powerful reagents.

More recently, R_nMLi type compounds where M is not an alkali metal have also been described for their unprecedented metallation ability. These species display a large range of reactivities, depending on both the metal M and the groups connected to it.[6] By combining soft organometallic compounds with lithium additives, bases allowing both efficient and chemoselective reactions have been prepared and used to generate functionalized aromatic compounds.[7]

We recently accomplished the deproto-metallation of a large range of aromatics including heterocycles using a newly developed lithium–cadmium base, $(TMP)_3CdLi$ (TMP = 2,2,6,6-tetramethylpiperidino),[8] and we here describe its use for the functionalization of thieno[2,3-*d*]- and thieno[3,2-*d*]pyrimidines. Some intermediates and target compounds were evaluated for their cytotoxic activity.

Results and discussions

Synthetic aspects

To this purpose, 4-chlorothieno[2,3-*d*]pyrimidine (**1**) was synthesized as described recently under microwave irradiation.[9] 4-Chloro-2-phenylthieno[3,2-*d*]pyrimidine (**2**) was prepared (as shown in Scheme 1) from commercially available methyl 3-aminothiophene-2-carboxylate by adapting a described procedure.[10] Isomeric 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**) was similarly synthesized, but from a known 2-aminothiophene-3-carboxamide.[11] Starting from 4-chlorothieno[2,3-*d*]pyrimidine (**1**), a subsequent functionalization at the 4 position was easily achieved either by nucleophilic substitution using sodium methoxide to afford the ether **4**, or by copper-catalyzed *N*-arylation of pyrazole by adapting described conditions[12] to furnish the derivative **5**. 4-Chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**) was readily converted by reaction with sodium methoxide or morpholine to the compounds **6** and **7**, respectively (Scheme 1).

In contrast to thiophene compounds which can be easily metallated using lithium bases,[13] pyrimidines are much more prone to nucleophilic attacks,[14] and their metallation can only be achieved at room temperature when softer bases are employed.[8,15] The lithium–cadmium base $(TMP)_3CdLi$ was chosen for the

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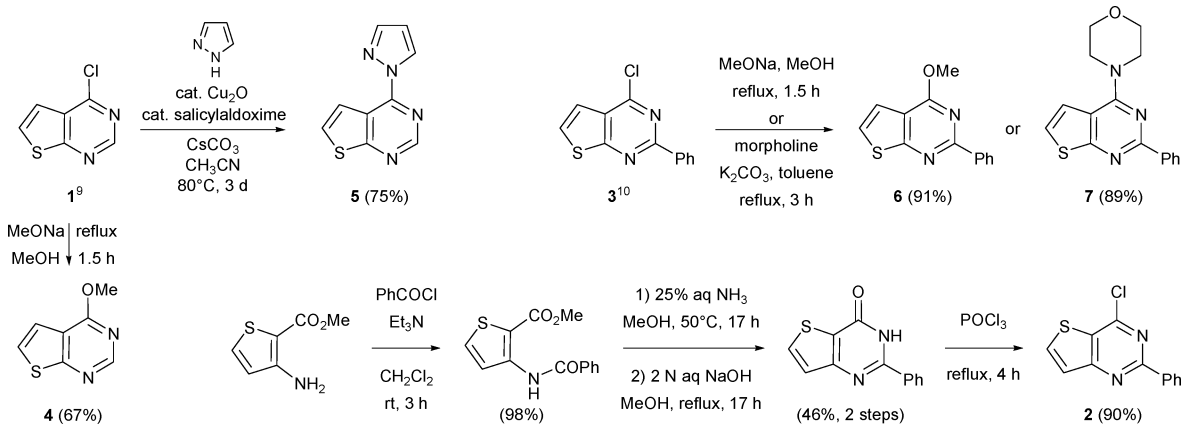
† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR data for methyl-3-benzoylaminothiophene-2-carboxylate, 2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one, and compounds **2**–**22**, and copies of ^1H and ^{13}C NMR spectra for methyl-3-benzoylaminothiophene-2-carboxylate, 2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one, and compounds **3**, **14**–**22**. CCDC reference number 729714. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b915274a



Scheme 1 Synthesis of the substrates **1–7**.

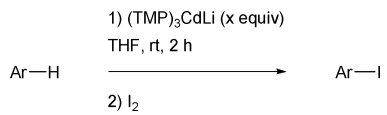
metallation of the thienopyrimidine substrates because of its high compatibility with sensitive heterocycles.[8]

Conducting the reaction from 4-chloro- (**1**) and 4-methoxythieno[2,3-*d*]pyrimidine (**4**) using 0.5 equivalent[8] of base in tetrahydrofuran (THF) at room temperature for 2 h resulted, after quenching with iodine, in the formation of the iodides **8** and **9**, respectively (48 and 90% yield). Unlike five-membered heterocycles, for which protons adjacent to heteroatoms have the strongest acidity, six-membered heterocycles have the weakest acidic protons adjacent to nitrogens, a result of the more important repulsion between the lone electron pair of nitrogen and the negative charge of the carbanion for the latter, due to the smaller angle between the two electron clouds.[16] As a consequence, the substrates **1** and **4** are regioselectively deprotonated at the most acidic 6 position (Table 1, entries 1,2). Upon treatment with 0.5 equiv of $(TMP)_3CdLi$ under the same reaction conditions, 4-(pyrazol-1-yl)thieno[2,3-*d*]pyrimidine (**5**) did not undergo regioselective deprotonation, and only a mixture of several inseparable derivatives was obtained (entry 3). Starting from 4-chloro-2-phenylthieno[3,2-*d*]pyrimidine (**2**), metallation still took place next to the sulfur atom, as evidenced by trapping with iodine to afford the 6-iodo derivative **10a** in 70% yield (compound **10a** was identified unequivocally by X-ray structure analysis).† Turning to 1 equivalent of base (instead of 0.5) allowed a slight yield improvement (76%) but also, more importantly, the formation of the diiodide **10b** in 18% yield. The latter could result from a dideprotonation due to the activation of the site at the 7 position by the close nitrogen atom (entries 4,5). Isomeric 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**) was similarly functionalized in an excellent yield (entry 6). By replacing the chloro group with a methoxy or a morpholino group[17] (substrates **6** and **7**), the conditions were optimized, and the best yields were obtained using 1 equivalent of base (entries 7,8).

It also proved possible to trap the metallated 4-(morpholin-4-yl)-2-phenylthieno[2,3-*d*]pyrimidine prepared from the substrate **7** in a palladium-catalyzed cross-coupling reaction with 2-bromopyridine. Indeed, by using catalytic amounts of palladium acetate and 1,1′-bis(diphenylphosphino)ferrocene (dppf), the expected product **14** was isolated in 70% yield (Scheme 2).

In order to progress towards molecules with potential antitumor activities, we decided to prepare thiophene analogues of 2-(6-iodo-2-phenylquinazolin-4-ylamino)-3-phenylpropanoic acid,

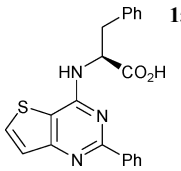
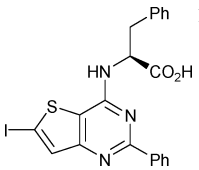
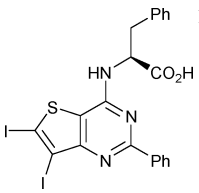
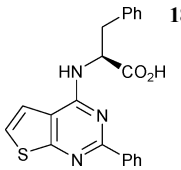
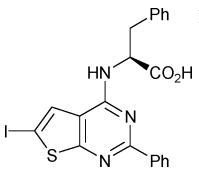
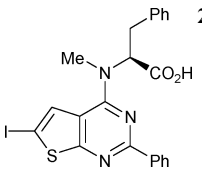
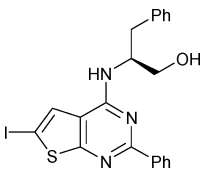
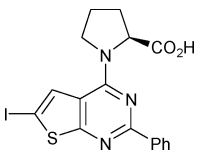
Table 1 Deproto-cadmiation (using $(TMP)_3CdLi$)-iodination



Entry	Substrate	*x*	Product	Yield (%)
1	**1**	0.5	**8**	48
2	**4**	0.5	**9**	90
3	**5**	0.5	Mixture	—[a]
4	**2**	0.5	**10a**	70
5	**2**	1	**10a** + **10b**	76 + 18
6	**3**	0.5	**11**	97
7	**6**	1	**12**	81
8	**7**	1	**13**	83

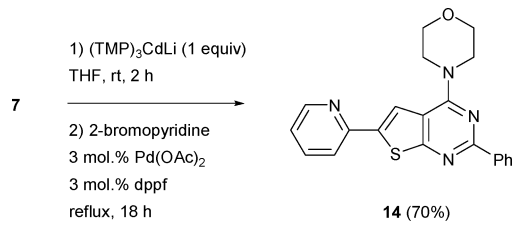
[a] Metallation took place but not regioselectively, and the products formed could not be separated.

Table 2 Nucleophilic substitution of the 4-chlorothienopyrimidines by an amino acid or derivative

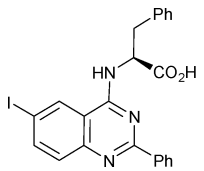
Entry	Substrate	RR′NH	Product		Yield (%)
1	**2**	L-Phenylalanine		**15**	66
2	**10a**	L-Phenylalanine		**16**	55
3	**10b**	L-Phenylalanine		**17**	48
4	**3**	L-Phenylalanine		**18**	53
5	**11**	L-Phenylalanine		**19**	80
6	**11**	*N*-Methyl-L-phenylalanine		**20**	74
7	**11**	L-Phenylalaninol		**21**	40
8	**11**	L-Proline		**22**	38

which proved to exhibit anticancer activity (cytotoxic) against U937 leukemia cell lines (Scheme 3).[18]

To this purpose, different 2-phenyl-4-chlorothienopyrimidines, iodinated or not, were involved in the nucleophilic substitution reaction of the chloride by L-phenylalanine, under the conditions described in the quinazoline series[18] to afford the analogues **15–19** (Table 2, entries 1-5). *N*-methyl-L-phenylalanine, L-phenylalaninol and L-proline were then used in order to evidence the importance of the secondary amine and carboxylic acid functions (entries 6–8, compounds **20–22**).



Scheme 2 Deproto-cadmiation (using $(TMP)_3CdLi$) followed by cross-coupling.



Scheme 3 Cytotoxic activity of 2-(6-iodo-2-phenylquinazolin-4-ylamino)-3-phenylpropanoic acid.

Table 3 [a]*In vitro* cytotoxic activity (IC_{50}) [b]of the newly synthesized compounds and doxorubicin against a liver carcinoma cell line (HEPG2), a human breast carcinoma cell line (MCF7), and a cervix carcinoma cell line (HELA)[c]

Entry	Compound	HEPG2/μg mL^{-1}	MCF7/μg mL^{-1}	HELA/μg mL^{-1}
1	**6**	3.4	1.6	2.8
2	**7**	2.5	1.3	4.4
3	**12**	2.6	2.1	3.5
4	**13**	1.2	2.7	4.0
5	**14**	4.3	2.2	4.7
6	**16**	2.7	1.5	5.0
7	**19**	0.40	0.94	1.3
8	**20**	1.6	1.6	3.0
9	**21**	1.7	2.4	5.6
10	Doxorubicin	0.60	0.70	0.85

[a] The cadmium presence in the tested compounds was not evaluated since $CdCl_2$·TMEDA was used at the last-step-but-one, but in case of further development these compounds would have to be synthesized using a safe base and tested again. [b] IC_{50} is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor. [c] Pharmacological tests have also been performed for compounds **15**, **17**, **18** and **22**, but they failed to give logical results.

Pharmacology

The compounds **6**, **7**, **12–14**, **16**, **19**, **20** and **21** were tested against a liver carcinoma cell line (HEPG2), a human breast carcinoma cell line (MCF7), and a cervix carcinoma cell line (HELA) (Table 3). The compounds **6**, **7**, **12**, **14** and **16** showed a low activity towards the liver carcinoma cell line HEPG2 compared to a reference drug (doxorubicin); the compounds **13**, **20** and **21** showed a moderate activity whereas the compound **19** was found to have a very potent activity towards HEPG2 with a IC_{50} of 0.40 μg mL^{-1} (the IC_{50} value

for the reference drug, doxorubicin, is 0.70 μg mL^{-1}). Concerning the activity towards the human breast carcinoma cell line MCF7, all the compounds showed moderate activities compared to the reference drug doxorubicin except the compound **19** which turned out to be promising. For the cervix carcinoma cell line HELA, all the compounds have low activities compared to the reference drug doxorubicin, except the compounds **6** and **19** which were found to be more effective towards this kind of carcinoma cell line (HELA) than other compounds.

Conclusions

A series of thieno[2,3-*d*]- and thieno[3,2-*d*]pyrimidines have been easily synthesized. The key step is a deprotonation–trapping sequence using a mixed lithium–cadmium base. Most of the compounds were screened in order to evidence an anticancer activity. Some of them such as (*S*)-2-(6-iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)-3-phenylpropanoic acid (**19**) showed significant cytotoxic activities towards the tested cell lines, in particular with the liver and human breast carcinoma cell lines.

Experimental

Syntheses: general methods

Metallation reactions were performed under argon atmosphere. THF was distilled over sodium/benzophenone. Column chromatography separations were achieved on silica gel (40–63 μm). Melting points were measured on a Kofler apparatus. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX-200 spectrometer at 200 and 50 MHz, respectively, on a Bruker Avance III spectrometer at 300 and 75 MHz, respectively, or on a Bruker AC-400 spectrometer at 300 and 75 MHz, respectively. ^{1}H chemical shifts (δ) are given in ppm relative to the solvent residual peak, and ^{13}C chemical shifts relative to the central peak of the solvent signal.[19] Mass spectra (HRMS) measurements were performed at the CRMPO (Centre Régional de Mesures Physiques de l'Ouest) of Rennes using either a Waters Q-TOF 2 or a Bruker micrOTOF Q II instrument in positive or negative elestrospray CI mode, respectively, or a Micromass MS/MS ZABSpec TOF instrument in EI mode. Elemental analyses were performed at the CRMPO using a Thermo-Finnigan Flash EA 1112 CHNS analyzer.

4-Chlorothieno[2,3-*d*]pyrimidine (**1**)[9] and 2-aminothiophene-3-carboxamide[11] were prepared according to described procedures. 2-Benzoylaminothiophene-3-carboxamide and 2-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one[20] were prepared by adapting a described procedure.[10]

Methyl-3-benzoylaminothiophene-2-carboxylate was prepared by adapting a described procedure.[10] To a mixture of methyl-3-aminothiophene-2-carboxylate (2.2 g, 14 mmol) and Et_3N (2.4 mL, 17 mmol) in CH_2Cl_2 (18 mL) at 0 °C was added dropwise benzoyl chloride (2.0 mL, 17 mmol). After stirring at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting solid was purified by column chromatography on silica gel (eluent: heptane–Et_2O 96 : 4 to 80 : 20) to give methyl-3-benzoylaminothiophene-2-carboxylate (3.7 g, 98%) as a white solid: mp 98 °C (lit.[21] 98–99 °C).

2-Phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one[22] was prepared by adapting a described procedure.[10] To a solution of methyl-3-benzoylaminothiophene-2-carboxylate (2.0 g, 7.6 mmol) in MeOH (80 mL) was added a 25% aqueous NH_3 solution (200 mL). After stirring at 50 °C for 17 h, the reaction mixture was concentrated to one-half of its initial volume and the resulting solid was collected to give a mixture of 3-benzoylamino-thiophene-2-carboxamide and 2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (0.84 g). To this mixture in MeOH (34 mL) was added a 2 N aqueous NaOH solution (12 mL). After stirring at reflux for 17 h, the reaction mixture was acidified with 1 M HCl until pH 4 and the resulting precipitate was collected to give 2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (0.80 g, 46%) as a white solid: mp > 260 °C. HRMS: calcd for $C_{12}H_8N_2OS$ (M^+) 228.0357, found 228.0348.

4-Chloro-2-phenylthieno[3,2-*d*]pyrimidine (2)[22] was prepared by adapting a described procedure.[10] A mixture of 2-phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (1.6 g, 7.0 mmol) and $POCl_3$ (16 mL, 0.17 mol) was heated at reflux for 4 h. $POCl_3$ was then distilled under reduced pressure. The residue was diluted in CH_2Cl_2, and neutralized with a cooled saturated $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The resulting solid was purified by column chromatography on silica gel (eluent: heptane–CH_2Cl_2 100 : 0 to 0 : 100) to give **2** (1.5 g, 90%) as a white solid: mp 118–119 °C. Anal. calcd for $C_{12}H_7N_2ClS$: C, 58.42; H, 2.86; N, 11.35; S, 13.00. Found: C, 58.08; H, 3.00; N, 11.28; S, 12.98%.

4-Chloro-2-phenylthieno[2,3-*d*]pyrimidine (3) was prepared as before but starting from 2-phenyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (1.6 g), and was obtained after recrystallization from AcOEt in a quantitative yield as a white solid: mp 148 °C (lit.[23] 146 °C).

4-Methoxythieno[2,3-*d*]pyrimidine (4)[24]. To a solution of MeONa in MeOH (0.18 g, 8.0 mmol of Na in 40 mL of MeOH) was added 4-chlorothieno[2,3-*d*]pyrimidine (**1**, 0.68 g, 4.0 mmol). After stirring at reflux for 1.5 h, the reaction mixture was quenched with water, extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated. The resulting solid was purified by column chromatography on silica gel (eluent: heptane–AcOEt 70 : 30) to give **4** (0.45 g, 67%) as a white solid: mp 51 °C. Anal. calcd for $C_7H_6N_2OS$: C, 50.59; H, 3.64; N, 16.86; S, 19.29. Found: C, 50.89; H, 3.73; N, 16.46; S, 19.21%.

4-Methoxy-2-phenylthieno[2,3-*d*]pyrimidine (6) was prepared as before from 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**, 0.98 g). Column chromatography on silica gel (eluent: CH_2Cl_2–heptane 50 : 50) afforded **6** (2.1 g, 91%) as a pale yellow solid: mp 90–92 °C. Anal. calcd for $C_{13}H_{10}N_2OS$: C, 64.44; H, 4.16; N, 11.56; S, 13.23. Found: C, 64.53; H, 4.26; N, 11.36; S, 12.74%.

4-(Pyrazol-1-yl)thieno[2,3-*d*]pyrimidine (5) was prepared from 4-chlorothieno[2,3-*d*]pyrimidine (**1**, 0.68 g) by adapting a described procedure.[12] Purification by column chromatography on silica gel (eluent: heptane/Et_2O 80 : 20) afforded **5** (0.61 g, 75%) as

a white solid: mp 115 °C. Anal. calcd for $C_9H_6N_4S$: C, 53.45; H, 2.99; N, 27.70; S, 15.86. Found: C, 53.77; H, 2.99; N, 27.52; S, 15.77%.

4-(Morpholin-4-yl)-2-phenylthieno[2,3-*d*]pyrimidine (7). To a solution of morpholine (0.38 g, 4.4 mmol) and 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**, 1.0 g, 4.0 mmol) in dry toluene (25 mL) was added K_2CO_3 (1.1 g, 8.0 mmol). After stirring at reflux for 1 h, one more equivalent of morpholine was added (0.35 g, 4.0 mmol). The mixture was stirred again for 2 h at reflux, filtered through celite®, and the filtrate was concentrated under reduced pressure. The resulting solid was purified by column chromatography on silica gel (eluent: CH_2Cl_2) to give **7** (1.0 g, 89%) as a yellow solid: mp 140 °C (lit.[23] 139 °C). Anal. calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.79; H, 5.15; N, 14.08; S, 10.43%.

General procedure for the deproto-cadmiation (using 0.5 equiv $(TMP)_3CdLi$)–iodination

To a stirred, cooled (0 °C) solution of 2,2,6,6-tetramethylpiperidine (0.25 mL, 1.5 mmol) in THF (5 mL) was added BuLi (about 1.6 M hexanes solution, 1.5 mmol). After 15 min at 0 °C, $CdCl_2$·TMEDA (0.15 g, 0.5 mmol) was added, and the mixture was stirred for 15 min at this temperature before introduction of the substrate (1.0 mmol). After 2 h at room temperature, a solution of I_2 (0.37 g, 1.5 mmol) in THF (5 mL) was added. The mixture was stirred overnight before addition of an aqueous saturated solution of $Na_2S_2O_3$ (10 mL) and extraction with Et_2O (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure before purification by column chromatography on silica gel (the eluent is given in the product description).

4-Chloro-6-iodothieno[2,3-*d*]pyrimidine (8) was prepared from 4-chlorothieno[2,3-*d*]pyrimidine (**1**, 0.17 g) following the general procedure. After purification by column chromatography on silica gel (eluent: CH_2Cl_2), compound **8** was obtained as a white solid (0.14 g, 48%): mp 168 °C. Anal. calcd for $C_6H_2ClIN_2S$: C, 24.30; H, 0.68; N, 9.45; S, 10.81. Found: C, 24.68; H, 1.06; N, 9.12; S, 10.73%.

6-Iodo-4-methoxythieno[2,3-*d*]pyrimidine (9) was prepared from 4-methoxythieno[2,3-*d*]pyrimidine (**4**, 0.17 g) following the general procedure. After purification by column chromatography on silica gel (eluent: heptane–AcOEt 90 : 10), compound **9** was obtained as a white solid (0.26 g, 90%): mp 152 °C. Anal. calcd for $C_7H_5IN_2OS$: C, 28.78; H, 1.73; N, 9.59; S, 10.98. Found: C, 29.09; H, 1.82; N, 9.43; S, 11.43%.

4-Chloro-6-iodo-2-phenylthieno[3,2-*d*]pyrimidine (10a) was prepared from 4-chloro-2-phenylthieno[3,2-*d*]pyrimidine (**2**, 0.25 g) following the general procedure. After purification by column chromatography on silica gel (eluent: heptane–CH_2Cl_2 98 : 2 to 74 : 26), compound **10a** was obtained as a white solid (0.26 g, 70%): mp 138 °C. Anal. calcd for $C_{12}H_6ClIN_2S$: C, 38.68; H, 1.62; N, 7.52; S, 8.61. Found: C, 38.69; H, 1.73; N, 7.71; S, 8.32%.

4-Chloro-6-iodo-2-phenylthieno[2,3-*d*]pyrimidine (11) was prepared from 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**, 0.25 g) following the general procedure. After purification by column chromatography on silica gel (eluent: CH_2Cl_2–heptane 50 : 50), compound **11** was obtained as a white solid (0.36 g, 97%): mp 148 °C. Anal. calcd for $C_{12}H_6ClIN_2S$: C, 38.68; H, 1.62; N, 7.52; S, 8.61. Found: C, 39.01; H, 1.92; N, 7.37; S, 8.70%.

General procedure for the deproto-cadmiation (using 1 equiv. $(TMP)_3CdLi$)–iodination

To a stirred, cooled (0 °C) solution of 2,2,6,6-tetramethylpiperidine (0.25 mL, 1.5 mmol) in THF (5 mL) was added BuLi (about 1.6 M hexanes solution, 1.5 mmol). After 15 min at 0 °C, $CdCl_2$·TMEDA (0.15 g, 0.5 mmol) was added, and the mixture was stirred for 15 min at this temperature before introduction of the substrate (0.5 mmol). After 2 h at room temperature, a solution of I_2 (0.37 g, 1.5 mmol) in THF (5 mL) was added. The mixture was stirred overnight before addition of an aqueous saturated solution of $Na_2S_2O_3$ (10 mL) and extraction with Et_2O (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure before purification by column chromatography on silica gel (the eluent is given in the product description).

4-Chloro-6,7-diiodo-2-phenylthieno[3,2-*d*]pyrimidine (10b) was prepared from 4-chloro-2-phenylthieno[3,2-*d*]pyrimidine (**2**, 0.12 g) following the general procedure. After purification by column chromatography on silica gel (eluent: heptane–CH_2Cl_2 98 : 2 to 74 : 26), compound **10b** was obtained as a white solid (45 mg, 18%): mp 176 °C. Anal. calcd for $C_{12}H_5ClI_2N_2S$: C, 28.91; H, 1.01; N, 5.62; S, 6.43. Found: C, 33.23; H, 2.12; N, 5.21; S, 6.13% (partial loss of iodine was observed upon storage). Note that compound **10a** was also obtained in 76% yield.

6-Iodo-4-methoxy-2-phenylthieno[2,3-*d*]pyrimidine (12) was prepared from 4-methoxy-2-phenylthieno[2,3-*d*]pyrimidine (**6**, 0.12 g) following the general procedure. After purification by column chromatography on silica gel (eluent: CH_2Cl_2–heptane 50 : 50), compound **12** was obtained as a white solid (0.15 g, 81%): mp 144–146 °C. Anal. calcd for $C_{13}H_9IN_2OS$: C, 42.41; H, 2.46; N, 7.61; S, 8.71. Found: C, 42.44; H, 2.42; N, 7.95; S, 8.43%.

6-Iodo-4-(morpholin-4-yl)-2-phenylthieno[2,3-*d*]pyrimidine (13) was prepared from 4-(morpholin-4-yl)-2-phenylthieno[2,3-*d*]pyrimidine (**7**, 0.15 g) following the general procedure. After purification by column chromatography on silica gel (eluent: CH_2Cl_2), compound **13** was obtained as a yellow solid (0.18 g, 83%): mp 206–208 °C. Anal. calcd for $C_{16}H_{14}IN_3OS$: C, 45.40; H, 3.33; N, 9.93; S, 7.85. Found: C, 45.11; H, 3.46; N, 9.57; S, 7.36%.

4-(Morpholin-4-yl)-2-phenyl-6-(pyridin-2-yl)thieno[2,3-*d*]-pyrimidine (14). To a stirred, cooled (0 °C) solution of 2,2,6,6-tetramethylpiperidine (0.26 mL, 1.5 mmol) in THF (2 mL) was added BuLi (about 1.6 M hexanes solution, 1.5 mmol). After 15 min at 0 °C, $CdCl_2$·TMEDA (0.15 g, 0.5 mmol) was added, and the mixture was stirred for 15 min at this temperature before introduction of 4-(morpholin-4-yl)-2-phenylthieno[2,3-*d*]pyrimidine

(**7**, 0.15 g, 0.5 mmol). After 2 h at room temperature, $Pd(OAc)_2$ (3.4 mg, 15 μmol), 1,1′-bis(diphenylphosphinoferrocene) (9 mg, 15 μmol) and 2-bromopyridine (0.48 mL, 0.5 mmol) were added successively, and the mixture was heated at reflux overnight. The mixture was filtered through celite®, and the cake was washed with CH_2Cl_2. After purification by column chromatography on silica gel (eluent: CH_2Cl_2–AcOEt 100 : 0 to 90 : 10), and crystallization from acetonitrile, compound **14** was obtained as a yellow solid (0.13 g, 70%): mp > 260 °C. HRMS: calcd for $C_{21}H_{19}N_4OS$ ($[M+H]^+$) 375.1280, found 375.1282.

General procedure for the nucleophilic substitution of 4-chlorothienopyrimidines by an amino acid or derivative

A previously described procedure was adapted.[18] A mixture of the required 4-chlorothienopyrimidine (0.13 mmol) and amino acid or derivative (0.14 mmol), and potassium carbonate (0.59 mmol) in DMSO (0.6 mL) was heated overnight at 100 °C. After cooling to room temperature, 1 N HCl was added until pH 4. The resulting solid was collected and purified by column chromatography on silica gel (AcOEt–MeOH 80 : 20).

(*S*)-3-Phenyl-2-(2-phenylthieno[3,2-*d*]pyrimidin-4-ylamino)propanoic acid (15) was prepared from 4-chloro-2-phenylthieno[3,2-*d*]pyrimidine (**2**, 32 mg) and using L-phenylalanine (23 mg) following the general procedure, and was obtained as a pale yellow solid (32 mg, 66%): mp > 240 °C. HRMS: calcd for $C_{21}H_{16}N_3O_2S$ ($[M - H]^-$) 374.0969, found 374.0971.

(*S*)-2-(6-Iodo-2-phenylthieno[3,2-*d*]pyrimidin-4-ylamino)-3-phenylpropanoic acid (16) was prepared from 4-chloro-6-iodo-2-phenylthieno[3,2-*d*]pyrimidine (**10a**, 48 mg) and using L-phenylalanine (23 mg) following the general procedure, and was obtained as a pale yellow solid (36 mg, 55%): mp > 240 °C. HRMS: calcd for $C_{21}H_{15}IN_3O_2S$ ($[M - H]^-$) 499.9935, found 499.9947.

(*S*)-2-(6,7-Diiodo-2-phenylthieno[3,2-*d*]pyrimidin-4-ylamino)-3-phenylpropanoic acid (17) was prepared from 4-chloro-6,7-diiodo-2-phenylthieno[3,2-*d*]pyrimidine (**10b**, 65 mg) and using L-phenylalanine (23 mg) following the general procedure, and was obtained as a pale yellow solid (39 mg, 48%): mp > 240 °C. HRMS: calcd for $C_{21}H_{14}I_2N_3O_2S$ ($[M - H]^-$) 625.8902, found 625.8916.

(*S*)-3-Phenyl-2-(2-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)propanoic acid (18) was prepared from 4-chloro-2-phenylthieno[2,3-*d*]pyrimidine (**3**, 32 mg) and using L-phenylalanine (23 mg) following the general procedure, and was obtained as a pale yellow solid (31 mg, 53%): mp > 240 °C. HRMS: calcd for $C_{21}H_{16}N_3O_2S$ ($[M - H]^-$) 374.0969, found 374.0968.

(*S*)-2-(6-Iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)-3-phenylpropanoic acid (19) was prepared from 4-chloro-6-iodo-2-phenylthieno[2,3-*d*]pyrimidine (**11**, 48 mg) and using L-phenylalanine (23 mg) following the general procedure, and was obtained as a pale yellow solid (52 mg, 80%): mp > 240 °C. HRMS: calcd for $C_{21}H_{17}IN_3O_2S$ ($[M+H]^+$) 502.0086, found 502.0088.

(*S*)-2-[(6-Iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-yl)(methyl)-amino]-3-phenylpropanoic acid (20) was prepared from 4-chloro-6-iodo-2-phenylthieno[2,3-*d*]pyrimidine (**11**, 48 mg) and using *N*-methyl-L-phenylalanine (25 mg) following the general procedure, and was obtained as a pale yellow solid (50 mg, 74%): mp > 240 °C. HRMS: calcd for $C_{22}H_{17}IN_3O_2S$ ($[M - H]^-$) 514.0092, found 514.0085.

(*S*)-2-(6-Iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-ylamino)-3-phenylpropan-1-ol (21) was prepared from 4-chloro-6-iodo-2-phenylthieno[2,3-*d*]pyrimidine (**11**, 48 mg) and using L-phenylalaninol (21 mg) following the general procedure, and was obtained as a pale yellow solid (25 mg, 40%): mp > 240 °C. HRMS: calcd for $C_{14}H_{11}IN_3OS$ ($[M-C_7H_7]^+$) 395.9668, found: 395.9626.

(*S*)-1-(6-Iodo-2-phenylthieno[2,3-*d*]pyrimidin-4-yl)pyrrolidine-2-carboxylic acid (22) was prepared from 4-chloro-6-iodo-2-phenylthieno[2,3-*d*]pyrimidine (**11**, 48 mg) and using L-proline (16 mg) following the general procedure, and was obtained as a pale yellow solid (22 mg, 38%): mp > 240 °C. HRMS: calcd for $C_{16}H_{14}IN_3S$ ($[M-CO_2]^+$) 406.9953, found: 406.9925.

Pharmacology

The method applied is similar to that reported by Skehan *et al.*[25] using 20 Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1.0, 2.5, 5.0, and 10 μg ml^{-1}) were added to the cell monolayer in triplicate wells individual dose, and monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader, and the relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound, and the IC_{50} was calculated.

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