On the reactivity of 6-acetyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-*a*]pyrimidines with 1,3- and 1,4-bisnucleophiles

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Reaction of 6-acetyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-*a*]pyrimidine **1** with 1,3- and 1,4-bisnucleophiles has been investigated; obtainment of new polycyclic heterocyclic derivatives is reported. A convenient procedure leading to new pyrazolo[1,5-*a*]quinazolines is described; a modest bioactivity of these compounds against two human tumor cell lines was also ascertained.

Introduction

Fused heterocyclic compounds such as pyrazolo[1,5-*a*]pyrimidine and its derivatives are known to possess pharmacological activity and anxiolytic properties.[1] Our research group has been involved for a long time in the chemistry of pyrazolo[1,5-*a*]pyrimidines with the aim of synthesizing new tricyclic systems as novel selective GABAA α1 receptor agonists or benzodiazepine receptor (BZR) ligands.[1–3] We have previously reported on the reactivity of compounds of type **1** towards some nucleophiles like ammonia,[4] hydroxylamine,[5] and hydrazine[6] to give pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidines **A**, pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine-7-oxides **B**, and 6-(pyrazol-3′-yl)pyrazolo[1,5-*a*]pyrimidines **C**, respectively (see Fig. 1).

Following these results we decided to investigate the behaviour of compound **1** towards various 1,3- and 1,4-bisnucleophiles in an effort to expand the scope of the above reaction.

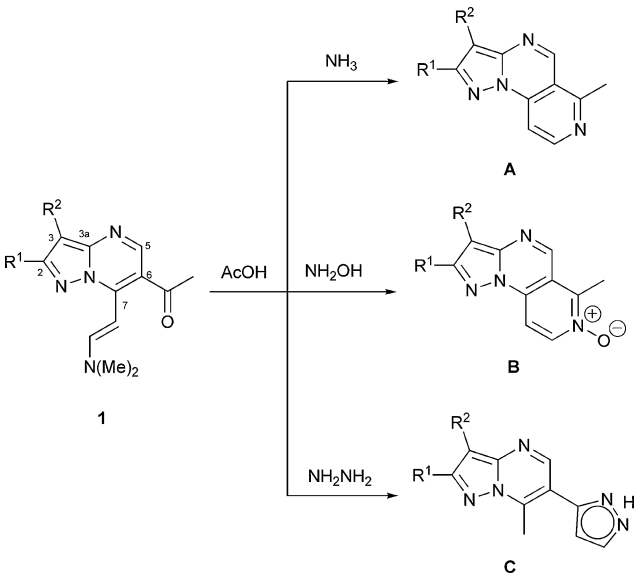

Fig. 1 Reactivity of **1** with ammonia, hydroxylamine and hydrazine.

Results and discussion

When the dimethylaminovinyl derivative **1** was allowed to react with 1,3-bisnucleophiles in glacial acetic acid in the presence of sodium acetate a complex mixture of products is obtained. Thus, we carried out the reaction in diglyme with the 1,3-bisnucleophile as free base. The reaction always afforded an unique product with moderate (*ca.* 60%) yields, except for when using *S*-methylthiourea. In this case the final product was obtained in low yield (18%) only when using a large excess of the reagent.

Although no intermediates have been isolated, it is possible to suggest that the reaction might proceed through compound **D** *via* two alternative pathways (Scheme 1): while route *a* should give rise to the pyrazolopyrimido[1,3]diazocine system **E**, route *b* rationalizes the formation of the isomeric compounds **2–5** through a nucleophilic attack of the NH group on the electron poor C-7 of the pyrimidine ring. In order to resolve this point with the aim to attribute the right structure to the reaction products, we examined the NMR spectra of the obtained compounds. 2D NMR spectra (gHSQC and gHMBC) show C–H connectivities that hold true for structures **E** and **2–5**. Thus, to distinguish between the two types of compound we performed some NOE experiments. To this end, we started assigning the pyrimidine and pyrazolo[1,5-*a*]pyrimidine proton signals on the basis of gHSQC and gHMBC experiments. Then, we noticed (using, for reference, compound **4**) that irradiation of the singlet at δ 8.7 ppm (attributed to H-5 of the unknown product) leads to a significant enhancement of the proton resonating at δ 7.32 ppm that appears as a doublet. Because at the same time no NOE is observed on the methyl group, we may attribute this signal to H-5′ of structures **2–5**. This attribution was then confirmed by irradiation of the singlet at δ 2.97 ppm (7-Me in structure **4** or 6-Me in the corresponding structure **E**) obtaining a significant enhancement only on the proton appearing as a doublet and that cannot be attributed to H-10 of structure **E**. These experimental data hold true for compounds **2–5**. Thus, we

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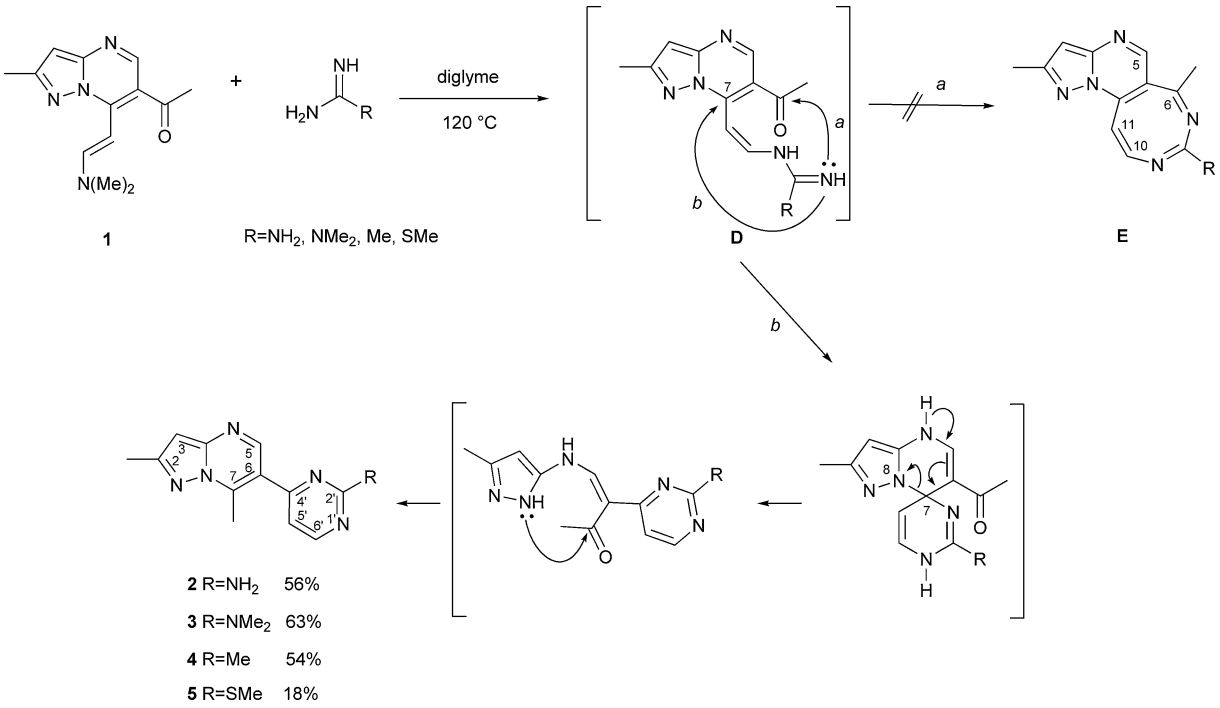
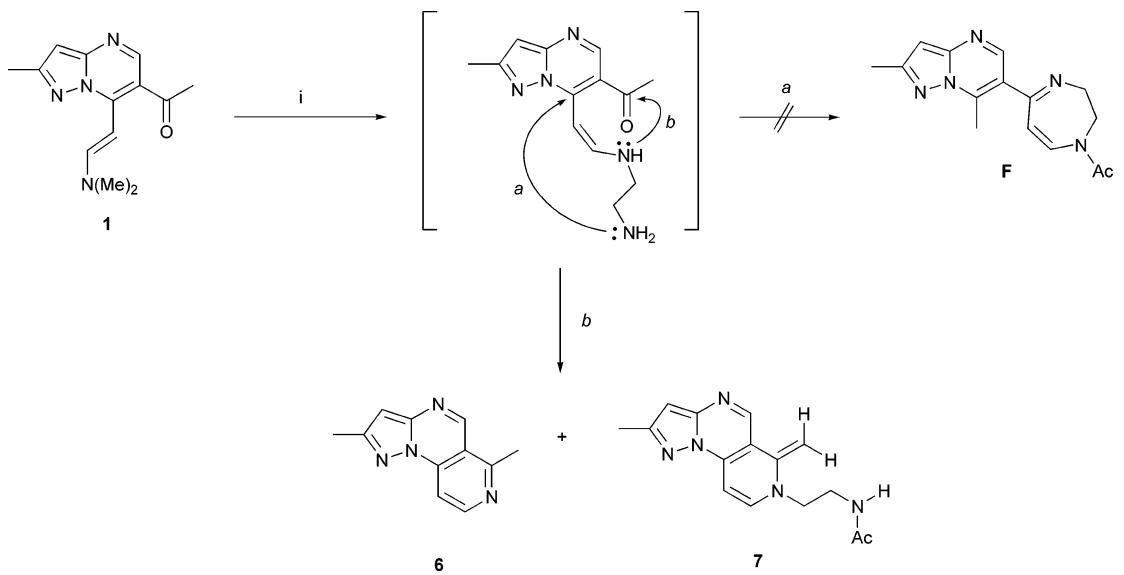


Scheme 1

established the pyrimidin-4-yl nature of compounds arising from the reaction of **1** with 1,3-bisnucleophiles, excluding the formation of the 1,3-diazocine ring system.

Following these results and being interested in new heterocycle-substituted pyrazolo[1,5-*a*]pyrimidines, we turned our attention to the reactivity of **1** with 1,4-bisnucleophiles. The reaction of **1** with *e.g.* ethane-1,2-diamine could give rise to a 1,4-diazepine system linked to position 6 of the pyrazolo[1,5-*a*]pyrimidine moiety according to that observed for 1,3-bisnucleophiles (Scheme 2, structure **F**). Reaction of compound **1** with ethane-1,2-diamine in acetic acid solution afforded a crude, mainly containing two products (*ca.* 2.5 : 1); work-up of this mixture (see Experimental) allowed us to isolate compounds **6** and **7**. The structure of the predominant compound **7** was derived as follows: first of all, the ^{1}H NMR spectrum clearly shows the presence of a terminal $C_{sp2}H_2$ group (two doublets at δ 4.66 and 3.96 ppm with a geminal coupling constant of 3.4 Hz), then the gHMBC spectrum contains two diagnostic correlations: (a) the carbon atom resonating at δ 140.0 ppm, that cannot be assigned to a C=N, is connected to the C_{sp2} protons and to those of a NCH_2 group, (b) the resonance at δ 170.0 ppm, clearly attributable to the C=O of the acetyl group, is connected to the exchangeable proton signal at δ 8.0 ppm



Scheme 2 *Reagents and conditions*: (i) ethane-1,2-diamine, AcOH, reflux, then NaOH, rt.

(NH) (Fig. 2). Moreover, the existence of a strong NOE effect between H-5 and only one of the C_{sp2} protons confirms the assigned pyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine structure and allows the exclusion of the formation of the 1,4-diazepine ring (Scheme 2, route *a*). Similarly, reaction of compound **1** with ethanolamine in the same experimental conditions gives rise to compound **8** (Scheme 3) whose structure was once again established by NMR experiments.

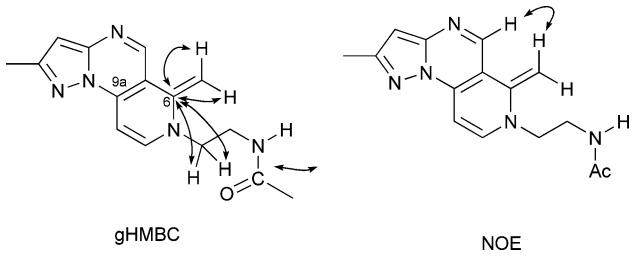
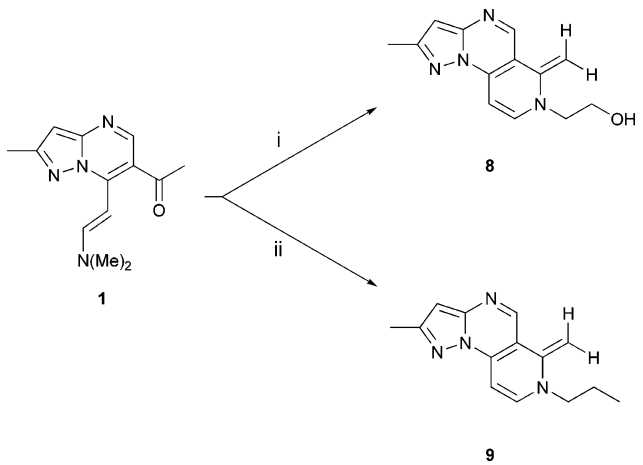


Fig. 2 Significant correlations in the gHMBC spectrum and NOE effect in compound **7**.

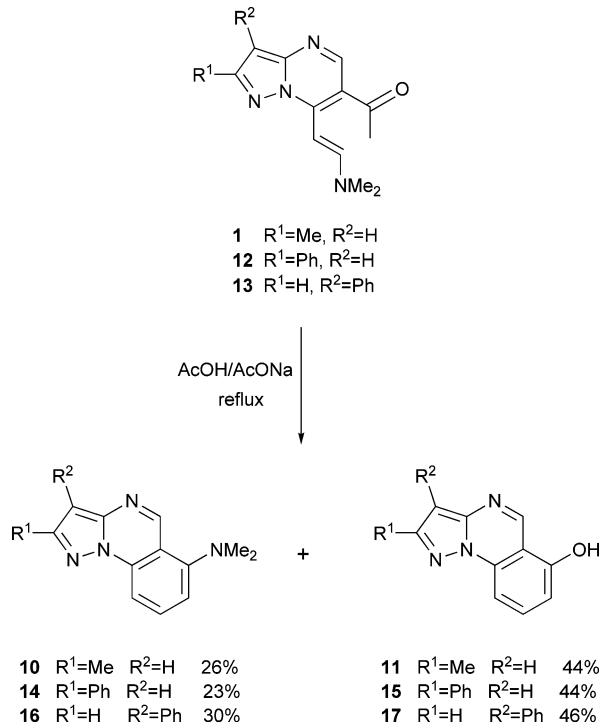


Scheme 3 *Reagents and conditions*: (i) ethanolamine, AcOH, reflux, then NaOH, rt, 85%; (ii) *n*-propylamine, AcOH, reflux, then NaOH, rt, 81%.

Thus, both ethane-1,2-diamine and ethanolamine do not react as 1,4-bisnucleophiles and to confirm this behaviour we carried out the reaction with *n*-propylamine. As expected, compound **1** reacts with this nucleophile in glacial acetic acid to give compound **9** in 81% yield.

A careful examination of the NMR spectra of the products originating from the reaction of **1** with ethane-1,2-diamine in glacial acetic acid gave evidence of the presence of a very small quantity of an heteroaromatic side product in the ^{1}H NMR spectrum of the separated, unpurified, compound **6**. In particular we noticed that the unknown compound possesses only one methyl group and showed, besides the signals of H-3 and H-5 of the pyrazolopyrimidine system, a clear pattern of three contiguous aromatic protons.

On this basis, we tentatively attributed a pyrazolo[1,5-*a*]quinazoline structure to this product that could arise from loss of dimethylamine owing to the attack by the enolic form of the acetyl group. To confirm this hypothesis compound **1** was refluxed in an acetic buffer solution to give, after separation, the expected 2-methylpyrazolo[1,5-*a*]quinazolin-6-ol **11** together with a minor amount of the corresponding 6-amino derivative **10** (Scheme 4). Compounds **12** and **13** showed an analogous reactivity, originating the corresponding derivatives. This procedure allowed us to obtain new pyrazoloquinazolines functionalized at position 6, starting from a suitable 6-acetyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-*a*]pyrimidine, thus opening a new synthetic pathway to the pyrazolo[1,5-*a*]quinazoline system.



Scheme 4

In the light of the reported biological properties of some pyrazolo[1,5-*a*]quinazolines,[7,8] we evaluated the antiproliferative activity of the new compounds. The cytotoxicity of compounds **10**, **11**, and **14–17**, was investigated on two cell lines of human tumor such as Jurkat (human lymphoblastoid leukaemia) and MCF-7 (human breast adenocarcinoma). Table 1 shows the extent of cell survival expressed as IC_{50} which is the concentration, expressed in μM, which induces 50% inhibition of cell growth, after

Table 1 Antiproliferative activity of tested compounds against two human tumor cell lines

	IC_{50}/μM[a] of cell lines	
Compound	Jurkat	MCF-7
10	>50	>50
11	36.3 ± 6.2	>50
14	>50	>50
15	35.5 ± 9.8	42.4 ± 6.5
16	18.5 ± 6.2	31.2 ± 4.3
17	>50	>50

[a] IC_{50} concentration of the compound required to inhibit the cellular growth by 50% after 72 hours of drug exposure, as determined by the MTT assay as described in the Experimental. Values are expressed as mean ± SEM of three experiments.

incubation for 72 h. The results indicate that these compounds present a modest reduction of the cell growth: the 6-dimethylamino derivative **16** is the most active compound. From a structure–activity relationship point of view the presence of a phenyl group in position 3 seems to be important for the activity in the series of compounds bearing the dimethylamino group (compounds **10**, **14**, and **16**) since its presence strongly increases the antiproliferative activity.

On the contrary, the same substitution in the series of compounds bearing the OH group in position 6 (**11**, **15**, and **17**) reduces the activity.

Conclusions

To summarise, we described the reactivity of 6-acetyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-*a*]pyrimidine **1** with 1,3- and 1,4-bisnucleophiles, showing that no pyrazolopyrimido[1,3]-diazocines or 2,3-dihydro-1*H*-1,4-diazepin-5-yl 6-substituted pyrazolo[1,5-*a*]pyrimidines can be obtained, and the reactions give rise to the new 6-pyrimidin-4-ylpyrazolo[1,5-*a*]pyrimidine system or to 6-methylidenepyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidines, respectively. Furthermore, from the same starting materials, we established a general synthetic procedure for the obtainment of variously substituted pyrazolo[1,5-*a*]quinazolines whose bioactivity has been tested against two cell lines of human tumor.

Experimental

General

Melting points were taken on a Büchi 510 apparatus and are uncorrected. IR spectra were obtained with a Perkin-Elmer 881 spectrophotometer after dispersion in KBr. Elemental analysis were obtained with Elemental Analyzer Perkin-Elmer 240C apparatus. Mass spectra were registered with a Carlo Erba QMD 1000 instrument at 70 eV. Compounds **1**, **12** and **13** were obtained as reported in the literature.[4,9] Silica gel plates (Merck F_{254}) and silica gel 60 (Merck 230–400 mesh) were used for analytical TLC and for column chromatography, respectively. Solvents were removed under reduced pressure. All 1D and 2D NMR experiments were performed on a Varian Mercuryplus-400 spectrometer (399.95 MHz for ^{1}H, 100.57 MHz for ^{13}C), with a 5 mm indirect detection probe equipped with a gradient coil, at 298 K. Chemical shifts (δ in ppm) were referenced to the solvent $CDCl_3$ (7.26 for ^{1}H and 77.00 ppm for ^{13}C NMR) or DMSO-d_6 (2.50 for ^{1}H and 39.50 ppm for ^{13}C NMR). All coupling constants are in Hz. Assignments are made using ^{1}H, ^{13}C, DEPT and NOESY 1D experiments and gHSQC, gHMBC, gHMQC, and gCOSY 2D experiments. All pulse sequences were used as provided by Varian and processing was done using standard Varian methods. ^{1}H NMR spectra were acquired using 4.6 kHz spectral width with 32k data points (4.5 μs 90° pulse width, 0.28 Hz/point digital resolution).

General procedure for the synthesis of compounds 2–5

A solution of 1-{7-[(*E*)-2-(dimethylamino)ethenyl]-2-methylpyrazolo[1,5-*a*]pyrimidin-6-yl}ethanone **1** (245 mg, 1 mmol) in diglyme (8 mL) containing sodium methoxide (64.8 mg, 1.2 mmol) and guanidine nitrate (146.5 mg, 1.2 mmol) or 1,1-dimethylguanidine sulfate (163.3 mg, 0.6 mmol) or acetamidine hydrochloride (113.4 mg, 1.2 mmol) or *S*-methylthiourea sulfate (167.0 mg, 0.6 mmol) was heated for 4 h at 120 °C. After cooling, the precipitate was filtered, washed with water and crystallised.

4-(2,7-Dimethylpyrazolo[1,5-*a*]pyrimidin-6-yl)pyrimidin-2-amine 2

Colourless crystals, mp 148–149 °C (from isopropanol); δ_H (400 MHz, $CDCl_3$) 8.55 (1 H, s, 5-H), 8.40 (1 H, d, ^3J = 5.0 Hz, 6′-H), 6.81 (1 H, d, ^3J = 5.0 Hz, 5′-H), 6.53 (1 H, s, 3-H), 5.24 (2 H, br s, NH_2), 2.96 (3 H, s, 7-CH_3), 2.56 (3 H, s, 2-CH_3); δ_C (100.58 MHz, $CDCl_3$) 163.0 (s, C-2′ and C-4′), 158.8 (d, C-6′), 156.0 (s, C-2), 148.9 (s, C-3a), 148.8 (d, C-5), 144.95 (s, C-7), 117.8 (s, C-6), 111.75 (d, C-5′), 96.9 (d, C-3), 15.1 (q, 7-CH_3), 14.8 (q, 2-CH_3); *m*/*z* (EI) 240 (M^+), 225, 198, 120; ν_{max}(KBr)/cm^{-1} 3423, 1601, 1456, 1254. Anal. found: C, 60.1; H, 4.9; N, 35.1%. Calc. for $C_{12}H_{12}N_6$: C, 60,0; H, 5.0; N, 35.0%.

4-(2,7-Dimethylpyrazolo[1,5-*a*]pyrimidin-6-yl)-*N*,*N*-dimethylpyrimidin-2-amine 3

Colourless crystals, mp 218–219 °C (from ethanol); δ_H (400 MHz, $CDCl_3$) 8.59 (1 H, s, 5-H), 8.41 (1 H, d, ^3J = 5.0 Hz, 6′-H), 6.66 (1 H, d, ^3J = 5.0 Hz, 5′-H), 6.52 (1 H, s, 3-H), 3.24 [6 H, s, $N(CH_3)_2$], 2.99 (3 H, s, 7-CH_3), 2.56 (3 H, s, 2-CH_3); δ_C (100.58 MHz, $CDCl_3$) 162.2 (s, C-2′), 162.0 (s, C-4′), 158.1 (d, C-6′), 155.7 (s, C-2), 149.2 (d, C-5), 148.9 (s, C-3a), 145.0 (s, C-7), 118.5 (s, C-6), 108.7 (d, C-5′), 96.7 (d, C-3), 37.05 [q, $N(CH_3)_2$], 15.1 (q, 7-CH_3), 14.8 (q, 2-CH_3); *m*/*z* (EI) 268 (M^+), 253, 239, 224, 210; ν_{max}(KBr)/cm^{-1} 2932, 1611, 1600, 1407. Anal. found: C, 62.6; H, 6.1; N, 31.4%. Calc. for $C_{14}H_{16}N_6$: C, 62.7; H, 6.0; N, 31.3%.

2,7-Dimethyl-6-(2-methylpyrimidin-4-yl)pyrazolo[1,5-*a*]pyrimidine 4

Colourless crystals, mp 168–169 °C (from ethanol); δ_H (400 MHz, $CDCl_3$) 8.74 (1 H, d, ^3J = 5.2 Hz, 6′-H), 8.57 (1 H, s, 5-H), 7.32 (1 H, d, ^3J = 5.2 Hz, 5′-H), 6.54 (1 H, s, 3-H), 2.97 (3 H, s, 7-CH_3), 2.82 (3 H, s, 2′-CH_3), 2.56 (3 H, s, 2-CH_3); δ_C (100.58 MHz, $CDCl_3$) 168.5 (s, C-2′), 161.7 (s, C-4′), 157.4 (d, C-6′), 156.0 (s, C-2), 148.7 (s, C-3a), 148.8 (d, C-5), 144.1 (s, C-7), 118.0 (d, C-5′), 117.35 (s, C-6), 96.9 (d, C-3), 26.2 (q, 2′-CH_3), 14.9 (q, 7-CH_3), 14.8 (q, 2-CH_3); *m*/*z* (EI) 239 (M^+), 238, 224, 211, 197, 170; ν_{max}(KBr)/cm^{-1} 2928, 1610, 1576, 1557, 1254. Anal. found: C, 65.1; H, 5.3; N, 29.4%. Calc. for $C_{13}H_{13}N_5$: C, 65.25; H, 5.5; N, 29.3%.

2,7-Dimethyl-6-[2-(methylsulfanyl)pyrimidin-4-yl]pyrazolo[1,5-*a*]pyrimidine 5

Yellow needles, mp 167–168 °C (from ethanol); δ_H (400 MHz, $CDCl_3$) 8.63 (1 H, d, ^3J = 5.1 Hz, 6′-H), 8.60 (1 H, s, 5-H), 7.18 (1 H, d, ^3J = 5.1 Hz, 5′-H), 6.56 (1 H, s, 3-H), 3.00 (3 H, s, 7-CH_3), 2.62 (3 H, s, S-CH_3), 2.57 (3 H, s, 2-CH_3); δ_C (100.58 MHz, $CDCl_3$) 172.65 (s, C-2′), 161.4 (s, C-4′), 156.9 (d, C-6′), 155.8 (s, C-2), 148.3 (s, C-3a), 148.15 (d, C-5), 144.9 (s, C-7), 115.3 (d, C-5′), 116.5 (s, C-6), 96.6 (d, C-3), 14.9 (q, CH_3), 14.8 (q, CH_3), 14.7 (q, CH_3); *m*/*z* (EI) 271 (M^+), 256, 224, 197; ν_{max}(KBr)/cm^{-1} 3030, 2924, 1600, 1490. Anal. found: C, 60.5; H, 5.8; N, 24.5; S, 6.4%. Calc. for $C_{13}H_{13}N_5S$: C, 60.6; H, 5.7; N, 24.45; S, 6.2%.

Reaction of 1 with ethane-1,2-diamine

To a solution of compound **1** (245 mg, 1 mmol) in acetic acid (10 mL) was added ethane-1,2-diamine (72.10 mg, 1.2 mmol) and the mixture was heated under reflux for 3 h, after which it was cooled, diluted with water (30 mL) and extracted exhaustively with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a solid resulting in a mixture of compounds **6** (25%) and **10** (traces). An analytical sample of 2,6-dimethylpyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine **6** was obtained by crystallisation: colourless crystals, mp 163–164 °C (from ethanol).[4] The remaining acidic aqueous solution was made alkaline (pH 13) with solid NaOH, and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give *N*-[2-(2-methyl-6-methylidenepyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidin-7(6*H*)-yl)ethyl]acetamide **7**: yield 54%; red crystals, mp 175–176 °C (from ethyl acetate); δ_H (400 MHz, DMSO-d_6) 8.63 (1 H, s, 5-H), 8.03 (1 H, t, ^3J = 6.0 Hz, NH), 7.18 (1 H, d, ^3J = 7.6 Hz, 8-H), 6.36 (1 H, s, 3-H), 5.99 (1 H, d, ^3J = 7.6 Hz, 9-H), 4.66 (1 H, d, ^2J = 3.4 Hz, $C_{sp2}H$), 3.96 (1 H, d, ^2J = 3.4 Hz, $C_{sp2}H$), 3.59 (2 H, m, 2′-CH_2), 3.31 (2 H, m, 1′-CH_2), 2.37 (3 H, s, 2-CH_3), 1.79 (3 H, s, $COCH_3$); δ_C (100.58 MHz, DMSO-d_6) 170.2 (s, CO), 154.5 (s, C-2), 148.3 (s, C-3a), 147.5 (d, C-8), 145.5 (d, C-5), 140.1 (s, C-6/C-9a), 140.0 (s, C-9a/C-6), 107.2 (s, C-5a), 96.3 (d, C-3), 87.1 (d, C-9), 77.1 (t, $C_{sp2}H_2$), 51.7 (t, C-2′), 34.9 (t, C-1′), 23.0 (q, $COCH_3$), 14.8 (q, 2-CH_3); *m*/*z* (EI) 283 (M^+), 225, 198; ν_{max}(KBr)/cm^{-1} 3287, 1596, 1548, 1435, 1347. Anal. found: C 63.8, H 6.2, N, 24.6%. Calc. for $C_{15}H_{17}N_5O$: C, 63.6, H 6.05, N 24.7%.

2-(2-Methyl-6-methylidene-6,7-dihydropyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine)ethanol 8

This compound was obtained from compound **1** with ethanolamine as described for **7**. Red crystals, mp 155–156 °C (from ethyl acetate); δ_H (400 MHz, DMSO-d_6) 8.62 (1 H, s, 5-H), 7.25 (1 H, d, ^3J = 7.6 Hz, 8-H), 6.35 (1 H, s, 3-H), 5.98 (1 H, d, ^3J = 7.6 Hz, 9-H), 4.92 (1 H, t, OH), 4.62 (1 H, d, ^2J = 3.0 Hz, $C_{sp2}H$), 3.81 (1 H, d, ^2J = 3.0 Hz, $C_{sp2}H$), 3.66–3.58 (4 H, m, 1′-CH_2 and 2′-CH_2), 2.37 (3 H, s, 2-CH_3); δ_C (100.58 MHz, DMSO-d_6) 154.5 (C-2), 148.3 (C-3a), 148.2 (C-8), 145.5 (C-5), 140.4 (C-6), 140.2 (C-9a), 107.0 (C-5a), 96.2 (C-3), 86.5 (C-9), 76.8 ($C_{sp2}H_2$), 56.0 (C-2′), 54.9 (C-1′), 14.8 (2-CH_3); *m*/*z* (EI) 242 (M^+), 227, 198, 183; ν_{max}(KBr)/cm^{-1} 3500–3000, 1637, 1556, 1525, 1347, 1123. Anal. found: C, 64.6; H, 6.0; N, 23.0%. Calc. for $C_{13}H_{14}N_4O$: C, 64.45; H, 5.8; N, 23.1%.

2-Methyl-6-methylidene-7-propyl-6,7-dihydropyrazolo[1,5-*a*]pyrido[3,4-*e*]pyrimidine 9

This compound was obtained from compound **1** with *n*-propylamine as described for **7**. Red crystals, mp 125–126 °C (from ethyl acetate); δ_H (400 MHz, DMSO-d_6) 8.62 (1 H, s, 5-H), 7.35 (1 H, d, ^3J = 7.5 Hz, 8-H), 6.35 (1 H, s, 3-H), 5.99 (1 H, d, ^3J = 7.5 Hz, 9-H), 4.64 (1 H, d, ^2J = 3.0 Hz, $C_{sp2}H$), 3.81 (1 H, d, ^2J = 3.0 Hz, $C_{sp2}H$), 3.50 (2 H, m, 1′-CH_2), 2.37 (3 H, s, 2-CH_3), 1.65 (2 H, m, 2′-CH_2), 0.89 (3 H, s, 3′-CH_3); δ_C (100.58 MHz, DMSO-d_6) 154.5 (s, C-2), 148.3 (s, C-3a), 147.4 (d, C-8), 145.5 (d, C-5), 140.2 (s, C-6/C-9a), 140.0 (s, C-9a/C-6), 107.0 (s, C-5a), 96.3 (d, C-3), 86.7 (d, C-9), 77.0 (t, $C_{sp2}H_2$), 54.0 (t, C-1′), 18.9 (t, C-2′), 14.8 (q, 2-CH_3), 11.2 (q, 2′-CH_3); *m*/*z* (EI) 240 (M^+), 225, 198; ν_{max}(KBr)/cm^{-1} 1640, 1553, 1522, 1341, 1126. Anal. found: C, 70.2; H, 6.5; N, 23.5%. Calc. for $C_{14}H_{16}N_4$: C, 70.0; H, 6.7; N, 23.3%.

Treatment of compounds 1, 12 and 13 with AcOH–AcONa

The dimethylaminovinyl derivative (1 mmol) was heated under reflux in glacial acetic acid (10 mL) containing anhydrous sodium acetate (393.8 mg, 4.8 mmol) for 4 h. After cooling, the solution was diluted with water (30 mL) and extracted exhaustively with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a solid. The obtained material resulted in a mixture of *N*,*N*-dimethylpyrazolo[1,5-*a*]quinazolin-6-amine and pyrazolo[1,5-*a*]quinazolin-6-ol derivatives that were separated by flash chromatography (CH_2Cl_2–MeOH = 20 : 1, as eluent).

N,*N*,2-Trimethylpyrazolo[1,5-*a*]quinazolin-6-amine 10

First running band starting from **1**: yellow needles, mp 104–105 °C (from ethanol); δ_H (400 MHz, $CDCl_3$) 9.15 (1 H, s, 5-H), 7.98–7.69 (2 H, m, 8-H and 9-H), 6.98 (1 H, dd, ^3J = 8.0 Hz, ^4J = 0.8 Hz, 7-H), 6.54 (1 H, s, 3-H), 2.99 [6 H, s, $N(CH_3)_2$], 2.55 (3 H, s, 2-CH_3); δ_C (100.58 MHz, $CDCl_3$) 153.4 (s, C-6), 152.7 (s, C-2), 149.1 (d, C-5), 146.2 (s, C-3a), 137.5 (s, C-9a), 134.3 (d, C-8), 112.9 (d, C-7), 112.0 (s, C-5a), 107.8 (d, C-9), 98.6 (d, C-3), 45.6 [q, $N(CH_3)_2$], 14.6 (q, 2-CH_3); *m*/*z* (EI) 226 (M^+), 210, 184, 112; ν_{max}(KBr)/cm^{-1} 2864, 1594, 1487, 1430. Anal. found: C, 69.2; H, 6.1; N, 24.85%. Calc. for $C_{13}H_{14}N_4$: C, 69.0; H, 6.2; N, 24.8%.

2-Methylpyrazolo[1,5-*a*]quinazoline-6-ol 11

Second running band starting from **1**: yellow needles, mp 272–273 °C (from ethanol); δ_H (400 MHz, DMSO-d_6) 11.16 (1 H, br s, OH), 9.09 (1 H, s, 5-H), 7.76–7.63 (2 H, m, 8-H and 9-H), 6.92 (1 H, dd, ^3J = 8.0 Hz, ^4J = 1.2 Hz, 7-H), 6.59 (1 H, s, 3-H), 2.43 (3 H, s, 2-CH_3); δ_C (100.58 MHz, DMSO-d_6) 156.9 (s, C-6), 152.3 (s, C-2), 147.4 (d, C-5), 146.4 (s, C-3a), 136.6 (s, C-9a), 136.2 (d, C-8), 110.2 (d, C-7), 108.6 (s, C-5a), 104.25 (d, C-9), 98.9 (d, C-3), 14.8 (q, 2-CH_3); *m*/*z* (EI) 199 (M^+), 198, 146, 76; ν_{max}(KBr)/cm^{-1} 3100–2000, 1595, 1480. Anal. found: C, 66.5; H, 4.65; N, 21.0%. Calc. for $C_{11}H_9N_3O$: C, 66.3; H, 4.55; N, 21.1%.

N,*N*-Dimethyl-2-phenylpyrazolo[1,5-*a*]quinazolin-6-amine 14

First running band starting from **12**: yellow needles, mp 120–121 °C (from ethanol); δ_H (400 MHz, $CDCl_3$) 9.22 (1 H, s, 5-H), 8.08–8.03 (2 H, m, 2′-H), 8.14 (1 H, d, ^3J = 8.0 Hz, 9-H), 7.75 (1 H, pt, ^3J = 8.0 Hz, 8-H), 7.51–7.45 (2 H, m, 3′-H), 7.42–7.36 (1 H, m, 4′-H), 7.08 (1 H, s, 3-H), 7.04 (1 H, dd, ^3J = 8.0 Hz, 7-H), 3.03 [6 H, s, $N(CH_3)_2$]; δ_C (100.58 MHz, $CDCl_3$) 154.5 (s, C-2), 153.2 (s, C-6), 148.5 (d, C-5), 145.1 (s, C-3a), 137.8 (s, C-9a), 135.3 (d, C-8), 132.9 (s, C-1′), 128.8 (d, C-3′ and C-4′), 126.4 (d, C-2′), 113.6 (d, C-7), 111.6 (s, C-5a), 108.3 (d, C-9), 95.7 (d, C-3), 45.7 [q, $N(CH_3)_2$]; *m*/*z* (EI) 288 (M^+), 144, 77; ν_{max}(KBr)/cm^{-1} 2839, 1605, 1594, 1469, 1321. Anal. found: C, 75.2; H, 5.7; N, 19.5%. Calc. for $C_{18}H_{16}N_4$: C, 75.0; H, 5.6; N, 19.4%.

2-Phenylpyrazolo[1,5-*a*]quinazoline-6-ol 15

Second running band starting from **12**: yellow needles, mp 299–300 °C (from ethanol); δ_H (400 MHz, DMSO-d_6) 11.20 (1 H, br s, OH), 9.15 (1 H, s, H-5), 8.12–8.02 (2 H, m, 2′-H), 7.85–7.74 (2 H, m, 8-H and 9-H), 7.55–7.44 (2 H, m, 3′-H), 7.44–7.36 (1 H, m, 4′-H), 6.98 (1 H, dd, ^3J = 7.6 Hz, ^4J = 1.0 Hz, 7-H), 7.30 (1 H, s, 3-H); δ_C (100.58 MHz, DMSO-d_6) 157.0 (s, C-6), 153.5 (s, C-2), 148.0 (d, C-5), 147.0 (s, C-3a), 136.7 (s, C-9a), 136.4 (d, C-8), 133.1 (s, C-1′), 129.3 (d, C-3′), 129.2 (d, C-4′), 126.4 (d, C-2′), 110.8 (d, C-7), 109.0 (s, C-5a), 104.5 (d, C-9), 96.6 (d, C-3); *m/z* (EI) 261 (M^+), 232, 130, 103, 77; ν_{max}(KBr)/cm^{-1} 3100–2000, 1621, 1465, 1372, 1307. Anal. found: C, 73.6; H, 4.2; N, 16.2%. Calc. for $C_{16}H_{11}N_3O$: C, 73.55; H, 4.2; N, 16.1%.

***N*,*N*-Dimethyl-3-phenylpyrazolo[1,5-*a*]quinazolin-6-amine 16**

First running band starting from **13**: yellow needles; mp 115–116 °C (ethanol); δ_H (400 MHz, $CDCl_3$) 9.35 (1 H, s, 5-H), 8.38 (1 H, s, 2-H), 8.10 (1 H, d, ^3J = 8.4 Hz, 9-H), 8.08–8.04 (2 H, m, 2′-H), 7.76 (1 H, pt, ^3J = 8.4 Hz, 8-H), 7.51–7.44 (2 H, m, 3′-H), 7.33–7.27 (1 H, m, 4′-H), 7.09 (1 H, d, ^3J = 8.4 Hz, 7-H), 3.06 [6 H, s, $N(CH_3)_2$]; δ_C (100.58 MHz, $CDCl_3$) 152.95 (s, C-6), 149.25 (d, C-5), 141.8 (s, C-3a), 140.7 (d, C-2), 137.8 (s, C-9a), 134.55 (d, C-8), 132.2 (s, C-1′), 128.8 (d, C-3′), 126.65 (d, C-2′), 126.4 (d, C-4′), 113.6 (d, C-7), 113.1 (s, C-3), 112.3 (s, C-5a), 108.05 (d, C-9), 45.6 [q, $N(CH_3)_2$]; *m/z* (EI) 288 (M^+), 144, 77; ν_{max}(KBr)/cm^{-1} 2840, 1595, 1538, 1420. Anal. found: C, 74.8; H, 5.7; N, 19.3%. Calc. for $C_{18}H_{16}N_4$: C, 75.0; H, 5.6; N, 19.4%.

3-Phenylpyrazolo[1,5-*a*]quinazoline-6-ol 17

Second running band starting from **13**: yellow needles, mp >300 °C (from ethanol); δ_H (400 MHz; DMSO-d_6) 11.30 (1 H, br s, OH), 9.24 (1 H, s, 5-H), 8.66 (1 H, s, 2-H), 8.18–8.12 (2 H, m, 2′-H), 7.84–7.74 (2 H, m, 8-H and 9-H), 7.50–7.48 (2 H, m, 3′-H), 7.29–7.21 (1 H, m, 4′-H), 7.01 (1 H, d, ^3J = 7.5 Hz, 7-H); δ_C (100.58 MHz, DMSO-d_6) 157.0 (s, C-6), 148.1 (d, C-5), 141.9 (s, C-3a), 141.2 (d, C-2), 137.0 (s, C-9a), 136.6 (d, C-8), 132.4 (s, C-1′), 129.1 (d, C-3′), 126.7 (d, C-4′), 126.55 (d, C-2′), 112.3 (s, C-3), 111.0 (d, C-7), 109.1 (s, C-5a), 104.5 (d, C-9); *m/z* (EI) 261 (M^+), 205, 117, 102; ν_{max}(KBr)/cm^{-1} 3100–2000, 1620, 1604, 1473, 1298. Anal. found: C, 73.4; H, 4.3; N, 16.2%. Calc. for $C_{16}H_{11}N_3O$: C, 73.5; H, 4.2; N, 16.1%.

Growth inhibitory activity

Human lymphoblastoid leukaemia cells (Jurkat) were grown in RPMI-1640 medium, (Sigma Co., MO, USA) human breast adenocarcinoma (MCF-7) were grown in DMEM medium (Sigma Co., MO, USA), all supplemented with 115 units mL^{-1} of penicillin G (Invitrogen, Milano, Italy), 115 μg mL^{-1} streptomycin (Invitrogen, Milano, Italy) and 10% fetal calf serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 μL of complete medium containing 8×10^3 Jurkat cells or 5×10^3 MCF-7 cells. The plates were incubated at 37 °C in a humidified 5% incubator for 18 h prior to the experiments. After medium removal, 100 μL of the drug solution, dissolved in DMSO and diluted with complete medium, were added to each well and incubated at 37 °C for 72 h. Cell viability was assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test as described previously.[10] Cell growth at each drug concentration was expressed as a percentage of untreated controls and the concentration resulting in 50% (IC_{50}) growth inhibition was determined by linear regression analysis.

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Notes and references

1 S. Selleri, F. Bruni, C. Costagli, A. Costanzo, G. Guerrini, G. Ciciani, P. Gratteri, F. Besnad, B. Costa, M. Montali, C. Martini, J. Fohlin, G. De Siena and P. Malmberg Aiello, *J. Med. Chem.*, 2005, **48**, 6756–6760.
2 S. Selleri, F. Bruni, C. Costagli, A. Costanzo, G. Guerrini, G. Ciciani, P. Gratteri, C. Bonaccini, P. Malmberg Aiello, F. Besnard, S. Renard, B. Costa and C. Martini, *J. Med. Chem.*, 2003, **46**, 310–313.
3 S. Selleri, F. Bruni, C. Costagli, A. Costanzo, G. Guerrini, G. Ciciani, B. Costa and C. Martini, *Bioorg. Med. Chem.*, 1999, **7**, 2705–2711.
4 F. Bruni, S. Chimichi, B. Cosimelli, A. Costanzo, G. Guerrini and S. Selleri, *Heterocycles*, 1990, **31**, 1141–1149.
5 F. Bruni, S. Chimichi, B. Cosimelli, A. Costanzo, G. Guerrini and S. Selleri, *Heterocycles*, 1990, **31**, 1635–1640.
6 S. Chimichi, B. Cosimelli, F. Bruni, S. Selleri, A. Costanzo, G. Guerrini and G. Valle, *J. Chem. Soc., Perkin Trans. 2*, 1994, 1657–1660.
7 S. M. Siddiqi, X-d. Ji, N. Melman, M. E. Olah, R. Jain, P. Evans, M. Glashofer, W. L. Padgett, L. A. Cohen, J. W. Daly, G. L. Stiles and K. A. Jacobson, *Nucleosides Nucleotides*, 1996, **15**, 693–718.
8 S. Schenone, F. Manetti and M. Botta, *Curr. Pharm. Des.*, 2007, **13**, 2118–2128.
9 F. Bruni, B. Cosimelli, A. Costanzo, G. Guerrini and S. Selleri, *Heterocycles*, 1993, **36**, 87–99.
10 G. Viola, L. Facciolo, M. Canton, F. Di Lisa, S. Dall'Acqua, D. Vedaldi, O. Tabarrini, A. Fravolini and V. Cecchetti, *Toxicol. in Vitro*, 2004, **18**, 581–594.

