Pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine and Pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine. New Tricyclic Ring Systems of Biological Interest

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Derivatives of the new ring system pyrrolo[3,4-*e*][1,2,3] triazolo[1,5-*a*]pyrimidine **6** were prepared in high yields in one step by reaction of 3-azidopyrrole **3** and substituted acetonitriles. Compound **6b** rearranged, upon heating in dimethyl sulfoxide in the presence of water, to pyrrolo[3,4-*d*][1,2,3]triazolo-[1,5-*a*]pyrimidine **7**.

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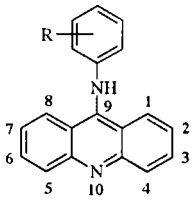
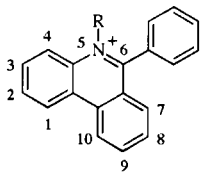
For several years we have been interested in the synthesis and evaluation of the biological activity of several flat tetracyclic heteroaromatic systems that can potentially intercalate into double-stranded DNA. In this context we have prepared and reported the interesting antiproliferative activity of indolo[3,2-*c*]cinnolines [1], indolo[1,2-*c*]benzo-[1,2,3]triazines [2], and pyrrolo[1,2-*f*]phenanthridines [3], that can be related to the well known classes of intercalators with linear (acridines of type **1**) or angular (phenanthridines of type **2**) structure. These compounds possess such a property whose principal driving forces are stacking and charge-transfer interactions as well as hydrogen bonding and electrostatic forces [4]. In particular, it has been demonstrated that the biological activity of 9-acridinylmethanesulfonanilide derivatives **1** (R = $NHSO_2Me$) correlates with their DNA association constants, the more active compounds being those that more tightly bind to DNA [5]. Ethidium derivatives **2** (R = Et) also tightly bind to DNA and show a strong specificity for GC residues [6]. Moreover compounds of type **1** are well known inhibitors of topoisomerase II [7].



1



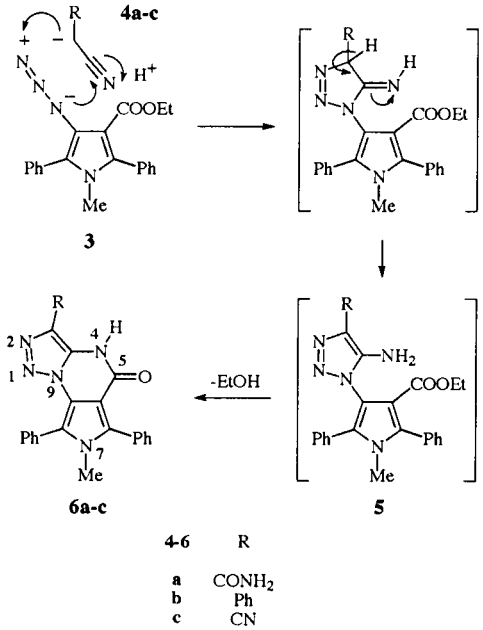
2

In connection with our studies on polycondensed nitrogen heterocycles, we now report the synthesis of a new tricyclic angular system, namely pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine and its rearrangement to pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine, a new tricyclic linear heterocycle.

The key intermediate for the preparation of the title compounds is the 3-azidopyrrole **3**. This derivative was chosen because of the presence of an ethoxycarbonyl group adjacent to the azido function. This last can act as a 1,3-dipolar group in cycloaddition reactions with dipolarophiles such as the anions obtained from suitable methylene active compounds of type **4**. Although this type of reaction is well described in aromatic series, only in a few cases it has been applied to pentatomic heterocycles, mainly thiophene derivatives [8,9]. Moreover in azole series the only two examples reported so far [10,11] have demonstrated that the nature of the substrate and the reaction conditions can widely influence the nature of the reaction products. To the best of our knowledge there are no examples of the use of azidopyrroles. The product resulting from the cycloaddition reaction could be the 3-(triazol-1-yl)pyrrole of type **5** which bears an amino group susceptible of further reactions.

The 3-azidopyrrole **3** was prepared starting from ethyl 4-nitro-2,5-diphenylpyrrole-3-carboxylate which was methylated in position 1 with methyl iodide, using a procedure already established in our laboratory [12]. Reduction of the nitro function with hydrogen and Palladium followed by

Scheme 1

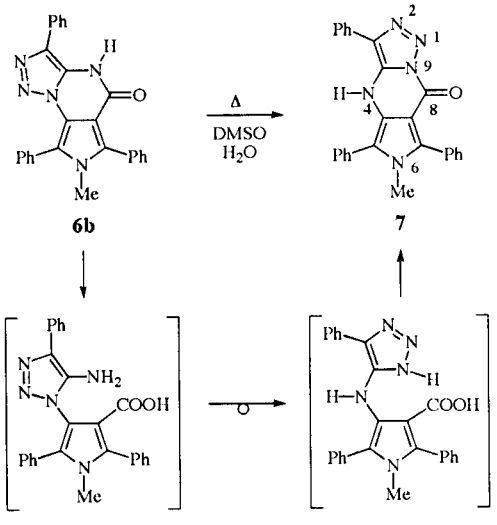


diazotization of the resulting amino group and treatment with sodium azide gave rise to derivative **3** in high overall yields. The azide **3** was reacted with the sodium salt of acetonitriles **4a-c** in ethanol, at room temperature for 24 hours. Under these conditions the 3-(triazol-1-yl)pyrrole of type **5** was indeed formed but the cycloaddition reaction was immediately followed by further condensation between the amino group and the ester function. Therefore the pyrrolo[3,4-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidin-5-ones **6a-c** were directly isolated in high yields (Scheme 1).

The structure of these compounds was confirmed by spectroscopic data. In particular in the IR spectra bands were observed that are attributable to a cyclic-amide structure. In the ^1H NMR spectra the signal corresponding to the amide NH was found in the range 10.2-12.7 ppm whereas in the ^{13}C NMR spectra the chemical shift of the amide carbonyl appeared at 156.1-157.4 ppm.

Considering that either triazole [13] and pyrimidine [14] derivatives easily undergo rearrangements following ring opening-ring closure reactions under a variety of conditions, we decided to explore whether this kind of reactivity is also shared by the new polycondensed ring system of type **6**. Derivatives **6a-c** did not undergo rearrangement under classical Dimroth conditions (*i.e.*, in 20% aqueous potassium hydroxide) probably because of its poor water solubility, nor upon heating in ethanol [13]. However compound **6b** was readily and nearly quantitatively converted into the isomeric 6-methyl-3,5,7-triphenyl-4,6-dihydro-8*H*-pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine-8-one (**7**) by heating under reflux for 1 hour in dimethylsulfoxide, in the presence of traces of water (Scheme 2). 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 well acknowledged literature report on Dimroth rearrangement in pyrimidine series [15]. Probably the lack of reactivity of derivatives **6a** and **6c** has to be ascribed to their high melting points.

Scheme 2



The direct rearrangement involving breakage of N_1-N_9 bond, immediately followed by ring closure on N_4, in our opinion, has to be ruled out because of the failure of the transformation under thermal conditions, as widely demonstrated in the presence of the NNN linkage in cyclic arrangement in five [13] and six [16] membered rings.

The structure of derivative **7** was assigned principally on the basis of its NMR spectra. In the proton spectrum it was possible to note a downfield shift (about 0.3 ppm) for the two *ortho* protons on the phenyl in position 3, that are on average closer to the NH than that of derivative **6b** [17]. In the ^{13}C NMR the carbonyl carbon (C-8) was deshielded by about 6.9 ppm with respect to the corresponding signal in derivative **6b**. We also found a good correlation with the calculated chemical shifts for all the carbon resonances [18]. Moreover in the IR spectrum the NH stretching band was found at 3366 cm^{-1}, a higher frequency than that of derivative **6b**, due to the fact that the imine proton is less accessible for hydrogen bonding.

To evaluate the potential ability of the two new planar polycyclic ring systems to interact with DNA, we calculated [17] 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 Amsacrine (AMSA, **1**, *o*-methoxy-*p*-methanesulfonanilide) or Doxo-rubicin (DOXO).

Table

LUMO and HOMO Energy Values (eV)

Compound	LUMO	HOMO
6a	-0.64	-8.79
6b	-0.80	-8.86
6c	-0.58	-8.56
7	-0.64	-8.33
AMSA	-1.15	-8.48
DOXO	-1.57	-9.10

EXPERIMENTAL

All melting points were taken on a Büchi-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 respectively in DMSO-d_6 solution (TMS as internal reference), at 200 and 50.3 MHz, using a Bruker AC-E series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM.

Ethyl 4-azido-1-methyl-2,5-diphenyl-1*H*-pyrrole-3-carboxylate (**3**).

To a cold suspension of ethyl 4-nitro-2,5-diphenyl-1*H*-pyrrole-3-carboxylate [19] (3.36 g, 10 mmoles) in absolute acetonitrile (20 ml) sodium hydride (60% dispersion in oil, 0.6 g, 15 mmoles)

was added in portions. After 30 minutes methyl iodide (1.3 ml, 20 mmoles) was added dropwise and the mixture was stirred at room temperature for 3 hours. The precipitate was filtered off, and the resulting solution was evaporated to give an oil residue which crystallized when purified by column chromatography using dichloromethane as eluant and which was identified as ethyl 1-methyl-4-nitro-2,5-diphenylpyrrole-3-carboxylate: Yield 94%, mp 146°C; IR: 1720 (CO), 1504 and 1343 (NO_2) cm^{-1}; ^1H NMR δ: 1.03 (3H, t, J = 6.4 Hz, CH_2CH_3), 3.21 (3H, s, CH_3), 4.08 (2H, q, J = 6.4 Hz, CH_2CH_3), 7.51-7.56 (10H, m, 2 x C_6H_5); ^{13}C NMR δ: 13.59 (q), 33.62 (q), 60.56 (t), 128.09 (s), 128.43 (d), 128.57 (d), 128.82 (s), 129.27 (d), 129.69 (d), 130.35 (d), 130.49 (d), 130.49 (s), 133.66 (s), 134.83 (s), 135.45 (s), 162.60 (s, CO).

Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.55; H, 5.18; N, 8.00. Found: C, 68.67; H, 5.21; N, 8.10.

A solution of ethyl 1-methyl-4-nitro-2,5-diphenyl-1*H*-pyrrole-3-carboxylate (3.5 g, 10 mmoles) in ethanol (20 ml) was reduced overnight with hydrogen over 10% Pd on charcoal (5.0 g) in a Parr apparatus at 50 psi at room temperature. Removal of the catalyst and evaporation of the solvent under reduced pressure gave a residue which was purified by column chromatography using dichloromethane as eluant and identified as ethyl 4-amino-1-methyl-2,5-diphenylpyrrole-3-carboxylate, an uncrystallizable oil: Yield 90%, IR: 3330 and 3310 (NH_2), 1697 (CO) cm^{-1}; ^1H NMR δ: 0.90 (3H, t, J = 6.4 Hz, CH_2CH_3), 3.20 (3H, s, CH_3), 3.93 (2H, q, J = 6.4 Hz, CH_2CH_3), 4.60 (2H, bs, NH_2), 7.40-7.47 (10H, m, 2 x C_6H_5); ^{13}C NMR δ: 13.72 (q), 33.36 (q), 58.30 (t), 101.55 (s), 116.23 (s), 126.20 (d), 127.58 (d), 127.84 (d), 128.54 (d), 129.04 (d), 130.71 (d), 131.31 (s), 132.26 (s), 132.78 (s), 136.13 (s), 164.98 (s, CO).

Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 74.96; H, 6.30; N, 8.75. Found: C, 75.02; H, 6.25; N, 8.83.

To a solution of ethyl 4-amino-1-methyl-2,5-diphenyl-1*H*-pyrrole-3-carboxylate (3.2 g, 10 mmoles) in acetic acid (30 ml) and water (4 ml), sodium nitrite (0.83 g, 12 mmoles) in water (4 ml) was added at 0°C, under vigorous stirring. After 50 minutes sodium azide (3.25 g, 50 mmoles) was added in portions and the reactants were stirred for another 3 hours at room temperature. The solid was filtered off and air dried to give **3** as a yellow precipitate: Yield 89%, mp 97°C; IR: 2118 (N_3), 1694 (CO) cm^{-1}; ^1H NMR δ: 0.96 (3H, t, J = 7.4 Hz, CH_2CH_3), 3.23 (3H, s, CH_3), 4.00 (2H, q, J = 7.4 Hz, CH_2CH_3), 7.40-7.55 (10H, m, 2 x C_6H_5); ^{13}C NMR δ: 13.65 (q), 33.37 (q), 59.13 (t), 106.34 (s), 119.85 (s), 126.02 (s), 127.88 (d), 128.16 (d), 128.46 (2d), 129.03 (s), 130.28 (d), 130.61 (d), 131.16 (s), 137.66 (s), 162.81 (s, CO).

Anal. Calcd for $C_{20}H_{18}N_4O_2$: C, 69.34; H, 5.24; N, 16.18. Found: C, 69.54; H, 5.30; N, 16.22.

General method for the preparation of 3-substituted 7-Methyl-6,8-diphenyl-5,7-dihydro-4*H*-pyrrolo[3,4-*e*][1,2,3]triazolo-[1,5-*a*]pyrimidin-5-ones **6a-c**.

To a solution of sodium (0.09 g, 3.9 mmoles) in absolute ethanol (10 ml) substituted acetonitriles **4a-c** (3.9 mmoles) in absolute ethanol (20 ml) were added at room temperature. After being stirred for 15 minutes a solution of azido-pyrrole **3** (0.74 g, 3.6 mmoles) in absolute ethanol (10 ml) was added and the mixture was stirred for further 24 hours 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.

7-Methyl-5-oxo-6,8-diphenyl-5,7-dihydro-4*H*-pyrrolo[3,4-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide (**6a**).

Compound **6a** was prepared from **3** and 2-cyano-acetamide (**4a**), (Yield 95%) had mp >325°C; IR: 3450-3330 (NH_2 and NH), 1658 (broad CO) cm^{-1}; ^1H NMR δ: 3.45 (3H, s, CH_3), 7.52-7.73 (10H, m, 2 x C_6H_5), 7.73-7.85 (2H, d, NH_2), 10.17 (1H, bs, NH); ^{13}C NMR δ: 33.99 (q, CH_3), 101.39 (s, C-5a), 118.73 (s, C-1'), 120.29 (s, C-1'), 122.28 (s, C-8a), 128.01 (d, 2 x C-2'), 128.27 (d, 2 x C-2'), 128.65 (d, C-4'), 128.96 (d, C-4'), 128.99 (s, C-3), 129.01 (s, C-3a), 131.14 (d, 2 x C-3'), 131.51 (d, 2 x C-3'), 135.31 (s, C-6), 136.34 (s, C-8), 156.05 (s, C-5), 162.50 (s, CO).

Anal. Calcd for $C_{21}H_{16}N_6O_2$: C, 65.60; H, 4.20; N, 21.87. Found: C, 65.63; H, 4.17; N, 21.90.

7-Methyl-3,6,8-triphenyl-5,7-dihydro-4*H*-pyrrolo[3,4-*e*][1,2,3]-triazolo[1,5-*a*]pyrimidine-5-one (**6b**).

Compound **6b** was prepared from **3** and 2-phenylacetamide (**4b**), (Yield 60%) had mp 225°C; IR: 3179 (NH), 1673 (CO) cm^{-1}; ^1H NMR δ: 3.43 (3H, s, CH_3), 7.28-7.73 (13H, m, 2 x C_6H_5 and 3-$C_6H_{5(meta\ and\ para)}$), 7.79-7.83 (2H, m, 3-$C_6H_{5(ortho)}$) 11.55 (1H, bs, NH); ^{13}C NMR δ: 33.85 (q, CH_3), 101.40 (s, C-5a), 118.94 (s, C-8a) , 120.03 (s, C-1'), 126.41 (d, 2 x C-2'), 126.97 (s, C-1'), 127.14 (d, C-4'), 127.92 (d, 2 x C-2'), 128.17 (d, 2 x C-2'), 128.45 (d, 2 x C-3'), 128.77 (d, C-4'), 128.81 (d, C-4'), 128.99 (s, C-3), 129.22 (s, C-6), 129.91 (s, C-3a), 131.12 (d, 2 x C-3'), 131.54 (d, 2 x C-3'), 134.85 (s, C-8), 157.38 (s, C-5).

Anal. Calcd for $C_{26}H_{19}N_5O$: C, 74.79; H, 4.59; N, 16.78. Found: C, 74.83; H, 4.61; N, 16.81.

7-Methyl-5-oxo-6,8-diphenyl-5,7-dihydro-4*H*-pyrrolo[3,4-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidine-3-carbonitrile (**6c**).

Compound **6c** was prepared from **3** and malononitrile (**4c**), (Yield 70%) had mp >325°C; IR: 3136 (NH), 2236 (CN), 1686 (CO) cm^{-1}; ^1H NMR δ: 3.45 (3H, s, CH_3), 7.52-7.73 (10H, m, 2 x C_6H_5), 12.73 (1H, bs, NH); ^{13}C NMR δ: 34.05 (q, CH_3), 101.45 (s, C-5a), 111.95 (s, CN), 118.47 (s, C-8a), 120.56 (s, C-3), 128.08 (d, 2 x C-2'), 128.40 (d, 2 x C-2'), 128.44 (s, 2 x C-1'), 128.82 (s, C-3a), 129.11 (d, C-4'), 129.15 (d, C-4'), 131.13 (d, 2 x C-3'), 131.45 (d, 2 x C-3'), 135.18 (s, C-6), 139.17 (s, C-8), 156.63 (s, C-5).

Anal. Calcd for $C_{21}H_{14}N_6O$: C, 68.83; H, 3.85; N, 22.95. Found: C, 68.89; H, 3.93; N, 23.01.

6-Methyl-3,5,7-triphenyl-4,6-dihydro-8*H*-pyrrolo[3,4-*d*][1,2,3]-triazolo[1,5-*a*]pyrimidin-8-one (**7**).

A solution of pyrrolo[3,4-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivative **6b** (0.5 mmoles) was refluxed in dimethylsulfoxide (10 ml) for 1 hour. The cooled reaction mixture was then poured onto crushed ice and the solid was filtered off, air dried to give derivative **7**: Yield 98%, mp 194°C; IR: 3366 (NH), 1674 (CO) cm^{-1}; ^1H NMR δ: 3.70 (3H, s, CH_3), 7.41-7.77 (13H, m, 2 x C_6H_5 and 3-$C_6H_{5(meta\ and\ para)}$), 8.19-8.23 (2H, m, 3-$C_6H_{5(ortho)}$) 11.27 (1H, bs, NH); ^{13}C NMR δ: 34.87 (q, CH_3), 106.18 (s, C-7a), 127.81 (d, C-4') , 127.89 (d, 2 x C-2'), 128.09 (d, 2 x C-2'), 128.35 (d, 2 x C-2'), 128.85 (s, C-1'), 129.35 (s, C-1'), 129.41 (d, C-4'), 129.66 (s, C-4a), 130.12 (d, 2 x C-3'), 130.93 (d, 2 x C-3'), 131.16 (d, 2 x C-3'), 131.76 (s, C-1'), 133.44 (d, C-4'), 133.51 (s, C-3), 134.80 (s, C-7), 145.69 (s, C-3a), 158.21 (s, C-5), 164.28 (s, C-8).

Anal. Calcd for $C_{26}H_{19}N_5O$: C, 74.79; H, 4.59; N, 16.78. Found: C, 74.86; H, 4.53; N, 16.83.

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