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An iminophosphorane-based approach for the synthesis of spiropyrrolidine–imidazole derivatives

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Abstract—A method based on the reaction of an *E*-phosphazide, an intermediate in the Staüdinger reaction between triphenylphosphine and an azide, with heterocumulenes allows the one-pot, two-component synthesis of a number of pyrrole–imidazole derivatives. The procedure, which involves sequential treatment of the appropriate α-azido ester with triphenylphosphine and isocyanate leads to the hydantoin product after aqueous work-up. The cyclization conditions can also be adapted for the synthesis of thiohydantoins by using isothiocyanates. These hybrids pyrrole–thiohydantoins undergo a novel oxidative spirocyclization by action of DDQ to give a tricyclic derivative (pyrrole–pyrrolidine–imidazole), which displays an interesting cytotoxic activity.

1. Introduction

Marine sponges have been recognized as a rich source of a structurally diverse and pharmacologically interesting class of $C_{11}N_5$ pyrrole–imidazole alkaloids.[1] Common structural features of this group of secondary metabolites are a brominated or nonbrominated pyrrole carboxamide unit connected to a functionalized imidazole ring either through a functionalized or unfunctionalized three-membered carbon bridge or spiroimidazoline ring. Among the former are midpacamide **1**[2] and dispacamide **2**,[3] which have been isolated from marine sponges of the genus *Agelas* and display antihistaminic activity.[3] In midpacamide **1** the imidazole ring appears in the guise of an unusual 3-methylhydantoyl moiety, whereas in dispacamide **2** it is present as a 2-aminoimidazolinone ring. In the second group, the spirocyclic guanidine molecular framework defines many biologically active sponge metabolites as exemplified by dibromophakellin **3**[4] and dibromoisophakellin[5] **4**. It is therefore not surprising that their general biological activity, antibacterial action against *Bacillus subtilis* and *Escherichia coli*,[4] combined with their exquisite structure, has made them a formidable challenge to syntheses[6] (Fig. 1).

In conjunction with our synthetic efforts on the synthesis of a number of imidazole-containing alkaloids of marine origin,[7] we have devised a reliable approach to the synthesis of the tricyclic ACD ring system of the dibromophakellin **3** and dibromoisophakellin **4**.[8]

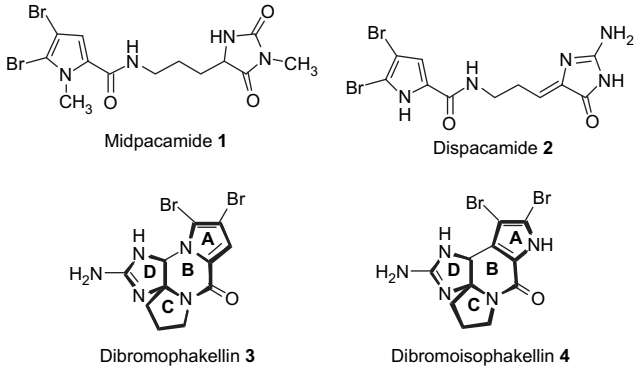

Figure 1. Biologically active molecules containing the pyrrole–imidazole (**1** and **2**) and spirocyclic guanidine moieties (**3** and **4**).

2. Results and discussion

The *N*-acylated amino ester **5** was prepared in 90% yield from the reaction of 2-pyrrolyltrichloromethyl ketone with ethyl 5-aminovalerate hydrochloride in acetonitrile in the presence of triethylamine. The common intermediate α-azido ester **6** was prepared in 70% yield from **5** by the enolate azidation procedure[9] using LDA/2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide)/HMPA. Staüdinger reaction between the α-azido ester **6** and triphenylphosphine was very slow with no detectable nitrogen evolution. When ethyl isocyanate was added to this mixture and the resulting solution was stirred at room temperature no triphenylphosphine oxide was detected. Surprisingly, after aqueous work-up the

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hydantoin **10a**, instead of the expected urea derivative, was formed cleanly in 75% yield. The assignment of **10a** as the product was determined by spectroscopic methods and mass spectrometry. Similar results were obtained when propyl-isopropyl, and benzyl isocyanate were used, and the corresponding hydantoins **10b–d** were obtained in yields ranging from 55 to 65%.

We were intrigued by our inability to observe the uncyclized urea intermediates despite the mild and neutral reaction conditions employed. Because in the reaction of α-amino acid esters with isocyanates to give urea derivatives cyclization usually involves heating, acid catalysis,[10] or prolonged treatment with triethylamine at room temperature.[11]

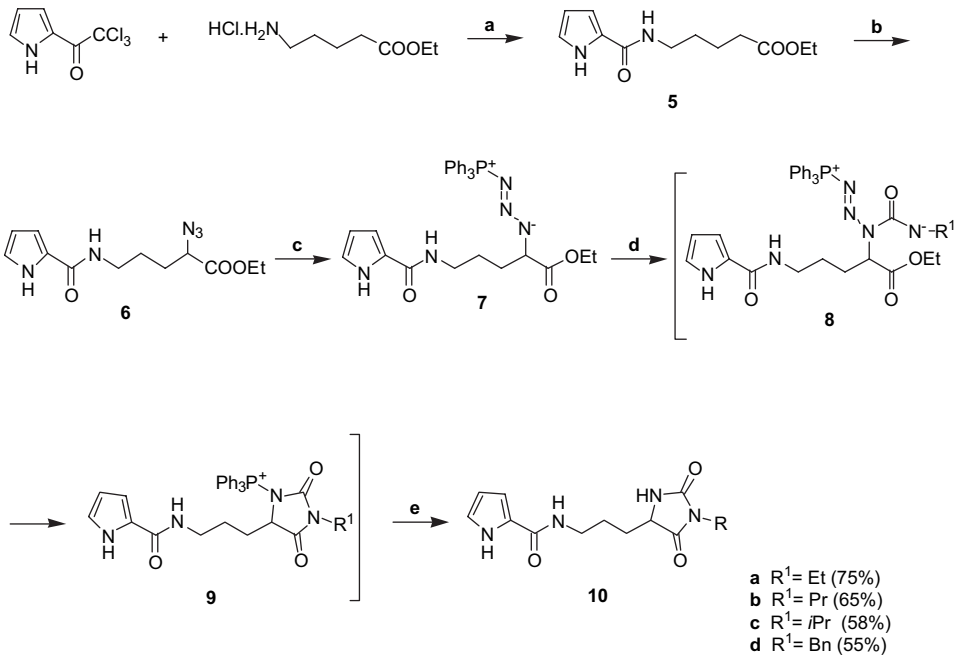
Monitoring the reaction sequence by ^{31}P NMR revealed a number of details: (a) the spectrum recorded on the mixture of triphenylphosphine and compound **6** showed a signal at δ=17.70 ppm, which is in good agreement with the previously reported values for phosphazides,[12] (b) the spectrum recorded after the addition of ethyl isocyanate exhibited a signal at δ=42.40 ppm characteristic of aminotriphenylphosphonium salts, and (c) the spectrum recorded on the final solution after aqueous work-up showed a signal at δ=26.12 ppm due to triphenylphosphine oxide. In addition, the IR spectrum of the solution after addition of ethyl isocyanate did not show the characteristic carbodiimide absorption band.

Taking into account these observations, we believe that this process is likely to take place through the initially formed *E*-phosphazide **7**, which reacts with ethyl isocyanate to give a betaine **8**, which undergoes cyclization across the ester functionality with concomitant nitrogen evolution. Eventually, hydrolytic cleavage of the resulting cyclized phosphonium salt **9** provided the final product **10** and triphenylphosphine oxide (Scheme 1). The *Z*-phosphazide required for elimination of nitrogen and concurrent iminophosphorane formation is essentially never formed, perhaps due to steric hindrance.

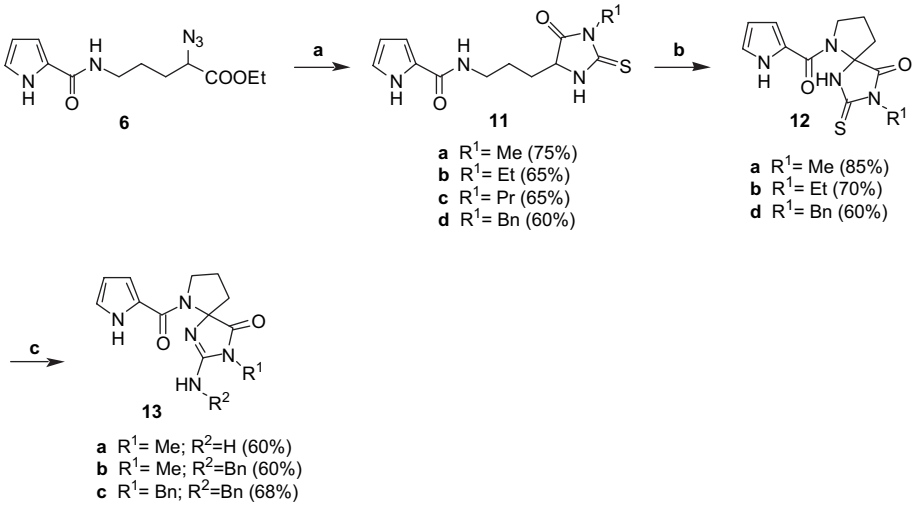
Mechanistic studies on the Staüdinger reaction, formation of iminophosphoranes from a tertiary phosphine and an organic azide, revealed that the nucleophilic attack of the tertiary phosphine on the azide occurs with the formation of an *E*-phosphazide, which displays zwitterionic character. After *E* to *Z* isomerization this decomposes rather easily into the iminophosphorane and nitrogen.[13] In spite of the important role of iminophosphoranes in organic synthesis, they can react with carbonyl compounds either via aza-Wittig[14] or abnormal aza-Wittig[15] pathways, little attention has been paid to their alusive precursors phosphazides, primarily due to their rapid conversion to iminophosphoranes. It has only been reported[11] that an isolated *Z*-phosphazide reacts with isocyanates in a 'normal' aza-Wittig type fashion to give the expected carbodiimide. So, the results given here describe a case where a phosphazide intermediate may participate in a new cyclization process, thus bypassing the usual Staüdinger pathway: nitrogen elimination to give the iminophosphorane.

It has been described that oxidation with bromine,[7d] NBS,[7e] and PhI(CN)OTf[16] of 2-substituted imidazoles (amino, hydroxy, phenylthio) takes place with concomitant cyclization of a tethered nucleophile giving rise to spirocyclic 4,4-disubstituted imidazoles, a molecular framework that defines many biologically active sponge metabolites.[17]

In this context, we decided to prepare the tricyclic ACD system of the dibromophakellin **3** and dibromoisophakellin **4**, by oxidative spirocyclization of the readily available hydantoins **10**. However, all attempts to promote the



Scheme 1. (a) CH_3CN, Et_3N, rt (90%); (b) i: LDA, THF, −30 °C, ii: trisyl azide, HMPA, −78 °C, iii: AcOH, rt (70%); (c) Ph_3P, Et_2O, 0 °C→rt; (d) R^1–N=C=O, Et_2O, 0 °C→rt; and (e) THF/H_2O (9:1), rt.



Scheme 2. (a) i: Ph_3P, Et_2O, 0 °C→rt; ii: R^1–N═C═S, Et_2O, 0 °C→rt, iii: THF/H_2O (9:1), rt; (b) DDQ, THF, rt (60–85%); (c) R^2–NH_2, TBHP, MeOH, rt (60–68%).

spirocyclization of **10** using the previously mentioned oxidizing agents failed and only complex mixtures were obtained.

Therefore, the thiohydantoins **11** were the starting materials of choice, which were prepared in 60–75% yields by sequential treatment of the α-azido ester **2** with triphenylphosphine, and isothiocyanates under the same conditions used for the preparation of hydantoins **10**. These results clearly show the wide scope of the iminophosphorane-promoted (thio)-hydantoin cyclization process. When compounds **11** were treated with DDQ in THF at room temperature spirocyclization took place and spirothiohydantoins **12** were isolated in yields ranging from 60 to 85% (Scheme 2).

The conversion of **11**→**12** could be understood by initial dehydrogenation of the thiohydantoin ring followed by concomitant nucleophilic attack of the NH group on the C═N bond formed. Finally, replacement of the sulfur atom in **12** by a nitrogen functionality to afford **13** was achieved by oxidation with *tert*-butylhydroperoxide (TBHP) followed by treatment of the resulting sulfinic acid[18] with ammonia or alkylamines.

These compounds have been characterized by means of standard spectroscopic techniques (^{1}H NMR and ^{13}C NMR), mass spectrometry, and elemental analyses, all data being in agreement with the proposed structure. Compound **12** was unambiguously determined through a single-crystal X-ray diffraction analyses[19] (Fig. 2).

The structure of **12a** (R′=Me) consists of three-ring systems, two of them with a common spiro carbon atom C(6) (Tables 1 and 2). The dihedral angle between the mean plane of the pyrrolidine and the imidazole rings is 96.1°. With the exception of N(2)–C(6) [1.4522(19) Å], which is a little bit longer than the mean value reported for C_{sp^3}–N in tetrahydropyrrole (1.475 Å)[20] the bond lengths are normal. The intermolecular contact N(1)–H(01)···O(1) (−*x*, −*y*+1, −*z*) [N(1)···O(1)=2.8130(18) Å, N(1)–H(01)···O(1)=156(2)°] may indicate hydrogen bonding, these contacts link the molecules into dimers (Fig. 2b) (Table 3).

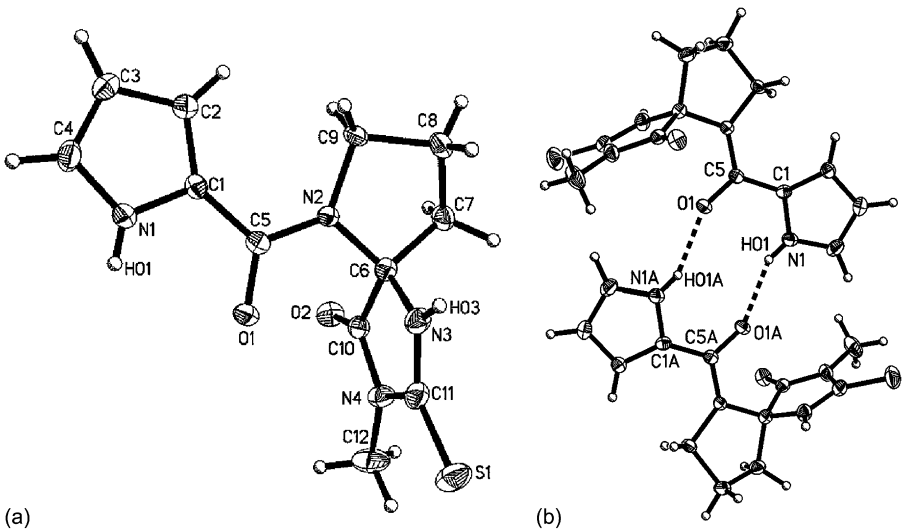


Figure 2. ORTEP view of the molecular structure of **12a** showing the atom labeling. Thermal ellipsoids are drawn at 50% probability level.

Table 1. Crystal data and structure refinement of compound **12a**

Empirical formula	$C_{12}H_{14}N_4O_2S$
Formula weight	278.33
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	*P*2(1)/*c*
Unit cell dimensions	a=8.9217(5) Å; α=90°
	b=12.5387(7) Å; β=102.282(5)°
	c=11.5862(7) Å; γ=90°
Volume	1266.44(13) Å^3
Z	4
Density (calculated)	1.460 mg m^{-3}
Absorption coefficient	0.260 mm^{-1}
F(000)	584
Crystal size	0.60×0.42×0.20 mm^3
Theta range for data collection	3.09–25.00°
Index ranges	$-10\leq h\leq 10$, $-14\leq k\leq 14$, $0\leq l\leq 13$
Reflections collected	4570
Independent reflections	2233 [R(int)=0.0177]
Completeness to theta=25.00°	99.9%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2233/0/181
Goodness-of-fit on F^2	1.028
Final R indices [I>2sigma(I)]	$R1$=0.0308, $wR2$=0.0752
R indices (all data)	$R1$=0.0377, $wR2$=0.0781
Largest diff. peak and hole	0.246 and −0.231 eÅ^{-3}

Table 2. Hydrogen bonds [Å and °]

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
N(1)–H(01)···O(1)[a]	0.83(2)	2.04(2)	2.8130(18)	156(2)

[a] Symmetry transformations used to generate equivalent atoms: $-x$, $-y+1$, $-z$.

Due to the wide array of biological activity associated with the pyrrolidine moiety as well as the 2-aminoimidazole ring, the biological activity of this hybrid template has been studied. A panel of 13 human tumor cell lines was used to evaluate the cytotoxic potential of compounds **12a** and **13a**: prostate carcinoma tumor cells (DU-145 and LN-CaP), SKOV-3 ovary adenocarcinoma, ovarian cells sensitive (IGROV) or resistant (IGROV-ET) to ET-743, SK-BR-3 breast adenocarcinoma, MEL-28 malignant melanoma, H-MEC-1 endothelium cells, A-549 lung carcinoma NSCL, PANC-1 pancreatic epitheloid carcinoma, HT-29 colon carcinoma cells, and LoVo lymph node metathesis cells and the corresponding LoVo-Dox cells resistant to Doxorubicin. A conventional colorimetric assay was set up to estimate GI_{50} values, i.e. the drug concentration, which causes 50% cell growth inhibition after 72 h of continuous exposure to the test molecule. The results obtained are shown in Table 3.

In general, compounds **12a** and **13a** have cytotoxic activity against the 13 human tumor cell lines being **13a** more active than **12a**. Perhaps the presence of the 2-aminoimidazole ring in **13a** explained the difference in the activity. Surprisingly, compound **13a** has the best inhibitory activity against A-549 (lung carcinoma NSCL) cells.

3. Conclusion

In conclusion a method based on unprecedented reaction of an *E*-phosphazide, an intermediate in the Staüdinger reaction, between a tertiary phosphine and organic azide with isocyanates or isothiocyanates allows the formation of hydantoins or thiohydantoins under mild reaction conditions. The prepared thiohydantoin derivatives undergo a new oxidative spirocyclization reaction promoted by DDQ leading to a spiropyrrolidine–imidazole hybrids, which display interesting cytotoxic activity.

4. Experimental

4.1. General

All reactions were carried under N_2 and solvents were dried by standard procedures. Column chromatography purifications were performed using silica gel (60 Å C-C, 70–200 μm, SDS) as the stationary phase. All melting points were determined on a hot-plate melting point apparatus and are uncorrected. NMR spectra were recorded at 200, 300 or 400 MHz using $CDCl_3$ or DMSO-d_6. Chemical shifts were reported in parts per million (δ scale) relative to Me_4Si as an internal standard, and all J values were in Hertz, assignments were made by DEPT or two-dimensional NMR experiments. The FAB positive mass spectra were recorded using 3-nitrobenzylalcohol as matrix.

4.1.1. Ethyl 5-(1*H*-pyrrole-2-carboxamido)pentanoate (5). A mixture of ethyl 5-aminovaleriato hydrochloride (2.68 g, 14.7 mmol), 2-trichloroacetylpyrrole (3.92 g, 18.5 mmol), and triethylamine (1.87 g, 18.5 mmol) in anhydrous acetonitrile (80 mL), was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel, using CH_2Cl_2/MeOH (9.5:0.5) as an eluent, to give compound **5** (3.15 g, 90% yield); mp 74–75 °C (white prisms from EtOAc/*n*-hexane). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (t, J=7.1 Hz, 3H), 1.50–1.75 (m, 4H), 2.34 (t, J=6.9 Hz, 2H), 3.41 (dt, 2H, J=6.3, 6.1 Hz, 2H), 4.12 (q, J=7.1 Hz, 2H), 6.10–6.25 (m, 1H), 6.43 (t, J=5.6 Hz, 1H), 6.60–6.65 (m, 1H), 6.85–6.95 (m, 1H), 10.34 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 22.0, 29.0, 33.7, 38.8, 60.3, 109.0, 109.4, 121.6, 125.9, 161.4, 173.6. EIMS *m*/*z* (%): 238 (M^+, 7), 144 (12), 110 (11), 94 (pyrroleCO$^+$, 100). Anal. Calcd for $C_{12}H_{18}N_2O_3$: C, 60.49; H, 7.61; N, 11.76. Found: C, 60.65; H, 7.73; N, 11.64.

Table 3. Data of in vitro cytotoxicity (GI_{50} μM) of compounds **12a** and **13a**

CELL LINE	**12a**	**13a**	CELL LINE	**12a**	**13a**	CELL LINE	**12a**	**13a**
DU-145	35.9	38.3	IGROV-ET	35.9	33.9	A-549	31.0	0.1
LN-CaP	9.7	6.3	SK-BR-3	22.7	7.2	PANC-1	21.7	12.8
SKOV-3	28.7	11.4	MEL-28	35.9	18.4	LoVo	7.8	1.7
IGROV	15.1	9.2	H-MEC-1	35.9	19.8	LoVo-Dox	35.9	25.0

4.1.2. Ethyl 5-(1*H*-pyrrole-2-carboxamido)-2-azidopentanoate (6). To a −78 °C cooled solution of diisopropylamine (4.9 mL, 34.6 mmol) in anhydrous THF (75 mL), *n*-butyllithium (20.87 mL, 33.4 mmol, 1.6 M in *n*-hexane) was added dropwise under N_2. The solution was stirred at −78 °C for 45 min, then a solution of amidoester **5** (1 g, 4.2 mmol) in THF (50 mL) was added, and the reaction mixture was allowed to warm to −30 °C, stirred for 1 h and recooled to –78 °C. Hexamethylphosphoric triamide (HMPA) (11.44 mL, 64.5 mmol) was added in one portion and then a precooled (−78 °C) solution of trisyl azide (5.20 g, 16.8 mmol) in THF (20 mL) was added. The reaction mixture was stirred at −78 °C for 1 h, and then was quenched with glacial acetic acid (7.2 mL, 126 mmol), allowed to warm to room temperature, stirred for 12 h, treated with an saturated solution of $NaHCO_3$ (300 mL) and extracted with dichloromethane (3×100 mL). The combined organic layers were washed with a solution of NaCl (1×100 mL) and H_2O (2×100 mL), dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using EtOAc/*n*-hexane (3:2) as an eluent to give **6** (0.82 g, 70% yield). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (t, J=7.4 Hz, 3H), 1.60–2.00 (m, 4H), 3.44 (td, J=6.2, 5.8 Hz, 2H), 3.90 (dd, J=7.6, 5.6 Hz, 1H), 4.24 (q, J=7.4 Hz, 2H), 6.21 (m, 1H), 6.32 (t, J=5.8 Hz, 1H), 6.61 (m, 1H), 7.08 (m, 1H), 10.12 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 26.0, 28.6, 38.5, 61.6, 61.9, 109.2, 109.6, 121.8, 125.6, 161.5, 170.3. MS: *m*/*z* (%) (FAB positive) 302 ($(M+Na)^+$, 82), 280 ($(M+H)^+$, 85), 267 (43), 251 ($(M-N_2)^+$, 31), 235 ($(M-EtOH+H)^+$, 63), 94 (pyrroleCO$^+$, 100). Anal. Calcd for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.08. Found: C, 51.68; H, 6.20; N, 25.01.

4.1.3. General procedure for the preparation of hydantoins (10) and thiohydantoins (11). To a solution of triphenylphosphine (0.47 g, 1.79 mmol) in dry diethyl ether (15 mL) a solution of the α-azido ester **6** (0.5 g, 1.79 mmol) in the same solvent (15 mL) at 0 °C was added dropwise under N_2. The resultant solution was allowed to warm to room temperature and stirred for 12 h. Then, it was cooled to 0 °C and a solution of the appropriate iso(thio)cyanate (1.79 mmol) was added. The resultant mixture was stirred at room temperature for 48 h and then THF/H_2O (9:1) (10 mL) was added and stirring was continued for 1 h. The solution was concentrated to dryness and the residue was chromatographed on a silica gel column using EtOAc as an eluent to give **10** and **11**.

4.1.3.1. Compound 10a. Yield 75% (R^1=Et, X=O), mp: 138 °C (from EtOAc/EtOH/*n*-hexane (4:1:5)). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.05 (t, J=7.2 Hz, 3H), 1.49–1.60 (m, H-9, 4H), 3.10–3.28 (m, 2H), 3.32 (q, J=7.2 Hz, 2H), 4.04 (t, J=4.8 Hz, 1H), 6.03–6.07 (m, 1H), 6.70–6.73 (m, 1H), 6.80–6.83 (m, 1H), 8.00 (t, J=5.5 Hz, 1H), 8.12 (s, 1H), 11.27 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.7, 25.2, 29.3, 32.9, 38.4, 56.4, 108.9, 110.0, 121.5, 126.7, 157.1, 161.0, 174.5. MS: *m*/*z* (%) (FAB positive) 279 (M+H, 100), 278 (M^+, 24). Anal. Calcd for $C_{13}H_{18}N_4O_3$: C, 56.10; H, 6.52; N, 20.13. Found: C, 56.17; H, 6.46; N, 20.06.

4.1.3.2. Compound 10b. Yield 65% (R^1=Pr, X=O), mp: 143–144 °C (from EtOAc/EtOH/*n*-hexane (5:1:4)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81 (t, J=7.5 Hz, 3H), 1.44–1.59 (m, 6H), 3.31–3.22 (m, 2H), 3.29 (t, J=7.2 Hz, 2H), 4.04 (t, J=4.5 Hz, 1H), 6.04–6.07 (m, 1H), 6.70–6.72 (m, 1H), 6.80–6.81 (m, 1H), 7.87 (t, J=5.5 Hz, 1H), 8.10 (s, 1H), 11.25 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.0, 20.9, 24.8, 28.9, 38.3, 39.5, 56.0, 108.4, 109.6, 121.1, 126.3, 156.8, 160.6, 174.3. MS: *m*/*z* (%) (FAB positive) 293 (M+H, 19), 292 (M^+, 100). Anal. Calcd for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.17. Found: C, 57.58; H, 6.84; N, 19.23.

4.1.3.3. Compound 10c. Yield 58% (R^1=*i*-Pr, X=O), mp: 116–117 °C (from EtOAc/EtOH/*n*-hexane (4:1:5)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (d, J=6.9 Hz, 3H), 1.28 (d, J=6.9 Hz, 3H), 1.48–1.73 (m, 4H), 3.18–3.19 (m, 2H), 3.98 (t, J=6.0 Hz, 1H), 4.10 (septet, J=6.9 Hz, 1H), 6.04–6.06 (m, 1H), 6.70–6.72 (m, 1H), 6.80–6.81 (m, 1H), 7.99 (t, J=6 Hz, 1H), 8.17 (s, 1H), 11.38 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.88, 19.78, 24.8, 29.1, 38.4, 42.6, 55.7, 108.6, 109.8, 121.3, 126.5, 156.8, 160.8, 174.3. MS: *m*/*z* (%) (FAB positive) 293 (M+H, 100), 292 (M^+, 24). Anal. Calcd for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.17. Found: C, 57.60; H, 6.81; N, 19.25.

4.1.3.4. Compound 10d. Yield 55% (R^1=Bn, X=O), mp: 157–158 °C (from EtOAc/EtOH/*n*-hexane (4:1:5)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52–1.74 (m, 4H), 3.20 (t, J=5.7 Hz, 2H), 4.15 (t, J=5.3 Hz, 1H), 4.50 (s, 2H), 6.04–6.06 (m, 1H), 6.71–6.72 (m, 1H), 6.80–6.82 (m, 1H), 7.20–7.31 (m, 5H), 7.97 (t, J=5.7 Hz, 1H), 8.35 (s, 1H), 11.36 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.4, 29.4, 38.3, 41.4, 56.6, 108.9, 109.0, 121.5, 126.7, 127.2, 127.7, 128.9, 137.2, 157.0, 161.0, 174.6. MS: *m*/*z* (%) (FAB positive) 341 (M+H, 100), 340 (M^+, 27). Anal. Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.46; H, 5.96; N, 16.52.

4.1.3.5. Compound 11a. Yield 75% (R^1=Me, X=S), mp: 200–202 °C (from EtOAc). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50–1.90 (m, 4H), 3.06 (s, 3H), 3.22 (td, J=6.0, 5.55 Hz, 2H), 4.24–4.33 (m, 1H), 6.05–6.09 (m, 1H), 6.74 (m, 1H), 6.83 (d, J=0.9 Hz, 1H), 7.98 (t, J=5.5 Hz, 1H), 10.34 (s, 1H), 11.36 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.8, 26.8, 28.3, 37.9, 58.7, 108.5, 109.7, 121.2, 126.3, 160.7, 174.8, 182.9. EIMS *m*/*z* (%): 281 (M+1, 6), 280 (M, 44), 213 (24), 94 (100). Anal. Calcd for $C_{12}H_{16}N_4O_2S$: C, 51.41; H, 5.75; N, 19.98. Found: C, 51.35; H, 5.82; N, 19.91.

4.1.3.6. Compound 11b. Yield 65% (R^1=Et, X=S), mp: 177–178 °C (from EtOAc/EtOH/*n*-hexane (7:0.5:2.5)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (t, J=7.1 Hz, 3H), 1.45–1.75 (m, 4H), 3.19 (m, 2H), 3.65 (q, J=7.1 Hz, 2H), 4.26 (t, J=5.7 Hz, 1H), 6.04–6.06 (m, 1H), 6.71 (m, 1H), 6.81 (m, 1H), 7.99 (t, J=5.7 Hz, 1H), 10.34 (s, 1H), 11.38 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.9, 24.6, 28.3, 35.1, 37.9, 58.4, 108.4, 109.7, 121.1, 126.3, 160.6, 174.4, 182.2. MS: *m*/*z* (%) (FAB positive) 295 (M+H, 100). Anal. Calcd for $C_{13}H_{18}N_2O_4S$: C, 53.04; H, 6.16; N, 19.03. Found: C, 53.11; H, 6.10; N, 18.98.

4.1.3.7. Compound 11c. Yield 60% (R^1=Pr, X=S), mp: 129–130 °C (from EtOAc/EtOH/*n*-hexane (9:1)). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81 (t, J=7.4 Hz, 3H), 1.45–1.60 (m, 4H), 3.17 (m, 2H), 3.59 (t, J=7.4 Hz, 2H), 4.28

(t, J=5.7 Hz, 1H), 6.03–6.07 (m, 1H), 6.70–6.73 (m, 1H), 6.80–6.83 (m, 1H), 8.0 (t, J=5.7 Hz, 1H), 10.35 (s, 1H), 11.38 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.9, 20.9, 25.1, 28.7, 38.5, 39.7, 58.8, 108.8, 110.0, 121.5, 126.7, 161.0, 175.2, 183.0. MS: *m/z* (%) (FAB positive) 309 (M+H, 100), 308 (M^+, 26). Anal. Calcd for $C_{14}H_{20}N_4O_2S$: C, 54.52; H, 6.54; N, 18.17. Found: C, 54.57; H, 6.50; N, 18.10.

4.1.3.8. Compound 11d. Yield 56% (R^1=Bn, X=S), mp: 162–163 °C (from EtOAc/EtOH/*n*-hexane (9:1)).^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49–1.78 (m, 4H), 3.18–3.25 (m, 2H, H-8), 4.36 (t, J=5.7 Hz, 1H), 4.86 (s, 2H), 6.04–6.07 (m, 1H), 6.71–6.72 (m, 1H), 6.81–6.83 (m, 1H), 7.20–7.30 (m, 5H), 7.93 (t, J=5.7 Hz, 1H), 10.43 (s, 1H), 11.30 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.7, 28.34, 37.8, 43.2, 58.5, 108.3, 109.5, 121.0, 126.3, 127.2, 127.3, 128.2, 136.3, 160.6, 174.6, 182.4. EIMS *m/z* (%): 358 (M+2, 11), 356 (M^+, 91). Anal. Calcd for $C_{18}H_{20}N_2O_4S$: C, 60.65; H, 5.66; N, 15.72. Found: C, 60.59; H, 5.62; N, 15.67.

4.1.4. Preparation of spiro-compounds (12). To a solution of the appropriate thiohydantoin **11a** (0.3 g, 1.07 mmol) in anhydrous THF (30 mL), DDQ (0.3 g, 1.34 mmol) was added under N_2. After stirring at room temperature for 24 h, the same amount of DDQ was added and the mixture was stirred for additional 48 h. The solvent was removed under reduced pressure and the residue was treated with EtOAc (100 mL) and filtered. The organic phase was washed with an aqueous saturated solution of $NaHCO_3$ (2×30 mL) and water (30 mL). The combined organic layers were dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel, using EtOAc/*n*-hexane (3:2) as an eluent, to give the title compound **12a** (R_f=0.54, 0.25 g, yield 85%).

4.1.4.1. 3-Methyl-6-(1*H*-pyrrole-2-carbonyl)-2-thioxo-1,3,6-triazaspiro[4.4]nonan-4-one (12a). Mp >264 °C (dec), yellow prisms (EtOAc/*n*-hexane). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.01–2.27 (m, 4H), 3.12 (s, 3H), 3.74–3.94 (m, 2H), 6.19 (dt, J=3.6, 2.4 Hz, 1H), 6.74 (m, 1H), 6.97 (m, 1H), 10.60 (s, 1H), 11.75 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.6, 27.1, 35.6, 48.5, 78.9, 109.6, 113.5 (C-4), 123.1, 124.0, 159.4, 172.7, 182.3. EIMS *m/z* (%): 279 (M^++1, 2), 278 (M^+, 10), 168 (M^+−Me−pyrrole–CO, 9), 94 (pyrroleCO$^+$, 100), 66 (pyrrole$^+$, 26). Anal. Calcd for $C_{12}H_{14}N_4O_2S$: C, 51.78; H, 5.07; N, 20.13. Found: C, 51.66; H, 5.22; N, 20.26.

4.1.4.2. 3-Ethyl-6-(1*H*-pyrrole-2-carbonyl)-2-thioxo-1,3,6-triazaspiro[4.4]nonan-4-one (12b). Yield 70%, mp: 118–120 °C (from Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (t, J=7.1 Hz, 3H), 2.09–2.39 (m, 4H), 3.79–3.72 (m, 2H), 3.86–4.03 (m, 2H), 6.18–6.20 (m, 1H), 6.57–6.59 (m, 1H), 6.84–6.86 (m, 1H), 8.66 (s, 1H), 10.44 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.1, 24.2, 36.2, 36.6, 48.9, 78.9, 109.7, 113.3, 122.7, 124.5, 161.0, 172.1, 183.1. EIMS *m/z* (%): 293 (M^++1, 4), 292 (M^+, 22), 94 (pyrroleCO$^+$, 100), 66 (pyrrole$^+$, 28). Anal. Calcd for $C_{13}H_{16}N_4O_2S$: C, 53.42; H, 5.48; N, 19.18. Found: C, 53.36; H, 5.40; N, 20.05.

4.1.4.3. 3-Benzyl-6-(1*H*-pyrrole-2-carbonyl)-2-thioxo-1,3,6-triazaspiro[4.4]nonan-4-one (12d). Yield 60%, mp: 218–220 °C (from Et_2O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.11–2.19 (m, 4H), 3.84–3.90 (m, 2H), 4.81 (d, J=15.3 Hz, 1H), 5.05 (d, J=15.3 Hz, 1H), 6.19 (m, 1H), 6.74 (m, 1H), 6.98 (m, 1H), 7.24–7.37 (m, 5H), 10.68 (s, 1H), 11.7 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.6, 38.4, 43.6, 48.6, 78.9, 109.5, 113.5, 123.1, 124.0, 127.1, 127.3, 128.3, 136.5, 159.4, 170.3, 181.8. LC/MS: *m/z* (%) 355 (M+H, 100). Anal. Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.01; H, 5.12; N, 15.81. Found: C, 59.91; H, 5.00; N, 15.76.

4.1.5. Synthesis of spiro-2-aminohydantoin (13). To a solution of the corresponding spirothiohydantoin **12** (0.36 mmol) in methanol (14 mL), the appropriate amine (0.47 mmol) or aqueous ammonia (30%, 4 mL) was added. After stirring at room temperature for 15 min TBHP (70%, 0.2 mL, 1.44 mmol) was added. The resulting reaction mixture was stirred for 36 h at room temperature, then the same amount of TBHP was added and stirring was continued for an additional 36 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/EtOH/aqueous NH_3 (7:2.5:0.5)) in deactivated silica gel with a EtOH/NH_3 mixture (30%) (9:1), to give the title compound **13**.

4.1.5.1. 2-Amino-3-methyl-6-(1*H*-pyrrole-2-carbonyl)-1,3,6-triazaspiro[4.4]non-1-en-4-one (13a). Yield 60%, mp: 212–214 °C (from Et_2O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75–2.15 (m, 4H), 2.75 (s, 3H), 3.60–3.95 (m, 2H), 6.10–6.20 (m, 1H), 6.57–6.67 (m, 1H), 6.89–6.99 (m, 1H), 7.15–7.55 (br s, 1H), 7.75–8.20 (br s, 1H), 11.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.5, 27.8, 36.9, 48.8, 80.7, 109.1, 112.5, 122.1, 125.4, 158.9, 169.8. MS: *m/z* (%) (FAB positive) 523 (2M+H$^+$, 15), 262 (M+H$^+$, 100). Anal. Calcd for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.79; N, 26.80. Found: C, 55.10; H, 5.73; N, 26.85.

4.1.5.2. 2-Benzylamino-3-methyl-6-(1*H*-pyrrole-2-carbonyl)-1,3,6-triazaspiro[4.4]non-1-en-4-one (13b). Yield 60%, mp: 122–124 °C (from Et_2O). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.92–2.27 (m, 4H), 3.0 (s, 3H), 3.85–4.01 (m, 2H), 4.29 (d, J=14.4 Hz, 1H), 4.58 (d, J=14.4 Hz, 1H), 6.14 (m, 1H), 6.54 (m, 1H), 6.75 (m, 1H), 7.16–7.28 (m, 5H), 9.21 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 25.7, 37.3, 46.2, 49.2, 84.9, 110.0, 113.2, 121.7, 125.1, 127.6, 128.1, 128.6, 138.2, 155.8, 160.0, 178.5. EIMS *m/z* (%): 352 (M^++1, 4), 351 (M^+, 13), 241 (55), 150 (51), 94 (pyrroleCO$^+$, 67), 91 (100), 66 (pyrrole$^+$, 19). Anal. Calcd for $C_{19}H_{21}N_5O_2$: C, 64.94; H, 6.02; N, 19.93. Found: C, 64.87; H, 5.58; N, 20.01.

4.1.5.3. 2-Benzylamino-3-benzyl-6-(1*H*-pyrrole-2-carbonyl)-1,3,6-triazaspiro[4.4]non-1-en-4-one (13c). Yield 68%, mp: 208–210 °C (from Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04–2.05 (m, 1H), 2.17–2.26 (m, 3H), 3.91–3.95 (m, 1H), 3.99–4.01 (m, 1H), 4.16 (d, J=14.5 Hz, 1H), 4.56 (d, J=16.4 Hz, 1H), 4.61–4.66 (m, 1H), 4.97 (d, J=16.4 Hz, 1H), 6.12 (m, 1H), 6.54 (m, 1H), 6.60 (m, 1H), 6.90 (m, 2H), 7.09–7.22 (m, 8H), 8.44 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 37.8, 36.9, 42.7, 45.3, 49.4, 85.9, 109.9, 113.1, 121.3, 125.1, 127.0, 127.3, 127.4, 127.7, 128.4, 128.9, 135.8, 138.3, 156.1, 159.9, 179.5. MS: *m/z* (%) (FAB positive) 428 (M+H$^+$, 100), 427 (M^+, 40), 361, 335. Anal. Calcd for $C_{25}H_{25}N_5O_2$: C, 70.24; H, 5.89; N, 16.38. Found: C, 70.19; H, 5.82; N, 16.31.

4.2. Cell growth inhibition assay: screening

A colorimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[21] Cells were seeded in 96 well microtiter plates, at 5×10^3 cells per well in aliquots of 195 μL of RPMI medium, and they are allowed to attach to the plate surface by growing in drug free medium for 18 h. Afterward, samples are added in aliquots of 5 μL (dissolved in DMSO/H_2O (3:7)). After 72 h exposure, the antitumor effect is measured by the SRB methodology: cells are fixed by adding 50 μL of cold 50% (w/v) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates are washed with deionized H_2O and dried; 100 μL of SRB solution (0.4% w/v in 1% acetic acid) is added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air-dried and bound stain is solubilized with Tris buffer. Optical densities are read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses are generated automatically by LIMS implementation. Using control OD values (C), test OD values (T), and time zero OD values (T_0), the drug concentration that causes 50% growth inhibition (GI_{50} value) was calculated from the equation: $100[(T-T_0)/(C-T_0)]=50$.

4.3. X-ray structure determinations

The crystal and molecular structures of compound **12a** have been determined by X-ray diffraction studies (Table 1). A crystal was mounted on glass fibers and transferred to the cold gas stream of the diffractometer Siemens P4. Data were recorded with Mo Kα radiation (l=0.71073 Å) in w-scan mode. Structure of **12a** was solved by the direct method and refined anisotropically on F^2 (program SHELXL-97).[22] The hydrogens at N were located in the Fourier difference maps and refined freely. Methyl groups were refined using rigid groups and other hydrogens were refined using a riding method.

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Supplementary data

Crystallographic data and structure refinement of compound **12a**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.12.021.

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