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A NEW TETRACYCLIC RING SYSTEM OF BIOLOGICAL INTEREST. INDOLO[3,2-*e*][1,2,3]TRIAZOLO[1,5-*a*]PYRIMIDINES THROUGH DOMINO REACTIONS OF 2-AZIDOINDOLE

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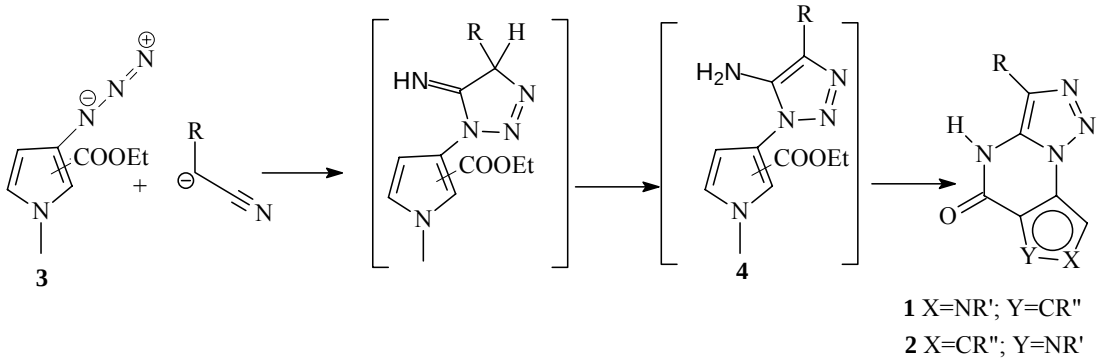
Abstract – Derivatives of the new ring system indolo[3,2-*e*][1,2,3]triazolo-[1,5-*a*]pyrimidine were easily prepared from 2-azidoindole (**7**) and substituted acetonitriles. Such a cycliczation represents the first example of an anionic domino reaction in the indole series. The tetracycle of type (**10**) presents suitable requirements to interact with DNA.

INTRODUCTION

Our researches have been devoted for several years to the development of new classes of polycondensed nitrogen systems incorporating either the pyrrole or the indole moiety.[1] In this context we have studied the synthesis and explored the biological activity of several flat heteroaromatic compounds, either tricyclic or tetracyclic, which can potentially intercalate into double-stranded DNA.[2] In particular we prepared and reported the interesting antiproliferative activity of cinnolines and 1,2,3-benzotriazines annelated with the indole nucleus,[3] that can be related to the well known classes of intercalating drugs such as acridines and anthracyclines. More recently we explored the synthetic access to the classes of annelated pyrrolo-triazolo-pyrimidines (**1**) and (**2**).[4] Routes to these ring systems were provided by domino reactions between substituted acetonitriles and 3-azidopyrroles (**3**) under basic conditions (Scheme 1). The azido moiety acted as a 1,3-dipolar compound in cycloaddition reactions with dipolarophiles such as the anions obtained from the methylene active derivatives. The intermediates resulting from the cycloaddition reaction, the 3-(triazol-1-yl)pyrroles (**4**), bear an amino group susceptible to further reactions. In the presence of a vicinal carboxylate function, intermediates (**4**) further cyclized to provide the pyrimidine ring.

Although this type of reaction was well described in the aromatic series, but only in a few cases has it been applied to pentatomic heterocycles,[5] in our hands it showed to be a versatile entry to annelated

1,2,3-triazolo[1,5-*a*]pyrimidines. However minor modifications of the experimental procedure were necessary for the reaction to become suitable for a general application in the pyrrole series.



Scheme 1

In this paper we discuss the use of this type of domino reaction on indole substrate and report its application for the synthesis of the new ring system indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5-one.

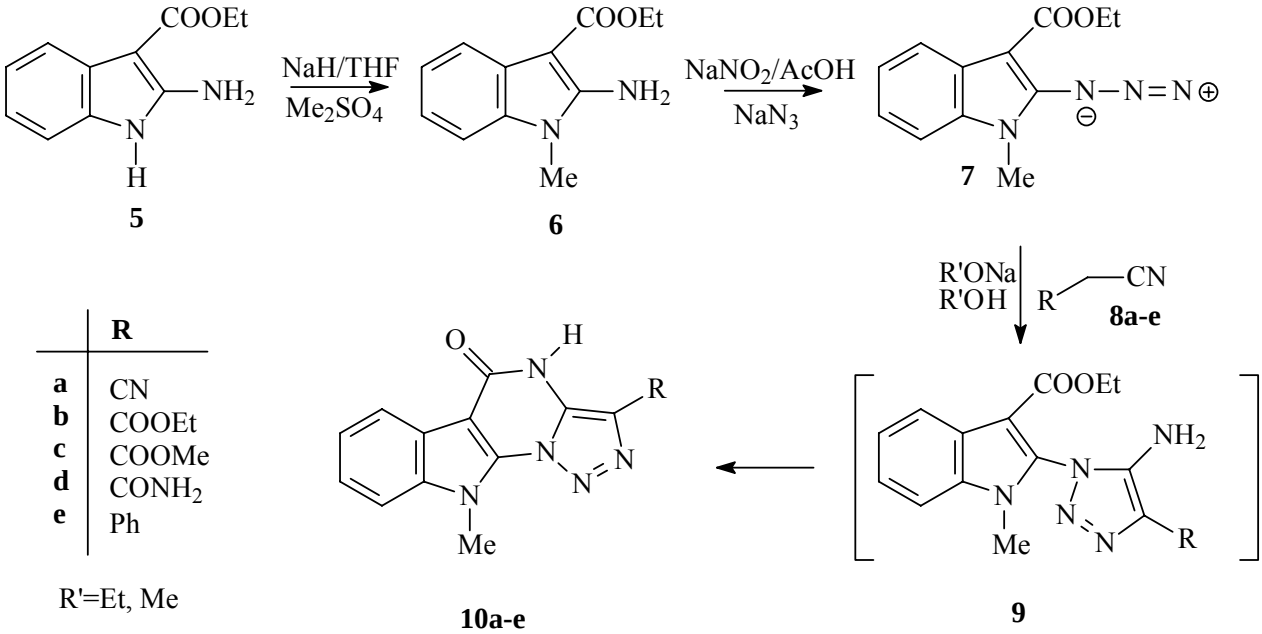
RESULTS AND DISCUSSION

In literature only a few examples of preparation of 2-azidoindoles by direct introduction *via* azido group transfer reaction with p-toluenesulfonyl azide were reported.[6] On the other hand 2-aminoindoles are not easy available since they are unstable and difficult to handle unless they bear an electron withdrawing group in the 3 position.[7] Therefore their reactivity was poorly studied. However our findings on protonation of aminoindoles[8] demonstrated that 2-aminoindoles could behave as aromatic amines and allowed to be confident on the feasibility of the diazotization reaction suggesting the use of weakly acid conditions. In fact the diazotization reaction of 2-aminoindoles was carried out in acetic acid under strict control of the temperature to give 2-diazo-2*H*-indoles in reasonable yields.[9] Taking into account these premises we were able to prepare 2-azidoindole from the corresponding 2-amino derivative.

Thus, the key intermediate 2-azidoindole (**7**) was prepared in two steps from ethyl 2-aminoindole-3-carboxylate (**5**),[10] which was first methylated to avoid unwanted side reactions in the base catalyzed step. When the reaction was carried out using methyl iodide, compound (**6**) was obtained as main product (60% yield) together with of 1-methyl-2-methylaminoindole (30%). However upon treatment of **5** with dimethyl sulfate, according to the procedure successfully employed in the case of 2-aminopyrroles,[11] selective methylation of the position 1 of the indole nucleus was achieved (Scheme 2). Diazotization of aminoindole (**6**) with sodium nitrite in acetic acid followed by treatment with sodium azide afforded to the key synthetic intermediate (**7**) in 90% yield.

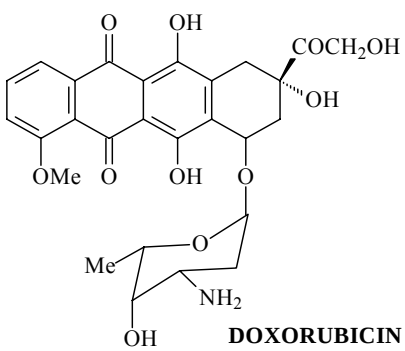
The azide (**7**) was reacted with the sodium salts of acetonitriles (**8**) in alcohol at rt for 3 h. The polycyclic compounds (**10a-e**) were directly isolated in good yields (80-90%). This sequence can be envisaged as an

anionic domino reaction in which the azidoindole acts as a 1,3-dipolar compound in cycloaddition reaction with the anion generated from the active methylene derivatives. Our findings in the pyrrole series[4] further support the only two examples in azole series reported so far in literature.[12] The nature of the substrate, the reaction conditions and the bond-forming economy can widely influence the nature of the reaction products. 2-Azido-3-ethoxycarbonylindole (**7**) was very prone to the cyclization reactions and the annelation reaction between the 2 and 3 positions of the indole ring was easier than that in the case of pyrrole derivatives.

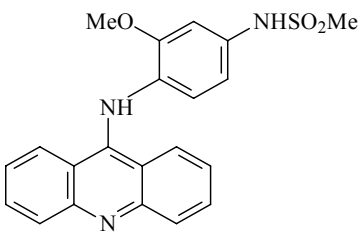


Scheme 2

To evaluate the potential ability of the new planar polycyclic derivatives (**10**) to interact with DNA, we calculated[13] the LUMO and HOMO energies, considering that these variables are of importance when two molecules with π electron systems form charge-transfer complexes. An analysis of the data reported in the table shows that these values are comparable with those of well known intercalating agents such as Doxorubicin (DOXO) or Amsacrine (AMSA). Moreover preliminary docking data to DNA fragments allow to be confident on the binding capability of our derivatives.



DOXORUBICIN



AMSACRINE

	E_{LUMO} (eV)	E_{HOMO} (eV)
a	-1.2012	-9.1428
b	-0.9880	-8.9717
c	-0.8793	-8.7432
d	-0.9007	-8.8954
e	-1.0552	-9.0346
DOXO	-1.5663	-8.0789
AMSA	-1.1653	-8.4858

In conclusion, our findings represent the first example of this type of domino reaction on indole substrates and in the case of 2-azidoindoles it resulted to be faster than in the pyrrole series. The reaction allows an useful access to the new ring indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5-one.

EXPERIMENTAL

Mps (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 DMSO-d_6 solution 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).

Ethyl 2-Amino-1-methyl-1*H*-indole-3-carboxylate (**6**).

A solution of ethyl 2-amino-1*H*-indole-3-carboxylate[10] (**5**) (1.35 g, 6.6 mmol) dissolved in anhydrous tetrahydrofuran (13 mL) was stirred in an ice bath while sodium hydride (a 60% mineral oil dispersion; 0.28 g, 7 mmol) was slowly added. After all bubbling had ceased, dimethyl sulfate (0.88 g, 7 mmol) was added, and the ice bath removed. The mixture was stirred at rt for 15 min and then heated under reflux for 2 h. This mixture was poured over crushed ice and diluted with water. The insoluble solid was filtered, air dried and purified by column chromatography using dichloromethane:ethyl acetate 98:2 as eluant. Compound (**6**) was isolated as white crystals, 1.29 g (90%), mp 152 °C (from ethanol). IR 3459 and 3347 (NH_2), 1650 (C=O) cm^{-1}. ^{1}H NMR δ: 7.62 (d, *J*=7.4 Hz, 1H, H-4); 7.19 (d, *J*=7.4 Hz ,1H, H-7); 6.95-7.06 (m, 2H, H-5 and H-6); 6.92 (s, 2H, NH_2); 4.24 (q, *J*=7.4 Hz, 2H, CH_2); 3.54 (s, 3H, CH_3); 1.34 (t, *J*=7.4 Hz, 3H, CH_3). ^{13}C NMR δ: 165.8 (s), 153.6 (s), 133.9 (s), 125.9 (s), 120.9 (d), 119.2 (d), 118.0 (d), 108.0 (d), 83.0 (s), 58.0 (t), 28.0 (q), 14.7 (q). Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.94; H, 6.52; N, 12.92.

Ethyl 2-Azido-1-methyl-1*H*-indole-3-carboxylate (**7**).

To a solution of ethyl 2-amino-1-methyl-1*H*-indole-3-carboxylate (**6**) (0.83 g, 3.8 mmol) in acetic acid (30 mL) and water (5 mL), sodium nitrite (0.53 g, 7.6 mmol) in water (4 mL) was added at 0°C, under vigorous stirring. After 30 min sodium azide (0.96 g, 15 mmol) was added in portions and the reactants were stirred 1h further at rt. The solid was filtered off and air dried to give **7** as orange precipitate: 0.83 g, (90%), mp 100 °C (decomp). IR 2134 (N_3), 1681 (C=O) cm^{-1}. ^{1}H-NMR δ: 8.00 (d, *J*=7.3 Hz, 1H, H-4); 7.43 (d, *J*=7.3, 1H, H-7); 7.16-7.32 (m, 2H, H-5 and H-6); 4.34 (q, *J*=7.4 Hz, 2H, CH_2); 3.60 (s, 3H, CH_3); 1.36 (t, *J*=7.4 Hz, 3H, CH_3). ^{13}C-NMR: 165.6 (s), 153.3 (s), 134.0 (s), 126.4 (s), 120.7 (d), 119.0 (d), 117.8

(d), 108.4 (d), 83.4 (s), 57,8 (t), 28.1 (q), 14.8 (q). Anal. Calcd for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95; N, 22.94. Found: C, 58.80; H, 4.99; N, 22.81.

General Method for the Preparation of Indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines **10a-e**.

To a solution of 0.56 M sodium ethoxide in ethanol, or sodium methoxide in methanol in the case of **8c**, (5.7 mL) substituted acetonitriles (**8a-e**) (0.16 mmol) in dry alcohol (10 mL) was added at rt. After being stirred for 15 min a solution of azidoindole (**7**) (0.2 g, 0.8 mmol) in dry alcohol (10 mL) was added and the mixture was stirred for a further 3 h at rt. The solid was filtered off, air dried and washed with dichloromethane to give **10a-e**.

10-Methyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carbonitrile (**10a**).

From **7** and malononitrile, white solid (0.19 g, 90%), mp>300 °C (from ethanol). IR 3390 (NH), 2245 (CN), 1650 (C=O) cm^{-1}. ^{1}H-NMR δ: 7.99 (d, *J*=7.3 Hz, 1H, H-6); 7.44 (d, *J*=8.1 Hz, 1H, H-9); 7.15-7.32 (m, 2H, H-7 and H-8); 3.75 (s, 3H, CH_3). ^{13}C-NMR δ: 156.3 (s), 152.3 (s), 148.9 (s), 137.9 (s), 122.8 (s), 122.7 (d), 120.4 (d), 119.3 (d), 115.2 (s), 108.6 (d), 103.3 (s), 90.8 (s), 27.6 (q). Anal. Calcd for $C_{13}H_8N_6O$: C, 59.09; H, 3.05; N, 31.80. Found: C, 59.00; H, 3.12; N, 31.93.

Ethyl 10-Methyl-5-oxo-5,10-dihydro-4*H*-indolo3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxylate (**10b**).

From **7** and ethyl cyanoacetate, white solid (0.2 g, 80%), mp>300 °C (from ethanol). IR 3421 (NH), 1677 (C=O), 1650 (C=O) cm^{-1}. ^{1}H-NMR δ: 7.96 (d, *J*=7.3 Hz, 1H, H-6); 7.39 (d, *J*=7.3 Hz, 1H, H-9); 7.15-7.24 (m, 2H, H-7 and H-8); 4.29 (q, *J*=7.3 Hz, 2H, CH_2); 3.75 (s, 3H, CH_3); 1.35 (t, *J*=7.3 Hz, 3H, CH_3). ^{13}C-NMR δ: 165.2 (s), 161.5 (s), 156.5 (s), 152.5 (s), 146.8 (s), 137.9 (s), 123.0 (s), 122.2 (d), 119.9 (d), 119.0 (d), 108.2 (d), 90.6 (s), 58.7 (t), 27.3 (q), 14.5 (q). Anal. Calcd for $C_{15}H_{13}N_5O_3$: C, 57.88; H, 4.21; N, 22.50. Found: C, 57.80; H, 4.29; N, 22.78.

Methyl 10-Methyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxylate (**10c**).

From **7** and methyl cyanoacetate, white solid (0.19 g, 80%), mp>300 °C (from methanol). IR 3330 (NH), 1680 (C=O), 1656 (C=O) cm^{-1}. ^{1}H-NMR δ: 7.97 (d, *J*=7.3 Hz, 1H, H-6); 7.39 (d, *J*=7.3 Hz, 1H, H-9); 7.12-7.28 (m, 2H, H-7 and H-8); 3.82 (s, 3H, CH_3); 3.76 (s, 3H, CH_3). ^{13}C-NMR δ: 161.9 (s), 156.6 (s), 152.5 (s), 146.7 (s), 137.9 (s), 123.0 (s), 122.2 (d), 121.2 (s), 120.0 (d), 119.1 (d), 108,3 (d), 90.6 (s), 50.4

(q), 27.5 (q). Anal. Calcd for $C_{14}H_{11}N_5O_3$: C, 56.57; H, 3.73; N, 23.56. Found: C, 56.70; H, 3.84; N, 23.71.

10-Methyl-5-oxo-5,10-dihydro-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide (**10d**).
From **7** and cyanoacetamide, white solid (0.2 g, 90%), mp>300 °C (from ethanol). IR 3353 and 3174 (NH_2 and NH), 1687 (C=O), 1650 (C=O) cm^{-1}. ^{1}H-NMR δ: 7.94 (d, *J*=7.4 Hz, 1H, H-6); 7.16-7.38 (m, 3H, H-7, H-8 and H-9); 3.76 (s, 2H, NH_2); 3.40 (s, 3H, CH_3). ^{13}C-NMR δ: 162.8 (s), 155.9 (s), 152.5 (s), 145.4 (s), 137.8 (s), 124.3 (s), 123.1 (s), 122.1 (d), 120.1 (d), 119.0 (d), 108.3 (d), 90.2 (s), 27.4 (q). Anal. Calcd for $C_{13}H_{10}N_6O_2$: C, 55.32; H, 3.57; N, 29.77. Found: C, 55.20; H, 3.60; N, 29.99.

10-Methyl-3-phenyl-4*H*-indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidin-5(10H)-one (**10e**).
From **7** and benzyl cyanide, white solid (0.2 g, 80%), mp>300 °C (from ethanol). IR 3340 (NH), 1656 (C=O) cm^{-1}. ^{1}H-NMR δ: 8.48 (d, *J*=7.4 Hz, 2H, H-2' and H-6'); 7.98 (d, *J*=7.4 Hz, 1H, H-6); 7.09-7.59 (m, 6H, H-7, H-8, H-9, H-3', H-4' and H-5'); 3.8 (s, 3H, CH_3). ^{13}C-NMR δ: 156.3 (s), 152.9 (s), 143.0 (s), 138.3 (s), 133.8 (s), 128.3 (d), 127.8 (s), 124.8 (d), 123.7 (d), 123.4 (s), 122.0 (d), 119.8 (d), 118.9 (d), 107.9 (d), 89.9 (s), 27.4 (q). Anal. Calcd for $C_{18}H_{13}N_5O$: C, 68.56; H, 4.16; N, 22.21. Found: C, 68.40; H, 4.22; N, 22.40.

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REFERENCES AND NOTE

1. G. Dattolo, G. Cirrincione, A. M. Almerico, G. Presti, and E. Aiello, *Heterocycles*, 1984, **22**, 2269; G. Dattolo, G. Cirrincione, A. M. Almerico, L. Lamartina, and E.Aiello, *J. Chem. Res.(s),* 1985, 164; G. Cirrincione, G. Dattolo, A. M. Almerico, and E. Aiello, *Heterocycles,* 1985, **23**, 2635; G. Cirrincione, G. Dattolo, A. M. Almerico, G. Presti, and E. Aiello, Heterocycles, 1986, **24**, 3403; G. Dattolo, G. Cirrincione, A. M.Almerico, and E. Aiello, *J. Heterocycl. Chem.*, 1989, **26**, 1747; G.Cirrincione, A. M. Almerico, E. Aiello, and G. Dattolo, *J. Heterocycl. Chem.*, 1990, **27**, 983; G. Cirrincione, A. M. Almerico, G. Dattolo, E. Aiello, P. Diana, and F. Mingoia, *J. Heterocycl. Chem.*, 1992, **29**, 1309; A. M. Almerico, G. Cirrincione, G. Dattolo, E. Aiello, and F. Mingoia, J. *Heterocycl. Chem.*, 1994, **31**, 193; A. M. Almerico, G.Cirrincione, P. Diana, S. Grimaudo, G. Dattolo, E. Aiello, F. Mingoia, and P. Barraja, *Heterocycles*, 1994, **37,** 1549; P. Diana, P. Barraja, A. Lauria, A. M. Almerico, G. Dattolo, and G. Cirrincione, *Eur. J. Med. Chem.*, 2002, **37**, 267.

2. E. Aiello, G. Dattolo, G. Cirrincione, A. M. Almerico, P. Diana, S. Grimaudo, F. Mingoia, and P. Barraja, *Il Farmaco*, 1995, **50**, 365; G. Cirrincione, A. M. Almerico, P. Barraja, P. Diana, S. Grimaudo, G. Dattolo, E. Aiello, and F. Mingoia, *Il Farmaco*, 1995, **50**, 849; F. Mingoia, P. Diana, P .Barraja, A. Lauria, and A. M. Almerico, *Heterocycles*, 2000, **53**, 1975; A. Montalbano, P. Diana, P. Barraja, A. Lauria, G. Cirrincione, G. Dattolo, and A. M. Almerico, *Arkivoc*, 2002, **3**, 264.
3. P. Barraja, P. Diana, A. Lauria, A. Passannanti, A. M. Almerico, C. Minnei, S. Longu, D. Congiu, C. Musiu, and P. La Colla, *Bioorg. Med. Chem.,* 1999, **7**, 1591; G. Cirrincione, A. M. Almerico, P. Barraja, P. Diana, A. Lauria, A. Passannanti, C. Musiu, A. Pani, P. Murtas, C. Minnei, M. E. Marongiu, and P. La Colla, *J. Med. Chem.,* 1999, **42**, 2561.
4. A. Lauria, P. Diana, P. Barraja, A. M. Almerico, G. Cirrincione, and G. Dattolo, *J. Heterocycl. Chem.,* 2000, **37**, 747; A. Lauria, P. Diana, P. Barraja, A. Montalbano, G. Cirrincione, G. Dattolo, and A. M. Almerico, *Tetrahedron*, 2002, **58**, 9723.
5. C. Westerlund, *J. Heterocycl. Chem*., 1980, **27**, 1765; C. Westerlund, *J. Heterocycl. Chem.*, 1980, **27**, 1771.
6. E. Foresti, M. T. Di Gioia, D. Nanni, and P. Zanirato, *Gazz. Chim. Ital*., 1995, **125**, 151; E. Foresti, P. Spagnolo, and P. Zanirato, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1354.
7. R. A. Jones, 'Comprehensive Heterocyclic Chemistry: Pyrroles and their Benzo derivatives: Reactivity,' Vol. 4, ed. by C. W. Birds and G. W. H. Cheeseman, Pergamon, Oxford, 1984, p. 298.
8. P. Diana, P. Barraja, A. Lauria, A. M. Almerico, G. Dattolo, and G. Cirrincione, *Tetrahedron*, 2000, **56**, 5177.
9. P. Barraja, P. Diana, A. Lauria, A. M. Almerico, G. Dattolo, and G. Cirrincione, *Helv. Chim. Acta*, 2001, **84**, 2212.
10. C. A. Grob and O. Weissbach, *Helv. Chim. Acta,* 1961*,* **44,** 1748.
11. R. J. Mattson and J. W. Sowell, Sr, *Synthesis,* 1979, 217.
12. M. A. Khan and A. C. Carreira Freitas, *J. Heterocycl. Chem.,* 1980, **17**, 1603; A. P. Freitas, M. F. J. R. P. Proenca, and B. L. Booth, *J. Heterocycl. Chem.,* 1995, **32**, 457.
13. Structure optimization *in vacuo* and in DMSO was carried out, by using the software PIMMS (V 1.43) and Vamp (V 6.1), supplied by Oxford Molecular-Accelrys, to obtain the interatomic distances and molecular orbital energies.