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Synthesis and BZR Affinity of Pyrazolo[1,5-*a*]pyrimidine Derivatives. Part 1: Study of the Structural Features for BZR Recognition

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Abstract—Examination of the pharmacophoric points of the pyrazolo[1,5-*a*]pyrimidine derivatives, ligands for BZR, previously published led us to the design of a novel class of 3,6-diaryl-4,7-dihydro-pyrazolo[1,5-*a*]pyrimidin-7-ones and to determine the groups involved in the BZR recognition.

Introduction

A rational design of new benzodiazepine receptor (BZR) ligands based on reliable pharmacophore models has been proposed on the basis of binding data displayed at multiple BZR subtypes by various structural families of BZR ligands. Such models could be useful starting points for identification of the molecular determinants of recognition and activation of the BZRs. An examination of the structure–activity relationships of affinity (SAFIR) and efficacy of many classes of compounds at the BZR has assisted in the development of several pharmacophoric models for ligand–receptor interaction at the BZR. All these models are characterized by a number of points of lipophilic and hydrogen-bonding ligand–receptor interaction[1–5] and in some cases areas of steric hindrance have also been defined.[6–8] In order to obtain a better level of molecular understanding, much emphasis over the past few years has been paid to the synthesis of small molecules as BZR ligands. With a similar goal, we are developing a SAR study to identify new flexible 3,6-substituted pyrazolo[1,5-*a*]pyrimidine derivatives with high binding potency and low intrinsic activity.

We have previously reported the syntheses and BZR affinities of many 3-aryl-4,7-dihydro-6-pyrazol-3′(5′)-yl-pyrazolo[1,5-*a*]pyrimidin-7-ones[9] ($5.1 \leqslant Ki(nM) \leqslant 1880$) and we have proposed the alignment of this class according to the model reported by Zhang and co-workers.[10] In this model the anchoring of the ligand (lead compound of 3-aryl-6-pyrazolyl derivatives) to the BZR (Fig. 1) involves $C^7{=}O$, N^1 and N^4–H with hydrogen bonding sites H_1 (donor site) and H_2/A_3 (donor/acceptor site) on the receptor protein, respectively. The 3-aromatic substituent is proposed to occupy the lipophilic regions L_1/L_2 and/or L_3. Moreover the 6-pyrazol-3′(5′)-yl moiety was located in the area of hydrogen bonding acceptor site A_2, whose role in BZR interaction has roused much controversy.[11–13] Receptor descriptors S are regions of negative steric/electrostatic repulsion.

With the aim of supporting this hypothesis in the BZR recognition, it seemed worthwhile to investigate the role of the tautomeric group N^4H-C^7O and of the pyrazole substituent at the 6-position with respect to the receptor protein. In view of the uncertain function of A_2 in the hydrogen bonding ligand–receptor interaction, we synthesized new molecules devoid of the hydrogen bonding donor site. Therefore 3,6-diphenyl-4,7-dihydro-pyrazolo[1,5-*a*]pyrimidin-7-one **1** was synthesized and investigated by a binding assay using [^{3}H]RO15-1788 as radioligand and bovine brain membranes from brain tissues as receptor source.[14] We have tried to predict the in vitro efficacy of the most active compounds by

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determining the GABA ratio (IC_{50} without GABA/IC_{50} with GABA). The resulting affinity and efficacy were quite comparable with the reference compound of the 6-pyrazol-3′(5′)-yl series,[9] thus identifying a potential new lead molecule **1** (Fig. 2).

Since it is well known that structural requirements for interaction and activation of the BZR involve the pharmacophoric points model mentioned above, we synthesized several new derivatives bearing substantial modifications either on $C^7=O$ group or concerning the N^1 and N^4H. As a consequence, the enol form of compound **1** was blocked as 7-methoxy derivative (**2**), the oxygen was replaced either by a sulfur atom (**3**) or with an amino group (**4**); the carbonyl group by a methine (**5**) or a methylene group (**6**). As regards N^1 and N^4, alkylderivatives **7**–**9** were prepared (Fig. 3).

As an extension of this study, the phenyl groups at 3- and 6-positions were in turn replaced either by 3′-methoxyphenyl or by a thienyl ring to verify the maintenance of BZR recognition and possibly to identify a potential bioisosteric series (**10**–**17**) (Scheme 1). The choice of these substituents at the 3-position is justified by high affinity values observed in the 6-pyrazol-3′(5′)-yl series as appeared in our previous paper.[9]

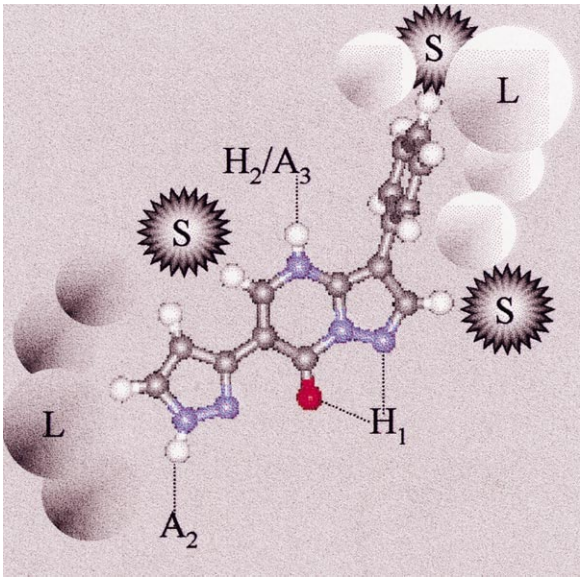


Figure 1. Schematic representation of the interaction between 4,7-dihydro-3-phenyl-6-pyrazol-3′-yl-pyrazolo-[1,5-*a*]pyrimidin-7-one (lead compound) and BZR.

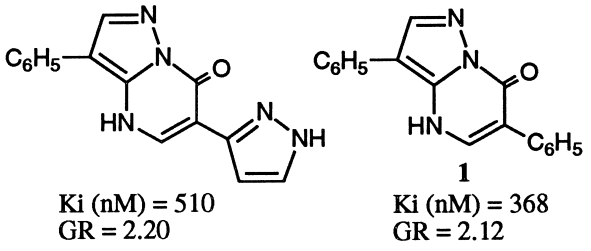


Figure 2. Molecular formulas and biological data of lead molecules.

Chemistry

The syntheses of 4,7-dihydro-3,6-substituted pyrazolo[1,5-*a*]pyrimidin-7-ones (**1**, **10**–**17**) were performed by a one-step reaction between 3(5)-amino-4-aryl pyrazoles (4-phenyl **A**, 4-thien-3′-yl **B**, 4,3′-methoxyphenyl **C**) and ethyl 2-aryl-3-hydroxypropenoates (2-phenyl- **D**, 2-thien-3′-yl-**E**, 2-thien-2′-yl **F**, ethyl 2,3′-methoxyphenyl- **G**) at reflux in diglyme or ethylene glycol (see Experimental).

7-Chloro-3,6-diphenyl-pyrazolo[1,5-*a*]pyrimidine, obtained from compound **1** by reaction with $POCl_3$, proved to be a useful intermediate for the synthesis of compounds **2** and **3**. In fact, by means of this reagent, compound **2** was obtained using sodium methoxide. Compound **3** was obtained, via the same 7-chloro derivative by treatment with thiourea and subsequent acidic hydrolysis, since compound **1** was unaffected either by Lawesson reagent or by phosphorus pentasulfide (P_2S_5) (see Experimental).

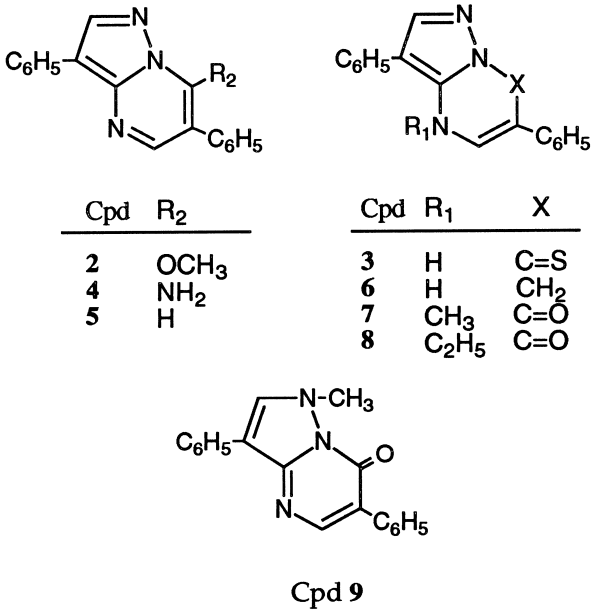
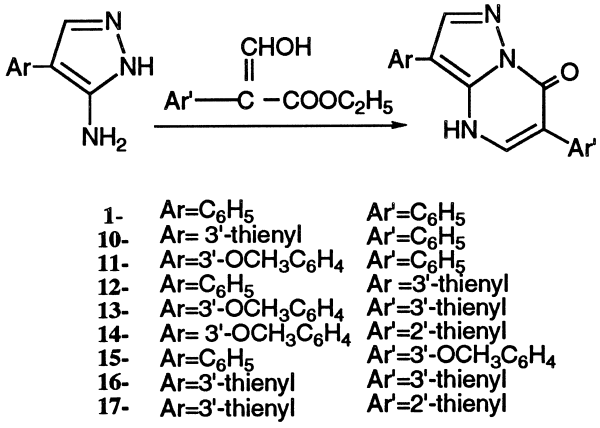


Figure 3. Molecular formulas of compounds **2**–**9**.



Scheme 1. One-step reaction for the synthesis of compounds **1** and **10**–**17**.

Following described procedures compounds **4**[15] and **5**[16] were prepared. Both of them lack the carbonyl oxygen and N^4H groups, even if the former could exist as N^4H-7-imino form in tautomeric equilibrium of compound **4**.

The replacement of the 7-carbonyl group with methylene group was accomplished by reaction of **5** with $NaBH_4$, which reduced the pyrimidine moiety[17] to give the 4,7-dihydro derivative **6**.

Alkylation of compound **1** with iodomethane in anhydrous dimethylformamide (DMF) in the presence of potassium carbonate (K_2CO_3) unexpectedly afforded compound **9** instead of compound **7**, unlike our previous experiences[18] according to which the above alkylation method selectively gave N^4-alkyl derivatives.

In order to verify unequivocally the structure of the former, 3-amino-1-methyl-4-phenylpyrazole[19] was reacted with ethyl 3-hydroxy-2-phenylpropenoate giving a compound chromatographically and spectroscopically identical to compound **9**.

So far as the role of N^4H group in the BZR recognition is concerned, the *N*-alkyl derivatives (compounds **7** and **8**) were prepared by reaction of ethyl 3-hydroxy-2-phenylpropenoate with 3-aminomethyl or 3-aminoethyl-4-phenylpyrazole, respectively. Those intermediates were, in turn, obtained starting from 3-formamido-4-phenylpyrazole or 3-acetamido-4-phenylpyrazole, which were reduced with borane methylsulfide ($BH_3(CH_3)_2S$).

Results and Discussion

The low overall BZR affinities (Table 1) of compounds **2**–**9** reported here and their lack of consistent SAR suggest that they do not possess the structural features required for binding to the BZR. In particular compounds **2**–**6**, which are devoid of the carbonyl function, do not possess any BZR recognition, whereas *N*-alkylderivatives (compounds **7** and **8**) maintain the recognition, although to a lesser extent of affinity. On the other hand a remarkable decrement of BZR affinity was shown by compound **9**, bearing a methyl group at the nitrogen 1. This result agrees with our previous hypothesis, according to which the essential anchoring of the ligand to the hydrogen bond donor site H_1 on the receptor protein is effected by the carbonyl group and the unsubstituted N^1 in a three-centred interaction. The above findings could be clarified by the observation of the negative affinity trend ranging from K_i value 369 nM for compound **1**, through 863 nM for compound **7**, to 3090 nM for compound **9**. In fact, whereas compound **7** is still able to interact with a three-centred hydrogen bond with H_1 losing its ability to bind with H_2/A_3, for compound **9** a complete reverse situation is observed.

The 3-substitution by a 3-thienyl ring or a 3-methoxyphenyl group which resulted in successfully improving the BZR affinity in the 6-pyrazol-3′(5′)-yl series[9] afforded again as many new ligands with an enhanced (compounds **10**–**13**, **16**–**17**) or similar potency (compounds **14,15**) compared with the lead molecule **1**.

As regards the intrinsic activity, the compounds of this new class, with the exception of full agonists **1, 7** and **11**, display in vitro properties consistent with a partial agonist efficacy. More importantly, compounds **13** and **16** which exhibit the best affinity values, possess the lowest values of intrinsic activity. This feature appeared intriguing since the clinical interest in partial agonists, due to their ability to act as modulators of BZR, is well known.[20]

Table 1. Biological data of compounds **1**–**17**

Compound	Inhibition(%)[a]	K_i (nM)[b]	GABA ratio[c]
1	91 ± 4.2	369 ± 2.4	2.12
2	60 ± 1.2	—	—
3	24 ± 6.4	—	—
4	2.7 ± 0.3	—	—
5	14 ± 3.5	—	—
6	6 ± 1.9	—	—
7	71 ± 3.6	867 ± 8.2	1.97
8	80 ± 2.7	1860 ± 9.7	—
9	89.6 ± 3.6	3090 ± 240	—
10	95 ± 5.6	72.2 ± 3.2	1.67
11	93 ± 3.8	192 ± 2.5	3.08
12	94 ± 6.2	111 ± 4.2	1.71
13	92 ± 0.8	42.2 ± 1.7	1.25
14	88 ± 4.3	363 ± 5.3	1.46
15	84 ± 4.2	597 ± 0.8	1.62
16	100 ± 6.7	16.3 ± 1.3	1.30
17	96 ± 2.5	87.7 ± 0.9	1.56
Flunitrazepam	100 ± 5.2	2.17 ± 0.2	1.57
βCC-E	100 ± 4.2	0.44 ± 0.08	0.80
RO 15–87	99 ± 1.1	1.01 ± 0.3	0.98

[a] Percent of inhibition of specific [^{3}H]RO15-1788 binding at 10 μM concentration are means ± SEM of five determinations (the tests were carried out using DMSO as solvent).
[b] K_i values are means ± SEM of five determinations.
[c] GABA ratio = IC_{50} compound/IC_{50} compound + 10 μM GABA. Flunitrazepam (agonist), βCC-E (inverse agonist), RO 15–87 (antagonist) are used as reference compounds.

Conclusion

The removal of the 6-pyrazolyl moiety in the previous parent compounds[9] and its substitution with a phenyl or a thienyl ring, proved the marginal role of the interaction with the acceptor site A_2. Taking into account this observation, four possible orientations, by which the interaction of the new ligands with the receptor protein might occur, can be drawn, as depicted in Figure 4.

Since interaction with the receptor site H_1 is more critical than binding to site H_2,[6] we believe that the present work demonstrates the essential function of C^7-carbonyl group and N_1 in the anchoring to the H_1 receptor protein through a three-centred hydrogen bond (as highlighted by the loss of BZR recognition exhibited by compounds **2**–**6** and **9** in comparison with compounds **7** and **8**). In fact, as regards the role of N^4H, it appears to affect the binding affinity potency involving the H_2/A_3 (donor/acceptor hydrogen bond) receptor site, which is well known as being less critical to affinity binding than the H_1.[6]

Therefore, the two orientations of the ligands 4b, 4b′ are to be ruled out on the basis of the above considerations, while in our opinion, the guide criterion for choosing between 4a, 4a′ orientations could be the definition of

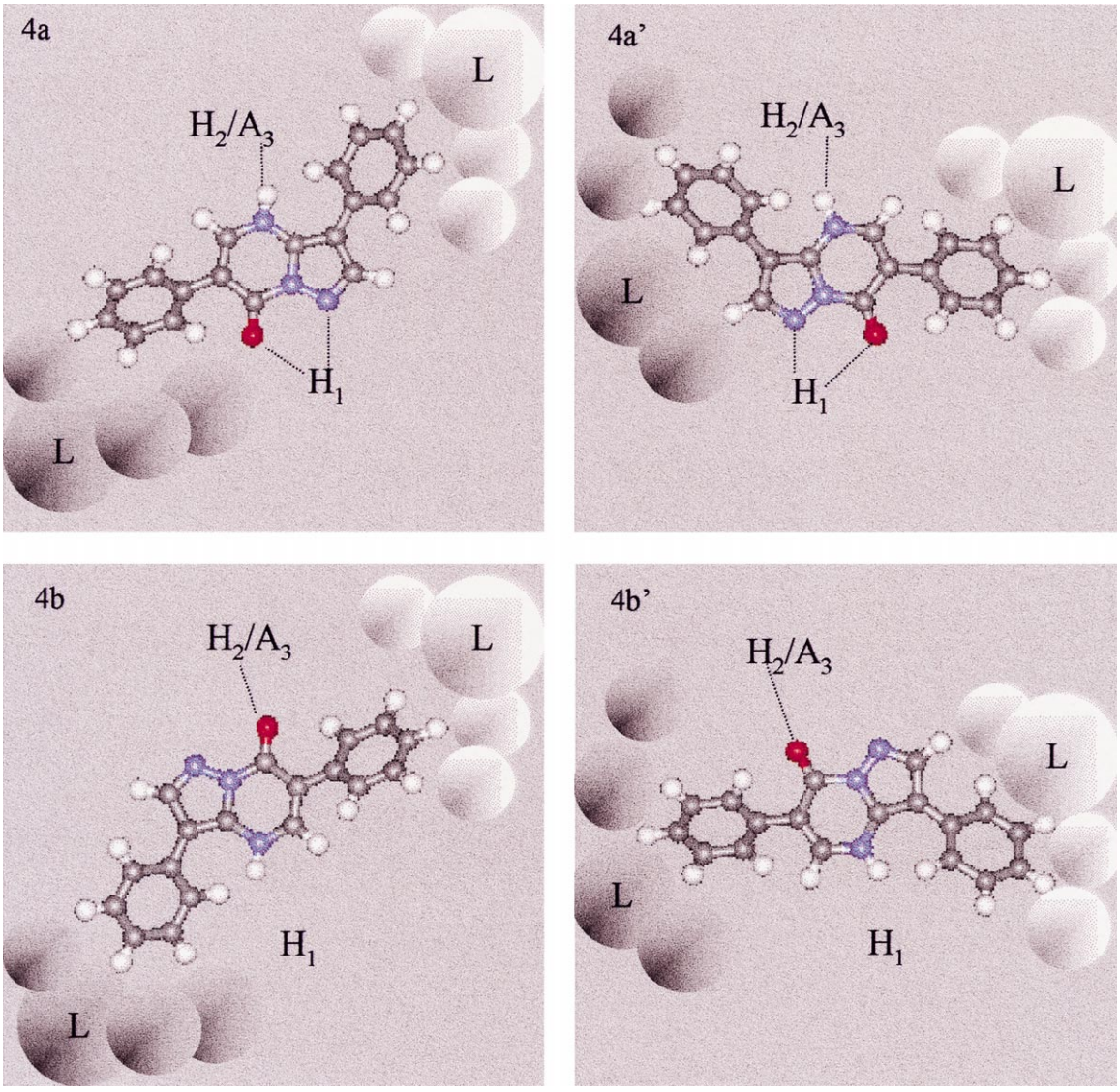


Figure 4. Schematic representation of four possible orientations (4a, 4a′; 4b, 4b′) of compound **1** respect to BZR.

the receptor lipophilic regions (as L_1/L_2 and L_3) by means of suitable 3- and 6-substitutions.

Further work concerning 3- and 6-substitution is planned in order to carry out structure activity studies on the BZR. In fact these substitutions, combined together, open up the way to a large variety of possible modifications for obtaining new ligands with high affinity and low intrinsic activity; in vivo investigations on the efficacy of these new partial agonists is currently in progress.

Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded with a Varian Gemini 200 instrument; chemical shifts are reported in δ (ppm) high frequency from tetramethylsilane as secondary reference standard and coupling constants in Hz. Silica gel plates (Merk F_{254}) were used for analytical TLC. Solvents were removed under reduced pressure. Microanalyses were performed by Laboratories of Dipartimento Farmaco Chimico Tecnologico of University of Siena, Italy with a Perkin–Elmer Model 240 C Elemental Analyzer and values are within ±0.4% of the theoretical values.

General procedure for obtaining compounds 1 and 10–17. A suspension of 3-amino-4-arylpyrazole (**A**–**C**) (10 mmol) and ethyl-2-formyl-2-aryl acetate (10 mmol) (**D**–**G**) in the appropriate solvent (20 mL) was refluxed under magnetic stirring for 8 h (Table 2). The precipitate was collected by filtration from the reaction mixture and used without further purification as judged pure by TLC.

4,7-Dihydro-3,6-diphenyl-pyrazolo[1,5-*a*]pyrimidin-7-one (1). White crystals. ^{1}H NMR (DMSO-d_6) δ 7.34–7.52 (m, 6H, ArH), 7.63–7.72 (m, 4H, ArH), 7.99 (s, 1H, H-2), 8.30 (s, 1H, H-5), 12.56 (bs, 1H, NH). Anal. calcd for $C_{18}H_{13}N_3O$: C, 75.25; H, 4.56; N, 14.62. Found: C, 75.03; H, 4.77; N, 14.26.

Table 2. Reaction conditions, physical and chemical data of compounds **1** and **10–17**[a]

Compound	Reagents	Reaction solvent	Yield %	mp (°C)
1	**A + D**	Dyglime	25	> 300
10	**B + D**	Dyglime	30	> 300
11	**C + D**	Dyglime	28	290–291
12	**A + E**	Dyglime	45	> 300
13	**C + E**	Ethylene glycol	28	> 300
14	**C + F**	Ethylene glycol	15	289–290
15	**A + G**	Dyglime	15	> 300
16	**B + E**	Ethylene glycol	46	> 300
17	**B + F**	Dyglime	50	> 300

[a] **A** = 3-amino-4-phenylpyrazole, **B** = 3-amino-4-thien-3′-yl pyrazole, **C** = 3-amino-4-(3′-methoxyphenyl)pyrazole, **D** = ethyl-2-phenyl-3-hydroxypropenoate, **E** = ethyl-2-thien-3′-yl-3-hyeroxypropenoate, **F** = ethyl-2-thien-2′-yl-3-hydroxypropenoate, **F** = ethyl-2-(3′-methoxyphenyl)-3-hydroxypropenoate.

3,6-Diphenyl-7-methoxy-pyrazolo[1,5-*a*]pyrimidine (2). To 100 mL of methanol in a two-necked flask, clean metallic sodium (0.6 mmol) was slowly added under magnetic stirring. After dissolution of sodium 3,6-diphenyl-7-chloro-pyrazolo[1,5-*a*]pyrimidine 0.18 g (0.6 mmol), synthesized according to a procedure described for a similar compound[21] (yellow crystals, mp 157–158°C, 65% yield. ^{1}H NMR (DMSO-d_6) δ 7.46–7.70 (m, 8H, ArH), 8.18–8.22 (pseudo d, 2H, ArH), 8.72 (s, 1H, H-2), 8.95 (s, 1H, H-5)), was added and refluxed for 5 h. The solvent was reduced under vacuum and crude product was filtered off; compound **2** was obtained by column chromatography (cyclohexane:ethyl acetate 1:1).

Pale yellow crystals, mp 99–100°C, 35% yield. ^{1}H NMR ($CDCl_3$) δ 4.19 (s, 3H, OCH_3), 7.29–7.32 (m, 2H, ArH), 7.44–7.56 (m, 6H, ArH), 8.04–8.09 (m, 2H, ArH), 8.48 (s, 1H, H-2), 8.58 (s, 1H, H-5). Anal. calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 75.53; H,4.99; N, 13.81.

4,7-Dihydro-3,6-diphenyl-pyrazolo[1,5-*a*]pyrimidin-7-thione (3). 3,6-Diphenyl-7-chloro-pyrazolo[1,5-*a*]pyrimidine 0.15 g (0.5 mmol), was suspended in ethanol and refluxed for 3 h in presence of thiourea 0.76 g (1 mmol). A solution of KOH (5%) 5 mL was added and refluxed for 10 min. The suspended solid was filtered off and the solution was acidified with AcOH. The yellow precipitate was collected and used without further purification as judged sufficiently pure (TLC analysis). mp > 300°C, 53% yield. ^{1}H NMR (DMSO-d_6) δ 7.22–7.46 (m, 6H, ArH), 7.63–7.67 (m, 2H, ArH), 7.93–8.02 (m, 3H: 2H, ArH; 1H, H-2), 8.50 (s, 1H, H-5). Anal. calcd for $C_{18}H_{13}N_3S$: C, 71.26; H, 4.32; N, 13.85. Found: C, 71.68; H,4.70; N, 13.45.

3,6-Diphenyl-7-amino-pyrazolo[1,5-*a*]pyrimidine (4). [15]

3,6-Diphenyl-pyrazolo[1,5-*a*]pyrimidine (5). Phenylmalondialdehyde (5 mmol),[16] was added in one batch to a stirred solution of 3-amino-4-phenylpyrazole (5 mmol) in glacial acetic acid (15 mL) and the reaction mixture was refluxed for 1 h. The disappearance of starting materials was monitored by TLC analysis. After cooling, compound **5** was obtained as a yellow solid in 90% yield by filtration and recrystallized from ethanol; mp 197–198°C; ^{1}H NMR (DMSO-d_6) δ 7.25–7.33 (m, 1H, ArH), 7.42–7.63 (m, 5H, ArH), 7.87 (pseudo d, 2H, ArH), 8.20 (pseudo d, 2H, ArH), 8.83 (s, 1H, H-2), 9.09 (d, ^4J = 2.2 Hz, 1H, H-5), 9.54 (d, ^4J = 2.2 Hz, 1H, H-7). Anal. calcd for $C_{18}H_{13}N_3$: C, 79.68; H, 4.83; N, 15.49. Found: C, 79.55; H,4.80; N, 15.33.

4,7-Dihydro-3,6-diphenyl-pyrazolo[1,5-*a*]pyrimidine (6). To a suspension of compound **5** (1 mmol) in methanol (40 mL) $NaBH_4$ (50 mmol) was added and kept under magnetic stirring at room temperature for 2 h. After evaporation of the solvent, compound **6** was collected by filtration and accurately washed with water to remove any inorganic materials. Pale yellow solid in 51% yield from isopropanol; mp 242–243°C; ^{1}H NMR (DMSO-d_6) d 5.08 (bs, 2H, CH_2), 7.00 (d, ^3J = 5.0 Hz, 1H, H-5), 7.20–7.51 (m, 10H, ArH), 7.74 (s, 1H, H-2), 9.20 (d, ^3J = 5.0 Hz 1H, NH, exchangeable). Anal.calcd for $C_{20}H_{19}N_3$: C, 79.70; H, 6.35; N, 13.94. Found: C, 79.52; H, 6.15; N, 13.71.

General procedure for obtaining compounds 7 and 8. Compounds **7** and **8** were obtained via boranemethylsulfide reduction from 3-formamido-4-phenylpyrazole and 3-acetamido-4-phenylpyrazole according to a general method.[22]

4,7-Dihydro-3,6-diphenyl-4-methyl-pyrazolo[1,5-*a*]pyrimidin-7-one (7). White crystals in 69% yield from ethanol; mp > 229°C; ^{1}H NMR (DMSO-d_6) δ 3.54 (s, 3H, N–CH_3) 7.25–7.52 (m, 8H, ArH), 7.60–7.75 (m, 2H, ArH), 7.99 (s, 1H, H-2), 8.22 (s, 1H, H-5). Anal.calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 75.70; H, 5.15; N, 14.06.

4,7-Dihydro-3,6-diphenyl-4-ethyl-pyrazolo[1,5-*a*]pyrimidin-7-one (8). White crystals in 38% yield from ethanol; mp 188–189°C; ^{1}H NMR (DMSO-d_6) 0.97 (t, 3H, NH–CH_2CH_3,), 3.99 (q, 2H, NH–CH_2CH_3), 7.30–7.55 (m, 8H, ArH), 7.65–7.75 (m, 2H, ArH), 7.98 (s, 1H, H-2), 8.27 (s, 1H, H-5). Anal. calcd for $C_{20}H_{17}N_3O$: C, 76.17; H, 5.43; N, 13.32. Found: C, 76.02; H, 5.27; N, 13.50.

1,7-Dihydro-3,6-diphenyl-1-methyl-pyrazolo[1,5-*a*]pyrimidin-7-one (9). Method A Iodomethane (2 mmol) was added to a stirred solution of **1** (2 mmol) in dimethylformamide (20 mL) containing potassium carbonate (2 mmol) and the reaction mixture was maintained at 80°C for 3 h. After cooling, the insoluble materials were filtered off and a minimum amount of water was added to the solution. A white solid, consisting of a mixture of compound **9** with compound **7** in trace precipitated. Compound **9** was purified via chromatography by eluting with CH_2Cl_2:MeOH, 100:1. The expected compound was obtained as a pale pink solid in 50% yield; mp 211–212°C; ^{1}H NMR ($CDCl_3$) δ 4.43 (s, 3H, NCH_3), 7.28–7.48 (m, 6H, ArH), 7.70–7.78 (m, 3H: 2H ArH, 1H H-2), 7.93–8.05 (m, 2H, ArH), 8.36 (s, 1H, H-5). Anal. calcd for $C_{19}H_{15}N_3O$: C, 75.73; H, 5.02; N, 13.94. Found: C, 76.11; H, 5.40; N, 14.30.

Method B: A suspension of 3-amino-1-methyl-4-phenylpyrazole[19] (1 mmol) and ethyl-2-formyl-2-phenyl acetate (1 mmol) in diglyme (5 mL) was refluxed under

magnetic stirring for 10 h. The solvent was reduced under vacuum and the crude product was purified by column chromatography (cyclohexane:ethyl acetate 1:1). The expected compound **9** was obtained in 50% yield.

4,7-Dihydro-3-thien-3′-yl-6-phenyl-pyrazolo[1,5-*a*]pyrimidin-7-one (10). Pale pink crystals. ^{1}H NMR (DMSO-d_6) δ ppm: 7.34–7.80 (m, 8H: 5H, ArH, 3H, thienyl), 8.00 (s, 1H, H_2), 8.32 (s, 1H, H-5). Anal. calcd for $C_{16}H_{11}N_3OS$: C, 65.51; H, 3.78; N, 14.32. Found: C, 65.58; H, 3.75; N, 14.11.

4,7-Dihydro-3-(3′methoxyphenyl)-6-phenyl-pyrazolo[1,5-*a*]pyrimidin-7-one (11). White crystals; ^{1}H NMR (DM SO-d_6) δ ppm: 3.86 (s, 3H, OCH_3) 6.85–6.95 (m, 1H, ArH) 7.20–7.25 (m, 2H, ArH) 7.30–7.50 (m, 4H, ArH), 7.70–7.82 (m, 2H, ArH) 7.97 (s, 1H, H-2) 8.31 (s, 1H, H-5) 12.51 (bs, 1H, NH exch.). Anal. calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.82; H, 4.55; N, 13.33.

4,7-Dihydro-3-phenyl-6-thien-3′-yl-pyrazolo[1,5-*a*]pyrimidin-7-one (12). White crystals; ^{1}H NMR (DMSO-d_6) δ ppm: 7.30–7.40 (m, 1H, thienyl) 7.45–7.56 (m, 2H, ArH) 7.60–7.74 (m,4H: 3H ArH,1H thienyl) 8.08–8.15 (m, 1H, thienyl) 8.24 (s, 1H, H-2) 8.30 (s, 1H, H-5) 12.85 (bs, 1H, NH exch.). Anal. calcd for $C_{16}H_{11}N_3OS$: C, 65.51; H, 3.78; N, 14.32. Found: C, 65.53; H, 3.89; N, 14.25.

4,7-Dihydro-3-3′-methoxyphenyl-6-thien-3′-yl-pyrazolo[1,5-*a*]pyrimidin-7-one (13). Light grey crystals; ^{1}H NMR (DMSO-d_6) δ ppm: 3.85 (s, 3H, OCH_3), 6.86–6.96 (m, 1H, thienyl), 7.14–7.24 (m, 2H, ArH), 7.36–7.46 (m, 1H, ArH), 7.58–7.68 (m, 2H: 1H ArH, 1H thienyl), 8.00–8.12 (m, 1H, thienyl), 8.22 (s, 1H, H-2), 8.32 (s, 1H, H-5), 12.64 (bs, 1H, NH exch.). Anal. calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99. Found: C, 63.02; H, 4.31; N, 13.05.

4,7-Dihydro-3-3′-methoxyphenyl-6-thien-2′-yl-pyrazolo[1,5-*a*]pyrimidin-7-one (14). Light green crystals; ^{1}H NMR (DMSO-d_6) δ ppm: 3.85 (s, 3H, OCH_3), 6.88–6.95 (m, 1H, thienyl), 7.10–7.25 (m, 3H, ArH), 7.35–7.52 (m, 2H: 1H ArH, 1H thienyl), 7.63–7.67 (m, 1H, thienyl), 8.32 (s, appears as pseudo d, 2H: 1H, H-2, 1H, H-5), 12.82 (bs, 1H, NH exch.). Anal. calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99. Found: C, 63.21; H, 3.99; N, 12.79.

4,7-Dihydro-3-phenyl-6-3′-methoxyphenyl-pyrazolo[1,5-*a*]pyrimidin-7-one (15). Ivory crystals; ^{1}H NMR (DM SO-d_6) δ ppm: 3.81 (s, 3H, OCH_3) 6.90–6.95 (m, 1H, ArH) 7.21–7.40 (m, 4H, ArH) 7.42–7.56 (m, 2H, ArH) 7.62–7.70 (m, 2H, ArH) 8.00 (s, 1H, H-2) 8.30 (s, 1H, H-5) 12.65 (bs, 1H, NH exch.). Anal. calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.82; H, 4.89; N, 13.38.

4,7-Dihydro-3,6-dithien-3′-yl-pyrazolo[1,5-*a*]pyrimidin-7-one (16). Light yellow crystals; ^{1}H NMR (DMSO-d_6) δ ppm: 7.52–7.72 (m, 4H: 2H, thienyl, 2H, thienyl), 7.80 (s, 1H, thienyl), 8.10 (s, 1H, thienyl), 8.24 (s, 1H, H-2), 8.33 (s, 1H, H-5), 12.53 (s, 1H, NH, exch.). Anal. calcd for $C_{14}H_9N_3OS_2$: C, 56.17; H, 3.03; N, 14.04. Found: C, 56.22; H, 2.98; N, 13.95.

4,7-Dihydro-3-thien-3′-yl-6-thien-2′-yl-pyrazolo[1,5-*a*]pyrimidin-7-one (17). Light green crystals; ^{1}H NMR (DM SO-d_6) δ ppm: 7.09–7.18 (m, 1H, thienyl), 7.37–7.59 (m, 2H, thienyl), 7.63–7.68 (m, 1H, thienyl), 7.69–7.76 (m, 1H, thienyl), 7.78–7.81 (m, 1H, thienyl), 8.36 (s, appears as pseudo d, 2H: 1H, H-2, 1H, H-5), 12.55–12.85 (bs,1H, NH, exch.). Anal. calcd for $C_{14}H_9N_3OS_2$: C, 56.17; H, 3.03; N, 14.04. Found: C, 55.95; H, 2.99; N, 14.11.

Molecular modelling

The low energy conformations of the ligands were calculated by AM1 routine in MOPAC, and the graphic presentations were obtained using Insight II software by Molecular Simulation Inc.

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