Pergamon

Tetrahedron 58 (2002) 9723–9727

TETRAHEDRON

New tricyclic systems of biological interest. Annelated 1,2,3-triazolo[1,5-*a*]pyrimidines through domino reaction of 3-azidopyrroles and methylene active nitriles

Antonino Lauria, Patrizia Diana, Paola Barraja, Alessandra Montalbano, Girolamo Cirrincione, Gaetano Dattolo and Anna Maria Almerico*

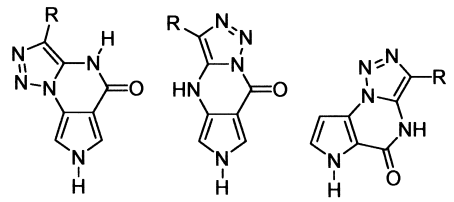
Dipartimento Farmacochimico, Tossicologico e Biologico, Università degli Studi, Via Archirafi 32, I-90123 Palermo, Italy

Received 27 June 2002; revised 12 September 2002; accepted 3 October 2002

Abstract—Anionic hetero-domino reaction of 3-azidopyrroles and acetonitriles constitutes the synthetic entry to annelated 1,2,3-triazolo[1,5-*a*]pyrimidines. Upon slight variations of the experimental conditions the method is of general application either with 2 or 4 substituted pyrroles. Thus derivatives of the new ring system pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine, isomers of the previously synthesized pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine, were prepared in high yields. The condensed pyrrolo-triazolo-pyrimidines, although only moderately active, can be used as model for the design of DNA-interactive compounds. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

In the course of our researches devoted to the development of new classes of heteroaromatic systems which incorporated the pyrrole/indole moiety, we have been interested in the synthesis and evaluation of the biological activity of several flat polycondensed nitrogen heterocycles, either tricyclic and tetracyclic, that can potentially intercalate into double-stranded DNA. In this context we have prepared and reported the interesting antiproliferative activity of indolo[3,2-*c*]cinnolines,[1] indolo [1,2-*c*][1,2,3]benzotriazines,[2] and pyrrolo[1,2-*f*]phenanthridines,[3] that can be related to the well known classes of intercalators with linear or angular structure such as acridines, anthracyclines and phenanthridines. These compounds possess such a property whose principal driving forces are π-stacking and charge-transfer interactions as well as hydrogen bonding and electrostatic forces.[4] More recently we explored the synthetic access to angular heterotricycles and we reported the preparation[5] of the new system, pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine of type **1** and its rearrangement to pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine of type **2**. The above systems can both be related to DNA-interactive drugs (Fig. 1).



1 **2** **3**

Figure 1.

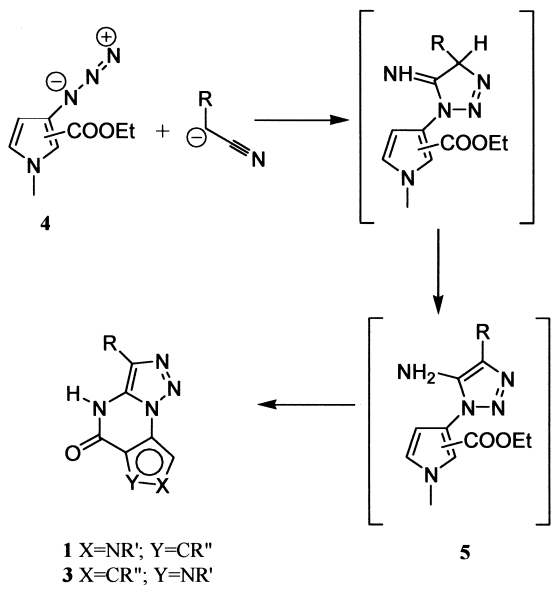
In connection with these studies in this paper we discuss the synthetic approach to a new tricyclic angular system, namely pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine of type **3**, isomer of derivatives of type **1**, and report the results of preliminary antitumor screening tests carried out on all these classes of annelated pyrrolo-triazolo-pyrimidines.

Routes to ring systems **1** and **3** can be provided by domino reactions between acetonitriles and azidopyrroles **4** under basic conditions (Scheme 1). These last through the azido moiety and can act as a 1,3-dipolar compound in cycloaddition reactions with dipolarophiles such as the anions obtained from the methylene active derivatives. Although this type of reaction is well described in aromatic series, only in a few cases has it been applied to pentatomic heterocycles.[6] Moreover in azole series the only two examples reported so far have demonstrated that the nature of the substrate and the reaction conditions can widely influence the nature of the reaction products.[7] The intermediate resulting from the cycloaddition reaction

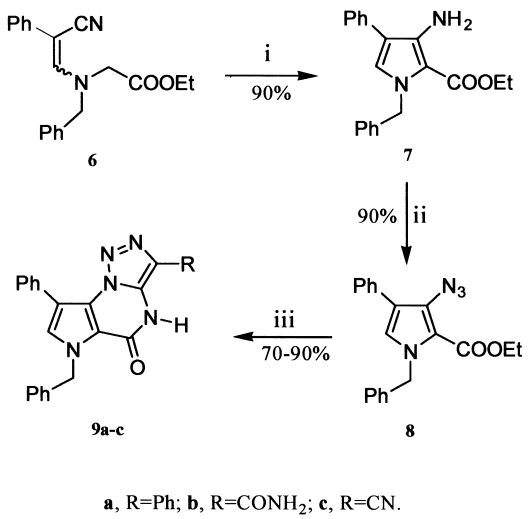
Keywords: heterotricyclic systems; pyrrolo-triazolo-pyrimidine; domino reaction; antiproliferative activity.

* Corresponding author. Tel.: +39-091-6161606; fax: +39-091-6169999; e-mail: almerico@unipa.it

0040–4020/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0040-4020(02)01245-0



Scheme 1.



a, R=Ph; **b**, R=CONH$_2$; **c**, R=CN.

Scheme 2. (i) EtONa, EtOH, 70°C, 6 h; (ii) $NaNO_2$, AcOH, 0°C; NaN_3, 0°C–rt; (iii) RCH_2CN, EtONa, rt.

could be the 3-(triazol-1-yl)pyrrole of type **5** which bears an amino group susceptible to further reactions. In the presence of a vicinal carboxylate function, intermediate **5** further cyclizes to provide the pyrimidine ring.

This type of anionic hetero-domino reaction has been successful when the starting compound was ethyl 3-azido-pyrrole-4-carboxylate, which represented the first example of such a sequence in pyrrole series.[5] However, since the reactivity of pyrrole derivatives varies greatly switching the positions of the substituents or moving them from a β to an α position,[8] and is strongly dependent on the reaction conditions, we thought to explore whether ethyl 3-azidopyrrole-2-carboxylate compounds behave similarly.

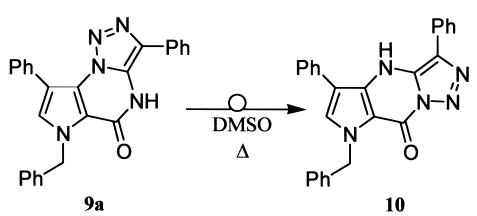
2. Results and discussion

The starting material **8** was easily prepared from ethyl 3-amino-1-benzyl-4-phenylpyrrole-2-carboxylate (**7**), obtained in turn by cyclization of **6Z** under basic conditions according to literature procedure[9] (Scheme 2). The 3-aminopyrrole **7** was diazotized with sodium nitrite in acetic acid. Addition of sodium azide to the intermediate diazonium salt led to the 3-azidopyrrole **8** in high yield.

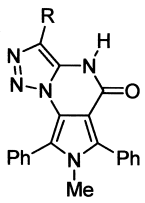
The azide **8** was added at room temperature to the sodium salts of acetonitriles in ethanol, under the identical experimental conditions successfully used in the case of the isomeric ethyl 3-azidopyrrole-4-carboxylate. In this case after 24 h at room temperature the TLC analysis showed mainly unreacted starting compounds. However, since ethanol is eliminated in the final step of this domino sequence, we decided to drive the reaction to completeness by removing the solvent under reduced pressure. In fact upon reduction of the volume of the reaction solution, the appearance of the new compounds was observed (TLC monitoring). Therefore it seems that in this case the bond-forming efficiency depends on the amount of ethanol present in the reaction environment in contrast with what was observed in the case of ethyl 3-azidopyrrole-4-carboxylate.

The new compounds **9a–c**, derivatives of the new ring system 4*H*-pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine, were generally isolated in high yields (70–90%). Their structure was confirmed by spectroscopic data; in particular by the presence in the IR spectra of the typical absorption bands (at 3450–3221 and 1667–1649 cm^{-1}) due to the presence of a cyclic-amide structure. In the ^1H NMR the amide NH signal was found at 11.15–11.17 ppm, and in the ^{13}C NMR the carbonyl peak was found at 148.6–158.5 ppm.

As already demonstrated in the case of pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives of type **1**, which rearranged to pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine of type **2**,[5] compound **9a** was heated under reflux in aqueous DMSO to give 7-benzyl-3,5-diphenyl-4,7-dihydro-8*H*-pyrrolo[3,2-*d*][1,2,3]triazolo[1,5-*a*]pyrimidin-8-one (**10**) in nearly quantitative yield (Scheme 3). This conversion reaction can be envisaged as a ring-opening ring-closure of the pyrimidine and triazole rings in the presence of water.[5] The participation of water in this rearrangement came from the observation that the reaction does not occur in dry solvents and is in agreement with a well acknowledged literature report on the Dimroth rearrangement in pyrimidine series.[10] In our opinion, the direct rearrangement involving only opening of the triazole ring has to be ruled out because of the failure of the transformation under thermal conditions.



Scheme 3.



11a-c

a R=Ph; **b** R=$CONH_2$; **c** R=CN

Figure 2.

All the derivatives of the annelated 1,2,3-triazolo[1,5-*a*] pyrimidine ring systems, obtained by using this type of domino reaction, compounds **9a–c**, **10**, and **11a–c**[5] (Fig. 2) were investigated for their biological activity.

Prior to the evaluation of the potential ability of the new annelated triazolo-pyrimidine ring systems to interact with DNA, we calculated[11] the LUMO and HOMO energies, considering that these variables are of importance when two molecules with π electron systems form charge-transfer complexes, and also the values of some molecular descriptors [MR (molar refractivity), ASA (accessible surface area)].

An analysis of the data reported in Table 1 shows that these values are comparable with those of well known DNA-intercalators of the acridine or anthracycline classes such as Amsacrine (AMSA) and Doxorubicin (DOXO). Therefore, these pyrrolo-triazolo-pyrimidine derivatives appear to be good candidate as antitumor drugs.

Compounds **9a–c**, **10**, and **11a–c** were screened at the NCI (Bethesda) in the Developmental Therapeutics Program. In the primary anticancer screening assay,[12] when tested against a 3-cell line panel consisting of the MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) the pyrrolo-triazolo-pyrimidine resulted inactive up to 10^{-4} M concentrations. All the derivatives were also tested against the human intestinal adenocarcinoma (LoVo), however, only compounds **11a–c** exhibited a moderate antiproliferative activity with GI_{50}=24.0–37.5 μM.[13]

Table 1. Molecular descriptors for annelated 1,2,3-triazolo[1,5-*a*]pyrimidines

Compound	LUMO (eV)	HOMO (eV)	MR[a]	ASA (Å^2)
9a	−0.7437	−8.7851	124.70	388.52
9b	−0.8271	−9.2677	108.15	346.72
9c	−1.0409	−9.4766	105.76	333.26
10	−0.7452	−8.4161	124.78	379.05
11a	−0.5561	−8.5358	125.11	364.84
11b	−0.6535	−9.0393	108.55	334.79
11c	−0.8288	−9.2694	106.16	323.32
AMSA	−1.2638	−8.1826	107.77	353.31
DOXO	−1.3378	−8.8961	134.02	467.29

These calculations were performed with the software TSAR (V 3.2), running on an Indigo II Silicon Graphics work station.

[a] Predicted values were calculated as a sum of the atomic values determined according to Viswanadhan et al.[15]

3. Conclusions

In conclusion, domino reactions between 3-azidopyrroles and acetonitriles constitute a versatile entry to annelated 1,2,3-triazolo[1,5-*a*]pyrimidines. Although in the presence of the same type of complexity (two rings formation in sequence) for this type of domino reaction the bond-forming economy greatly depends on the nature of the starting material. However, minor modification of the experimental procedure allows the reaction to become suitable for a general application in pyrrole series.

Some of the variously condensed pyrrolo-triazolo-pyrimidine ring systems showed only a moderate antiproliferative activity. However, because of their structural features they can constitute a model suitable for the design of new potential DNA-interactive molecules worthy of further developments.

4. Experimental

4.1. General

Melting points (uncorrected) were taken on a Buchi-Tottoli capillary apparatus; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively, in $(CD_3)_2SO$ solution, unless otherwise specified, using a Bruker AC-E series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with a Biotage FLASH40i chromatography module (prepacked cartridge system).

4.1.1. Ethyl 3-amino-1-benzyl-4-phenyl-1*H*-pyrrole-2-carboxylate (7). This aminopyrrole was prepared according to the literature procedure.[9] Thus a solution of 3-oxo-2-phenylpropanenitrile[14] (1.0 g, 6.9 mmol) and *N*-benzyl-glicine ethyl ester (1.6 g, 8.3 mmol) in benzene (25 mL) was heated to reflux under a water separator (Dean-Stark trap) for 10 h and then evaporated under reduced pressure. Trituration of the residue with ethyl ether afforded a white crystalline solid (900 mg) identified as **6Z**. After filtration, the ethereal mother liquid was chromatographed using dichloro-methane/methanol 30:1 as eluant. The first product to be eluted was **6Z** (380 mg). Further elution gave a syrup identified as **6E** (230 mg). A solution of **6Z** (770 mg, 2.4 mmol) in 0.28 M sodium ethoxide in ethanol (10 mL) was heated at 70°C for 6 h. The reaction mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The residue was partitioned between water and ethyl acetate. After separation of the organic phase, the water layer was neutralized with acetic acid and extracted twice with ethyl acetate. The organic layer and washings were combined, dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using dichloromethane/methanol 30:1 as eluant to give derivative **7** as a colorless oil which solidified on standing (690 mg, yield 90%), mp 65°C (lit.,[9] mp 67°C).

4.1.2. Ethyl 3-azido-1-benzyl-4-phenyl-1*H*-pyrrole-2-carboxylate (8). To a solution of **7** (3.2 g, 10 mmol) in acetic acid (30 mL) and water (4 mL), sodium nitrite

(0.83 g, 12 mmol) in water (4 mL) was added at 0°C, under vigorous stirring. After 50 min sodium azide (3.25 g, 50 mmol) was added in portions and the reactants were stirred for a further 3 h at room temperature. The solid was filtered off and air dried to give **8** as a yellow precipitate: 3.1 g, yield 90%, mp 110°C; IR: 2120 (N_3), 1693 (CO) cm^1; ^1H NMR δ: 1.23 (3H, t, J=6.4 Hz, CH_2CH_3), 4.22 (2H, q, J=6.4 Hz, CH_2CH_3), 5.54 (2H, s, CH_2), 7.12–7.71 (11H, m, 2×C_6H_5, H-5); ^{13}C NMR δ: 14.0 (q), 52.5 (t), 60.1 (t), 108.7 (s), 112.9 (s), 124.2 (d), 126.4 (d), 126.5 (d), 127.0 (d), 127.2 (d), 127.3 (s), 128.5 (d), 129.1 (d), 132.1 (s), 138.2 (s), 159.3 (s). Anal. Calcd for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.25; H, 5.29; N, 16.25.

4.2. General method for the preparation of 3-substituted 6-benzyl-5-oxo-8-phenyl-5,6-dihydro-4*H*-pyrrolo[2,3-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidines (9a–c)

To a solution of 1 mM sodium ethoxide in ethanol (3.9 mL) substituted acetonitriles (3.9 mmol) in absolute ethanol (10 mL) were added at room temperature. After being stirred for 15 min a solution of azido-pyrrole **8** (1.2 g, 3.6 mmol) in absolute ethanol (10 mL) was added and the mixture was stirred for a further 24 h at room temperature. Evaporation of the solvent under reduced pressure gave a solid which was purified by column chromatography using dichloromethane/ethyl acetate 95:5 as eluant.

4.2.1. 6-Benzyl-3,8-diphenyl-4*H*-pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(6*H*)-one (9a). From **8** and 2-phenylacetamide, white solid (1.15 g, yield 80%) mp>300°C; IR: 3350 (NH), 1649 (CO) cm^{-1}; ^1H NMR δ: 5.71 (2H, s, CH_2), 7.13–7.42 (11H, m, 2×H-3′, H-4′, 2×H-3″, H-4″, 2×H-3‴, H-4‴, 2×H-2′), 8.09 (1H, s, H-7), 8.34 (2H, d, J=7.4 Hz, 2×H-2″), 8.52 (2H, d, J=7.3 Hz, 2×H-2‴), 11.15 (1H, s, H-4); ^{13}C NMR δ: 50.9 (t), 110.6 (s), 111.9 (s), 123.2 (d), 124.1 (d), 124.4 (d), 125.1 (d), 126.6 (s), 127.2 (d), 127.3 (d), 128.2 (2d), 128.4 (d), 130.7 (d), 134.4 (s), 135.2 (s), 139.6 (s), 143.2 (s), 147.5 (s), 148.6 (s). Anal. Calcd for $C_{26}H_{19}N_5O$: C, 74.80; H, 4.59; N, 16.78. Found: C, 74.76; H, 4.65; N, 16.80.

4.2.2. 6-Benzyl-5-oxo-8-phenyl-5,6-dihydro-4*H*-pyrrolo [2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide (9b). From **8** and 2-cyanoacetamide, white solid (1.24 g, yield 90%) mp>325°C; IR: 3450–3250 (NH_2 and NH), 1662 (CO) cm^{-1}; ^1H NMR δ: 5.72 (2H, s, CH_2), 7.16–7.40 (9H, m, H-7, NH_2, 2×H-3′, H-4′, 2×H-3″, H-4″), 8.08–8.11 (4H, m, 2×H-2′, 2×H-2″), 11.17 (1H, bs, H-4); ^{13}C NMR δ: 51.0 (t), 110.8 (s), 112.0 (s), 123.2 (s), 124.9 (d), 125.2 (d), 127.3 (2d), 128.3 (d), 128.5 (d), 131.1 (d), 134.1 (s), 139.3 (s), 145.0 (s), 146.5 (s), 148.6 (s), 163.1 (s). Anal. Calcd for $C_{21}H_{16}N_6O_2$: C, 65.62; H, 4.20; N, 21.86. Found: C, 65.59; H, 4.22; N, 21.83.

4.2.3. 6-Benzyl-5-oxo-8-phenyl-5,6-dihydro-4*H*-pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carbonitrile (9c). From **8** and malononitrile, white solid (922 mg, yield 70%) mp>325°C; IR: 3221 (NH), 2236 (CN), 1667 (CO) cm^{-1}. ^1H NMR δ: 5.10 (2H, s, CH_2), 6.63–6.79 (8H, m, 2×H-2′, 2×H-3′, H-4′, 2×H-3″, H-4″), 7.58 (1H, s, H-7), 7.67 (2H, d, J=7.8 Hz, 2×H-2″), 11.17 (1H, s, H-4); ^{13}C NMR δ: 51.0 (t), 115.7 (s), 116.5 (s), 125.8 (s), 127.3 (d), 127.5 (d), 128.3 (2d), 128.6 (d), 128.9 (d), 129.1 (d), 133.4 (s), 138.5 (s), 142.0 (s), 142.8 (s), 154.2 (s), 158.5 (s). Anal. Calcd for $C_{21}H_{14}N_6O$: C, 68.84; H, 3.85; N, 22.94. Found: C, 68.87; H, 3.80; N, 22.79.

4.2.4. 7-Benzyl-3,5-diphenyl-4,7-dihydro-8*H*-pyrrolo-[3,2-*d*][1,2,3]triazolo[1,5-*a*]pyrimidin-8-one (10). A solution of 6-benzyl-3,8-diphenyl-4*H*-pyrrolo[2,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(6*H*)-one (**9a**) (0.21 g, 0.5 mmol) was heated under reflux in dimethylsulfoxide (99.8%, 10 mL) for 1 h. The cooled reaction mixture was then poured onto crushed ice and the solid was filtered off, air dried to give derivative **10** (206 mg) as a light yellow solid. Yield 98%, mp 210°C; IR: 3295 (NH), 1685 (CO) cm^{-1}; ^1H NMR δ: 5.78 (s, 2H, CH_2), 7.50–7.33 (m, 11H, 2×C_6H_5, H-6), 7.79–7.97 (m, 5H, C_6H_5), 12.30 (s, 1H, H-4); ^{13}C NMR δ: 50.7 (t), 111.9 (s), 113.3 (s), 117.5 (s), 124.9 (d), 126.5 (d), 127.1 (d), 127.3 (d), 127.4 (d), 127.7 (d), 128.1 (d), 128.5 (d), 128.6 (d), 129.2 (d), 129.8 (s), 130.2 (s), 131.4 (s), 131.5 (s), 137.9 (s), 153.9 (s). Anal. Calcd for $C_{26}H_{19}N_5O$: C, 74.80; H, 4.59; N, 16.78. Found: C, 74.70; H, 4.55; N, 16.95.

Acknowledgements

This work was financially supported in part by Ministero dell'Istruzione, dell'Università e della Ricerca.

References

1. Barraja, P.; Diana, P.; Lauria, A.; Passannanti, A.; Almerico, A. M.; Minnei, C.; Longu, S.; Congiu, D.; Musiu, C.; La Colla, P. *Bioorg. Med. Chem.* **1999**, *7*, 1591–1596.
2. Cirrincione, G.; Almerico, A. M.; Barraja, P.; Diana, P.; Lauria, A.; Passannanti, A.; Musiu, C.; Pani, A.; Murtas, P.; Minnei, C.; Marongiu, M. E.; La Colla, P. *J. Med. Chem.* **1999**, *42*, 2561–2568.
3. Aiello, E.; Dattolo, G.; Cirrincione, G.; Almerico, A. M.; Diana, P.; Grimaudo, S.; Mingoia, F.; Barraja, P. *Il Farmaco* **1995**, *50*, 365–368.
4. Wakelin, L. P. G.; Waring, M. J. *Comprehensive Medicinal Chemistry*, Sammes, P. G., Ed.; Pergamon: Oxford, 1990; 2, pp 703–724.
5. Lauria, A.; Diana, P.; Barraja, P.; Almerico, A. M.; Cirrincione, G.; Dattolo, G. *J. Heterocycl. Chem.* **2000**, *37*, 747–750.
6. (a) Westerlund, C. *J. Heterocycl. Chem.* **1980**, *27*, 1765–1769. (b) Westerlund, C. *J. Heterocycl. Chem.* **1980**, *27*, 1771–1775.
7. (a) Khan, M. A.; Carreira Freitas, A. C. *J. Heterocycl. Chem.* **1980**, *17*, 1603–1604. (b) Freitas, A. P.; Proenca, M. F. J. R. P.; Booth, B. L. *J. Heterocycl. Chem.* **1995**, *32*, 457–462.
8. See for examples: (a) Jones, R. A.; Bean, G. B. *The Chemistry of Pyrroles*. Academic: London, 1977. (b) Jones, R. A. *Comprehensive Heterocyclic Chemistry*, Katritzky, R. A., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 4, pp 268–308.
9. Lim, M.-I.; Klein, R. S.; Fox, J. J. *J. Org. Chem.* **1979**, *44*, 3826–3829.
10. Perrin, D. D.; Pitman, I. H. *J. Chem. Soc.* **1965**, 7071–7082.

11. Structure optimization in vacuo and in $(CH_3)_2SO$ was carried out, by using the software PIMMS (V 1.43) and Vamp (V 6.1), supplied by Oxford Molecular, to obtain the interatomic distances and molecular orbital energies.
12. In the protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration and the culture incubated for 48 h. End-point determinations are made with alamar blue Gray, G. D.; Wickstrom, E. *Biotechniques* **1996**, *21*, 780–782, Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduce the growth of any one of the cell lines to approximately 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.
13. Test compounds, dissolved in $(CH_3)_2SO$ at an initial concentration of 200 mM and serially diluted in culture medium, were incubated in the presence of LoVo cells for 72 h at 37°C. The number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method Denizot, F.; Lang, R. *J. Immunol. Meth.* **1986**, *89*, 271–277, Tumor cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% (GI_{50}) growth inhibition was determined by linear regression analysis.
14. Anderson, E. L.; Casey, J. E.; Greene, L. C.; Lafferty, J. J.; Reiff, H. E. *J. Med. Chem.* **1964**, *7*, 259–268.
15. Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robin, R. K. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163–172.