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Pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine: a new ring system through Dimroth rearrangement

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ABSTRACT

Derivatives of the new ring system pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine were synthesized from the corresponding angular isomers, through a Dimroth rearrangement, in quantitative yields. Preliminary computational studies demonstrated that this class of compounds could be a good candidate as DNA intercalating agents.

Flat heterocyclic structures are fundamental moieties in anticancer compounds acting as DNA intercalating agents.[1] In fact, they play a determinant role in the π–π stacking interactions with the DNA base pairs. Consequently, the modulation of their electronic and steric features by the presence of opportune substituents or heteroatoms, could improve this capability. With the aim to extend the synthetic pathways to new planar heterocyclic ring systems we focused our studies on the pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine core structure **1** (Fig. 1), isomer of the angular pyrazolo[4,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine **2**, known in literature.[2]

All the above systems can be related to DNA-interactive drugs such as acridines, anthracyclines, and actinomycins. The principal driving forces for the intercalation into DNA are stacking, charge-transfer, as well as hydrogen bonding and electrostatic interactions.[3]

Recently, we have also reported on the synthesis[4] of other systems incorporating a triazolopyrimidine moiety, the indolo[3,2-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidine core structure **3** (Fig. 2), again related to the abovementioned DNA intercalators. The functionalization by chain shape in 4 position (**3**, R_1 = **B**–**H**, R_2 = Ph) has shown to give a remarkable anti-tumor activity in micromolar scale.[5]

We hypothesize that in a similar fashion to the pyrrolotriazolopyrimidine series,[6,7] the Dimroth rearrangement (DR), in basic conditions, could be applied to the angular isomer **4** in hopes of preparing polyheterocyclic ring **5** (Scheme 1).

It is clear that a crucial role in this approach is played by the starting angular isomer **4**, for two principal reasons. First, the reaction reported in literature for its preparation[2] is not of general

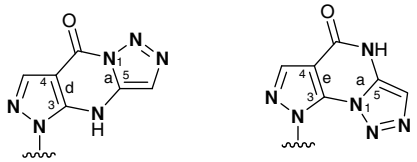


Figure 1. Designed heterocyclic ring systems.

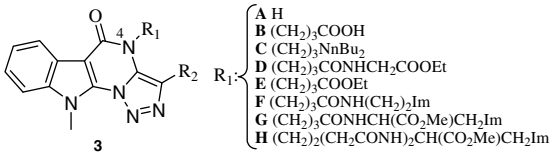
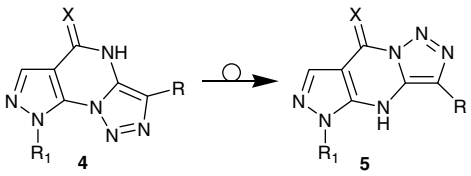


Figure 2. Indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine derivatives with anti-tumor activity.



Scheme 1. Synthetic route to pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine core structure.

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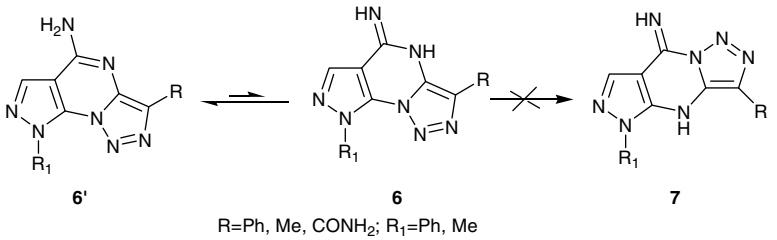
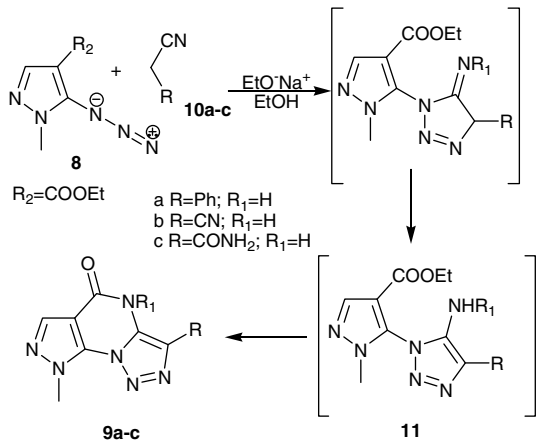


Scheme 2. Prototropic shift influences in the Dimroth rearrangement.



Scheme 3. Routes to pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine ring systems.

application, since it only allowed to isolate one derivative **4** (X = NH, R = R_1 = Ph) due to the other derivatives' instability during the work-up. Second, the DR applied onto the imino derivative **6** does not take place, due to the rapid prototropic shift to the more stable amino isomer **6′** (Scheme 2).

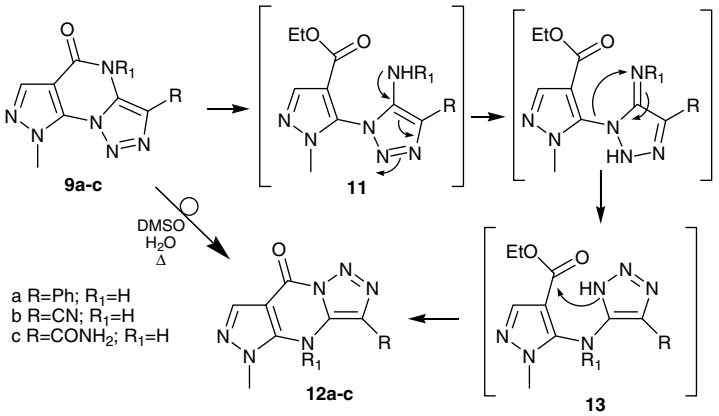
In fact the opening of pyrimidine ring and the following DR to get **7**, could allow only in the lactam form **6** and not in the amidine one **6′**.

Our experience on 1,3-cycloaddition reactions[4,6–8] led us to use the carboxyethyl derivative (**8**, R_2 = CO_2Et) instead of the carbonitrile one (**8**, R_2 = CN). In fact, routes to ring systems **9** can be provided by domino reactions[9] between acetonitriles **10** and azidopyrazole **8** under basic conditions (Scheme 3).

Thus, the pyrazole derivative **8** through the azido moiety can act as a 1,3-dipolar compound in cycloaddition reactions with dipolarophiles such as the anions obtained from the methylene active derivatives **10**. The intermediate resulting from the cycloaddition reaction could be the 3-(triazol-1-yl)pyrazole of type **11**, which bears an amino group susceptible to further reactions. In fact intermediate **11**, in the presence of a vicinal carboxylate function, further cyclizes to provide the pyrimidine ring.

The azidopyrazole (**8**, R_1 = CO_2Et) was prepared, in good yields, from the corresponding 2-amino derivative by diazotization with sodium nitrite in acetic acid and addition in situ of sodium azide to the intermediate diazonium salt.[10] The pyrazole **8** was added at room temperature to the sodium salts of acetonitriles in ethanol, under the same experimental conditions successfully used previously.[4,6–8]

In this case after 24 h at room temperature the TLC analysis showed mainly unreacted starting compounds. However, when the reaction was carried out under reflux the appearance of the new compounds was observed (TLC monitoring). Therefore, it seems that in this case the bond-forming efficiency depends on the first reaction step, consisting in the 1,3-dipolar cycloaddition to form the triazole moiety (Scheme 3). Probably this is due to a more electron withdrawing character of the pyrazole ring respect to the pyrrole one. In this context, it is worth mentioning that the HOMO–LUMO energy gap, calculated by the DFT B3LYP[11] method with the 6-31G(d,p)[12] basis set, by the GAUSSIAN 03 program package,[13] resulted to be 462.1 and 414.8 kJ/mol, for the optimized geometries of azidopyrazole and azidopyrrole, respectively. On the other hand, the energy of the azidopyrazole HOMO resulted to be 85.3 kJ/mol lower than that of azidopyrrole. Therefore, these results support the hypothesis that the interaction of the LUMO of the dipolarophile **10** with the HOMO of **8** should be less favored compared to that with the HOMO of the analogous pyrrole derivative. The new compounds **9**, derivatives of the ring system pyrazolo[4,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine, were generally isolated in high yields (70–90%).[10] Their structures were confirmed by spectroscopic data; in particular by the presence in the IR spectra of the typical absorption bands (at 3435–2926 and 1661–1644 cm^{-1}) due



Scheme 4. Dimroth rearrangement mechanism.

Table 1
DNA fragment docking results (GSCORE)

Entry	R_1							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
9a	−5.84	−5.76	−7.50	−6.22	−5.69	−7.80	−6.94	−7.54
9b	−6.01	−6.10	−7.14	−6.12	−5.91	−7.71	−7.29	−7.02
9c	−7.21	−7.03	−9.34	−8.33	−7.81	−8.48	−7.61	−6.96
12a	−5.62	−5.34	−6.69	−5.72	−5.22	−7.77	−7.47	−6.53
12b	−5.56	−5.74	−6.78	−5.75	−5.39	−7.05	−6.95	−6.50
12c	−5.82	−5.55	−6.41	−5.17	−5.44	−7.53	−6.81	−6.48
3 (R_2 = Ph)	−5.56	−6.19	−7.47	−6.18	−6.06	−8.19	−7.37	−7.16
Mean	−5.95	−5.96	−7.33	−6.21	−5.93	−7.79	−7.21	−6.88

A: H; **B**: $(CH_2)_3COOH$; **C**: $(CH_2)_3NnBu_2$; **D**: $(CH_2)_3CONHCH_2COOEt$; **E**: $(CH_2)_3COOEt$; **F**: $(CH_2)_3CONH(CH_2)_2Im$; **G**: $(CH_2)_3CONHCH(CO_2Me)CH_2Im$; **H**: $(CH_2)_2(CH_2CONH)_2$-$CH(CO_2Me)CH_2Im$.

to the presence of a cyclic-amide structure. Moreover, in the ^{13}C NMR the diagnostic signal of the amide carbonyl moiety was found in the range 154.8–155.1 ppm.

Thus, as already demonstrated in the case of pyrrolo[3,4-*e*]-[1,2,3]triazolo[1,5-*a*]pyrimidine derivatives which rearranged to pyrrolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine,[7] compounds **9** were heated under reflux in aqueous DMSO to give pyrazolo[3,4-*d*]-[1,2,3]triazolo[1,5-*a*]pyrimidines **12** in nearly quantitative yields (Scheme 4).[10]

This conversion reaction can be envisaged as a ring-opening ring-closure of the pyrimidine ring in the presence of water.[4] 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 DR in pyrimidine series.[14] The spectroscopic evidence for the rearranged structure, as observed in pyrrole series,[6,7] are a general increase in the IR frequencies for the amide carbonyl and the downfield shifts (both ^1H and ^{13}C) of the methyl moiety signal.

For a preliminary evaluation of the ability of the designed pyrazolotriazolopyrimidines **9** and **12** to interact with DNA, we performed docking calculations by using the GLIDE[15] software. GLIDE output consists of a score named GSCORE, where a more negative value indicates a higher binding affinity. The aim of the computational studies was also to analyze the effect of the chain shape functionalization on the DNA binding affinity, as already observed in indolotriazolopyrimidine series.[5]

The Protein Data Bank[16] was searched for DNA fragments bound with intercalators and the structure 1DSC (an octamer complexed with Actinomycin D) was selected. The original ligand was removed and the DNA sequence was utilized for the docking experiments.

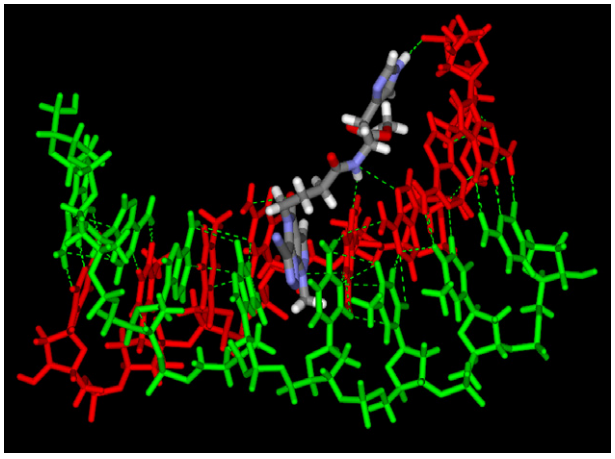


Figure 3. Docking mode of derivative **9bG** into DNA fragment.

At first glance, by analyzing the docking scores reported in Table 1, it emerged that the new derivatives **9** and **12**, when unsubstituted (R_1 = A), show a DNA binding affinity comparable to **3A**. Moreover, considering the same side chain substituents introduced in the indolotriazolopirimidine series (R_1 = **B**–**H**),[5] even in this case again their presence drastically improves the binding affinity if compared with the unsubstituted derivatives. The docking scores were found in the range from −5.17 to −9.34 and more negative than the value obtained by us[17] for Actinomycin D (GSCORE = −4.5). It is note to worth that the same trend observed in case of indolotriazolopirimidines **3**-substituted[5] was observed. All docked ligands revealed a common binding mode with the chromophore intercalated between the DNA GC base pairs, whereas the side chain lies along the minor groove (Fig. 3).

Therefore, the pyrazolotriazolopyrimidine derivatives **9** and **12** could be capable of forming stable complexes with DNA. Considering that the biological activity of Actinomycin D is related to a very slow dissociation rate of the complex with DNA, the docking results predict these compounds to be good candidates as antitumor drugs.

In conclusion, in this work we have generalized the synthetic route to the pyrazolo[4,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine ring system, previously successfully achieved only in one case. Moreover, by applying the DR it is possible to suitably synthetize derivatives of the new ring system pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine, a promising candidate as DNA intercalating compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.104.

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10. *Experimental*: Melting points (uncorrected) were taken on a Buchi–Tottoli capillary apparatus; 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 $(CD_3)_2SO$ 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 5-azido-1-methyl-1*H*-pyrazole-4-carboxylate (**8**, R = COOEt). To a solution of ethyl 5-amino-1-methyl-1*H*-pyrazole-4-carboxylate (1.7 g, 10 mmol) in acetic acid (20 mL) and water (4 mL), sodium nitrite (1.38 g, 20 mmol) in water (4 mL) was added at 0 °C, under vigorous stirring. After 50 min sodium azide (2.60 g, 40 mmol) was added in portions and the reactants were stirred for a further 3 h at room temperature. The mixture, poured into water/ice, was neutralized with $NaHCO_3$ and then it was extracted with ethyl acetate. The organic layers were combined, dried over sodium sulfate, and evaporated to dryness. The residue was purified by column chromatography using dichloromethane/ethyl acetate 95:5 as eluant to give derivative give **8** as a yellow oil: 1.17 g, yield 60%; IR: 2120 (N_3), 1693 (CO) cm^{-1}; ^1H NMR δ: 1.25 (3H, t, J = 6.4 Hz, CH_3), 3.69 (3H, s, CH_3), 4.31 (2H, q, J = 6.4 Hz, CH_2), 7.77 (1H, s, H-3); ^{13}C NMR δ: 14.4 (q), 35.4 (q), 60.3 (t), 104.7 (s), 139.1 (s), 140.8 (d), 162.4 (s). Anal. Calcd for $C_7H_9N_5O_2$: C, 43.08; H, 4.65; N, 35.88. Found: C, 43.19; H, 4.59; N, 36.08. HRMS: (M+) calcd for $C_7H_9N_5O_2$ 195.0756; found 195.0772.

General method for the preparation of 3-substituted 8-methyl-4*H*-pyrazolo[4,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines (**9a**–**c**). To a solution of 1.0 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 derivative **8** (3.6 mmol) in absolute ethanol (10 mL) was added and the mixture was stirred for a further 8 h under reflux. Evaporation of the solvent under reduced pressure gave a solid which was purified by column chromatography using dichloromethane/ethyl acetate 95:5 as eluant. 8-Methyl-3-phenyl-4*H*-pyrazolo[4,3-*e*][1,2,3]-triazolo[1,5-*a*]pyrimidin-5(8*H*)-one (**9a**). From **8** and 2-phenylacetamide (**10a**), a yellow solid (yield 90%) was obtained: mp >300 °C; IR: 3356–2926 (NH), 1644 (CO) cm^{-1}; ^1H NMR δ: 3.71 (3H, s, CH_3), 7.00 (1H, t, J = 6.0 Hz, *p*-Ph), 7.27 (2H, dd, J = 6.0 and 6.5 Hz, *m*-Ph), 7.81 (1H, s, H-6), 8.40 (2H, d, J = 6.5 Hz, *o*-Ph); ^{13}C NMR d:33.5 (q), 97.9 (s), 123.8 (d), 125.0 (d), 126.8 (s), 128.6 (d), 133.9 (d), 134.1 (s), 144.8 (s), 152.5 (s), 155.1 (s). Anal. Calcd for $C_{13}H_{10}N_6O$: C, 58.64; H, 3.79; N, 31.56. Found: C, 58.67; H, 3.71; N, 31.68. HRMS: (M+) calcd for $C_{13}H_{10}N_6O$ 266.0916; found 266.0925. 8-Methyl-5-oxo-5,8-dihydro-4*H*-pyrazolo[4,3-*e*][1,2,3]triazolo-[1,5-*a*]pyrimidine-3-carbonitrile (**9b**). From **8** and malononitrile (**10b**), a brown solid (yield 85%) was obtained: mp >300 °C; IR: 3424–3050 (NH), 2232 (CN), 1661 (CO) cm^{-1}; ^1H NMR δ: 3.80 (3H, s, CH_3), 7.98 (1H, s, H-6); ^{13}C NMR δ: 33.8 (q), 98.6 (s), 102.4 (s), 115.6 (s), 134.4 (d), 150.4 (s), 151.9 (s), 154.8 (s). Anal. Calcd for $C_8H_5N_7O$: C, 44.66; H, 2.34; N, 45.57. Found: C, 44.73; H, 2.31; N, 45.69. HRMS: (M+) calcd for $C_8H_5N_7O$ 215.0556; found 215.0544. 8-Methyl-5-oxo-5,8-dihydro-4*H*-pyrazolo[4,3-*e*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide (**9c**). From **8** and 2-cyanoacetamide (**10c**), a yellow solid (yield 70%) was obtained: mp >300 °C; IR: 3435–3070 (NH), 1654 (CO) cm^{-1}; ^1H NMR δ: 3.92 (3H, s, CH_3), 4.90 (2H, br s, NH_2), 7.84 (1H, s, H-6); ^{13}C NMR δ: 33.5 (q), 96.6 (s), 104.1 (s), 133.5 (d), 150.1 (s), 151.3 (s), 154.9 (s), 168.2 (s). Anal. Calcd for $C_8H_7N_7O_2$: C, 41.21; H, 3.03; N, 42.05. Found: C, 41.29; H, 3.15; N, 42.19. HRMS: (M+) calcd for $C_8H_7N_7O_2$ 233.0661; found 233.0679.

General method for the preparation of 3-substituted 5-methyl-4,5-dihydro-8*H*-pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine (**12a,c**). A solution of **9** 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 derivatives **12**. 5-Methyl-3-phenyl-4,5-dihydro-8*H*-pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidin-8-one (**12a**). From **9a** (0.5 g, 1.88 mmol) as a light yellow solid (yield 98%): mp 210 °C; IR: 3370–2965 (NH), 1698 (CO) cm^{-1}; ^1H NMR δ: 3.82 (3H, s, CH_3), 7.12 (1H, t, J = 6.0 Hz, *p*-Ph), 7.31 (2H, dd, J = 6.1 and 6.4 Hz, *m*-Ph), 7.92 (1H, s, H-7), 8.31 (2H, d, J = 6.4 Hz, *o*-Ph); ^{13}C NMR δ: 36.5 (q), 99.9 (s), 123.9 (d), 125.0 (d), 126.9 (s), 128.7 (d), 132.6 (d), 135.2 (s), 139.2 (s), 151.4 (s), 156.2 (s). Anal. Calcd for $C_{13}H_{10}N_6O$: C, 58.64; H, 3.79; N, 31.56. Found: C, 58.71; H, 3.75; N, 31.59. HRMS: (M+) calcd for $C_{13}H_{10}N_6O$ 266.0916; found 266.0928. 5-Methyl-8-oxo-5,8-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carbonitrile (**12b**). From **9b** (0.5 g, 2.32 mmol) as a light yellow solid (yield 95%): mp 214 °C; IR: 3389–3076 (NH), 2234 (CN), 1671 (CO) cm^{-1}; ^1H NMR δ: 3.91 (3H, s, CH_3), 8.09 (1H, s, H-7); ^{13}C NMR δ: 36.1 (q), 98.5 (s), 102.9 (s), 115.6 (s), 135.2 (d), 150.2 (s), 151.8 (s), 156.1 (s). Anal. Calcd for $C_8H_5N_7O$: C, 44.66; H, 2.34; N, 45.57. Found: C, 44.77; H, 2.39; N, 45.65. HRMS: (M+) calcd for $C_8H_5N_7O$ 215.0556; found 215.0541.

5-Methyl-8-oxo-5,8-dihydro-4*H*-pyrazolo[3,4-*d*][1,2,3]triazolo[1,5-*a*]pyrimidine-3-carboxamide (**12c**). From **9c** (0.5 g, 2.15 mmol) white solid (yield 95%): mp 203 °C; IR: 3411–3076 (NH), 1668 (CO) cm^{-1}; ^1H NMR δ: 3.95 (3H, s, CH_3), 4.70 (2H, br s, NH_2), 7.91 (1H, s, H-7); ^{13}C NMR δ: 35.9 (q), 97.5 (s), 103.9 (s), 134.3 (d), 152.7 (s), 152.9 (s), 155.8 (s), 168.4 (s). Anal. Calcd for $C_8H_7N_7O_2$: C, 41.21; H, 3.03; N, 42.05. Found: C, 41.38; H, 3.12; N, 42.39. HRMS: (M+) calcd for $C_8H_7N_7O_2$ 233.0661; found 233.0652.

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