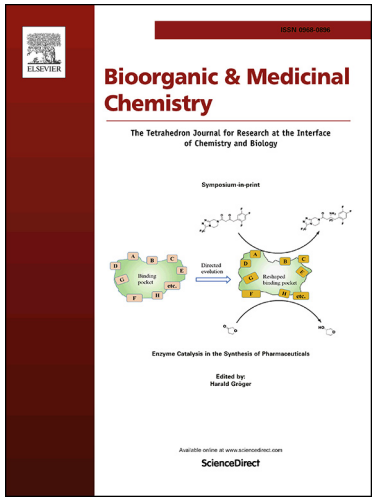
# Accepted Manuscript

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# A new class of pyrazolo[5,1-c][1,2,4]triazines as γ-aminobutyric type A ($GABA_A$) receptor subtype ligand: synthesis and pharmacological evaluation

Gabriella Guerrini,[a*] Giovanna Ciciani,[a] Simona Daniele,[b] Claudia Martini,[b] Camilla Costagli,[a] Chiara Guarino,[a] Silvia Selleri[a]

[a]Dipartimento NEUROFARBA, sezione Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Italy

[b]Dipartimento FARMACIA, Università degli Studi di Pisa, Italy

*Corresponding author: Guerrini Gabriella, Dipartimento NEUROFARBA, sezione Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Italy, Via Ugo Schiff, 6, Sesto Fiorentino Firenze, 50019; tel. +390554573766; e-mail: gabriella.guerrini@unifi.it

**Abstract**

A comparison between compounds with pyrazolo[1,5-a]pyrimidine structure (series 4-6) and pyrazolo[5,1-c][1,2,4]triazine core (series 9) as ligands at $GABA_A$-receptor subtype, was evaluated. Moreover, for pyrazolotriazines derivatives having binding recognition, the interaction on recombinant rat α(1-3,5) $GABA_A$ receptor subtypes, was performed. Among these latter, emerge compounds **9c**, **9k**, **9l**, **9m** and **9n** as α1-selective and **9h** as α2-selective ligands.

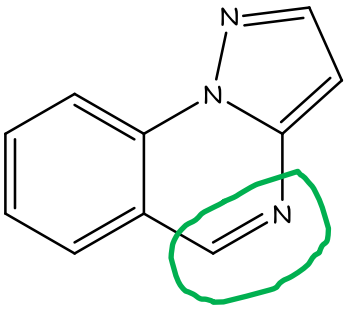
## 1. Introduction

The γ-aminobutyric acid type A receptors ($GABA_A$-Rs) are ligand-gated chlorine channels belonging to cys-loop receptors superfamily which also includes the nicotinic acetylcholine receptor, the $5HT_3$ and glycine receptors. The $GABA_A$-Rs are involved in neurological disorders and are interesting target for a variety of drugs for the treatment of insomnia, anxiety, epilepsy, pain, cognitive impairments, some forms of depression and schizophrenia. [1]

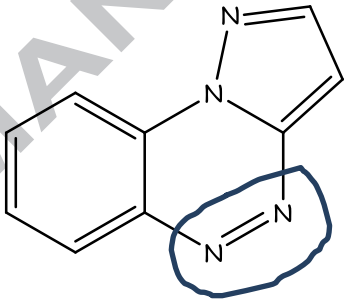
The $GABA_A$-R is a heteropentameric channel formed by subunits belonging to 8 families (α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3) and the subtypes mostly expressed in CNS are composed of three subunit (α, β, γ) in stoichiometric ratio 2α:2β:1γ, and γ-β-α-β-α arranged (if read counter clock-wise when view from extracellular domain). It is known that the two orthosteric GABA binding sites lie at the β+/α- interfaces in the extracellular domain, while an allosteric binding pocket, already known as 'benzodiazepine receptor site' and currently named '$GABA_A$-R subtype', exists at the α+/γ- interface.[2] The α-subunit type (1-3 and 5) constituting the pentamer expresses the benzodiazepine pharmacology and, from mouse subunit knock-in approach was evidenced that α1 it is responsible of sedation, α2/α3 of anxiolityc-like, antihiperalgesic and miorelaxant activity and, finally, the α5 subunit is implicated in learning and memory.[3] In the last decade the identification of separable key functions of $GABA_A$ receptor subtypes, suggests not only the overcoming limitations of classical benzodiazepines but also the opening of a fascinating scenario for new therapeutic indications in schizophrenia, chronic pain, cognitive diseases.[4]

Our research group has extensively investigated fused heterocyclic systems containing variable nitrogen atoms as potential $GABA_A$-R subtype ligands and very interesting results have stand out: opportunely substituted pyrazolo[5,1-c][1,2,4]benzotriazines (PBT), pyrazolo[1,5-a]quinazolines (PQ) and pyrazolo[1,5-a]pyrimidines (PP), exhibit high affinity to $GABA_A$-R subtype. Some compounds afforded in vivo interesting results, having promnemonic and/or dual activity (anxiolytic-like and antihyperalgesic), [5–8] Chart 1.

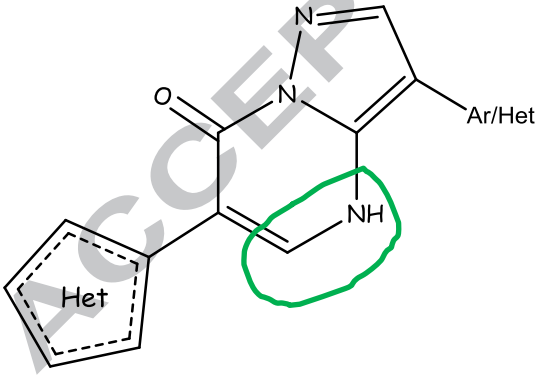
**Chart 1**



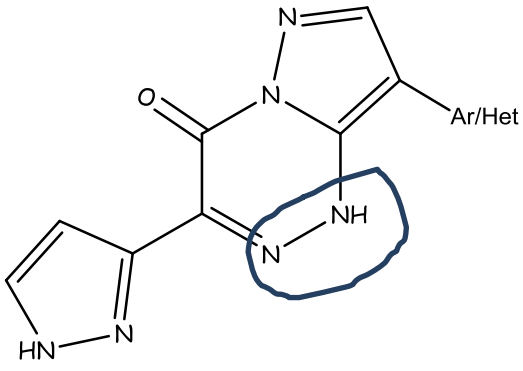

**PQ**




**PBT**




**PP**




**PT**

Deeping our study on bicycle systems, we have again taken up compounds with 6-(1H-pyrazol-3-yl)pyrazolo[1,5-a]pyrimidin-4-one (PP) scaffold and new substituents/ moieties at 3-/6-position were inserted. At the same time we performed the synthesis of a new series of pyrazolo[5,1-c][1,2,4]triazine (PT), as 5-azaisomers of PP. The affinity to $GABA_A$-R subtype of the two series of bicycles (PP and PT) was evaluated as well as subtype selectivity of PTs by using recombinant rat $\alpha$(1-3,5)$GABA_A$-R subtypes.

## 2. Chemistry

Chemical data of new derivatives here described are reported in SI and listed in Tables S1-S5.

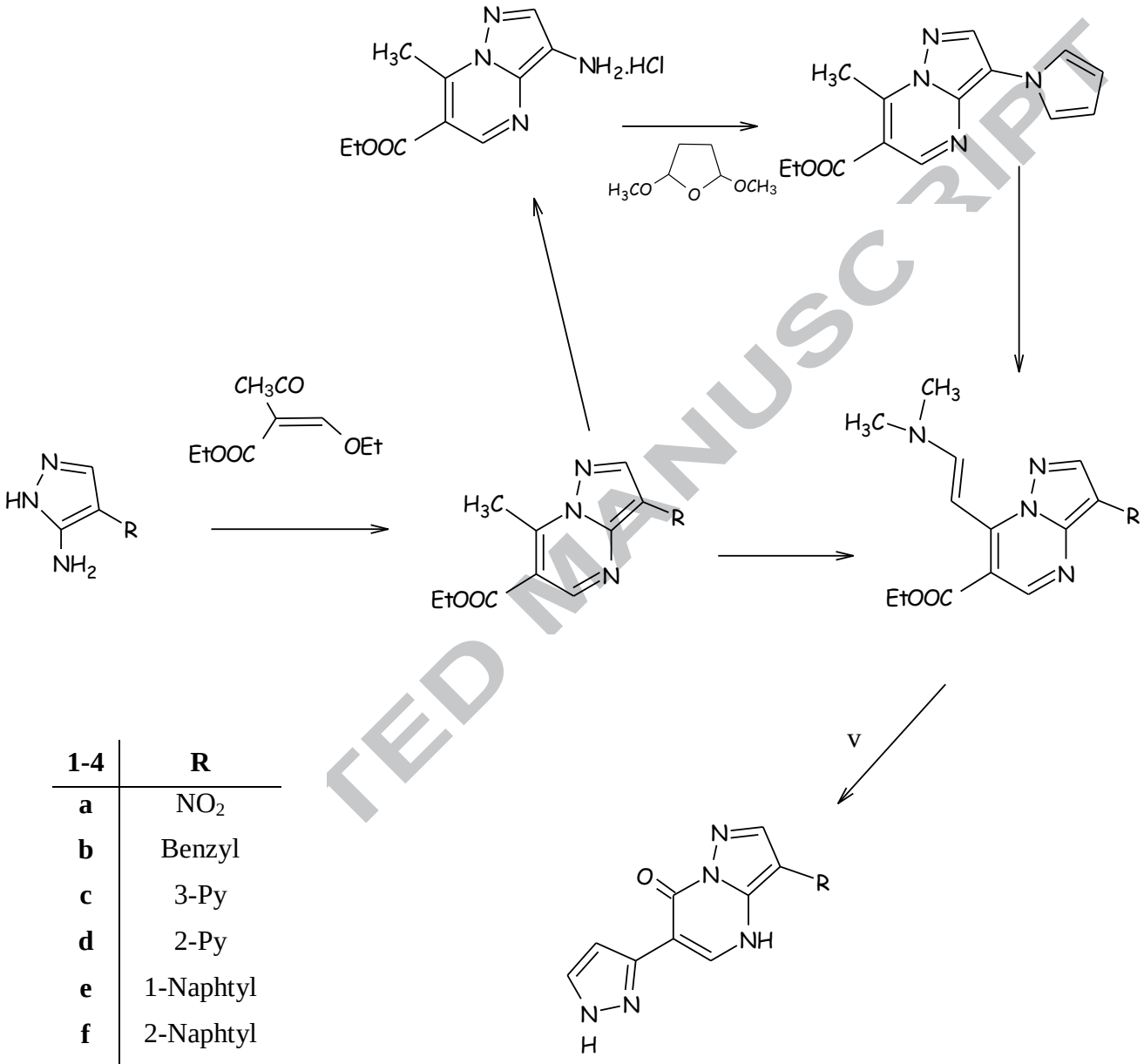
The 3-aminopyrazoles differently substituted at position 4, are used as starting material for the synthesis of both pyrazolopyrimidines (schemes 1, 2) and pirazolotriazines (schemes 3, 4).

Compounds 6-(1*H*-pyrazol-3-yl)pyrazolo[1,5-a]pyrimidin-7(4*H*)-ones) 3-aryl/heteroaryl substituted **4b-g**, were obtained following a previously described procedure [9–11] and depicted in Scheme 1. The 3(5)-aminopyrazoles 4-substituted (**1a-f**) were reacted with ethyl 2-acetyl-3-ethoxyacrylate and the intermediates ethyl 7-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate 3-substituted **2a-f** were obtained. The 3-nitro derivative **2a** was reduced to the corresponding 3-aminoderivative (**2a'**) which was reacted with 2,5-dimethoxytetrahydrofurane to give the 3-(pyrrol-1-yl)-derivative **2g**. Therefore compounds **2b-g** were first treated with DMF-DMA (affording **3b-g**) and then with hydrazine hydrate to yield compounds **4b-g** (Scheme 1).

The introduction at position 6 of the pyrazolo[1,5-a]pyrimidine core of a pyrazol-1-yl or imidazole-1-yl ring, was carried out through a one-step reaction between the ethyl 3-oxo-2-(1H-pyrazol-1-yl)propanoate **A** or ethyl 2-(1H-imidazol-1-yl)-3-oxopropanoate **B** and 3(5)-amino-4-(thien-2-yl)pyrazolo (**1h**) or 3(5)-amino-4-phenylpyrazolo (**1i**) in dyglime[9,11–15] obtaining the final compounds **5a, b** and **6a, b** (Scheme 2). Reagents **A** and **B** were in turn prepared by a formylation reaction, starting from the corresponding ethyl (pyrazol-1-yl)- and (imidazole-1-yl)acetate.[16]

The synthesis of final pyrazolo[5,1-c][1,2,4] triazin-4-(*1H*)-one derivatives type **9**, were obtained in a convenient manner as reported in Scheme 3.

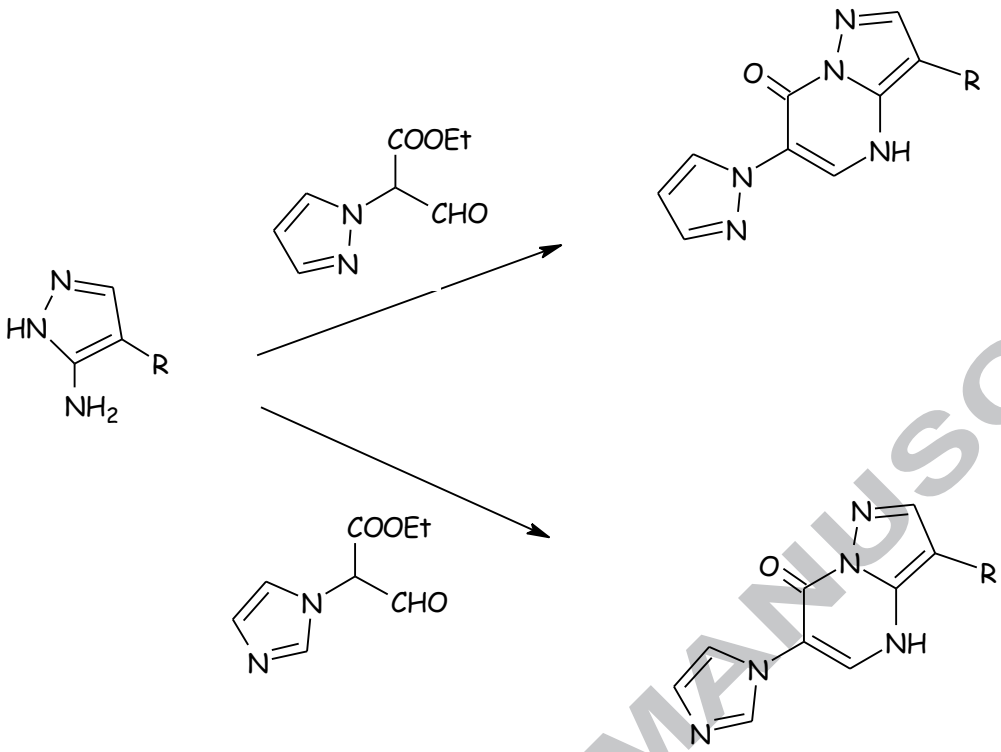
**Scheme 1**




| 1-4 | R |
|---|---|
| **a** | $NO_2$ |
| **b** | Benzyl |
| **c** | 3-Py |
| **d** | 2-Py |
| **e** | 1-Naphtyl |
| **f** | 2-Naphtyl |
| **g** | Pyrrol-1-yl |

Reagents and conditions: i) ethyl ethoxymethylacetoacetate/EtOH; ii) 10% Pd/C, $H_2$ 40 psi, EtOH, HCl; iii) Dimethoxytetrahydrofurane/dioxane; iv) DMF-DMA, toluene, piperidine cat.; v) hydrazine hydrate 60%; acetic acid, sodium acetate.

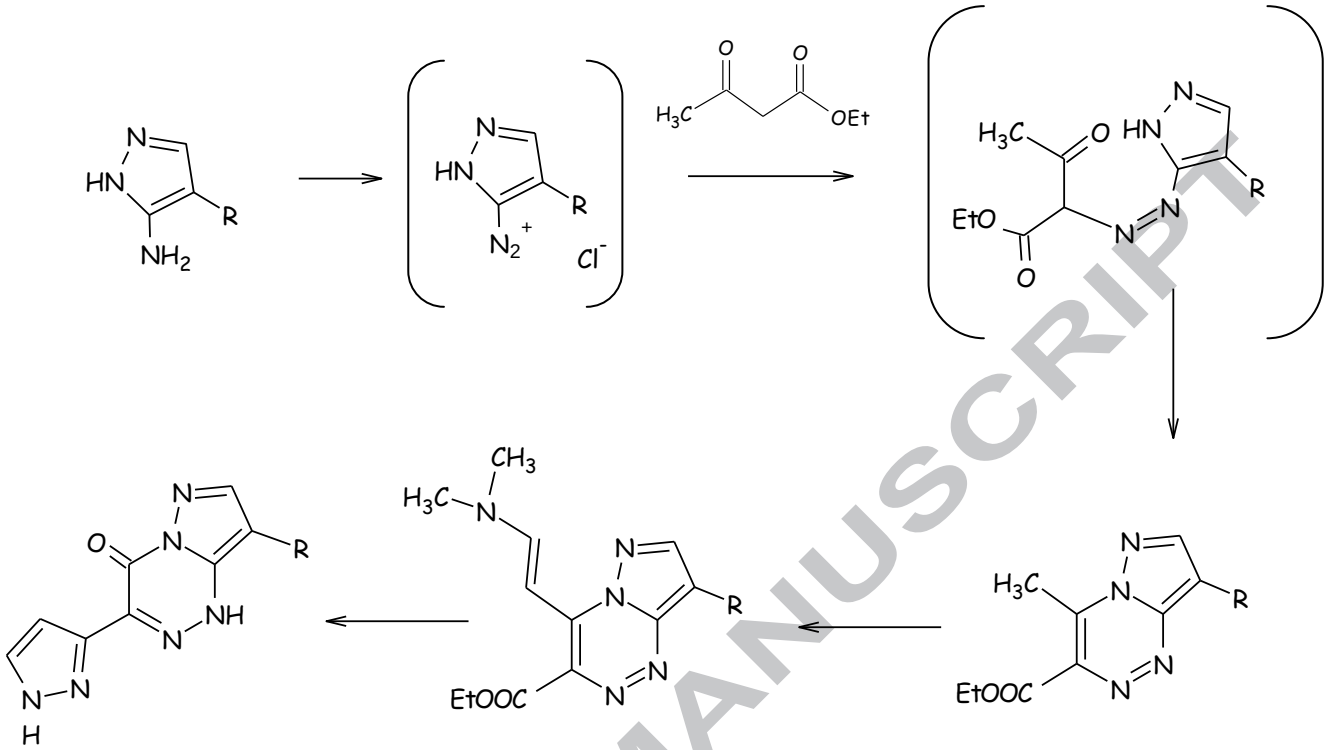
**Scheme 2**




| **Compds** | **R** |
|---|---|
| **1h, 5a, 6a** | 2-Thienyl |
| **1i, 5b, 6b** | Ph |

Reagents and conditions: i) dyglime reflux

**Scheme 3.**

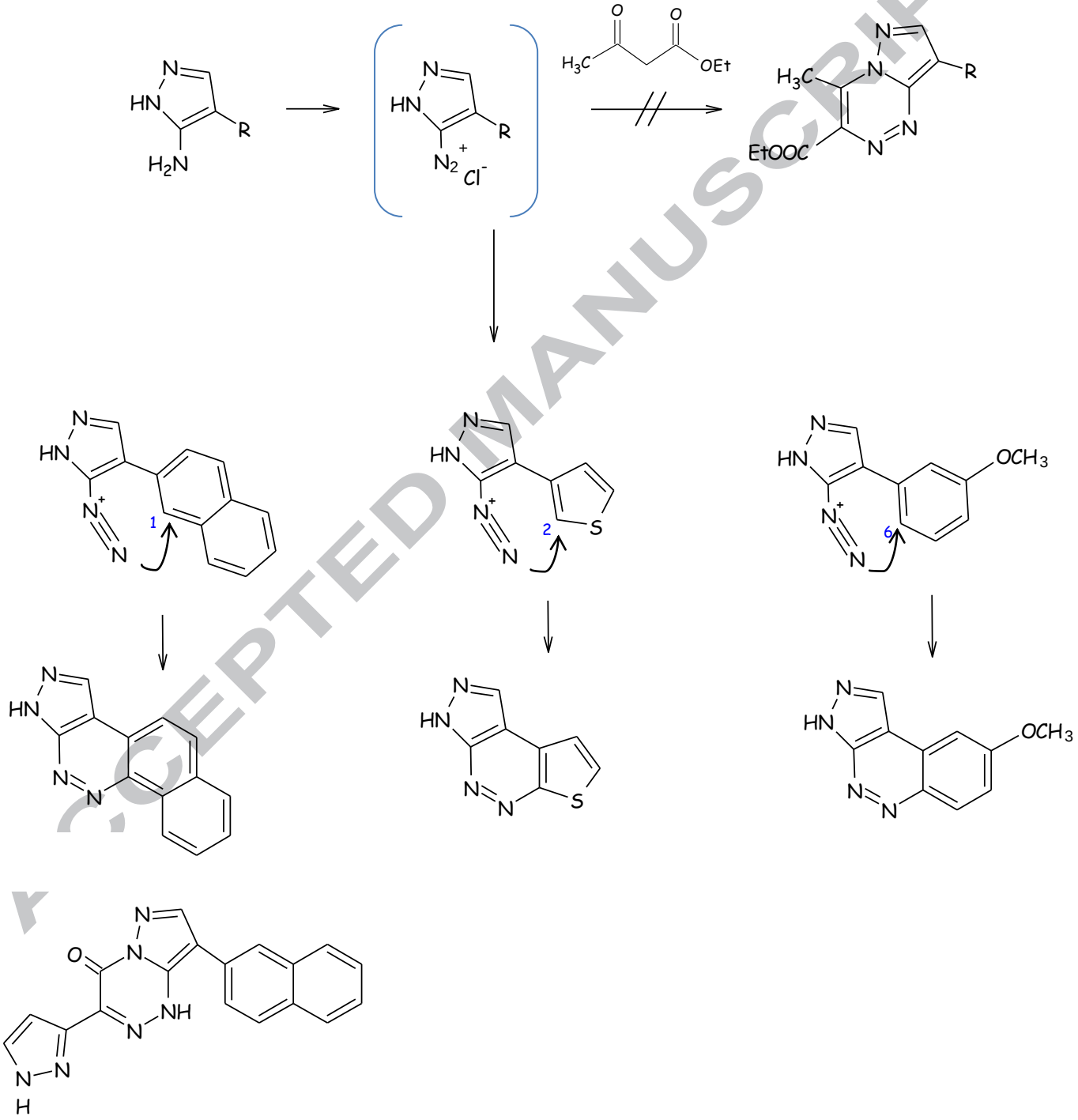


| **1, 7-9** | R |
|---|---|
| **c** | 3-Py |
| **d** | 2-Py |
| **e** | 1-Naphtyl |
| **f** | 2-Naphtyl |
| **h** | 2-Thienyl |
| **i** | Ph |
| **j** | 3-FPh |
| **k** | 4-FPh |
| **l** | 3-ClPh |
| **m** | 3-BrPh |
| **n** | 3-$CF_3$Ph |
| **o** | 4-$OCH_3$Ph |
| **p** | 4-$CH_3$Ph |

Reagents and conditions: i) HCl, $NaNO_2/H_2O$; ii) sodium acetate, ethyl acetoacetate iii) DMF-DMA, toluene, piperidine cat.; iv) hydrazine hydrate 60%; acetic acid, sodium acetate.

**Scheme 4**

| **1** | **R** |
|---|---|
| **f** | 2-Naphtyl |
| **q** | 3-Thienyl [9] |
| **r** | 3-$OCH_3$Ph[9] |



**9f** (80%)

Reagents and conditions: i) HCl, $NaNO_2/H_2O$;

The suitable 3(5)-aminopyrazoles 4-substituted (**1c-f, 1h-p**) were diazotized and then cyclized with ethyl acetoacetate to ethyl 4-methylpyrazolo[5,1-c][1,2,4]triazines 3-carboxylate 8-substituted, compound of type **7**. Since the methyl group at position 4 shows acid features, as evidenced by the relatively high value of its chemical shift (about 3.25 ppm), it can be easily transformed into a dimethylaminovinyl moiety with dimethylformamide-dimethylacetal (DMF-DMA) according to the Brederek reaction.[17] The obtained intermediate 4-dimethylaminovinyles (compounds of type **8**) showed two geometrical isomers as evidenced by TLC and $^1$H-NMR spectra. The high reactivity of the dimethylaminovinyl derivatives towards hydrazine hydrate easily leads to final compounds **9c-f, 9h-p** in a reaction manner already investigated in the reported for pyrazolo[1,5-a]pyrimidines.[9]

A special case is highlighted when at position 4 of the 3(5)-aminopyrazole a 2-naphtalene ring (**1f**), 3-thiofene[9] (**1q**) or 3-methoxyphenyl[9] (**1r**) are present; in fact, the corresponding highly reactive diazonium salts prefer the electrophilic intramolecular attack to 4-hetero/aryl ring electron rich position of the aminopyrazole, instead of the coupling reaction with ethyl acetoacetate. (Scheme 4)

In particular the positions 1, 2 and 6 of the naphthalene, thiofene and methoxyphenyl ring respectively, were attacked by diazo group affording the tricyclic derivatives **10-12**, whose structures were confirmed by crystallographic analysis (data not shown). Starting from 3-(thien-3-yl)- and 3-(3-methoxyphenyl)pyrazole, exclusively led to 3*H*-pyrazolo[3,4-c]thieno[3,2-e]pyridazine **11** and 8-methoxy-3*H*-pyrazolo[3,4-c]cinnoline **12**, respectively, while starting from 3-(2-naphtyl)pyrazole **1f**, preferentially is obtained the pyrazoletriazine **9f** in good yield (80%) and the tetracyclic compound, 1*H*-benzo[h]pyrazolo[3,4-c]cinnoline **10**, only as by-product (20%).

**Biological results and discussion**

The Bz site/$GABA_A$-R binding affinity of newly synthesized compounds was evaluated by their ability to displace [$^3$H]flumazenil (Ro15-1788) from its specific binding in bovine brain membrane and was expressed as $K_i$ value limited to those compounds inhibiting radioligand binding by more than 80% at 10$\mu$M. The binding data of the new pyrazolo[1,5-a]pyrimidines (**4b-g**, **5a**, **b** and **6a**, **b**) and of reference compounds **I** and **II**,[9,10] are reported in Table 1.

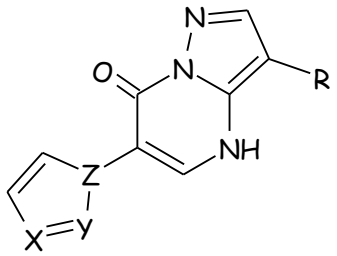



Table 1. Binding data for pyrazolo[1,5-a]pyrimidine derivatives **4b-g**, **5a**, **b** and **6a**,**b**.

| **N°** | **R** | **X** | **Y** | **Z** | **I% - $K_i$ (nM)**[a] |
|---|---|---|---|---|---|
| **4b** | $CH_2Ph$ | NH | N | C | 854±23 |
| **4c** | 3-Py | NH | N | C | 37.3±0.3 |
| **4d** | 2-Py | NH | N | C | 14.2±1.6 |
| **4e** | 1-Naphtyl | NH | N | C | 599±22 |
| **4f** | 2-Naphtyl | NH | N | C | 20% |
| **4g** | 1-Pyrrolyl | NH | N | C | 2670±25 |
| **5a** | 2-Thienyl | CH | N | N | 13.9% |
| **5b** | Ph | CH | N | N | 59.5% |
| **6a** | 2-Thienyl | N | CH | N | 14.7% |
| **6b** | Ph | N | CH | N | 2% |
| **I** | 2-Thienyl | NH | N | C | 533±27[b] |
| **II** | Ph | NH | N | C | 326.3±37[c] |

[a] Percent inhibition values of specific [$^3$H]Ro15-1788 binding at 10 μM concentration and $K_i$ values are means ±SEM of five determinations; [b]see ref [9]; [c]see ref [10]

Among the new synthesized compounds (**4b-4g**), the most promising are **4c** and **4d** both bearing a pyridine ring at position 3; looking at $K_i$ value (37 nM and 14.2 nM respectively) it can reasonably be said that the 2- or 3-position of nitrogen atom is irrelevant. All other compounds show poor affinity (**4b, e**) or no affinity (**4f, g**). When in the lead compounds **I** and **II** the pyrazole nucleus at position 6 was differently connected, as in the new compounds **5a, b** or replaced by imidazole ring

(**6a, b**), the binding affinity dramatically falls down, thus indicating that the presence of NH group on 6-substitutent is required for receptor binding, Table 1.

As concerning pyrazolo[5,1-c][1,2,4]triazine derivatives (**9c-f**, **9h-p**) designed and synthesized as 5-aza isomers of the pyrazolo[1,5-a]pyrimidines, the receptor affinity (Table 2) is generally fair ($K_i$ range 38-329 nM), with exception of compounds **9f**, **9o** and **9p** which show very poor or no affinity. Among the compounds belonging to the **9** series, emerged **9d** and **9n** bearing at position 3 a 2-pyridyl and 3-trifluoromethylphenyl ring respectively, they exhibit affinity values in the nanomolar range (**9d**, $K_i$ = 10.5 nM; **9n** $K_i$ = 7.2 nM).

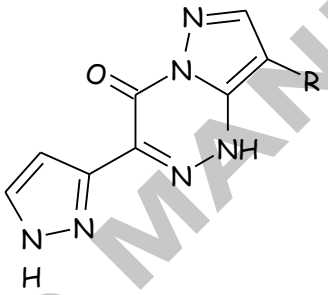



Table 2. Binding data for pyrazolo[5,1-c][1,2,4]triazines derivatives **9c-f** and **9h-p**

| N° | R | cortex | alpha1 | alpha2 | alpha3 | alpha5 |
|---|---|---|---|---|---|---|
| | | **I% - $K_i$ (nM)**[a] | **I% - $K_i$ (nM)**[a] | **I% - $K_i$ (nM)**[a] | **I% - $K_i$ (nM)**[a] | **I% - $K_i$ (nM)**[a] |
| **9c** | 3-Py | 73.6±2 | **51** | 5000 | 502 | 1236±26 |
| **9d** | 2-Py | 10.5±1.0 | **9.1±0.9** | **12.0±10** | - | 32% |
| **9e** | 1-Naphtyl | 77 | 150 | - | - | 159 |
| **9f** | 2-Naphtyl | 2298±96 | - | - | - | - |
| **9h** | 2-Thienyl | 236.9±18.5 | 284.8±25. | **41.2±0.4** | 28% | 662±60 |
| **9i** | Ph | 329±30 | 420±33 | 3000±25 | 2086 | 408±33 |
| **9j** | 3-FPh | 152±11 | 100±7 | 632±60 | 1080 | 23% |
| **9k** | 4-FPh | 88.3±8 | **77.3** | 2420±20 | 894 | 1111±37 |
| **9l** | 3-ClPh | 74±6 | **65.6** | 990±13 | 660 | 40% |
| **9m** | 3-BrPh | 38.0±8 | **21** | 50% | 570 | 1117±8 |
| **9n** | 3-$CF_3$Ph | 7.2 | **6.5±0.6** | >5000 | 501±22 | 144±16 |
| **9o** | 4-$OCH_3$Ph | 1609 | - | - | - | - |
| **9p** | 4-$CH_3$Ph | 32% | - | - | - | - |

[a] Percent inhibition values of specific [$^3$H]Ro15-1788 binding at 10 μM concentration and $K_i$ values are means ±SEM of five determinations.

For those compounds showing binding recognition, the investigation continued in the evaluation of their αn subtypes selectivity (α1, α2, α3, α5). While compound **9d** showed a comparable affinity value on α1 and α2 in nanomolar range ($K_i$ = 9.1±0.9 nM and 12.0±10 nM respectively) and shows no affinity for α5, it is noteworthy that compounds **9c**, **9k-n** bind preferentially the alpha1 subtype, which generally reflected the binding on cortex. In particular compounds **9m** and 9**n** display a marked α1 subtype selectivity with respect to α2 subtype and a lower selectivity with respect to the other α3- and α5-subtypes. Finally, compound **9h**, although showed α2 binding affinity value ($K_i$) of 41.2 nM, possessed moderate α1 and α5 subtypes selectivity, of about 7-folds and 16-folds respectively ($K_i$ α1 = 284.8 nM, $K_i$ α5 = 662.6 nM).

The affinity values of PP and PT derivatives were tabulated in Table 3, **4c-e**, **A-D** in column 2 and compounds **9c-e, k-n** in column 3, in order to highlight which could be the best pharmacophore.

The type of substituent (aryl or heteroaryl ring) on the byciclic system (position 3 for pyrazolo[1,5-a]pyrimidines and the position 8 for pyrazolo[5,1-c][1,2,4]triazines), seems to have the same influence on the binding of the two series of compounds, as demonstrated by similar affinity values. The only exception is represented by compounds **4e** and **9e** bearing the 1-naphtyl group at position 3 or 8 respectively; the affinity values differ of about 8-folds, being the pyrazolotriazine **9e** more affine than **4e**, ($K_i$= 77 nM vs $K_i$=599 nM). This difference of affinity could be ascribed to the extra nitrogen atom as in compound **9e**, which could result cooperative to binding with receptor protein also considering that the presence of rigid and bulky substituent at position 3 or 8 of the two scaffolds could shift the whole molecule in the receptor pocket.

Finally, the comparison between the PT (**9k-n**) and the previously published PP (**A-D**) bearing at position 8 and 3 respectively a substituted phenyl reing, clearly demonstrated that there is no difference in binding affinity, being the $K_i$ values in the same order of magnitude (range **A-D**: 19.4-95.4 nM and **9k-9n**: 7.2-88.3 nM).

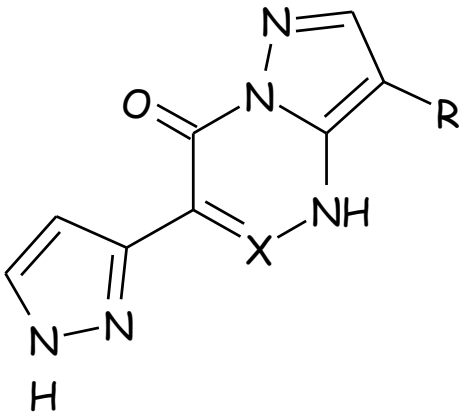


**Table 3.** Comparison between 6-(pyrazol-3-yl)pyrazolo[1,5-a]pyrimidine (PP) and corresponding 3-(pyrazol-3-yl)-pyrazolo[5,1-c][1,2,4]triazines (PT)

| R | Comp | X | $K_i$ (nM) cortex[a] | Comp | X | $K_i$ (nM) cortex[a] |
|---|---|---|---|---|---|---|
| 3-Py | **4c** | CH | 37.3 | **9c** | N | 73.6±2 |
| 2-Py | **4d** | CH | 14.2±1.6 | **9d** | N | 10.5±1.0 |
| 1-Naphtyl | **4e** | CH | 599±2.5 | **9e** | N | 77 |
| 4-FPh | **A** | CH | 68.3±2.1[b] | **9k** | N | 88.3±8 |
| 3-ClPh | **B** | CH | 95.4±6[c] | **9l** | N | 74±6 |
| 3-BrPh | **C** | CH | 54.5±0.8[b] | **9m** | N | 38.0±8 |
| 3-$CF_3$Ph | **D** | CH | 19.4[b] | **9n** | N | 7.2 |

[a] Percent inhibition values of specific [$^3$H]Ro15-1788 binding at 10 μM concentration and $K_i$ values are means ±SEM of five determinations. [b]see ref [11]. [c]see ref [9]

## 3. Conclusions

In this research we synthesized a series of PT as aza-analogues of potent PP[9,11,18] and, at the same time, we complete this latter series; as evidenced by the binding study the substitution of CH with N does not induce any affinity improvement.

Thus, the direct involvement to receptor interaction, by the nitrogen atom at position 2 of pyrazolotriazine core (position 5 of pyrazolopyrimidine core) is negligible, suggesting that this atom is not directly involved in the receptor protein anchoring in contrast with NH and the CO that

could act as 'dual hydrogen bond point' towards hydrogen bond donor (H1) and/or H2/A3 (hydrogen bond acceptor) site on receptor protein, already hypothesized. (Silvia Selleri et al. 1999) Moreover the presence at position 6 of pyrazolepyrimidine scaffold of the 1H-3-pyrazole ring results important for the interaction with receptor protein, as confirmed by the complete loss of affinity evidenced in compound bearing the 1-imidazole or 1-pyrazole ring.

## 4. Experimental section

Melting points were determined in open capillary tubes on a Büchi apparatus and were uncorrected. IR spectra were recorded (in KBr pellets in nujol mulls) on Perkin-Elmer 1420 spectrophotometer. $^1$H-NMR spectra were recorded with an Advance 400 Instrument (Bruker BiospinVersion 002 with SGU, Bruker AXS Inc., Madison, WI USA). Chemical shifts are reported in δ ppm using the solvent as internal standard. Extracted were dried over $Na_2SO_4$ and the solvent were removed under reduced pressure. Merk F-254 commercial plates (Merk-Gruppe, Darmstadt, Germany) were used for analytical thin layer chromatography (TLC) to follow the reaction course. Silica gel 60 (Merk 70-230 mesh) was used for column chromatography. Microanalyses were performed with a Perkin-Elmer elemental analyzer (Perkin-Elmer, Waltham, MA USA) for C, H, and N. Results within ±0.4% of the theoretical materials were commercially available. Experimental data of more representative compounds are reported.

### 5.1 General procedure for the synthesis of compounds 2a-f

A mixture of ethyl-2-acetyl-3-ethoxyacrylate (55 mmoles) and (3)5-aminopyrazoles 4-substituted (**1a-f**) (50 mmoles) in ethanol (100 mL) was refluxed under magnetic stirring until the starting material disappeared in TLC. After cooling a precipitate was separated and filtered.

#### 5.1.1 Ethyl 7-methyl-3-nitropyrazolo[1,5-a]pyrimidin-6-carboxylate, 2a

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.08 (s, 1H, H5); 8.80 (s, 1H, H-2); 4.36 (q, J = 7.2 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.45 (t, J = 7.2, 3H, $CH_3$). Anal C, H, N.

#### 5.1.2 Ethyl 3-amino-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate hydrochloride, 2a'

The 3-nitroderivative **2a** (1.5 g, 6.0 mmoles) was hydrogenated with $H_2$ and 5% Pd/C. At the end of the reaction after the filtration of catalyst the ethanol solution was added of HCl to obtain the corresponding 3-amino hydrochloride. Yellow crystals; TLC eluent: dichloromethane /methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 10.14 (bs, 2h, $NH_2$ exch.); 8.91 (s, 1H, H5); 8.44 (s, 1H, H-2); 4.36 (q, J = 7.2 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.45 (t, J = 7.2, 3H, $CH_3$). Anal C, H, N.

**5.1.3 Ethyl 3-benzyl-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2b**

Yellowish waxy solid; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 8.94 (s, 1H, H-5); 8.05 (s, 1H, H-2); 7.30 (m, 5H, benzyl); 4.47 (q, J = 7.0 Hz, 2H, $CH_2$); 4.18 (s, 2H, $CH_2$); 3.18 (s, 3H, $CH_3$); 1.48 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.1.4 Ethyl 3-(pyridin-3-yl)-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2c**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.41 (m, 1H, Py); 9.08 (s, 1H, H-5); 8.77-8.52 (m, 3H, H-2 and Py); 7.68-7.61 (m, 1H, Py); 4.47 (q, J = 7.2 Hz, 2H, $CH_2$); 3.27 (s, 3H, $CH_3$); 1.48 (t, J = 7.2 Hz, 3H, $CH_3$). Anal C, H, N.

**5.1.5 Ethyl 3-(pyridin-2-yl)-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2d**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.08 (s, 1H, H5); 8.96 (s, 1H, H-2); 8.64 (m, 1H, Py); 7.81-7.70 (m, 2H, Py); 7.18 (m, 1H, Py); 4.47 (q, J = 7.0 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.45 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.1.6 Ethyl 3-(napht-1-yl)-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2e**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.02 (s, 1H, H-5); 8.05 (s, 1H, H-2); 8.01-7.88 (m, 3H, naphtalene); 7.50-7.41 (m, 4H, naphthalene); 4.45 (q, J = 7.0 Hz, 2H, $CH_2$); 3.32 (s, 3H, $CH_3$); 1.45 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.1.7 Ethyl 3-(napht-2-yl)-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2f**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.09 (s, 1H, H-5); 8.67 (s, 1H, H-2); 8.58-8.51 (m, 1H, naphtalene); 8.20 (m, 1H, naphthalene); 7.95-7.82 (m,

3H, naphthalene); 7.51-7.46 (m, 2H, naphthalene); 4.45 (q, J = 6.9 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.45 (t, J = 6.9 Hz, 3H, $CH_3$). Anal C, H, N.

**5.1.8 Ethyl 3-(pyrrol-1-yl)-7-methylpyrazolo[1,5-a]pyrimidin-6-carboxylate, 2g**

A suspension of **2a'** (10 mmoles) was suspended in dioxane (40 mL) and 1.5 mL of 2,5-dimethoxytetrahydrofurane was added. The suspension was heated at 70 °C and after 1 hour the reaction was complete. The addition of water and sodium acetate to neutralize the final solution, the extraction with chloroform (25 mL x 2) and the usual work up gave a yellow solid, recrystallized from ethanol. TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 8.95 (m, 1H, (s, 1H, H-5); 8.35 (s, 1H, H-2); 7.35 (dd, J = 2.6, 2.1 Hz, 2H, pyrrole); 6.38 (dd, J = 3.5, 2.6 Hz, 2H, pyrrole); 4.45 (q, J = 7.0 Hz, 2H, $CH_2$); 3.21 (s, 3H, $CH_3$); 1.45 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2 General procedure for the synthesis of compounds 3b-g.**

Compounds **2b-g** (10 mmoles) in toluene (50 mL) containing dimethylformamide-dimethyl acetale (DMF-DMA) (20 mmoles) and catalytic amount of piperidine (1 mL) were warmed at 90 °C under magnetic stirring. The reaction was monitored by TLC and the final solution was evaporated to dryness obtained a solid residue that was recrystallized by suitable solvent.

**5.2.1 Ethyl 3-benzyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3b**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.16 (d, J = 12.82 Hz, 1H, CH=CH); 8.91 (s, 1H, H5); 7.88 (s, 1H, H-2); 7.20 (m, 5H, benzyl); 7.01 (d, J = 12.82 Hz 1H, CH=CH); 4.35 (q, J = 6.9 Hz, 2H, $CH_2$); 4.16 (s, 2H, CH2); 3.22 (bs, 3H, $N(CH_3)_2$); 3.06 (bs, 3H, $N(CH_3)_2$); 1.40 (t, J = 6.9 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2.2 Ethyl 7-(2-dimethylaminovinyl)-3-(pyridin-3-yl)-pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3c**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.70 (d, J = 12.70 Hz, 1H, CH=CH); 9.16 (s, 1H, Py); 8.98 (s, 1H, H5); 8.50-8.41 (m, 3H, H-2 and Py); 7.40-

7.31 (m, 1H, Py); 7.08 (d, J = 12.70 Hz, 1H, CH=CH); 4.37 (q, J = 7.0 Hz, 2H, $CH_2$); 3.30 (bs, 3H, $N(CH_3)_2$); 3.08 (bs, 3H, $N(CH_3)_2$); 1.40 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2.3 Ethyl 7-(2-dimethylaminovinyl)-3-(pyridin-2-yl)-pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3d**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.75 (d, J = 12.74 Hz, 1H, CH=CH); 9.01 (s, 1H, H5); 8.81 (s, 1H, H-2); 8.63-8.52 (m, 2H, Py); 7.79-7.71 (m, 1H, Py); 7.15 (m, 1H, Py); 7.11 (d, J = 12.74 Hz, 1H, CH=CH); 4.39 (q, J = 7.0 Hz, 2H, $CH_2$); 3.31 (bs, 3H, $N(CH_3)_2$); 3.09 (bs, 3H, $N(CH_3)_2$); 1.42 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2.4 Ethyl 7-(2-dimethylaminovinyl)-3-(napht-1-yl)-pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3e**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.80 (d, J = 12.65 Hz, 1H, CH=CH,); 8.94 (s, 1H, H5); 8.34 (s, 1H, H-2); 8.04 (m, 1H, naphtalene); 7.93-7.84 (m, 2H, naphtalene); 7.70 (m, 1H, naphthalene); 7.61-7.40 (m, 3H, naphthalene); 7.12 (d, J = 12.65 Hz 1H, CH=CH,); 4.39 (q, J = 7.0 Hz, 2H, $CH_2$); 3.32 (bs, 3H, $N(CH_3)_2$); 3.13 (bs, 3H, $N(CH_3)_2$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2.5 Ethyl 7-(2-dimethylaminovinyl)-3-(napht-2-yl)-pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3f**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 10.02 (d, J = 12.74 Hz, 1H, CH=CH,); 9.04 (s, 1H, H5); 8.53 (m, 2H, H-2 and naphtalene); 8.20 (m, 1H, naphtalene); 7.93-7.84 (m, 3H, naphtalene); 7.50-7.45 (m, 2H, naphthalene); 7.05 (d, J = 12.74 Hz, 1H, CH=CH,); 4.45 (q, J = 7.0 Hz, 2H, $CH_2$); 3.31 (bs, 3H, $N(CH_3)_2$); 3.11 (bs, 3H, $N(CH_3)_2$); 1.46 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.2.6 Ethyl 7-(2-dimethylaminovinyl)-3-(pyrrol-1-yl)-pyrazolo[1,5-a]pyrimidin-6-carboxylate, 3g**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.71 (d, J = 12.70 Hz, 1H, CH=CH,); 8.89 (s, 1H, H5); 8.17 (m, 1H, H-2); 7.29 (dd, J = 2.6, 2.1 Hz, 2H,

pyrrole); 7.22 (d, J = 12.70 Hz, 1H, CH=CH,); 6.35 (dd, J = 3.5, 2.6 Hz, 2H, pyrrole); 4.36 (q, 2H, $CH_2$); 3.29 (bs, 3H, $N(CH_3)_2$); 3.09 (bs, 3H, $N(CH_3)_2$); 1.40 (t, 3H, $CH_3$). Anal C, H, N.

**5.3 General procedure for the synthesis of compounds 4b-g.**

Hydrazine hydrate (10 mmoles) was added to a solution of ethyl 7-(2-dimethylaminovinyl)pyrazolo [1,5-a]pyrimidine-6-carboxylate 3-substituted (**3b-g**) (10 mmoles) and sodium acetate (24 mmoles) in acetic acid (50 mL). The mixture was refluxed under magnetic stirring monitoring by TLC, and until the solution became from yellow to colorless. After cooling or by addition of water a precipitate was recovered and filtered.

**5.3.1 3-Benzyl-4,7-dihydro-6-(1'*H*-pyrazolo-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4b**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.62 (bs, 1H, NH, exchange); 8.38 (s, 1H, H-5); 7.75 (s, 1H, H-2); 7.67-7.16 (m, 6H, benzyl and H-5' pyrazole); 6.78 (m, 1H, H-4' pyrazole); 3.97 (s, 2H, $CH_2$). LC-MS: 291.9 $[M+H]^+$. Anal C, H, N.

**5.3.2 4,7-dihydro-6-(1'*H*-pyrazolo-3'-yl)-3-(pyridin-3-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4c**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.91 (m, 1H, Py); 8.54 (m, 1H, Py); 8.40 (s, 1H, H-5); 8.38 (s, 1H, H-2); 8.11-8.07 (m, 1H, Py); 7.73 (m, 1H, H-5' pyrazole); 7.58-7.49 (m, 1H, Py); 6.92 (m, 1H, H-4' pyrazole). LC-MS: 278.8 $[M+H]^+$. Anal C, H, N.

**5.3.3 4,7-dihydro-6-(1'*H*-pyrazolo-3'-yl)-3-(pyridin-2-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4d**

Yellowish crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.75 (bs, 1H, NH, exchange); 12.35 (bs, 1H, NH exchange); 8.66 (m, 2H, H-2 and H-5); 8.54-8.49 (m, 1H, Py); 7.88 (m, 2H, Py); 7.74 (m, 1H, H-5' pyrazole); 7.25 (m, 1H, Py); 6.91 (m, 1H, H-4' pyrazole). LC-MS: 278.7 $[M+H]^+$. Anal C, H, N.

**5.3.4 4,7-dihydro-3-(napht-1-yl)-6-(1'*H*-pyrazolo-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4e**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 12.60 (bs, 1H, NH, exchange); 8.28 (s, 1H, H-5); 8.19 (s, 1H, H-2); 8.10-7.52

(m, 8H, naphtalene and H-5' pyrazole); 6.91 (m, 1H, H-4' pyrazole). LC-MS: 328.0 $[M+H]^+$. Anal C, H, N.

**5.3.5 4,7-dihydro-3-(napht-2-yl)-6-(1'*H*-pyrazolo-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4f**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.72 (bs, 1H, NH, exchange); 8.43 (s, 1H, H-5); 8.38 (s, 1H, H-2); 8.17 (d, J = 0.81 Hz, 1H, H-5'pyrazole); 8.06-7.52 (m, 7H, naphtalene); 6.92 (d, J = 0.81 Hz, 1H, H-4'pyrazole). LC-MS: 327.9 $[M+H]^+$. Anal C, H, N.

**5.3.6 4,7-dihydro-6-(1'*H*-pyrazolo-3'-yl)-3-(pyrrol-1-yl)pyrazolo[1,5-a]pyrimidin-7-one, 4g**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.82 (bs, 1H, NH, exchange); 8.31 (s, 1H, H-5); 8.20 (s, 1H, H-2); 7.71 (d, J = 1.59 Hz, 1H, H-5' pyrazole); 7.08 (m, 2H, pyrrole); 6.89 (d, J = 1.59 Hz, 1H, H-4' pyrazole); 6.30 (m, 2H, pyrrole). LC-MS: 268.3 $[M+H]^+$. Anal C, H, N.

**5.4 General procedure for the synthesis of compounds A and B.**

A suspension of 50% sodium hydride in mineral oil was added to anhydrous toluene (200 mL) in 1 l round bottomed flask. After addition of *t*-amyl alcohol the mixture was heated at 70 °C and a solution of the ethyl pyrazol-1-yl acetate or ethyl imidazole-1-yl acetate (162 mmoles, 25g) and ethyl formate (180 mmoles, 15 mL) in anhydrous toluene (50 mL) was added dropwise during an hours. After 6 hours of stirring and heating at 70-80 °C the resulting solid was left to stand overnight. The mixture was then treated with ice-water (400 mL) and the aqueous phase was separated and washed with diethyl ether. Acidification with HCl conc. causes the separation of oil which was extracted with diethyl ether. The extracted were dried over anhydrous sodium sulphate and evaporated to dryness, obtaining a solid.

**5.4.1 Ethyl 2-(pyrazol-1'-yl)-2-formylacetate A**

White crystals from cyclohexane; mp 78-79 °C; yield, 62%; TLC eluent: dichloromethane /methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 8.54 (d, J = 2.5 Hz, 1H, H-5); 7.76 (s, 1H, CH); 7.60

(d, J= 1.8 Hz, 1H, H-3); 6.38 (dd, J = 2.4, 1.8 Hz, 1H, H-4); 4.30 (q, J = 7.0 Hz, 2H, $CH_2$); 1.34 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.4.2 Ethyl 2-(imidazol-1'-yl)-2-formylacetate B**

White crystals from ethanol; mp 160-161 °C; yield, 78%; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 8.73 (s, 1H, CH); 8.21 (s, 1H, H-2); 7.10-7.06 (m, 2H, H-4 and H-5); 3.80 (q, J = 7.0 Hz, 2H, $CH_2$); 0.90 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.5 General synthesis of compounds 5a, b, 6a, b.**

A suspension of 3(5)-amino-4-(thien-2-yl)pyrazole (**1h**) or 3(5)-amino-4-phenylpyrazole (**1i**) [11,13] (10 mmoles) and ethyl 2-(pyrazol-1'-yl)-2-formylacetate (**A**) or ethyl 2-(imidazol-1'-yl)-2-formylacetate (**B**) (10 mmoles) in dyglime (20 mL) was refluxed under magnetic stirring until the starting material disappeared. The precipitate was collected by filtration from the reaction mixture and recrystallized by suitable solvent, obtaining compounds **5a, 5b, 6a** and **6b** respectively.

**5.5.1 4,7-dihydro-6-(pyrazol-1-yl)-3-(thien-2-yl)-pyrazolo[1,5-a]pyrimidin-7-one, 5a**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 10.54 (bs, 1H, NH, exchange); 8.97 (s, 1H, H-5); 8.43 (d, J = 2.73 Hz, 1H, H-5' pyrazole); 8.31 (s, 1H, H-2); 7.91 (m, 1H, thienyl); 7.78 (d, J = 1.46 Hz, 1H, H-3' pyrazole); 7.65 (m, 1H, thienyl); 7.56 (m, 1H, thienyl); 6.54 (m, 1H, H-4'pyrazole). Anal C, H, N.

**5.5.2 4,7-dihydro-3-phenyl-6-(pyrazol-1-yl)pyrazolo[1,5-a]pyrimidin-7-one, 5b**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 10.40 (bs, 1H, NH, exchange); 8.37 (s, 1H, H-5); 8.32 (s, 1H, H-2); 8.28 (d, J = 2.36 Hz, 1H, H-5'pyrazole); 7.72 (d, J = 1.75 Hz, 1H, H-3' pyrazole); 7.67 (m, 2H, Ph); 7.55-7.47 (m, 2H, Ph); 7.37 (m, 1H, Ph); 6.52 (m, 1H, H-4' pyrazole). Anal C, H, N.

**5.5.3 4,7-dihydro-6-(imidazol-1-yl)-3-(thien-2-yl)-pyrazolo[1,5-a]pyrimidin-7-one, 6a**

Yellowish crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 10.54 (bs, 1H, NH, exchange); 8.74 (s, 1H, H-2'imidazole); 8.27 (s, 1H, H-5); 8.21 (s, 1H,

H-2); 7.85 (m, 1H, thienyl); 7.72 (d, J = 1.56 Hz, 1H, H-5'imidazole); 7.69 (m, 1H, thienyl); 7.56 (m, 1H, thienyl); 7.48 (d, J = 1.56 Hz, 1H, H-4' imidazole). Anal C, H, N.

**5.5.4 4,7-dihydro-3-phenyl-6-(imidazol-1-yl)pyrazolo[1,5-a]pyrimidin-7-one, 6b**

White crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 10.30 (bs, 1H, NH, exchange); 8.98 (s, 1H, H-2'imidazole); 8.35 (s, 1H, H-5); 8.26 (s, 1H, H-2); 7.99 (m, 2H, Ph); 7.83 (d, J = 1.42 Hz, 1H, H-5' imidazole); 7.60 (d, J = 1.42 Hz, 1H, H-4' imidazole); 7.44-7.36 (m, 2H, Ph); 7.15 (m, 1H, Ph). Anal C, H, N.

**5.6 General procedure for the synthesis of compounds 7c-f, 7h-p.**

The suitable 4-substituted-5-aminopyrazoles (**1c-f, 1h-p**) (20 mmoles) were diazotized as usual and the solution of diazonium salt was treated with of sodium acetate (24 mmoles) at room temperature and stirred until to complete dissolution. At this solution was then added ethyl acetoacetate (20.0 mmoles) in ethanol and stirred for 1 hour at room temperature. The final compounds were recovered by filtration after addition of water or by extraction if a precipitate was not formed. Starting from **1f**, in this reaction condition, a byproduct is formed and identified as 1*H*-Benzo[h]pyrazolo[3,4-c]cinnoline, **10**. The unambiguous synthesis of this latter was obtained as described below.

**5.6.1 Ethyl 4-methyl-8-(pyridin-3-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7c**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.41 (d, J = 2.0 Hz, 1H, Py); 8.77 (s, 1H, H-2); 8.70 (dd, J = 7.8, 1.7 Hz, 1H, Py); 8.22 (dd, J = 4.9, 1.7 1H, Py); 7.49 (dd, J = 7.8, 4.9, 1H, Py); 4.60 (q, J = 7.0 Hz, 2H, $CH_2$); 3.27 (s, 3H, $CH_3$); 1.60 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.2 Ethyl 4-methyl-8-(pyridin-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7d**

Yellow crystals; TLC eluent: toluene/ethyl acetate/acetic acid 8:2:1 v/v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.16 (s, 1H, H-2); 8.71 (d, J = 5.0 Hz, 1H, Py); 8.64 (d, J = 8.0 Hz, 1H, Py); 8.02 (dd, J = 8.0, 7.7 Hz, 1H, Py); 7.40 (dd, J = 7.7, 5.0 Hz, 1H, Py); 4.51 (q, J = 7.0 Hz, 2H, $CH_2$); 3.13 (s, 3H, $CH_3$); 1.42 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.3 Ethyl 4-methyl-8-(napht-1-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7e**

Yellow crystals; TLC eluent: toluene/ethyl acetate 8:2 v/v; $^1$H-NMR ($CDCl_3$) δ 8.68 (s, 1H, H-2); 8.08 (d, J = 7.5 Hz, 1H, naphthalene); 7.98 (d, J = 7.5 Hz, 2H, naphthalene); 7.88 (d, J = 7.5 Hz, 1H, naphthalene); 7.68-7.46 (m, 3H, naphthalene); 4.62 (q, J = 7.0 Hz, 2H, $CH_2$); 3.26 (s, 3H, $CH_3$); 1.58 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.4 Ethyl 4-methyl-8-(napht-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7f**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 8.85 (s, 1H, naphthalene); 8.80 (s, 1H, H-2); 8.30 (d, J = 7.5 Hz, 1H, naphthalene); 8.00-7.90 (m, 3H, naphthalene); 7.55-7.48 (m, 2H, naphthalene); 4.60 (q, J = 7.0 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.55 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.5 Ethyl 4-methyl-8-(thien-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7h**

Orange crystals; TLC eluent: toluene/ethyl acetate 8:2 v/v; $^1$H-NMR ($DMSOd_6$) δ 9.06 (s, 1H, H-2); 7.85 (d, J = 2.8 Hz, 1H, thienyl); 7.66 (d, J = 4.8 Hz, 1H, thienyl); 7.24 (m, 1H, thienyl); 4.48 (q, J = 7.0 Hz, 2H, $CH_2$); 3.08 (s, 3H, $CH_3$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.6 Ethyl 4-methyl-8-phenylpyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7i**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; ) δ 9.09 (s, 1H, H-2); 8.24 (m, 2H, phenyl); 7.49 (m, 2H, phenyl); 7.34 (m, 1H, phenyl); 4.43 (q, J = 7.0 Hz, 2H, $CH_2$); 3.08 (s, 3H, $CH_3$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.7 Ethyl 4-methyl-8-(3-fluorophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7j**

Dark yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.20 (s, 1H, H-2); 8.19 (m, 2H, H-2' and H-4' Ph); 7.60 (m, 1H, H-5' Ph); 7.21 (m, 1H, H-6' Ph); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.10 (s, 3H, $CH_3$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.8 Ethyl 4-methyl-8-(4-fluorophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7k**

Dark yellow crystals; TLC eluent: dichloromethane/methanol 10:2 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.15 (s, 1H, H-2); 8.35 (m, 2H, H-3' and H-5' Ph); 7.40 (m, 2H, H-2' and H-6' Ph); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.10 (s, 3H, $CH_3$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.9 Ethyl 4-methyl-8-(3-chlorophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7l**

Yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.20 (s, 1H, H-2); 8.40 (d, J = 1.5 Hz, 1H, H-2' Ph); 8.28 (dd, J = 7.0, 1.5 Hz, 1H, H-4' Ph); 7.58 (t, J = 7.0 Hz, 1H, H-5' Ph); 7.45 (dd, J = 7.0, 1.5 Hz, 1H, H-6' Ph); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.10 (s, 3H, $CH_3$); 1.41 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.10 Ethyl 4-methyl-8-(3-bromophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7m**

Yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 8.70 (s, 1H, H-2); 8.45 (d, J = 1.5 Hz, 1H, H-2' Ph); 8.20 (dd, J = 8.0, 1.5 Hz, 1H, H-4' Ph); 7.52 (dd, J = 8.0, 1.5 Hz, 1H, H-6' Ph); 7.40 (t, J = 8.0 Hz, 1H, H-5' Ph); 4.60 (q, J = 7.0 Hz, 2H, $CH_2$); 3.25 (s, 3H, $CH_3$); 1.53 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.11 Ethyl 4-methyl-8-(3-trifluoromethylphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7n**

Red crystals; TLC eluent: toluene/ethyl acetate/acetic acid 8:2:1 v/v/v; $^1$H-NMR ($CDCl_3$) δ 8.75 (s, 1H, H-2); 8.55 (m, 1H, H-2' Ph); 8.48 (m, 1H, H-5' Ph); 7.68 (m, 2H, H-4' and H-6' Ph); 4.65 (q, J = 7.0 Hz, 2H, $CH_2$); 3.28 (s, 3H, $CH_3$); 1.55 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.12 Ethyl 4-methyl-8-(4-methoxyphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7o**

Yellow crystals; TLC eluent toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 8.50 (s, 1H, H-2); 8.08 (d, J = 8.0 Hz, 2H, H-2' and H-6' Ph); 7.28 (m, 2H, H-3' and H-5' Ph); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.80 (s, 3H, 4'-$OCH_3$Ph); 3.15 (s, 3H, $CH_3$); 1.45 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.6.13 Ethyl -4-methyl-8-(4-methylphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 7p**

Dark yellow crystals; TLC eluent toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 8.55 (s, 1H, H-2); 8.08 (d, J = 8.0 Hz, 2H, H-2' and H-6' Ph); 7.28 (m, 2H, H-3' and H-5' Ph); 4.50 (q, J = 7.0

Hz, 2H, $CH_2$); 3.15 (s, 3H, $CH_3$); 2.35 (s, 3H, 4'-$CH_3$Ph); 1.45 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7 General procedure for the synthesis of compounds 8c-f, 8h-p.**

Compounds **7c-f, 7h-p** (10 mmoles) in toluene (50 mL) containing dimethylformamide-dimethyl acetale (DMF-DMA) (20 mmoles) and catalytic amount of piperidine (1 mL) were warmed at 90 °C under magnetic stirring. The reaction was monitored by TLC and the final solution was evaporated to dryness obtaining a solid residue or the precipitate was filtered and recrystallized by suitable solvent.

**5.7.1 Ethyl 4-(2-dimethylaminovinyl)-8-(pyridin-3-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8c**

Yellow crystals; TLC eluent: choloform/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.75 (d, J = 12.74 Hz, 1H, CH=CH,); 9.30 (s, 1H, H-2); 8.75 (dd, J = 7.8, 1.7 Hz, 1H, Py); 8.50 (m, 2H, Py); 7.45 (dd, J = 7.8, 4.9, 1H, Py); 7.00 (d, J= 12.74 Hz, 1H, CH=CH,); 4.54 (q, J = 7.0 Hz, 2H, $CH_2$); 3.35 (s, 3H, $N(CH_3)_2$); 3.15 (s, 3H, $N(CH_3)_2$); 1.51 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.2 Ethyl 4-(2-dimethylaminovinyl)-8-(pyridin-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8d**

Yellow crystals; TLC eluent: choloform/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 10.99 (s, 1H, H-2); 9.91 (dd, J = 5.0 Hz, 1H, Py); 8.46 (d, 1H, CH=CH, $J_{trans}$ = 12.74 Hz); 8.39 (dd, 1H, Py); 8.31 (dd, 1H, Py); 7.58 (dd, 1H, Py); 6.80 (d, 1H, CH=CH, $J_{trans}$ = 12.74 Hz); 4.54 (q, 2H, $CH_2$); 3.72 (s, 3H, $N(CH_3)_2$); 3.46 (s, 3H, $N(CH_3)_2$); 1.51 (t, 3H, $CH_3$). Anal C, H, N.

**5.7.3 Ethyl 4-(2-dimethylaminovinyl)-8-(napht-1-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8e**

Dark yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.85 (d, J = 12.74 Hz, 1H, CH=CH); 8.45 (s, 1H, H-2); 8.15 (d, J = 7.5 Hz, 1H, naphthalene); 7.98-7.85 (m, 3H, naphthalene); 7.68-7.46 (m, 3H, naphthalene); 6.81 (d, J = 12.74 Hz, 1H, CH=CH,); 4.25 (q, J =

7.0 Hz, 2H, $CH_2$); 3.40 (s, 3H, $N(CH_3)_2$); 3.20 (s, 3H, $N(CH_3)_2$); 1.37 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.4 Ethyl 4-(2-dimethylaminovinyl)-8-(napht-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8f**

Brown crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.80 (d, J= 12.74 Hz, 1H, CH=CH); 8.90 (s, 1H, naphthalene); 8.58 (s, 1H, H-2); 8.30 (d, J = 7.5 Hz, 1H, naphthalene); 7.98-7.85 (m, 3H, naphthalene); 7.50 (m, 2H, naphthalene); 6.95 (d, J = 12.74 Hz, 1H, CH=CH); 4.55 (q, J = 7.0 Hz, 2H, $CH_2$); 3.40 (s, 3H, $N(CH_3)_2$); 3.15 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.5 Ethyl 4-(2-dimethylaminovinyl)-8-(thien-2-yl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8h**

Dark yellow crystals; TLC eluent: toluene/ethyl acetate/acetic acid 8:2:1 v/v/v; $^1$H-NMR ($CDCl_3$) δ 9.68 (d, J = 12.74 Hz, 1H, CH=CH); 8.76 (s, 1H, H-2); 7.75 (d, J = 2.8 Hz, 1H, thienyl); 7.55 (d, J = 4.8 Hz,1H, thienyl); 7.18 (m, 1H, thienyl); 6.81 (d, J = 12.74 Hz, 1H, CH=CH); 4.38 (q, J = 7.0 Hz, 2H, $CH_2$); 3.65 (m, 6H, $N(CH_3)_2$); 1.37 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.6 Ethyl 4-(2-dimethylaminovinyl)-8-phenylpyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8i**

Yellow crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.75 (d J= 12.74 Hz, 1H, CH=CH); 8.45 (s, 1H, H-2); 8.20 (d, J = 7.2 Hz, 2H, Ph); 7.45 (t, J = 7.2 Hz, 2H, Ph); 7.28 (t, J = 7.2 Hz, 1H, Ph); 6.85 (d, J = 12.74 Hz, 1H, CH=CH); 4.60 (q, J = 7.0 Hz, 2H, $CH_2$); 3.31 (bs, 3H, $N(CH_3)_2$); 3.09 (bs, 3H, $N(CH_3)_2$); 1.42 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.7 Ethyl 4-(2-dimethylaminovinyl)-8-(3-fluorophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8j**

Yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.80 (d, J = 12.74 Hz, 1H, CH=CH); 8.45 (s, 1H, H-2); 8.09 (m, 2H, H-2' and H-4' Ph); 7.45 (m, 1H, H-5' Ph); 7.12 (d, J = 12.74 Hz, 1H, CH=CH,); 7.00 (m, 1H, H-6' Ph); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.60 (m, 6H, $N(CH_3)_2$; 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.8 Ethyl 4-(2-dimethylaminovinyl)-8-(4-fluorophenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8k**

Dark yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.78 (d, J = 12.74 Hz, 1H, CH=CH,); 8.39 (s, 1H, H-2); 8.24 (m, 2H, H-3' and H-5' Ph); 7.20-705 (m, 3H, H-2' and H-6' Ph, CH=CH); 4.51 (q, J = 7.0 Hz, 2H, $CH_2$); 3.36 (s, 3H, $N(CH_3)_2$); 3.14 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.9 Ethyl 8-(3-chlorophenyl)-4-(2-dimethylaminovinyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8l**

Yellow crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.80 (d, J = 12.74 Hz, 1H, CH=CH,); 8.45 (d, J = 1.5 Hz, 1H, H-2' Ph); 8.30 (s, 1H, H-2); 8.15 (dd, J = 7.0, 1.5 Hz, 1H, H-4' Ph); 7.50 (t, J = 7.0 Hz, 1H, H-5' Ph); 7.25 (dd, J = 7.0, 1.5 Hz, 1H, H-6' Ph); 7.12 (d, J = 12.74 Hz, 1H, CH=CH,); 4.51 (q, J = 7.0 Hz, 2H, $CH_2$); 3.36 (s, 3H, $N(CH_3)_2$); 3.14 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.10 Ethyl 8-(3-bromophenyl)-4-(2-dimethylaminovinyl)pyrazolo[5,1-c][1,2,4]triazin-3carboxylate, 8m**

Yellow crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR ($CDCl_3$) δ 9.65 (d, J = 12.74 Hz, 1H, CH=CH,); 8.47 (d, J = 1.5 Hz, 1H, H-2' Ph); 8.38 (s, 1H, H-2); 8.15 (dd, J = 8.0, 1.5 Hz, 1H, H-4' Ph); 7.39-7.31 (m, 2H, H-5' and H-6' Ph); 6.85 (d, J = 12.74 Hz, 1H, CH=CH,); 4.51 (q, J = 7.0 Hz, 2H, $CH_2$); 3.34 (s, 3H, $N(CH_3)_2$); 3.10 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.11 Ethyl 4-(2-dimethylaminovinyl)-8-(3-trifluoromethylphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8n**

Yellow crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.70 (d, J = 12.74 Hz, 1H, CH=CH,); 8.50 (m, 3H, H-2, H-2' and H-4' Ph); 7.70 (m, 2H, H-5' and H-6' Ph); 7.10 (d, J = 12.74 Hz, 1H, CH=CH,); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.38 (s, 3H, $N(CH_3)_2$); 3.18 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.12 Ethyl 4-(2-dimethylaminovinyl)-8-(4-methoxyphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8o**

Red crystals; TLC eluent: toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.70 (d, J = 12.74 Hz, 1H, CH=CH); 8.40 (s, 1H, H-2); 8.20 (d, J = 7.5 Hz, 2H, H-2' and H-6' Ph); 7.00 (d, J = 7.5 Hz, 2H, H-3' and H-5' Ph); 6.80 (d, J = 12.74 Hz, 1H, CH=CH); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.80 (s, 3H,OCH3); 3.38 (s, 3H, $N(CH_3)_2$); 3.18 (s, 3H, $N(CH_3)_2$); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.7.13 Ethyl 4-methyl-8-(4-methylphenyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate, 8p**

Dark yellow crystals; TLC eluent toluene/ethyl acetate 8:3 v/v; $^1$H-NMR ($CDCl_3$) δ 9.70 (d, J = 12.74 Hz, 1H, CH=CH); 8.55 (s, 1H, H-2); 8.08 (d, J = 8.0 Hz, 2H, H-2' and H-6' Ph); 7.28 (m, 2H, H-3' and H-5' Ph); 6.80 (d, J = 12.74 Hz, 1H, CH=CH); 4.50 (q, J = 7.0 Hz, 2H, $CH_2$); 3.38 (s, 3H, $N(CH_3)_2$); 3.18 (s, 3H, $N(CH_3)_2$); 2.35 (s, 3H, 4'-$CH_3$Ph); 1.50 (t, J = 7.0 Hz, 3H, $CH_3$). Anal C, H, N.

**5.8 General procedure for the synthesis of compounds 9c-f, 9h-p.**

A solution of ethyl 4-(2-dimethylaminovinyl)pyrazolo[5,1-c][1,2,4]triazin-3-carboxylate 8-substituted (**7c-f, 7h-p**) (10 mmoles) in acetic acid (50 mL) was added of sodium acetate (24 mmoles) and hydrazine hydrate (10 mmoles) The mixture was refluxed under magnetic stirring monitoring by TLC until the disappeared of starting material. After cooling or by addition of water a precipitate was recovered and filtered.

**5.8.1 1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)-8-(pyridin-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9c**

Yellow crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.00 (s,1H, Py); 8.48 (m, 2H, H-2 and Py); 8.22 (m, 1H, Py); 7.70( m, 1H, H-5' pyz); 7.52 (m, 1H, Py); 6.90 (m, 1H, H-4'). LC-MS: 279.9 $[M+H]^+$. Anal C, H, N.

**5.8.2 1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)-8-(pyridin-2-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9d**

Yellow crystals; TLC eluent: ethyl acetate/methanol 10:2 v/v; $^1$H-NMR (DMSO-$d_6$) δ 9.06 (s, 1H, H-2); 8.72-8.68 (m, 2H, Py); 8.35 (d, J = 7.2 Hz, 1H, H-5' pyz); 8.02 (m, 1H, Py); 7.41 (m, 1H, Py);

7.12 (d, J = 7.2 Hz, 1H, H-4' pyz); 6.39 (s, 2H, NH exchange). LC-MS: 279.8 $[M+H]^+$. Anal C, H, N.

**5.8.3 1,4-dihydro-8-(napht-1-yl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9e**

Brown crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 14.10 (bs 1H, NH, exchange); 13.20 (bs, 1H, NH, exchange); 8.28 (s, 1H, H-2); 8.02-7.89 (m, 3H, naphthalene); 7.63-7.52 (m, 5H, naphthalene and H-5'); 7.02 (m, 1H, H-4'). LC-MS: 328.9 $[M+H]^+$. Anal C, H, N.

**5.8.4 1,4-dihydro-8-(napht-2-yl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9f**

Yellow-brown crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 8.56 (s, 1H, H-2); 8.26 (s, 1H, naphthalene); 8.02-7.86 (m, 4H, naphthalene); 7.73 (m, 1H, H-5'); 7.02 (m, 1H, H-4'). LC-MS: 329.0 $[M+H]^+$. Anal C, H, N.

**5.8.5 1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)-8-(thien-2-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9h**

Brown crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.50 (bs, 1H, NH, exchange); 8.23 (s, 1H, H-2); 7.54 (m, 2H, H-5' and thienyl); 7.36 (m, 1H, thienyl); 7.07 (m, 1H, thienyl); 6.89 (m, 1H, H-4'). LC-MS: 284.9 $[M+H]^+$. Anal C, H, N.

**5.8.6 1,4-dihydro-3-(1'*H*-pyrazolo-3'-yl)-8-phenylpyrazolo[5,1-c][1,2,4]triazin-4-one, 9i**

Yellowish crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.38 (s, 1H, H-2); 8.25 (m, 2H, H2' and H-6' Ph); 7.50 (bs, 1H, H-5' pyz); 7.38 (m, 2H, H3' and H-5' Ph); 7.25 (m, 1H, H-4' Ph); 6.88 (bs 1H, H-4' pyz). LC-MS: 278.8 $[M+H]^+$. Anal C, H, N.

**5.8.7 1,4-dihydro-8-(3-fluorophenyl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9j**

Red-brown crystals; TLC eluent: chloroform/methanol 10:0.5 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.44 (s, 1H, H-2); 8.15 (m, 1H, H-2' Ph); 8.00 (m, 1H, H-4' Ph); 7.45 (bs, 1H, H-5' pyz); 7.38 (m, 1H, H-5' Ph); 6.91 (m, 1H, H-4' Ph); 6.88 (bs 1H, H-4'pyz). LC-MS: 296.9 $[M+H]^+$. Anal C, H, N.

**5.8.8 1,4-dihydro-8-(4-fluorophenyl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9k**

Orange crystals; TLC eluent: chloroform/methanol/acetic acid 10:2:1 v/v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.44 (s, 1H, H-2); 7.80-7.65 (m, 3H, H-3' and H-5' Ph and H-5' pyz); 7.35 (m, 2H, H-2' and H-6' Ph); 6.90 (bs 1H, H-4'pyz). LC-MS: 296.8 $[M+H]^+$. Anal C, H, N.

**5.8.9 8-(3-chlorophenyl)-1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9l**

Yellow crystals; TLC eluent: chloroform/methanol 10:1.5 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.40 (m, 2H, H-2 and H-2' Ph); 8.05 (m, 1H, H-4' Ph); 7.50 (bs, 1H, H-5' pyz); 7.38 (m, 1H, H-5' Ph); 7.18 (m, 1H, H-6' Ph); 6.89 (bs 1H, H-4'pyz). LC-MS: 313.3 $[M+H]^+$. Anal C, H, N.

**5.8.10 8-(3-bromophenyl)-1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9m**

Yellow crystals; TLC eluent: chloroform/methanol 10:1.5 v/v; $^1$H-NMR (DMSO-$d_6$) δ 11.70 (bs, 1H, NH, exchange); 8.55 (m, 1H, H-2' Ph); 8.42 (s, 1H, H-2); 8.08 (m, 1H, H-4' Ph); 7.52 (bs, 1H, H-5' pyz); 7.35-7.28 (m, 2H, H-5' and H-6' Ph); 6.89 (bs 1H, H-4'pyz). LC-MS: 357.8 $[M+H]^+$. Anal C, H, N.

**5.8.11 1,4-dihydro-3-(1'*H*-pyrazolo-3-yl)-8-(3-trifluoromethyl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9n**

Orange crystals; TLC eluent: dichloromethane/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 8.50 (s, 1H, H-2); 8.34 (d, 1H, H-2' Ph); 8.19 (m, 1H, H-4' Ph); 7.69-7.54 (m, 3H, H-5', H-6' Ph and H-5' pyz); 6.98 (bs 1H, H-4'pyz). LC-MS: 346.8 $[M+H]^+$. Anal C, H, N.

**5.8.12 1,4-dihydro-8-(4-methoxyphenyl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9o**

Orange crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.19 (s, 1H, H-2); 8.07 (d, J = 7.5 Hz, 2H, H-2' and H-6' Ph); 7.37 (bs, 1H, H-5' pyz); 6.88 (d, J = 7.5 Hz, 2H, H-3' and H-5' Ph); 6.76 (bs 1H, H-4'pyz); 3.70 (s, 3H, $OCH_3$). LC-MS: 308.9 $[M+H]^+$. Anal C, H, N.

**5.8.13 1,4-dihydro-8-(4-methylphenyl)-3-(1'*H*-pyrazolo-3-yl)pyrazolo[5,1-c][1,2,4]triazin-4-one, 9p**

Orange crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 12.85 (bs, 1H, NH, exchange); 8.40 (s, 1H, H-2); 7.65 (bs, 1H, H-5' pyz); 7.61 (d, J = 7.5 Hz, 2H, H-2' and H-6' Ph); 7.27 (d, J = 7.5 Hz, 2H, H-3' and H-5' Ph); 6.89 (bs, 1H, H-4' pyz); 2.34 (s, 3H, $CH_3$). LC-MS: 292.9 $[M+H]^+$. Anal C, H, N.

**5.9 General procedure for synthesis of compounds 10-12.**

A solution of suitable 4-(naphthalene-2-yl)-3(5)-aminopyrazole **1f**, 4-(thien-2-yl)-3(5)-aminopyrazole **1q** and 4-(3-methoxyphenyl)-3(5)aminopyrazole **1r** [12,13] (10 mmoles) in acetic acid (16 mL) and hydrochloric acid (6 mL) was diazotized with sodium nitrite solution (10 mmoles/3 mL). After one hour at 0 °C the reaction was maintained under stirring at room temperature for other 30 min then, addition of water, give a precipitate that was filtered and recrystallized by suitable solvent.

**5.9.1 1*H*-Benzo[h]pyrazolo[3,4-c]cinnoline, 10**

White crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 13.4 (bs, 1H, NH exch.); 9.60 (s, 1H, H-2); 9.00-8.90 (s, 1H, naphthalene); 8.55-8.35 (m, 2H, naphthalene); 8.30-8.10 (s, 1H, naphthalene); 8.00-7.70 (m, 2H, naphthalene). LC-MS: 220.2 $[M+H]^+$. Anal C, H, N.

**5.9.2 3*H*-Pyrazolo[3,4-c]thieno[3,2-e]pyridazine, 11**

Cream crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 13.0 (bs, 1H, NH exch.); 8.62 (s, 1H, H-2); 8.53-8.48 (d, 1H, thienyl); 7.98-7.93 (d, 1H, thienyl). LC-MS: 176.8 $[M+H]^+$. Anal C, H, N.

**5.9.3 8-Methoxy-3*H*-Pyrazolo[3,4-c]cinnoline, 12**

Yellow crystals; TLC eluent: chloroform/methanol 10:1 v/v; $^1$H-NMR (DMSO-$d_6$) δ 13.5 (bs, 1H, NH exch.); 8.55-8.48 (m, 1H, Ph); 8.80 (s, 1H, H-2); 7.91-7.87 (m, 1H, Ph); 7.48-7.42 (m, 1H, Ph). LC-MS: 200.8 $[M+H]^+$. Anal C, H, N.

**6. Biological experimental section**

**6.1 Radioligand binding assay on bovine cortex.** [$^3$H]Ro15-1788 (specific activity 78.8 Ci/mmol) was obtained from Perkin Elmer. All the other chemicals, which were of reagent grade, were obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared as previously described.[19,20] The membrane preparations were diluted with 50 mM tris-citrate buffer pH 7.4, and used in the binding assay. Protein concentration was assayed using the method of Lowry et al.[21] [$^3$H]Ro 15-1788 binding studies were performed as previously reported.[22] At least six different concentrations of each compound were used. The data of n=5 experiments carried out in triplicate were analyzed by means of an iterative curve-fitting procedure (program Prism, GraphPad, San Diego, CA), which provided $IC_{50}$, Ki, and SEM values for tested compounds, the Ki values being calculated from the Cheng and Prusoff equation.[23]

**6.2 Radioligand Binding Studies at $GABA_A$ receptor subtypes**

Clonal mammalian cell lines, expressing $GABA_A$ receptor subtypes (α1β2γ2, α2β2γ2, α3β2γ2, α5β3γ2), were maintained in minimum essential medium Eagle's with EBSS, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 5% $CO_2$, 95% air at 37 °C.

The cells were collected by centrifugation at 500g; the crude membranes by differential centrifugation at 48,000g for 30 min at 4°C. The pellets were washed twice before final resuspension in 10 mM potassium phosphate, pH 7.4.

[$^3$H] Ro15-1788 binding assays to transfected cell membranes was carried out as described above. The Kd values of [$^3$H] Ro15-1788 were calculated in previous experiments, and determined as

follows. α1β2γ2: Kd=0.67 nM; α2β2γ2: Kd=0.90 nM; α3β2γ2: Kd=1.29 nM , α5β3γ2: Kd=0.68 nM ). [24]

**Supplementary data.**

Supplementary data associated with this article can be found, in the online version.

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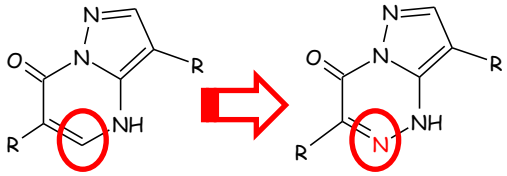
**A new class of pyrazolo[5,1-c][1,2,4]triazines as γ–aminobutyric type A ($GABA_A$) receptor subtype ligand: synthesis and pharmacological evaluation**

Gabriella Guerrini,* Giovanna Ciciani, Simona Daniele, Claudia Martini, Camilla Costagli, Chiara Guarino, Silvia Selleri,




The synthesis of the bicyclic core pyrazolotriazine (PT) strictly related to pyazolopyrimidine (PP), was realized. New compounds show α1 or α2 subtype selectivity.