Potential of Alkyl Orthoformates as Alkylating Agents of Non-electron Rich Nitrogen Heterocycles

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Findings and suggestions about the potential of the alkyl orthoformates as alkylating agents of non electron rich nitrogen heterocycles and unambiguous instrumental and chemical characterization of the alkylated compounds are reported.

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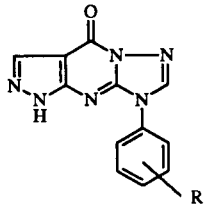
Introduction.

In connection with studies designed to produce pyrazolotriazolopyrimidin-4-one derivatives of pharmaceutical interest (Formula **A**) [1,2] the final formation of the condensed triazole ring using triethyl orthoformate as the C7 supplying agent constantly produced a byproduct having mass at M+ 28 with respect to the expected product.



A

The compound was observed in the crude solid separated by a rather intense peak in the mass spectrometric measurements and by a spot lower than that of the desired product (Figure 1, inset) [1,2,3] on tlc (ethyl acetate). Based upon a general review of the literature data, and by rule of thumb, we believed it to be such an impurity as the *N*-formyl derivative. We supposed the latter should result from the hydrolysis of the hemiketal intermediate formed by the reaction of the triethyl orthoformate with the heteroarene nitrogen. The byproduct could be converted into the stable desired nitrogen unsubstituted product by means of the thermal decomposition during the recrystallization [1]. Urged by the very interesting pharmacological properties of these and other related molecules [1-6] we decided to carry out a greater in depth investigation. Our aims were both the exact characterization of the reaction byproduct and the setting up of an accurate analytical method of general applicability for the chemical purity determination, and the identification of any impurity in polycondensed heterocycles [1-6]. Technical details of the instrumental study are reported elsewhere [S. Pucci, A. Raffaelli, F. Russo, S. Guccione, G. Romeo, forthcoming paper]. Within the series [1-3], we selected the 8-(4-bromophenyl) derivative as the investigation tool (Figure 1, inset) since the bromine is a better "structural probe" in mass spectrometry with respect to chlorine, due to its isotopic composition close to 1:1, and a sample with a high relatively concentration of the impurity was available.

The structural characterization of the impurity as the 2-ethyl-8-(4-bromophenyl)-2*H*,4*H*,8*H*-pyrazolo[3,4-*d*]-[1,2,4]triazolo[1,5-*a*]pyrimidin-4-one (**7b**) (Scheme 2) led us to survey the potential of the unexpected reaction as a new *N*-alkylation method of non electron rich nitrogen heterocycles. We believed it worthwhile to perform a thorough investigation in order to establish the more appropriate conditions at which the reaction occurs in an advantageous and specific way also comparing the effect of other ortho esters [7-10].

In fact, *N*-alkylation of an NH group is usually brought about by the action of either methyl iodide or dimethyl sulphate on the anion of the heterocycle, and this is sometimes conveniently achieved by the use of phase transfer conditions [11]. Although ortho esters and the di- and trialkoxycarbenium ions *in situ* generated from them by proton or Lewis acids action may constitute one of the longest and best studied class of the reactive intermediates in organic chemistry [7-10,12], little is known of their potential as alkylating agents, particularly in the area of the non electron rich nitrogen heterocycles [13,14]. The chemistry of ortho esters was recently presented in an exhaustive review by Pindur [12]. In this paper we report the unambiguous structural instrumental-chemical **7b** (Schemes 2 and 3) characterization of the target compound (Figure 1) and the identification of methyl or ethyl derivatives resulting from

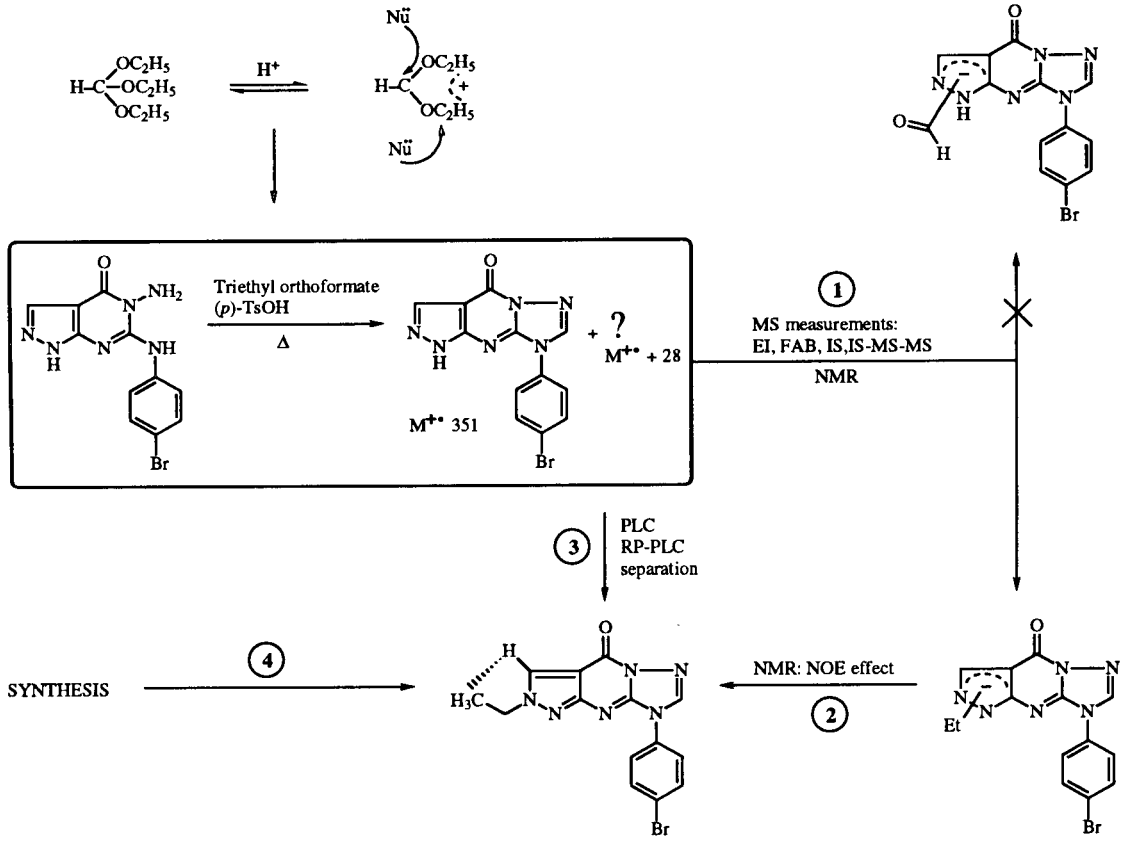


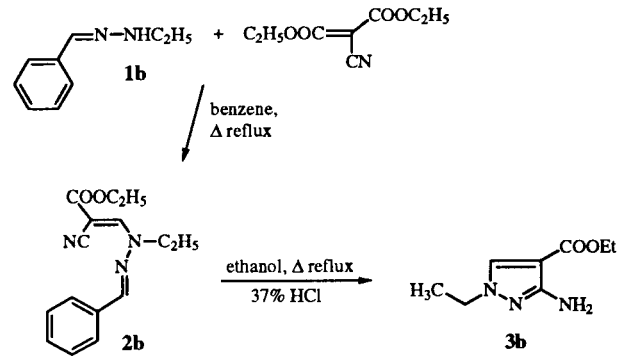
Figure 1. Chronological sequence of the investigation **1** → **4**.

the trimethyl- or triethyl orthoformate effect. *N*1 or *N*2 substituted samples obtained by synthesis of **7a** (Scheme 2) and **13a,b** (Scheme 4) were employed as controls. Preliminary suggestions about the synthetic potential of the unexpected reaction as "tandem" alkylating method exploiting the a_1 synthon properties of the alkyl orthoformate are discussed [11,12,15,16].

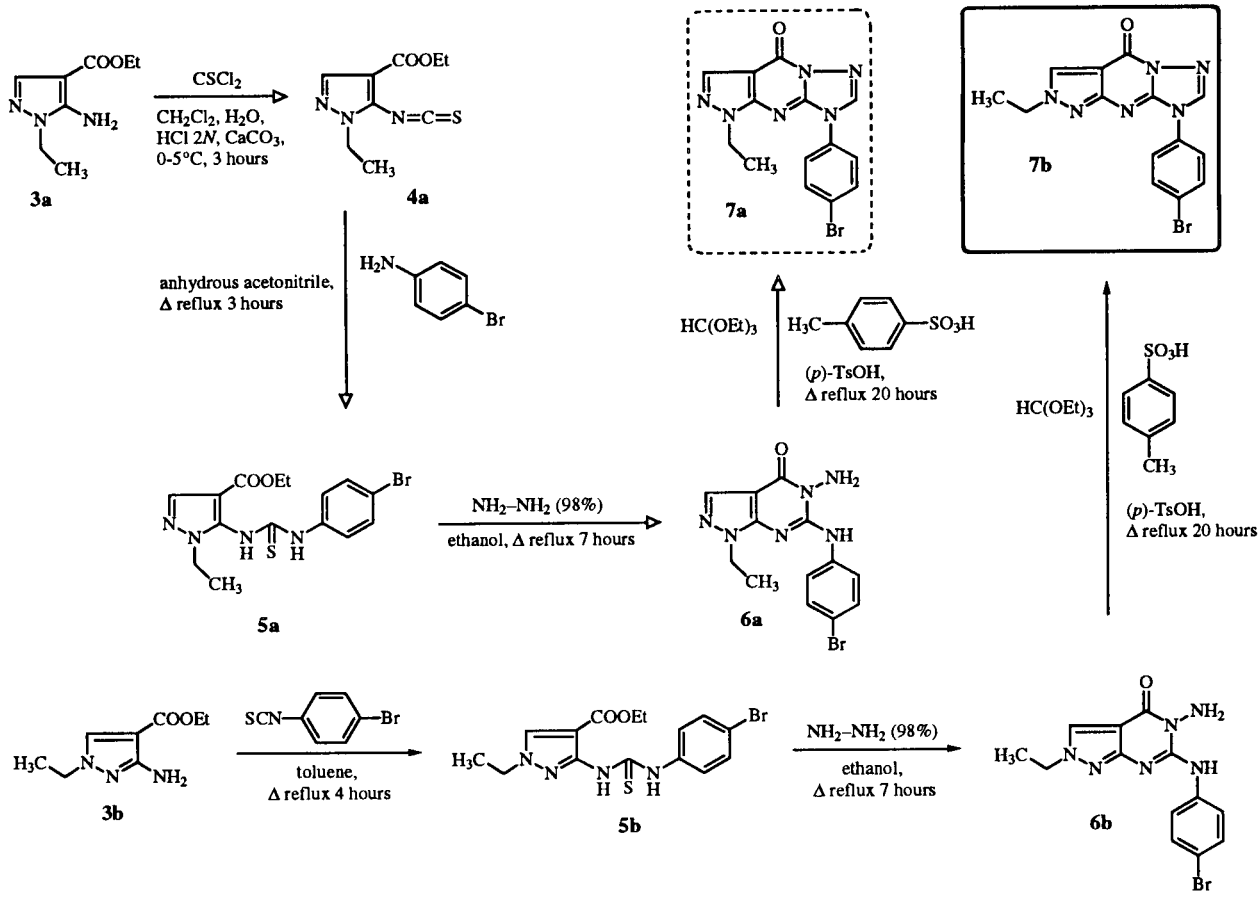
Chemistry.

The synthetic pathway (Scheme 2) we choose to obtain the isomeric 1-ethyl-8-(4-bromophenyl)-1*H*,4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-one **7a** and the target compound 2-ethyl-8-(4-bromophenyl)-2*H*,4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-one **7b** was the one previously applied for the synthesis of the 1*H*-pyrazolotriazolopyrimidine-4-one derivatives [1-3] *i.e.* by cyclization of the appropriate ethyl substituted-2-(4-bromophenyl)amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-ones **6a,b** with triethyl orthoformate, *p*-toluenesulfonic acid (*p*-TsOH) as the catalyst [1-3]. The starting compounds **6a,b** were in turn prepared by reaction of hydrazine monohydrate 98% with the ethyl-substituted *N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)thioureas **5a,b**. Compound **5a** was obtained by reaction of the 2-ethyl-3-isothiocyanato-pyrazole-4-carboxylic acid ethyl ester **4a** with 4-bromoaniline (Aldrich). Compound **5b** was obtained by reaction of the *N*1-ethyl-3-amino-4-carbethoxypyrazole **3b** [17-19] with the commercially available (4-bromophenyl)isothiocyanate (Aldrich) in toluene at reflux (Scheme 2). In the synthesis of **7a** the two initial "by pass" reactions, *i.e.* the reactions between the *N*2-ethyl-3-amino-4-carbethoxypyrazole **3a** [17-19] with thiophosgene (φ = 97%) solution (Aldrich), followed by that of the formed 2-ethyl-3-isothiocyanatopyrazole-4-carboxylic acid ethyl ester **4a** with 4-bromoaniline were necessary for the preparation of the 2-ethyl-*N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)thiourea

Scheme 1. Synthetic route to 1-ethyl-3-amino-4-carbethoxypyrazole.



Scheme 2. Synthetic routes to *N*1 (**a**) or *N*2 (**b**) ethyl-substituted 8-(*p*-bromophenyl)pyrazolotriazolopyrimidine derivatives **7a,b**.



5a (Scheme 2) because of the very low *N*2-substituted-3-amino-4-carbethoxypyrazoles reactivity with aliphatic or aromatic isothiocyanates [17,20].

The starting aminoesters **3a,b** respectively [17-19] were prepared by refluxing in ethanol the commercially available ethyl (ethoxymethylene)cyanoacetate (Aldrich) with ethylhydrazine or following the method of Schmidt *et al.*, by means of thermal decomposition in a mixture ethanol/37% hydrochloric acid of the appropriate α-cyanoacrilate ethyl ester **2b** (Scheme 1) [17-19].

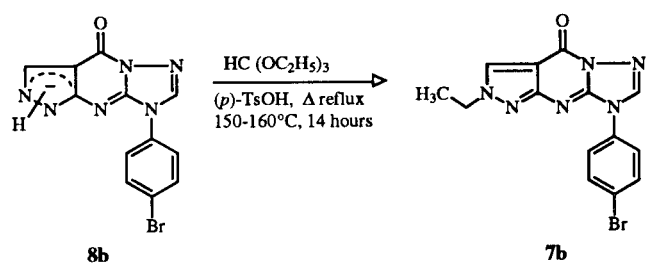
The latter intermediate was in turn prepared by reaction of the benzalethylhydrazine **1b** [21] with ethyl (ethoxymethylene)cyanoacetate (Aldrich) in benzene at reflux (Scheme 1).

A pure sample of the target compound 2-ethyl-8-(4-bromophenyl)-2*H*,4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo-[1,5-a]pyrimidin-4-one (Figure 1) was also obtained by direct alkylation with triethyl orthoformate of the 1*H* analog **8b** [5,6] (Scheme 3) or recovered from the crude product of the previously reported ring closure reaction [5,6] by plc or rp-plc separation using respectively ethyl acetate and methanol-water (φ = 30%)/0.5 triethylamine as the mobile phases (Figure 1).

The two isomeric tricyclic derivatives **7a,b** are well differentiated and labelled as **a** (upper, $R_f = 0.61$) or **b** (lower, $R_f = 0.41$) according to their r_f with relation to the unsubstituted compound ($r_f = 0.54$) [1,2]. For convenience, the two isomeric series **3a-6a** and **3b-6b** of their synthetic intermediates were labelled following a similar method (Schemes 2 and 3).

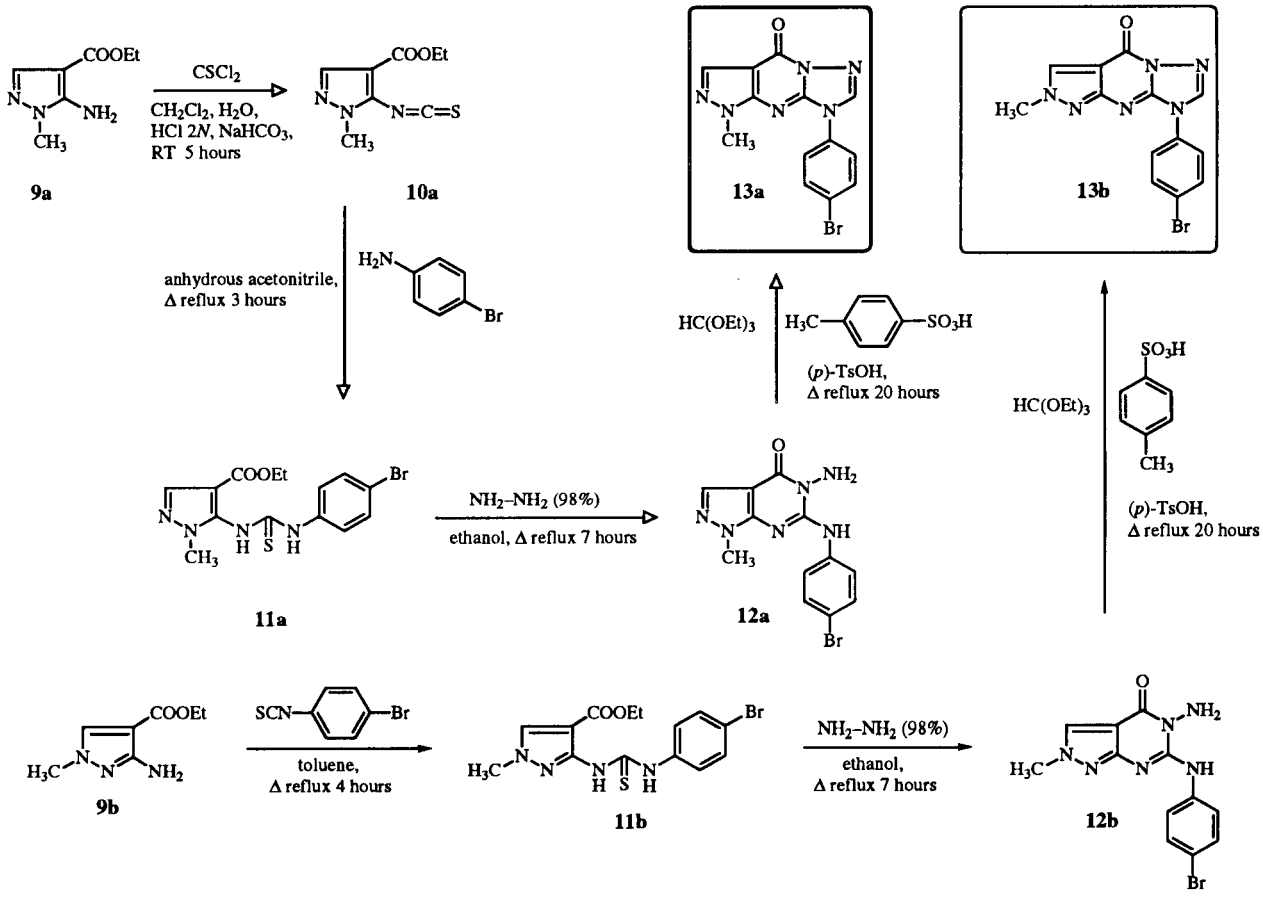
In accordance with the ethyl series, the methyl substituted tricyclic derivatives, when resulting from the alkyl donor effect of the trimethyl orthoformate, were identified as **a** ($r_f = 0.58$, upper) or **b** ($r_f = 0.27$, lower) as a consequence of their relative r_f in relation to the unsubstituted

Scheme 3. Synthetic route to **7b** by direct alkylation of its 1*H*–analog **8b**.



compound (r_f = 0.54) and to the samples obtained by synthesis **13a,b** (Scheme 4). The synthetic routes to the methyl-substituted 8-(4-bromophenyl)-1*H*(2*H*),4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-ones **13a,b** (Scheme 4) are parallel to those above outlined for their *N*-ethyl-substituted analogs **7a,b**. The starting aminoesters **9a** and **9b** were prepared according to the reported procedures [17,19,21].

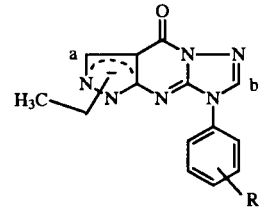
Scheme 4. Synthetic routes to *N*1 (**a**) or *N*2 (**b**) methyl-substituted 8-(*p*-bromophenyl)pyrazolotriazolopyrimidine derivatives **13a,b**.



All spectral data (ir, ^{1}H, ^{13}C nmr and mass measurements) are in accordance with the assigned structures and are consistent with the literature data for the compounds noted [17-19,21]. They are listed below for each of the new compounds synthesized. Yields of the alkylated methyl or ethyl derivatives obtained by the action of triethyl orthoformate or trimethyl orthoformate were determined based upon the ^{1}H nmr spectra.

The ir and nmr spectra of the isomers both in the ethyl and methyl substituted series **a,b** are very similar. Mass spectrometric measurements of the two ethyl substituted series **3a-7a** and **3b-7b** tend to be more and more similar following the synthetic pathway and only differ in the intensity of some signals, *e.g.* the relative abundance of the peak at m/z 319 ($M^{+\cdot}$ -29, ethyl group) for the isomers **6a** and **6b**. Mass spectra of the tricyclic compounds **7a,b** showed an intense peak corresponding to the molecular ion. The fragmentation produces only very low intensity peaks due to the high stability of the polycyclic cation. The only significative fragments are at m/z 343 ($M^{+\cdot}$ -Me of the ethyl group) and 330 ($M^{+\cdot}$ - ethylene group). The latter fragment produce the positively charged species.

^{1}H NMR Analysis.



The crude product of the ring closure reaction (Figure 1) was analyzed by ^1H nmr spectroscopy in DMSO-d_6 as the solvent. Its proton spectrum consisted into two groups of signals, arising from a major (unsubstituted derivative) and a minor component (Figure 2). The main unsubstituted product showed absorptions only in the low-field region of the spectrum: the signal centered at 7.84 ppm, corresponding to four protons, was assigned to the (4-bromo)-substituted aromatic protons. The singlets at 9.21 ppm and 8.14 ppm were the absorptions of the protons adjacent to the aromatic nucleus and in position a of the polycondensed ring respectively. Finally, the broad signal centered at 13.47 ppm originated from the fast exchanging NH proton. The minor component showed similar resonances in the low-field spectral region: two doublets at 7.92 ppm and 7.82 ppm and 9.16 ppm, each integrating for one proton. However, further signals were found in the high field portion of the spectrum; a triplet at 1.44 ppm due to a methyl group and a quartet at 4.27 ppm due to a methylene group. Therefore, the simple analysis of the spectrum revealed the impurity as a compound with a structure similar to the major component, but containing an ethyl group (Figure 2).

The structural assignment of the target 2-ethyl-8-(4-bromophenyl)-2*H*,4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo-[1,5-*a*]pyrimidin-4-ones (Figure 1) was performed by 1D noe measurements as follows: the saturation of the aromatic protons at 7.82-7.92 ppm produced enhancement at 9.16 ppm, thus allowing the assignment of the singlet at 9.16 ppm to the H_b proton adjacent to the aromatic substituent. As a consequence, the singlet at 8.67 ppm was assigned to the H_a proton, saturation of which produced a remarkable enhancement of the ethyl group resonances. It can be concluded that the impurity is alkylated where the proton in position a and the ethyl group are in close proximity. A similar scenario results from the ^1H nmr spectrum of a **7b** sample obtained by synthesis (Scheme 2) or plc/rp-plc separation (system: ethyl acetate or methanol-water (φ = 30%)/0.5 triethylamine as above reported) from the crude product of the ring closure reaction (Figure 1).

Further confirmation was obtained by recording the ^1H nmr spectrum of the 1-ethyl analog **7a** (Scheme 2), spectral parameters of which are very similar to **7b** except for the enhancement of the ethyl absorptions as a consequence of the proton saturation in position 3.

Conclusion.

Due to the interesting therapeutic potential of the new pyrazolotriazolopyrimidine derivatives [1,2], we believed it worthwile to clarify the nature of a constant impurity with a mass at M+ 28 formed in the final ring closure reaction to

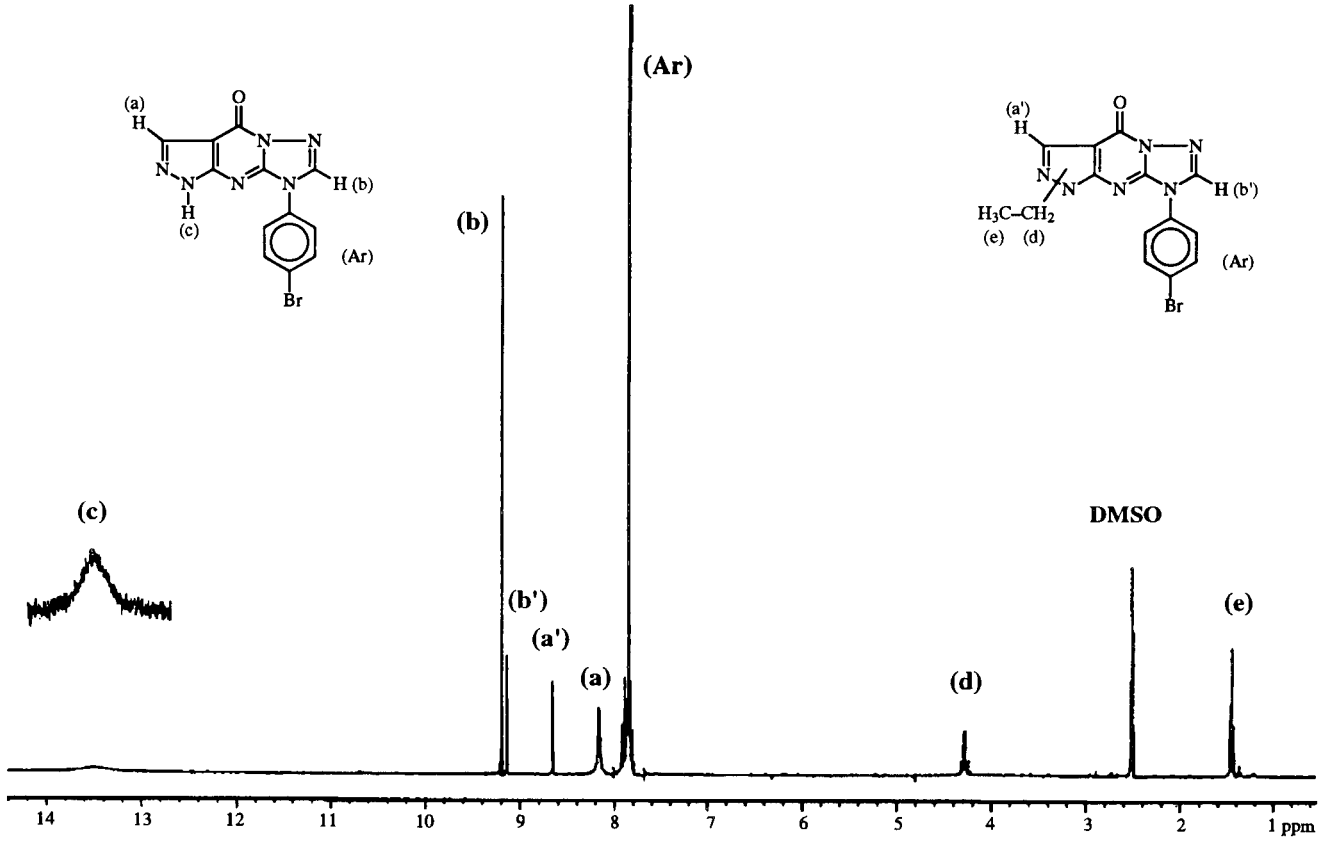


Figure 2

obtain the triazole ring by the C7 supplying action of the triethyl orthoformate. Our aim was the setting up of a precise and accurate analytical method of general applicability for this heterocyclic class in consideration of their potential in the new drug development [1-6]. The use of different ionization techniques, such as negative-positive mode fast atom bombardment (fab ms), negative-positive mode ion spray ionization (is-ms), tandem ion spray ionization (is-ms-ms) [S. Pucci, A. Raffaelli, F. Russo, S. Guccione and G. Romeo, forthcoming paper] provided a reliable identification of the impurity as the 2-ethyl substituted analog (Figure 1) together with a good rationalization of the fragmentation pathway. The adopted procedure overcame the inadequacy of the traditional electron ionization due to the very low fragmentation as a consequence of the high stability of the aromatic nucleus. The results obtained from the noe experiments and the different relative r_f point out the target compound either isolated from the reaction mixture (Figure 1) or obtained by synthesis of **7b** (Schemes 2 and 3) from its 1-substituted isomer **7a** (Scheme 2). The structural identification of the "ballast" as the 2-ethyl substituted analog (Figure 1) of the pyrazolotriazolopyrimidin-4-one derivatives (Formula A) may represent a rather new aspect in the chemistry of the alkyl orthoformates to exploit their ambident electrophilic reactivity [7-10,12,22] as selective alkylating agents of non electron rich nitrogen heterocycles. Under this aspect, preliminary and rough results of our investigation using both triethyl and trimethyl orthoformate showed the latter active as an alkylating agent under a wider range of temperatures. The trimethyl orthoformate methyl donor effect spans from 110° to 150° whereas the one of the triethyl orthoformate is detectable from 140° to 150°. In general, the SN_2 transfer of a methyl group in comparison with an ethyl group in the alkoxy carbenium ions is in general more easily feasible (Figure 3) [7-10,12,22].

The triethyl orthoformate effect is more selective at higher temperatures (150-160°), being the predominant formation (85%) of the *N*2-ethyl-substituted derivative. A mixture of *N*1 and *N*2 isomers in a variable percentage is formed by decreasing the temperature to 130°. Under the same range of temperatures, these preliminary tests showed that only the *N*1-methyl substituted analog can be recovered from the crude product of the reaction mixture by plc separation (ethyl acetate) or can be detected (35%) by ^1H nmr spectroscopy in the collected solid materials. At present, no other definable and analytically characterisable products were isolated in a pure form from the reaction mixture.

The specific *N*1-methyl or *N*2-ethyl substitution is difficult to explain. Perhaps an important role is played by the reverse thermodynamic stabilization effects of the *N*1- or *N*2-alkyl derivative and the NH tautomeric equilibrium of the pyrazole ring [11,13,14,20].

The SN_2 type reaction is markedly controlled by the nucleophilicity of the heterocycle, the temperature and the nature of the acid catalyst [9,10-13,20]. In general, the formylation is kinetically controlled whereas the alkylation

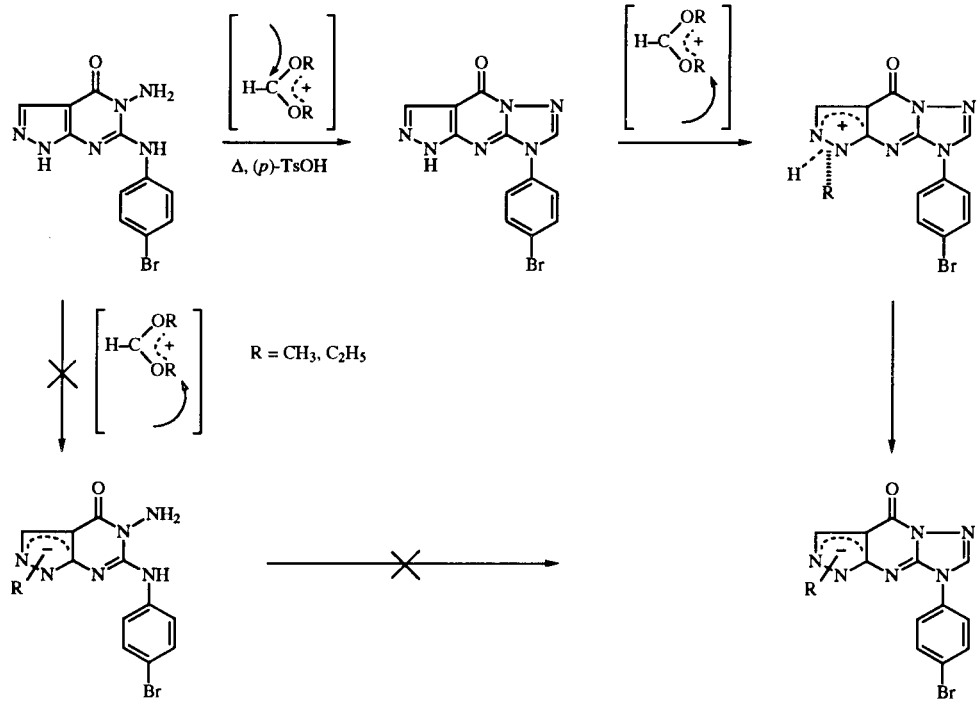


Figure 3. Reaction mechanism in the alkylating effect of trimethyl or triethyl orthoformate.

by alkoxy carbenium ions is thermodynamically controlled. A thermodynamic control can be reached at lower temperature during a longer reaction time [9,10-13]. In our tests no formation of tricyclic *N*-alkyl derivatives was detected on tlc (ethyl acetate) up to the reaction time of 10 hours in the final ring closure (Figure 1) using both trimethyl orthoformate and triethyl orthoformate as C7 supplying agent in comparison with the synthesized samples **7a,b** and **13a,b** as controls.

From a mechanistic viewpoint we suggest that the "formylation" for ring closure *i.e.* the formation of a formamide derivative (not shown) is the first step due to the common ambident reactivity of the formed alkoxy carbenium ion, respectively (Figure 5). *N*1- or *N*2-Alkyl substituted 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one derivatives were not detected by tlc monitoring of the ring closure reaction mixture (Figure 1) when compared with the samples **6a,b** and **12 a,b** synthesized as controls (Figure 3).

The reaction paths are indeed more complicated either by the special nature of the nucleophile as a tetra-ambident electrophilic system (four N atoms) or by that of the dual ambident electrophile together with the above mentioned pyrazole tautomerism that may be influenced by temperature [7-14,20,22].

Finally, we can conclude that the orthoesters reactivity mentioned by Hünig *et al.* [8] cannot be generalized at all. Although carbenium ions constitute one of the longest known and best studied class of reactive intermediates [7-14,22] in organic chemistry, there is still room for interesting applications in this field, *e.g.* we emphasize the use of their a_1 synthon properties [1,12,15,16] to set up new and not so tedious disconnection-alkylating methods of non electron rich nitrogen heterocycles [11-14]. Further experimental findings to rationalize the usefulness of the unexpected reaction as a synthetic procedure will be investigated also using semiempirical molecular orbital calculations about the stability (heat of formation, charge density, homo, lumo) as a predictive model [12].

EXPERIMENTAL

Melting points were determined on a Büchi capillary apparatus and are uncorrected. The ir spectra were obtained with potassium bromide discs or nujol films on a Perkin-Elmer 1600 FT-IR series spectrophotometer. Elemental combustion analyses were performed on a Carlo Erba Mod EA 1108 Analyzer instrument by Dr. S. Di Marco of the Microanalysis Laboratory of Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania. When analyses are indicated by the elements' symbols or functions, the analytical results were within ±0.40% of the theoretical values. The mp of the crude synthetic intermediates in both the series of alkyl isomers **a,b** were within ±0.3 if compared with the pure product and they might be used without further purification.

The ^1H and ^{13}C nmr spectra were respectively recorded at 300.13 MHz and 75.5 MHz on a Bruker AMX-R 300 spectrometer in DMSO-d_6 as a solvent at 298 K. The ^1H nmr spectrum of the thiourea derivative **5a** in DMSO-d_6 does not show the ethyl group, probably due to a decomposition resulting from the dissolution in the above solvent. The structure of the derived compound was not further investigated. The $^1H\{^1H\}$-noe experiments were performed on a Varian VXR-300 spectrometer operating at 300 MHz for ^1H in DMSO-d_6 as a solvent at 25° on carefully degassed samples under the difference mode. The decoupler was placed at the required frequency to saturate the proton involved. The decoupler power used was the minimum required to saturate the concerned spin. A period from 10-20s was used to allow the system to reach equilibrium. Each noe experiment was repeated at least 4 times. All ^1H and ^{13}C chemical shifts are given as δ values in parts per million (ppm) downfield tetramethylsilane (0.00 ppm) as the internal standard and coupling constants in Hz. Mass spectrometric measurements were performed on a VG 70-70E instrument operating in electron ionization (EI) mode (70 eV, 100 μA). The samples were introduced by a direct inlet probe at the minimum temperature which gave an adequate vapour pressure: source temperature 150°. The fab mass spectra were obtained on the same instrument using xenon atoms as the primary beam. Ion spray ms and ms-ms measurements were performed on a Perkin-Elmer Sciex API III, triple quadrupole mass spectrometer operating under atmospheric pressure ionization conditions.

The tlc was performed on plates RP 18 $F_{254}S$ Merck precoated 5-10 cm, layer thickness 0.25 mm. Column chromatography was performed on Silica gel 60, Merck (230-400 Mesh ASTM). Reactions were routinely followed by thin layer chromatography (tlc) on silica gel 60 F_{254} aluminium sheets (Merck); system: ethyl acetate, ethyl acetate/cyclohexane (φ = 10%, 20%, 40%, 50%, 60%) as an eluent, and similarly the purity of each compound was checked. The spots were detected by uv irradiation at 254-365 nm. All chemicals were purchased from Aldrich, Fluka, Merck and Carlo Erba Chemical Co and were used without further purification. Chromatographic eluents were of analytical grade or purified following the usual method.

β-(*N*2-Benzylidene-*N*1-ethylhydrazino)-α-cyanoacrilate Acid Ethyl Ester (**2b**).

To a solution of benzalethylhydrazine **1b** (13.4 g, 90.54 mmoles) (Ref *N*-Ethylbenzamidine) [21] dissolved in 20 ml of benzene, ethyl (ethoxymethylene)cyanoacetate (Aldrich) (17 g, 100 mmoles), was slowly added. After the addition was completed, the mixture was refluxed for 1 hour and the solvent was removed by rotary evaporation under reduced pressure. The yellow residue, after being triturated with ethanol and filtered off, was processed without further purification. An analytical pure sample can be obtained by recrystallization from ethanol, yield 60%, mp 138-140°; ir (potassium bromide): ν 2220 (CN), 1690 (C=O),1620 (C=N) cm^{-1}; ms: m/z 271 ($M^{+\cdot}$); tlc system: ethyl acetate, ethyl acetate-cyclohexane (φ =10%, 20%).

Anal. Calcd. for $C_{15}H_{17}N_3O_2$: C, 66.42; H, 6.27; N, 15.49. Found: C, 66.38; H, 6.57; N, 15.35.

1-Ethyl-3-amino-4-carboethoxypyrazole (**3b**).

To a suspension of β-(*N*2-benzyliden-*N*1-ethylhydrazino)-α-cyanoacrylic acid ethyl ester **2b** (10 g, 37.03 mmoles) in 30 ml of hot ethanol, 37% hydrochloric acid (4.5 ml) was added. The mixture was refluxed for 30 minutes then rotary evaporated under reduced pressure. The oil residue was treated with hot ether (30 ml) and the resultant solid was collected, solubilized in

8 ml of 5*N* sodium hydroxide and repeatedly extracted by chloroform. The pooled organics were dried (anhydrous sodium sulphate) and evaporated under reduced pressure to yield 3.24 g (48%) of **3b**. Physico-chemical properties of the compound are consistent with the reference data [17-19]; ms: m/z (relative abundance %) 183 ($M^{+\cdot}$, 75), 168 (7), 138 ($M^{+\cdot}$ -EtO, 45), 137 ($M^{+\cdot}$ -EtOH, 100), 122 (7), 110 ($M^{+\cdot}$ -COOEt, 15), 109 ($M^{+\cdot}$ -CO + EtOH, 18), 52 (17), 36 (35).

2-Ethyl-3-isothiocyanatopyrazole-4-carboxylic Acid Ethyl Ester (**4a**).

To a stirred and cooled (0°) mixture of dichloromethane (10 ml), water (8 ml), calcium carbonate (4 g, 40 mmoles) and thiophosgene (φ = 97%) (0.92 g, 8.00 mmoles) a solution of the aminoester **3a** [17,18] (1.2 g, 6.55 mmoles) dissolved in 37% hydrochloric acid (4 ml) was slowly added drop by drop for 2 hours. After being stirred for an additional 3 hours at 0-5° the reaction mixture was filtered and the solid material was twice washed with cold water (2 x 60 ml). The organic phase was separated and the aqueous solution extracted with dichloromethane (4 x 50 ml). The pooled organics, after being washed with 2*N* hydrochloric acid (2 x 50 ml) and as many times with cold water, were kept overnight at 0° over a mixture of anhydrous sodium sulfate/silica gel (1:1), then rotary evaporated under reduced pressure. The yellow residue was purified by heating in petroleum ether followed by solvent removal to drynessa *in vacuo* (2 x 50 ml) then it was directly processed. A pure analytical sample was obtained by column chromatography (cyclohexane - (φ = 40%) ethyl acetate), yield 60%; ir (Nujol): ν 1730 (C=O), 1200 (C=S) cm^{-1}; tlc system, cyclohexane-ethyl acetate (φ = 40%), ethyl acetate; ms: 225 ($M^{+\cdot}$, 76), 197 (10), 180 (100), 152 (43).

Anal. Calcd. for $C_9H_{11}N_3O_2S$: C, 48.00; H, 4.88; N, 18.66; S, 14.22. Found: C, 47.95; H, 4.75; N, 18.60; S, 13.98.

2-Ethyl-*N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)-thiourea (**5a**).

To a solution of 2-ethyl-3-isothiocyanatopyrazole-4-carboxylic acid ethyl ester **4a** (1 g, 4.44 mmoles) [17,19] dissolved in 6 ml of anhydrous acetonitrile, 4-bromoaniline (0.76 g, 4.41 mmoles) was added. The reaction mixture was heated under reflux for 3 hours and the resultant solution was rotary evaporated *in vacuo* to dryness. The residual semisolid was dissolved in hot ethanol and water was added drop by drop to the solution until a solid was formed. The latter precipitate was collected, heated in chloroform and directly processed without further purification. An analytically pure sample was obtained by column chromatography (ethyl acetate - (φ = 50%) cyclohexane) followed by recrystallization from acetonitrile-water or from a small amount of ethanol, yield 60%, mp 179-181°; ir (potassium bromide): ν 3220 (NH), 1645 (C=O) cm^{-1}; tlc system, ethyl acetate-cyclohexane (φ = 50%); ms: m/z (relative abundance %) 396 ($M^{+\cdot}$, 12), 398 (M+2, 14), 319 (7), 317 (8), 242 (7), 183 ($M^{+\cdot}$ -Br-$C_6H_4CNS^+$, 100), 180 (60), 173 (45), 171 (47), 152 (28), 137 (70), 109 (63).

Anal. Calcd. for $C_{15}H_{17}BrN_4O_2S$: C, 45.34; H, 4.82; N,14.10; S, 8.06. Found: C, 45.55; H, 4.48; N, 14.11; S, 8.00.

1-Ethyl-*N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)-thiourea (**5b**).

A solution of aminoester **3b** [17-19] (1 g, 5.46 mmoles) and (4-bromophenyl)isothiocyanate (1.16 g, 5.42 mmoles) dissolved in 5 ml of toluene was heated at reflux. After 4 hours the mixture was cooled and the solid was collected, dried and recrystallized from toluene or benzene. yield 50%; mp 166-167°; ir (potassium bromide): ν 3350 (NH), 1695 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 11.36 (v br, 1H, NH), 9.48 (v br, 1H, NH), 8.39 (s, 1H, pyrazole H3), 7.67 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 7.57 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 4.28 (q, ^3J = 7.1 Hz, 2H, methylene), 4.17 (q, ^3J = 7.2 Hz, 2H, methylene N), 1.40 (t, ^3J = 7.2 Hz, 3H, methyl), 1.29 (t, ^3J = 7.1 Hz, 3H, methyl N); tlc system, ethyl acetate; ms: m/z (relative abundance %) 396 ($M^{+\cdot}$, 24), 398 (M+2, 25), 319 (7), 317 (8), 242 (7), 183 (100), 180 (24), 173 (17), 171 (18), 152 (10), 137 (40), 109 (6).

Anal. Calcd. for $C_{15}H_{17}BrN_4O_2S$: C, 45.34; H, 4.82; N, 14.10; S, 8.06. Found: C, 45.55; H, 4.48; N, 14.11; S, 8 00.

Ethyl-substituted 2-(4-Bromophenyl)amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-ones **6a,b**.

To a stirred solution of the appropriate ethyl-substituted *N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)thiourea **5a,b** (2.5 g, 6.31 mmoles) dissolved in 15 ml of ethanol, hydrazine monohydrate 98% (3 ml) was slowly added and the mixture was refluxed for 7 hours. The solid which crystallized on cooling was collected, repeatedly washed with water and dried. The filtrate, after being added with water (100 ml) and gently heated, gave by cooling, an additional amount of compound **6a,b** which was collected and treated as above. The compounds **6a,b** can be processed without recrystallization. An analytically pure sample can be obtained by recrystallization from ethanol.

The yield of **6a** was 45%, mp 184-185°; ir (potassium bromide): ν 3300 (NH), 1695 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.57 (v br, 1H, NH), 7.87 (s, 1H, pyrazole H3), 7.82 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 7.52 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 5.49 (br s, 2H, NH_2), 4.17 (q, ^3J = 7.2 Hz, 2H, methylene), 1.36 (t, ^3J = 7.2 Hz, 3H, methyl); tlc system, ethyl acetate, ms: m/z (relative abundance %) 348 ($M^{+\cdot}$, 100), 350 (M+2, 99), 317 (24), 178 (42).

Anal. Calcd. for $C_{13}H_{13}BrN_6O{\cdot}H_2O$: C, 42.50; H, 4.08; N, 22.88. Found: C, 42.59; H, 4.13; N, 22.75.

The yield of **6b** was 90%, mp 225-227°; ir (potassium bromide): ν 3320 (NH), 1700 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.41 (v br, 1 H, NH), 8.37 (s, 1H, pyrazole H3), 7.86 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 7.49 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 5.47 (br s, 2H, NH_2), 4.18 (q, ^3J = 7.2 Hz, 2H, methylene), 1.42 (t, ^3J = 7.2 Hz, 3H, methyl); tlc system, ethyl acetate, ms: m/z (relative abundance %) 348 ($M^{+\cdot}$, 100), 350 (M+2, 97), 319 (64), 317 (64), 178 (10).

Anal. Calcd. for $C_{13}H_{13}BrN_6O$: C, 44.70; H, 3.72; N, 24.06. Found: C, 44.81; H, 3.70; N, 23.81

Method A.

Ethyl-substituted 8-(4-Bromophenyl)-1*H*,(2*H*),4*H*,8*H*-pyrazolo-[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-ones **7a,b**.

A suspension of the appropriate ethyl-substituted 2-(4-bromophenyl)amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **6a,b** (1.5 g, 4.29 mmoles) and *p*-toluenesulfonic acid (*p*-TsOH) (1.5 g, 8.72 mmoles) in triethyl orthoformate (45 ml) was refluxed under stirring for 20 hours. The solid material was collected by filtration, washed with water, dried and recrystallized from ethanol **7a** or dimethylformamide **7b**.

The yield of **7a** was 70%, mp 273-275°, ir (potassium bromide): ν 1720 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.21 (s, 1H, H7), 8.13 (s, 1H, H3), 7.90, 7.84 (m, J_{av} = 8.9 Hz, 4H, phenyl ring), 4.23 (q, ^3J = 7.2 Hz, 2H, methylene), 1.37 (t, ^3J = 7.2 Hz, 3H, methyl); ^{13}C

nmr (DMSO-d_6): δ 151.4 (1C), 151.0 (1C), 147.4 (1C), 141.1 (1C, CH), 134.4 (1C, CH), 132.4 (2C, CH phenyl ring), 132.1 (1C), 125.5 (2C, CH phenyl ring), 121.2 (1C), 101.0 (1C), 41.5 (1C, methylene), 14.3 (1C, methyl), tlc system, ethyl acetate, ms: m/z (relative abundance %) 358 ($M^{+\cdot}$, 99), 360 (M+2, 100), 345 (22), 343 (23), 332 (36), 330 (35), 280 (10), 157 (12), 155 (12).

Anal. Calcd. for $C_{14}H_{11}BrN_6O$: C, 46.79; H, 3.06; N, 23.39. Found: C, 46.66; H, 3.23; N, 23.25.

The yield of **7b** was 95%, mp 254-255°; ir (potassium bromide): ν 1720 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.13 (s, 1H, H7), 8.65 (s, 1H, H3), 7.91 (d, J_{av} = 9.0 Hz, 2H, phenyl ring), 7.81 (d, J_{av} = 9.0 Hz, 2H, phenyl ring), 4.28 (q, ^3J = 7.3 Hz, 2H, methylene), 1.45 (t, ^3J = 7.3 Hz, 3H, methyl); ^{13}C nmr (DMSO-d_6): δ 157.9 (1C), 152.3 (1C), 147.2 (1C), 140.7 (1C, CH), 132.6 (1C), 132.2 (2C, CH phenyl ring), 128.5 (1C, CH), 124.9 (2C, CH phenyl ring), 120.5 (1C), 102.6 (1C), 47.8 (1C, methylene), 15.0 (1C, methyl); tlc system, ethyl acetate, ms: m/z (relative abundance %) 358 ($M^{+\cdot}$, 100), 360 (M+2, 99), 345 (7), 343 (7), 332 (32), 330 (33), 280 (8), 157 (10), 155 (11).

Anal. Calcd. for $C_{14}H_{11}BrN_6O$: C, 46.79; H, 3.06; N, 23.39. Found: C, 46.89; H, 3.17; N, 23.11.

Method B.

2-Ethyl 8-(4-Bromophenyl)-2*H*,4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]-triazolo[1,5-*a*]pyrimidin-4-one (**7b**).

A suspension of 8-(4-bromophenyl)-1*H*,4*H*,8*H*-pyrazolo[3,4-*d*]-[1,2,4]triazolo[1,5-*a*]pyrimidin-4-one **8b** (1 g, 3.00 mmoles) [5,6] and *p*-toluenesulfonic acid (*p*-TsOH) (1 g, 5.81 mmoles) in 30 ml of triethyl orthoformate was refluxed under stirring at 150-160° for 14 hours. The solid material was collected, washed with hot ethanol (4 x 50 ml) dried and recrystallized from dimethylformamide, yield 45%. Physico-chemical data of an analytical sample were identical to those reported above (Method A).

2-Methyl-3-isothiocyanatopyrazole-4-carboxylic Acid Ethyl Ester (**10a**).

To a stirred mixture at room temperature of dichloromethane (50 ml), water (17.5 ml), sodium bicarbonate (2.95 g, 35.11 mmoles) and thiophosgene (φ = 97%) (3.45 g, 30.00 mmoles) a solution of the aminoester **9a** (4.25 g, 25.00 mmoles) [17,18] dissolved in 25 ml of dichloromethane was added drop by drop for 2 hours. The solid material was collected, washed with cold water (6 x 50 ml) and eliminated. The organic phase was separated and the aqueous solution extracted by dichloromethane (4 x 50 ml). The pooled organics, after being washed with 2*N* hydrochloric acid (2 x 50 ml) and as many times with cold water, were dried (sodium sulfate), filtered through silica gel and rotary evaporated to dryness under reduced pressure. The yellow residue was purified by double column chromatography, (cyclohexane-(φ = 40%) ethyl acetate; ethyl acetate), yield 40%, mp 38-40°; ir (Nujol): ν 1722 (C=O), 1203 (CS) cm^{-1}; tlc system, cyclohexane-ethyl acetate (φ = 40%); ethyl acetate.

Anal. Calcd. for $C_8H_9N_3O_2S$: C, 45.49; H, 4.26; N, 19.90; S, 15.16. Found: C, 45.52; H, 4.23; N, 19.86; S, 15.23.

2-Methyl-*N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)-thiourea (**11a**).

To a solution of 2-methyl-3-isothiocyanatopyrazole-4-carboxylic acid ethyl ester **10a** (1 g, 4.71 mmoles) dissolved in 3 ml of anhydrous acetonitrile, 4-bromoaniline (0.81 g, 4.70 mmoles) was added. The reaction mixture was refluxed for 3 hours and the resultant solution obtained may be treated as follows: i) separated from the formed oil and rotary evaporated *in vacuo*. The solid residue fractional recrystallized from acetonitrile/water gave **11a** as the second product; ii) allowed to stand 6-7 hours at room temperature, separated from the precipitate which formed and rotary evaporated under reduced pressure; the solid residue **11a** is homogeneus on tlc (single spot in the solvent system described), yield 40%, mp 153-154°; ir (potassium bromide): ν 3240 (NH), 1695 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 10.38 (br, 1H, NH), 9.46 (br, 1H, NH), 7.81 (s, 1H, pyrazole H3), 7.53 (m, 4H, phenyl ring), 4.16 (q, ^3J = 7.0 Hz, 2H, CH_2), 3.67 (s, 3H, methyl), 1.22 (t, ^3J = 7.0 Hz, 3H, CH_3); tlc system, ethyl acetate.

Anal. Calcd. for $C_{14}H_{15}BrN_4O_2S$: C, 43.86; H, 3.91; N, 14.62; S, 8.35. Found: C, 43.78; H, 4.02; N, 14.43; S, 8.42.

1-Methyl-*N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)-thiourea (**11b**).

A solution of **9b** [17,18] (1 g, 5.91 mmoles) and (4-bromophenyl)isothiocyanate (1.27 g, 5.93 mmoles) dissolved in 5 ml of toluene was heated at reflux. After cooling the solid was collected, dried and recrystallized from toluene, yield 98%, mp 177-178°; ir (potassium bromide): ν 3360 (NH), 1660 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 11.28 (br, 1H, NH), 9.47 (br, 1H, NH), 8.37 (s, 1H, pyrazole H3), 7.60 (m, 4H, phenyl ring), 4.26 (q, ^3J = 7.2 Hz, 2H, CH_2), 3.86 (s, 3H, methyl), 1.28 (t, ^3J = 7.2 Hz, 3H, CH_3); tlc system, ethyl acetate.

Anal. Calcd. for $C_{14}H_{15}BrN_4O_2S$: C, 43.86; H, 3.91; N, 14.62; S, 8.35. Found: C, 43.96; H, 4.20; N, 14.56; S, 8.29.

Methyl-substituted 2-(4-Bromophenyl)amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-ones **12a,b**.

To a stirred solution of the appropriate methyl substituted *N*-(4-carbethoxypyrazol-3-yl)-*N'*-(4-bromophenyl)thiourea **11a,b** (2.5 g, 6.52 mmoles) dissolved in 15 ml of ethanol, hydrazine monohydrate 98% (3 ml) was slowly added. The mixture was refluxed for 7 hours and the product crystallized on cooling was collected by filtration, repeatedly washed with water and dried. The filtrate was added with water (100 ml) and gently heated to give an additional amount of compound **12a,b** which was treated as above. The compounds **12a,b** can be processed without recrystallization. An analytically pure sample was obtained by recrystallization from ethanol/water **12a** or ethanol **12b**.

The yield of **12a** was 70%, mp 220-222°; ir (potassium bromide): ν 3308-3205 (NH), 1700 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.65 (br, 1H, NH), 7.88 (s, 1H, pyrazole H3), 7.86 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 7.51 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 5.53 (br s, 2H, NH_2), 3.77 (s, 3H, methyl); tlc system, ethyl acetate.

Anal. Calcd. for $C_{12}H_{11}BrN_6O{\cdot}H_2O$: C, 40.79; H, 3.68; N, 23.79. Found: C, 40.86; H, 3.85; N, 23.76.

The yield of **12b** was 70%; mp 248-249°; ir (potassium bromide): ν 3294-3190 (NH), 1695 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.47 (br, 1H, NH), 8.34 (s, 1H, pyrazole H3), 7.87 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 7.49 (d, J_{av} = 8.8 Hz, 2H, phenyl ring), 5.49 (br s, 2H, NH_2), 3.89 (s, 3H, methyl); tlc system, ethyl acetate.

Anal. Calcd. for $C_{12}H_{11}BrN_6O$: C, 42.98; H, 3.28; N, 25.07. Found: C, 42.80; H, 3.49; N, 24.80.

Methyl-substituted 8-(4-Bromophenyl)-1*H*(2*H*),4*H*,8*H*-pyrazolo[3,4-*d*][1,2,4]triazolo[1,5-*a*]pyrimidin-4-ones **13a,b**.

A suspension of the appropriate methyl-substituted 2-(4-bromophenyl)amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **12a,b** (1.5 g, 4.41 mmoles) and *p*-toluenesulfonic acid (*p*-TsOH) (1.5 g, 8.72 mmoles) in 45 ml of triethyl orthoformate was refluxed under stirring. After 20 hours the compound was recovered as a solid residue by solvent removal to dryness under reduced pressure, **13a** or as a precipitate **13b**, was washed with water, dried and recrystallized from dimethylformamide.

The yield of **13a** was 98%, mp >300° (260°, transformation); ir (potassium bromide): ν 1720 (C=O) cm^{-1}; tlc system, ethyl acetate.

Anal. Calcd. for $C_{13}H_9BrN_6O$: C, 45.21; H, 2.60; N, 24.34. Found: C, 45.12; H, 2.58; N, 24.00.

The yield of **13b** was 60%, mp 274-276°; ir (potassium bromide): ν 1710 (C=O) cm^{-1}; ^1H nmr (DMSO-d_6): δ 9.15 (s, 1H, H7), 8.62 (s, 1H, pyrazole H3), 7.86 (m, 4H, phenyl ring), 3.99 (s, 3H, methyl); tlc system, ethyl acetate.

Anal. Calcd. for $C_{13}H_9BrN_6O$•1/2DMF: C, 45.66; H, 3.28; N, 23.88. Found: C, 45.83; H, 3.36; N, 23.65.

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