PYRROLO[2,3-*d*][1,2,3]TRIAZOLES AS POTENTIAL ANTINEOPLASTIC AGENTS

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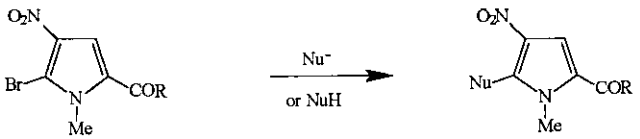
Abstract – Halonitropyrrole (**1a**) underwent nucleophilic substitution by substituted benzylamines (**7**) to give the intermediates (**8**), which by reduction and diazotization followed by an intramolecular coupling reaction led to derivatives of the title ring system (**10**).

We recently reported several examples of direct nucleophilic substitutions on halonitropyrroles.[1] Such nucleophilic substitutions were achieved either by charged and neutral carbon, nitrogen, oxygen and sulfur nucleophiles.



1 a R = Me
b R = H

2-6

2 a Nu = OMe, R = Me
b Nu = OMe, R = H
c Nu = OEt, R = Me
d Nu = OEt, R = H

3 a Nu = N_3, R = Me
b Nu = N_3, R = H

4 a Nu = CN, R = Me

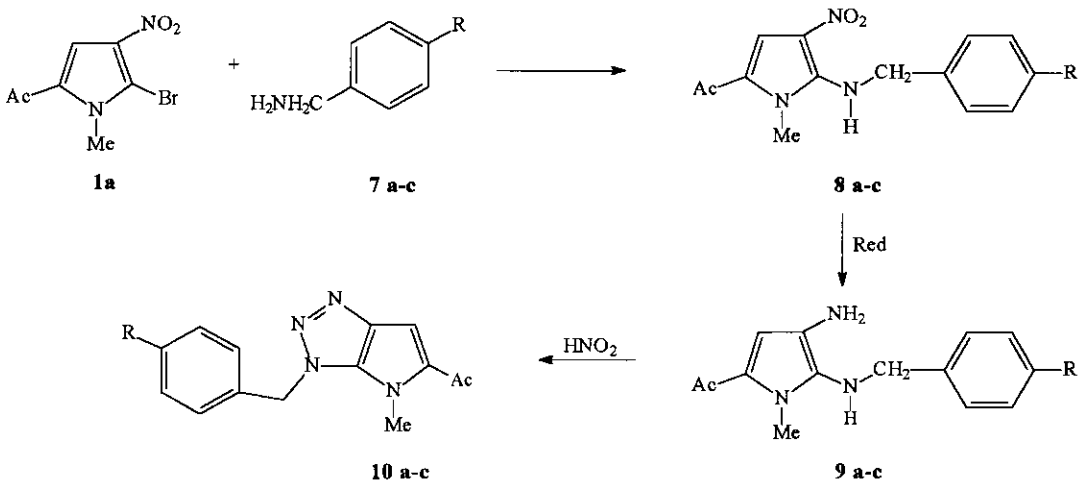
5 a Nu = Pyrrolidinyl, R = Me
b Nu = Pyrrolidinyl, R = H

6 a Nu = C_6H_5S, R = Me
b Nu = 4-MeC_6H_4S, R = Me

This type of reactions, achieved by neutral nitrogen, oxygen and nucleophiles, represents the first examples of direct nucleophilic substitution in pyrrole series by uncharged nucleophiles. In fact, in earlier reports nucleophilic reaction by primary amines on nitropyrroles afforded products derived from ring-opening ring-closure processes.[2]

Therefore, it is evident that nucleophilic reactions in pyrrole series represent a valuable and versatile instrument to prepare a wide variety of functionalized pyrroles and to obtain key intermediates for the

synthesis of polycyclic systems. In fact the choice of suitably functionalized nucleophiles allows the preparation of synthons that easily can undergo cyclization reactions. An example of the above statement is provided by the synthesis of derivatives of the pyrrolo[2,3-*d*][1,2,3]triazole ring system of type (**10**) described in this paper.



a R = H, **b** R = Me, **c** R = OMe

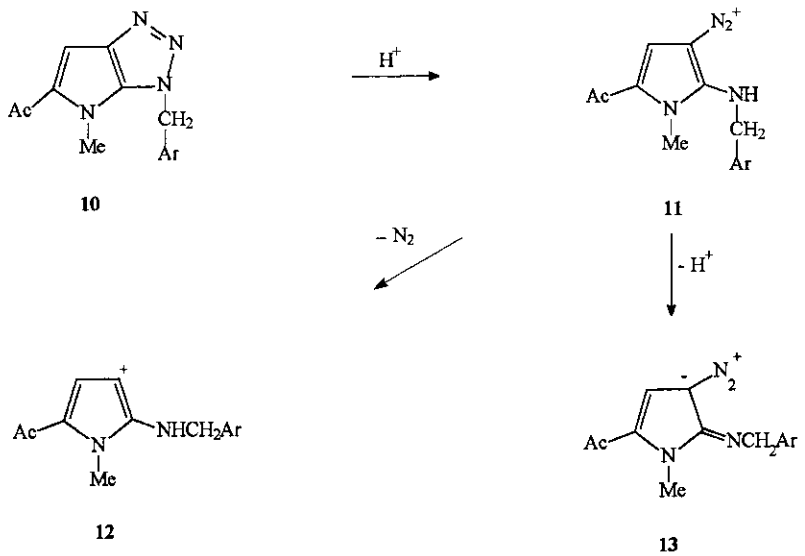
In fact the bromo derivative (**1a**) reacted with the substituted benzylamines (**7a-c**) to give the corresponding products (**8a-c**) of direct nucleophilic substitution. The reaction was carried out in refluxing dimethylformamide with an excess of amine to neutralize the hydrogen bromide developed during the reaction. The intermediates (**8a-c**) were thus obtained in quite good yield (80 - 90%). The structure of compounds (**8**) was confirmed on the basis of the IR spectra which showed a NH stretching at 3320 - 3337 cm^{-1} and of the ^{13}C NMR spectra which exibited, with respect to that of the bromo derivative (**1a**), a marked downfield shift of the pyrrole C-5 resonances (*ca.* 33 ppm) and a smaller but significant upfield shift of the pyrrole C-4 signals (*ca.* 6 ppm).[3] Such shifts are justified by the effects that either the amino and the bromo groups experience on the *ipso* carbon and on the adjacent position. In fact the presence of the amino group involves a strong downfield shift of the *ipso* carbon and a similar upfield shift of the adjacent carbon.[4] The bromo group exerts opposite but smaller effects on the above carbons.[5] The nitro derivatives (**8a-c**) were reduced to the corresponding amino derivatives (**9a-c**) with hydrogen and palladium on charcoal at room temperature (yield 80 - 85%). The formation of the 4-amino group was confirmed by the appearence in the IR spectra of a very broad absorption bands centered at 3407 - 3414 cm^{-1} and, in the ^{1}H NMR spectra, of an exchangeable signal for two protons at 3.64 - 4.82 ppm. Moreover the upfield shift of the C-5 resonance (12 - 15 ppm) gives account of the different effects on the adjacent carbon exerted by the nitro and amino groups.

The first attempt to cyclize the amino derivatives (**9**) was performed by diazotization with sodium nitrite in hydrochloric acid. The reactions led to a very complex mixture and extensive formation of tars.

Diazotization of derivatives (**9**) in acetic acid was smoother and it was possible to isolate pyrrolo[2,3-*d*][1,2,3]triazoles (**10**), in 75 – 80% yield, derived by the intramolecular coupling reaction of the diazonium group with the benzylamine nitrogen. However nitrosation of the benzyl nitrogen and subsequent attack on this latter by the pyrrole amino group cannot be ruled out. The structure was confirmed in the IR spectra, by the disappearence of the very broad NH and NH_2 absorptions and by the appearence of a band at 1483 - 1485 cm^{-1}due to the N=N group. In the ^1H NMR spectra the methylene group of compounds (**10**), bounded to the N-3 nitrogen, appeared as a singlet at 5.81 - 5.86 ppm, whereas in compounds (**8**) and (**9**) appeared as doublet because of the coupling with the benzylamine NH.

Compounds (**10**) represent the first aromatic derivatives of the title ring system. In fact the sole report dealing with pyrrole[2,3-*d*][1,2,3]triazole ring system concerns the isolation of hexahydro derivatives incidentally obtained by thermal reaction of *cis*-1,2-bis(arenoazo)ethylenes with acrylonitrile in which an unexpected rearrangement of the 1,3-cycloaddition products took place.[6]

The pyrrole[2,3-*d*][1,2,3]triazole ring system can be expected to behave towards biological substrates in a fashion similar to alkylating agents. In fact it is well know that the 1,2,3-triazole ring, under hydrolytic conditions, easily undergoes breakage of the N-1—N-2 bond leading to intermediates bearing a diazonium function. Such diazonium intermediates can covalently interact with nucleophiles by themselves, and/or upon loss of nitrogen can further originate an intermediate capable of covalent binding to nucleic acid.[7]



10 **11** **12** **13**

In the case of pyrrolotriazoles of type (**10**) the cleavage of the N-N bond should lead to a pyrrole- 3-diazonium intermediate (**11**) that can evolve to the structure (**12**) as mentioned above or, at physiological

pH, can give rise to the zwitterionic species (**13**) related to the structure of 3-diazopyrroles which have already shown good antineoplastic activity.[8]

Thus compounds (**10**), as well as the intermediates (**8**) and (**9**) were evaluated *in vitro* for antiproliferative activity against a panel of leukemia-, lymphoma-, carcinoma- and neuroblastoma-derived cell lines. Unfortunately none of the tested compounds showed antiproliferative activity even at concentrations of 200 μM.

In conclusion, in spite the discouraging biological results, we believe evident the versatility of the nucleophilic substitutions in pyrrole series as a method to prepare building blocks for the synthesis of pharmacologically interesting polycyclic heterocycles.

EXPERIMENTAL SECTION

All melting points were taken on a Buchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^1H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz respectively in DMSO-d_6 solution, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM.

Reaction of 2-acetyl-5-bromo-1-methyl-4-nitropyrrole (1a) with amines (7a-c).

To a solution of the pyrrole (**1a**)[1b] (2 mmol) in anhydrous DMF (20 mL), the amine (**7a-c**) (6 mmol) was added. The reaction mixture was refluxed for 8-10 h, cooled to rt and poured onto crushed ice. The solid precipitate was collected, air dried and purified by column chromatography (25 g; eluent: dichloromethane).

Reaction of (**1a**) with benzylamine (**7a**) (reaction time 10 h) gave **2-acetyl-5-benzylamino-1-methyl-4-nitropyrrole (8a)**: Yield 80%, mp 101-103°C from ethnol; IR: 3337 (NH), 1653 (CO), 1541 (NO_2) cm^{-1}; ^1H NMR (ppm): 2.33 (3H, s, CH_3), 3.69 (3H, s, NCH_3), 4.70 (2H, d, J = 5.9 Hz, CH_2), 7.31 (5H, s, ArH), 7.58 (1H, s, H-3), 7.79 (1H, t, J= 5.9 Hz, NH); ^{13}C NMR (ppm): 26.6 (q, CH_3), 34.6 (q, NCH_3), 48.6 (t, CH_2) 115.1 (d, C-3), 124.9 (s, C-4), 127.0 (d, C-3' and C-5'), 127.4 (d, C-4'), 128.6 (d, C-2' and C-6'), 139.0 (s, C-2 and C-1'), 147.4 (s, C-5), 187.9 (s, CO). Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 61.44; H, 5.50; N, 15.61.

Reaction of (**1a**) with 4-methylbenzylamine (**7b**) (reaction time 8 h) gave **2-acetyl-1-methyl-5-(4-methylbenzylamino)-4-nitropyrrole (8b)**: Yield 89%, mp 89-90°C from ethanol; IR: 3335 (NH), 1651 (CO), 1539 (NO_2) cm^{-1}; ^1H NMR (ppm): 2.35 (3H, s, CH_3), 2.39 (3H, s, CH_3), 3.75 (3H, s, NCH_3), 4.70 (2H, d, J = 5.9 Hz, CH_2), 7.20-7.23 (4H, m, ArH), 7.63 (1H, t, J = 5.9 Hz, NH), 7.82 (1H, s, H-3); ^{13}C NMR (ppm): 20.6 (q, CH_3), 26.5 (q, CH_3), 34.5 (q, NCH_3), 48.3 (t, CH_2), 115.0 (d, C-3), 121.1 (s,

C-1'), 124.9 (s, C-4), 127.0 (d, C-3' and C-5'), 129.2 (d, C-2' and C-6'), 135.9 (s, C-4'), 136.6 (s, C-2), 147.4 (s, C-5), 190.3 (s, CO). Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.70; H, 5.96; N, 14.63. Found: C, 62.54; H, 5.78; N, 14.49.

Reaction of (**1a**) with 4-methoxybenzylamine (**7c**) (reaction time 8 h) gave **2-acetyl-5-(4-methoxybenzylamino)-1-methyl-4-nitropyrrole** (**8c**): Yield 90%, mp 108-110°C from ethanol; IR: 3320 (NH), 1651 (CO), 1514 (NO_2) cm^{-1}; ^1H NMR (ppm): 2.32 (3H, s, CH_3), 3.70 (6H, s, NCH_3 and O CH_3), 4.61 (2H, d, J = 5.9 Hz, CH_2), 6.87 (2H, d, J = 7.3 Hz, H-3' and H-5'), 7.22 (2H, d, J = 7.3 Hz, H-2' and H-6'), 7.56 (1H, t, J = 5.9 Hz, NH), 7.68 (1H, s, H-3); ^{13}C NMR (ppm): 26.5 (q, CH_3), 34.6 (q, NCH_3), 48.1 (t, CH_2), 54.9 (q, OCH_3), 114.0 (d, C-3' and C-5'), 115.0 (d, C-3), 128.8 (s, C-4), 128.4 (d, C-2' and C-6'), 130.7 (s, C-2), 134.6 (s, C-1'), 147.3 (s, C-5), 158.5 (s, C-4'), 187.8 (s, CO). Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.39; H, 5.65; N, 13.86. Found: C, 59.21; H, 5.36; N, 13.63.

Preparation of 2-Acetyl-4-amino-5-(4-substituted benzylamino)-1-methylpyrroles (9a-c).

A solution of the nitro derivatives (**8a-c**) (5 mmol) in ethanol (100 mL) was reduced over 10% Pd on charcoal (0.1 g) in a Parr apparatus at 60 psi at rt for 24 h. Removal of the catalyst and evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography (25 g; eluent: dichloromethane:ethyl acetate 8:2). The amine derivatives were isolated as uncrystallizable oils.

2-Acetyl-4-amino-5-benzylamino-1-methylpyrrole (9a): Yield 85%; IR: 3410 (br NH and NH_2), 1640 (CO) cm^{-1}; ^1H NMR (ppm): 2.12 (3H, s, CH_3), 3.60 (3H, s, NCH_3), 3.64 (2H, br s, NH_2), 4.15 (2H, d, J = 7.4 Hz, CH_2), 5.19 (1H, t, J = 7.4 Hz, NH), 6.38 (1H, s, H-3), 7.21-7.38 (5H, m, ArH); ^{13}C NMR (ppm): 26.0 (q, CH_3), 31.9 (q, NCH_3), 50.1 (t, CH_2), 110.3 (d, C-3), 121.3 (s, C-4), 121.5 (s, C-2), 126.9 (d, C-4'), 127.7 (d, C-2' and C-6'), 128.2 (d, C-3' and C-5'), 135.6 (s, C-5), 140.6 (s, C-1'), 182.0 (s, CO). Anal. Calcd for $C_{14}H_{17}N_3O$: C, 69.11; H, 7.04; N, 17.27. Found: C, 68.97; H, 7.29; N, 17.48.

2-Acetyl-4-amino-1-methyl-5-(4-methylbenzylamino)pyrrole (9b): Yield 80%; IR: 3407 (br NH and NH_2), 1645 (CO) cm^{-1}; ^1H NMR (ppm): 2.12 (3H, s, CH_3), 2.26 (3H, s, CH_3), 3.60 (3H, s, NCH_3), 3.69 (2H, br s, NH_2), 4.10 (2H, d, J = 7.4 Hz, CH_2), 5.16 (1H, t, J = 7.4 Hz, NH), 6.38 (1H, s, H-3), 7.11 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.24 (2H, d, J = 8.8 Hz, H-3' and H-5'); ^{13}C NMR (ppm): 20.7 (q, CH_3), 26.0 (q, CH_3), 31.9 (q, NCH_3), 49.9 (t, CH_2), 110.4 (d, C-3), 121.3 (s, C-4), 121.5 (s, C-2), 127.8 (d, C-2' and C-6'), 128.8 (d, C-3' and C-5'), 135.7 (s, C-5), 135.9 (s, C-4'), 137.6 (s, C-1'), 182.9 (s, CO). Anal. Calcd for $C_{15}H_{19}N_3O$: C, 70.01; H, 7.44; N, 6.33. Found: C, 70.17; H, 7.29; N, 16.48.

2-Acetyl-4-amino-5-(4-methoxybenzylamino)-1-methylpyrrole (9c): Yield 85%; IR: 3414 (br NH and NH_2), 1639 (CO) cm^{-1}; ^1H NMR (ppm): 2.27 (3H, s, CH_3), 3.62 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 4.14 (2H, d, J = 7.3 Hz, CH_2), 4.82 (2H, br s, NH_2), 5.77 (1H, t, J = 7.3 Hz, NH), 6.90 (2H, d, J = 8.8 Hz, H-3' and H-5'), 6.99 (1H, s, H-3), 7.36 (2H, d, J= 8.8 Hz, H-2' and H-6'); ^{13}C NMR (ppm): 26.4 (q, CH_3), 32.4

(q, NCH_3), 50.5 (t, CH_2), 55.1 (q, OCH_3), 103.5 (s, C-4), 113.7 (d, C-3' and C-5'), 114.2 (d, C-3), 123.6 (s, C-2), 129.4 (d, C-2' and C-6'), 131.2 (s, C-5), 139.9 (s, C-1'), 158.5 (s, C-4'), 185.9 (s, CO). Anal. Calcd for $C_{15}H_{19}N_3O_2$: C, 65.91; H,7.01; N, 15.37. Found: C, 65.77; H, 722; N, 15.48.

Preparation of pyrrolo[2,3-*d*][1,2,3]triazoles (10a-c).

To a solution of amines (**9a-c**) (2.5 mmol) in acetic acid (30 mL) at 0°C, stoichiometric amount of sodium nitrite (173 mg; 2.5 mmol) dissolved in water (2 mL) was added. The reaction mixture was kept at 4°C overnight, poured onto crushed ice and extracted with ether (3 x 50 mL). The organic layer, evaporated under reduced pressure, gave a residue which was purified by column chromatography (25 g; eluent: dichloromethane: ethyl acetate 9:1).

5-Acetyl-3-benzyl-4-methylpyrrolo[2,3-*d*][1,2,3]triazole (10a): Yield 75%, mp 152-153°C from ethanol; IR: 1655 (CO), 1485 (N=N) cm^{-1}; ^1H NMR (ppm): 2.45 (3H, s, CH_3), 3.78 (3H, s, NCH_3), 5.89 (2H, s, CH_2), 7.18 (2H, d, J = 7.4 Hz, H-2' and H-6'), 7.29-7.36 (3H, m, H-3', H-4' and H-5'), 7.40 (1H, s, H-6); ^{13}C NMR (ppm): 27.2 (q, CH_3), 33.8 (q, NCH_3), 50.8 (t, CH_2), 104.8 (d,C-6), 126.8 (d, C-2' and C-6'), 128.1 (d, C-4'), 129.0 (d, C-3' and C-5'), 136.2 (s, C-6a), 136.4 (s, C-1'), 139.2 (s, C-5), 140.3 (s, C-3a), 191.1 (s, CO). Anal. Calcd for $C_{14}H_{14}N_4O$: C, 66.13; H, 5.55; N, 22.03. Found: C, 66.07; H, 5.49; N, 22.28.

5-Acetyl-4-methyl-3-(4-methylbenzyl)pyrrolo[2,3-*d*][1,2,3]triazole (10b): Yield 80%, mp 119°C from ethanol; IR: 1657 (CO), 1483-1485 (N=N) cm^{-1}; ^1H NMR (ppm): 2.29 (3H, s, CH_3), 2.48 (3H, s, CH_3), 3.82 (3H, s, NCH_3), 5.86 (2H, s, CH_2) 7.10 (2H, d, J = 8.8 Hz, H-3' and H-5'), 7.20 (2H, d, J = 8.8 Hz, H-2' and H-6'), 7.43 (1H, s, H-6); ^{13}C NMR (ppm): 20.6 (q, CH_3), 27.2 (q, CH_3), 33.8 (q, NCH_3), 50.6 (t, CH_2), 104.8 (d, C-6), 126.7 (d, C-2' and C-6'), 129.5 (d, C-3' and C-5'), 133.4 (s, C-4'), 136.1 (s, C-6a), 137.4 (s, C-1'), 139.2 (s, C-5), 140.3 (s, C-3a), 191.1 (s, CO). Anal. Calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.27; H, 6.09; N, 20.68.

5-Acetyl-3-(4-methoxybenzyl)-4-methylpyrrolo[2,3-*d*][1,2,3]triazole (10c): Yield 80%, mp 124°C from ethanol; IR: 1657 (CO), 1483 (N=N) cm^{-1}; ^1H NMR (ppm): 2.46 (3H, s, CH_3), 3.73 (3H, s, NCH_3), 3.82 (3H, s, OCH_3), 5.81 (2H, s, CH_2), 6.92 (2H, d, J = 7.4 Hz, H-3' and H-5'), 7.16 (2H, d, J = 7.4 Hz, H-2' and H-6'), 7.40 (1H, s, H-6); ^{13}C NMR (ppm): 27.7 (q, CH_3), 34.3 (q, NCH_3), 51.0 (t, CH_2), 55.5 (q, OCH_3), 105.2 (d, C-6), 114.8 (d, C-3' and C-5'), 128.8 (d, C-2' and C-6'), 136.6 (s, C-1'), 139.6 (s, C-6a), 140.8 (s, C-5), 148.8 (s, C-3a), 159.5 (s, C-4'), 191.6 (s, CO). Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.48; H, 5.79; N, 19.67.

Diazotization of the amine derivatives (**9a-c**) with sodium nitrite in 6N hydrochloric acid led to a complex mixture of products impossible to purify.

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