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Laboratory note

Unforeseen alkylating effect of triethylorthoformate in the synthesis of pyrazolotriazolopyrimidine derivatives

S Guccione[1], A Raffaelli[2], G Uccello Barretta[2], L Monsù Scolaro[3], S Pucci[2], F Russo[1]

[1]*Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania, viale Andrea Doria, 6, Ed 12 Città Universitaria, 95125 Catania;*
[2]*Centro di Studio del CNR per le Macromolecole Stereordinate ed Otticamente attive, Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento, 35, 56126 Pisa;*
[3]*Dipartimento di Chimica Inorganica e Struttura Molecolare, Università di Messina, Villaggio S Agata, Salita Sperone, 31, 98166 Messina, Italy*

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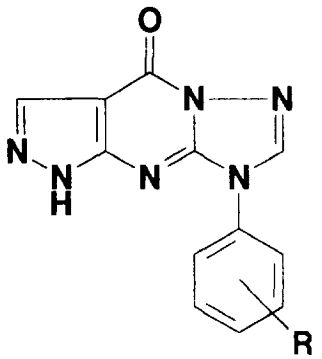
Summary — The final ring closure reaction of 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-ones with triethylorthoformate in the synthesis of 1*H*-pyrazolo[3,4-*d*][1,2,4]-8*H*-triazolo[2,3-*a*]-4*H*-pyrimidin-4-one derivatives, unexpectedly gave both the desired product and its N_2-ethyl analog. The structure of the latter, which arises from an unexpected alkylating effect of triethylorthoformate, was determined through a combined instrumental mass spectroscopy/NMR study reported elsewhere (S Pucci *et al*, manuscript in preparation). It was further defined using a comparison between a sample of **4** obtained by synthesis and a sample of **4** isolated by PLC or RP-PLC of the crude reaction product.

pyrazolotriazolopyrimidin-4-one derivative / triethylorthoformate / alkylating effect

Introduction

Pyrazolotriazolopyrimidin-4-ones **A** form part of the extensive investigations by F Russo *et al* in the area of polycondensed heterocycles [1–5] as biologically active compounds. These compounds have previously been reported as potential antiinflammatory–analgesic agents [1, 2], which probably act *via* a mechanism that is different from that of common non-steroidal anti-inflammatory drugs (NSAIDs), hence the absence of ulcerogenic effects.



(A)

Mass spectrometric measurements of the crude tricyclic compounds showed a rather intense peak at M + 28 as a constant impurity (fig 1, 2). TLC (ethylacetate) revealed a spot lower than that of the desired product [1, 2]. As an extension of an earlier work published in this journal [1], using the 8-(4-bromophenyl) derivative (R_f = 0.54) as an investigative tool, the byproduct was identified as the 2-ethyl analog (R_f = 0.41, TLC system: ethylacetate) of the desired 1*H* compounds. We report here the $^1H\{^1H\}$-NOE structural characterization of the 'ethyl ballast' in the crude product of the ring-closure reaction using triethylorthoformate as a C7-supplying agent, and confirmation of the assigned structure. A sample obtained by synthesis **4** (scheme 1) and a sample isolated by PLC or RP-PLC (scheme 1, inset) were used as controls.

Chemistry

The synthetic pathway (scheme 1) we chose to obtain the target compound 2-ethyl-8-(4-bromophenyl)pyrazolo[3,4-*d*][1,2,4]-8*H*-triazolo[2,3-*a*]-4*H*-pyrimidin-4-one **4** was previously applied for the synthesis of 1*H*-pyrazolotriazolopyrimidine-4-ones [1, 2]. This involves cyclizing the 6-ethyl-2-(4-bromophenyl)-

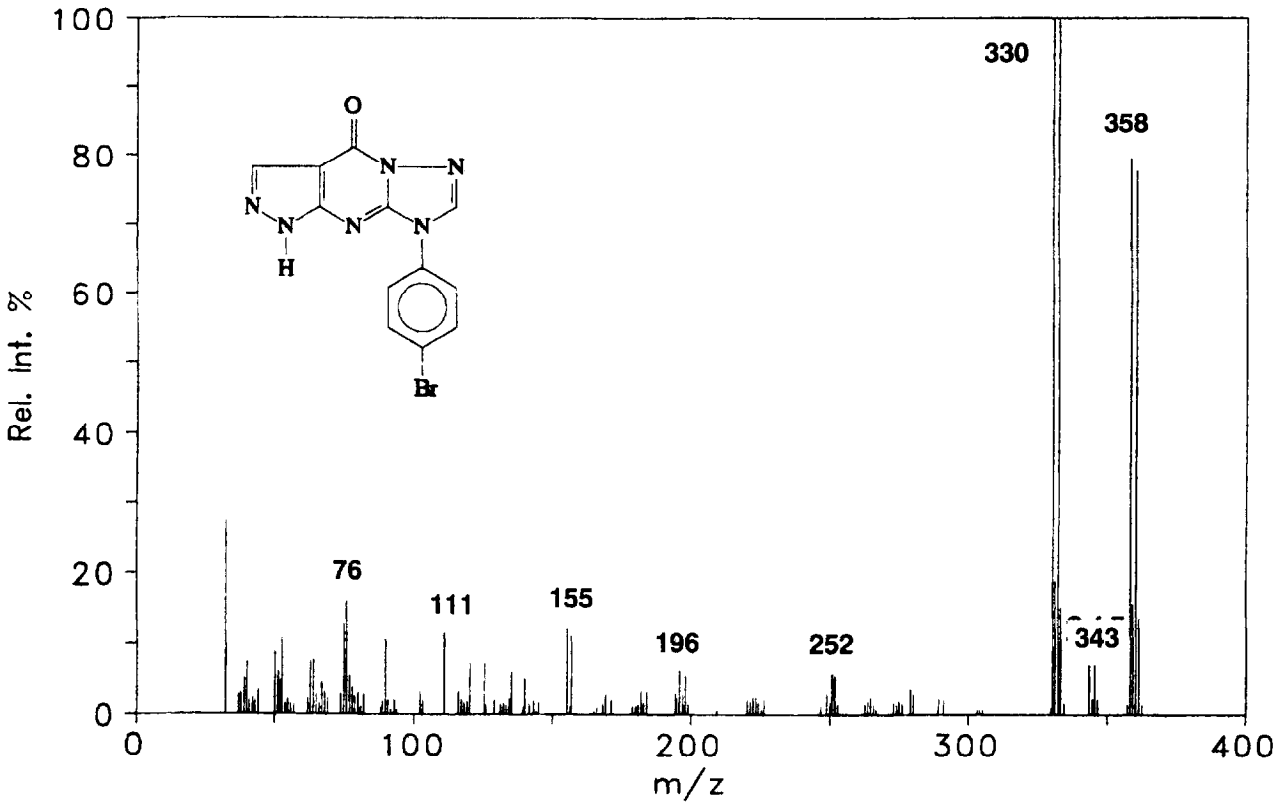


Fig 1. Mass spectrum of compound **4** as a byproduct.

amino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **3** with triethylorthoformate, using *p*-toluenesulfonic acid (*p*-TsOH) monohydrate as a catalyst [1, 2]. The starting compound **3** was prepared by reaction of hydrazine monohydrate 98% with 1-ethyl-*N*-(4-carboethoxypyrazol-3-yl)*N'*-(4-bromophenyl)thiourea **2**. The latter was obtained by reaction of the N_1-ethyl-3-amino-4-carboethoxypyrazole **1** [6–8] with the commercially available (4-bromophenyl)isothiocyanate (Aldrich) in toluene at reflux (scheme 1). A pure sample of **4** was recovered from the crude product of the above-mentioned ring closure reaction [1, 2] by PLC or RP-PLC separation (scheme 1, inset) using ethyl acetate or methanol/ water (φ = 30%)/0.5 triethylamine, respectively, as mobile phases.

All the spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopic measurements) comply with the assigned structures and are listed below for each newly synthesized compound.

^{1}H-NMR analysis

A simple analysis of the spectrum of the crude reaction product revealed its impurity as a compound with a structure similar to the major unsubstituted nitrogen component, but containing an ethyl group (fig 3).

The structural assignment of **4** (fig 3) was performed by 1D-NOE measurements as follows. The saturation of the aromatic protons at 7.82–7.92 ppm produced an enhancement at 9.16 ppm, thus allowing the assignment of the singlet at 9.16 ppm to the H_b

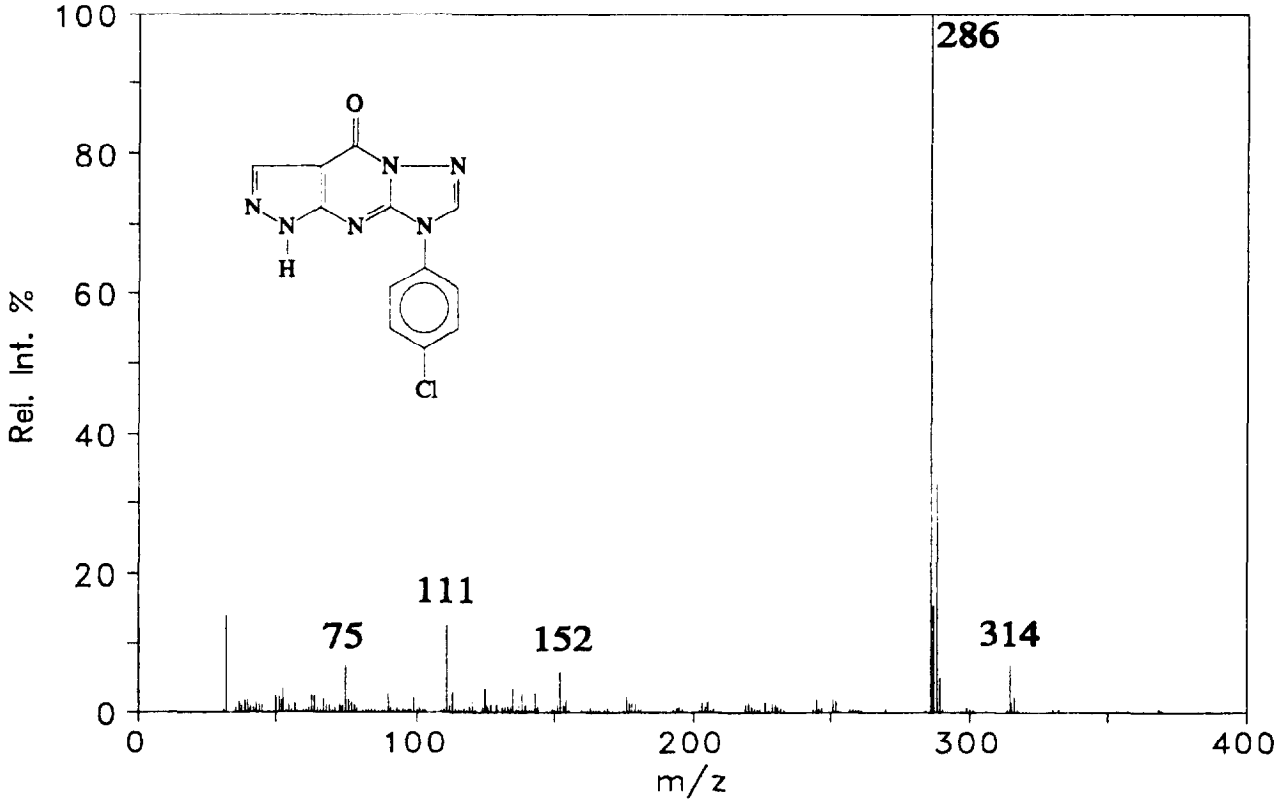


Fig 2. Mass spectrum of the chloro analog of compound **4** as a byproduct.

proton adjacent to the aromatic substituent. As a consequence, the singlet at 8.67 ppm was assigned to the H_a, the saturation of which produced a remarkable enhancement of the ethyl group resonances. It can be concluded that the impurity is alkylated when the proton in position a and the ethyl group are in close proximity. A similar scenario results in the ^{1}H-NMR spectra of samples of **4** obtained by synthesis (scheme 1) or by PLC or RP-PLC separation from the crude product of the ring closure reaction (scheme 1, inset) (see *Experimental protocols*).

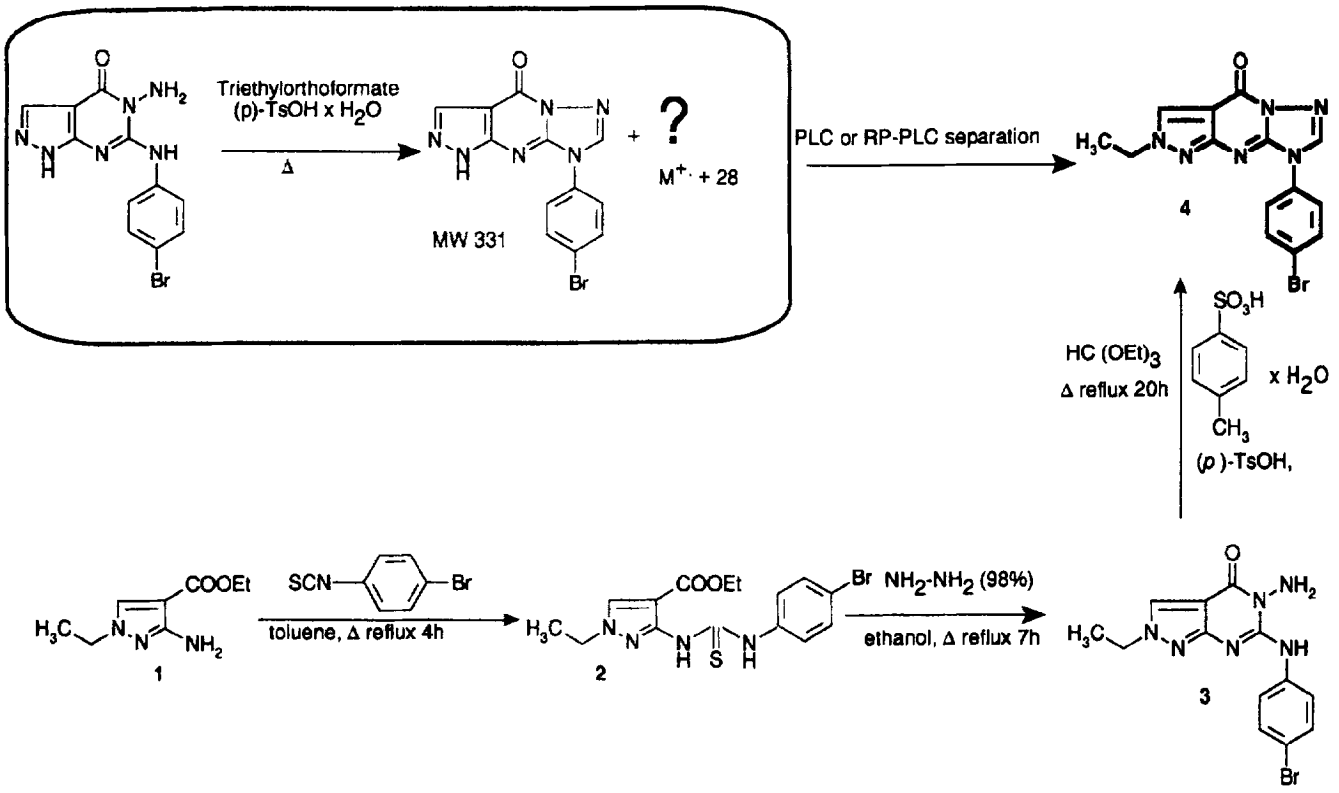
Experimental protocols

Chemical methods

Melting points were determined on a Büchi capillary apparatus and are uncorrected. IR spectra were obtained with potassium bromide discs on a Perkin-Elmer 1600 FTIR series spectrophotometer. Elemental combustion analyses were performed on a Carlo Erba Mod EA 1108 Analyzer instrument by S Di Marco at the Microanalysis Laboratory of Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania. The data are within ±0.40% of the theoretical values. ^{1}H and ^{13}C-NMR spectra were recorded at 300.13 and 75.5 MHz, respectively, on a Bruker AMX-R 300 spectrometer in DMSO-d_6 as a solvent at 298 K. ^{1}H{^{1}H}-NOE experiments were performed on a Varian VXR-300 spectrometer operating at 300 MHz for ^{1}H in DMSO-d_6 as a solvent at 25°C using carefully degassed samples under the difference mode.

^{1}H and ^{13}C chemical shifts are given as δ values in parts per million (ppm) downfield Me_4Si (0.00 ppm) as the internal standard and coupling constants in Hz. Mass spectrometric measurements were performed on a VG 70-70E instrument operating under electron ionization (EI) mode (70 eV, 100 μA). TLC was performed on plates RP 18 F_{254}S Merck precoated 5–10 cm, layer thickness of 0.25 mm. PLC was performed on plates 60 F_{254} pre-coated 20 x 20 cm, layer thickness of 1 mm (system: ethyl/acetate) and RP 18 pre-coated 20 x 20 cm, layer thickness of 1 mm. The RP-mobile phase was methanol/water (φ = 30%) and 0.5 triethylamine. 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. The purity of each compound was similarly checked. The spots were detected by UV irradiation at 254–365 nm. All chemicals were purchased from Aldrich,



Scheme 1. Synthetic route to N_2-ethyl-8-(4-bromophenyl)pyrazolotriazolopyrimidin-4-one **4**.

Fluka, Merck and Carlo Erba Chemical Co and were used without further purification. Chromatographic eluents were of analytical grade or were purified following the usual method.

1-Ethyl-N-(4-carboethoxypyrazol-3-yl)-N'-(4-bromophenyl)-thiourea 2

A solution of aminoester **1** [6–8] (1 g, 0.0054 mol) and (4-bromophenyl)isothiocyanate (1.16 g, 0.0054 mol) in 5 ml toluene was heated for 4 h at reflux. The precipitate was collected, dried and recrystallized from toluene or benzene.

Yield 50%; mp 166–167°C. IR (KBr) 3350 (NH), 1695 (C=O). ^{1}H-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. Anal $C_{15}H_{17}BrN_4O_2S$ (C, H, N, S). MS (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).

6-Ethyl-2-(4-bromophenyl)amino-3-aminopyrazolo[3,4-d]pyrimidin-4-one 3

To a solution of the appropriate ethyl substituted *N*-(4-carboethoxypyrazol-3-yl)*N'*-(4-bromophenyl) thiourea **2** (2.5 g, 0.0063 mol) in 15 ml ethanol, hydrazine monohydrate 98% (3 ml) was slowly added under stirring and the mixture was heated for 7 h at reflux. After cooling the solid was collected, washed with water, dried and recrystallized from ethanol. The filtrate was diluted with 100 ml water, heated and filtered to yield additional product **3**. The compound can be processed without recrystallization (single spot on TLC in the described mobile phase).

Yield 90%; mp 225–227°C. IR (KBr) 3320 (NH), 1700 (C=O). ^{1}H-NMR (DMSO-d_6) δ 9.41 (v br, 1H, NH); 8.37 (s, 1H, pyrazole H_3); 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. Anal $C_{13}H_{13}BrN_6O$ (C, H, N). MS (relative abundance): 348 ($M^{+\cdot}$, 100), 350 (M + 2, 97), 319 (64), 317 (64), 178 (10).

2-Ethyl-8-(4-bromophenyl)-pyrazolo[3,4-d][1,2,4]8H-triazolo[2,3-a]4H-pyrimidin-4-one 4

A suspension of the appropriate ethyl-substituted 2-(4-bromophenyl)amino-3-aminopyrazolo[3,4-*d*] pyrimidin-4-one **3** (1.5 g, 0.0043 mol) in 45 ml triethylorthoformate was refluxed under stirring, using *p*-TsOH monohydrate (1.5 g, 0.0078 mol) as a catalyst. After 20 h the solid was collected, washed with water, dried and recrystallized from dimethylformamide.

Yield 95%; mp 254–255. IR (KBr) 1720 (C=O). ^{1}H-NMR (DMSO-d_6) δ 9.13 (s, 1H, H7); 8.65 (s, 1H, H3); 7.91 (d, J_{av} =

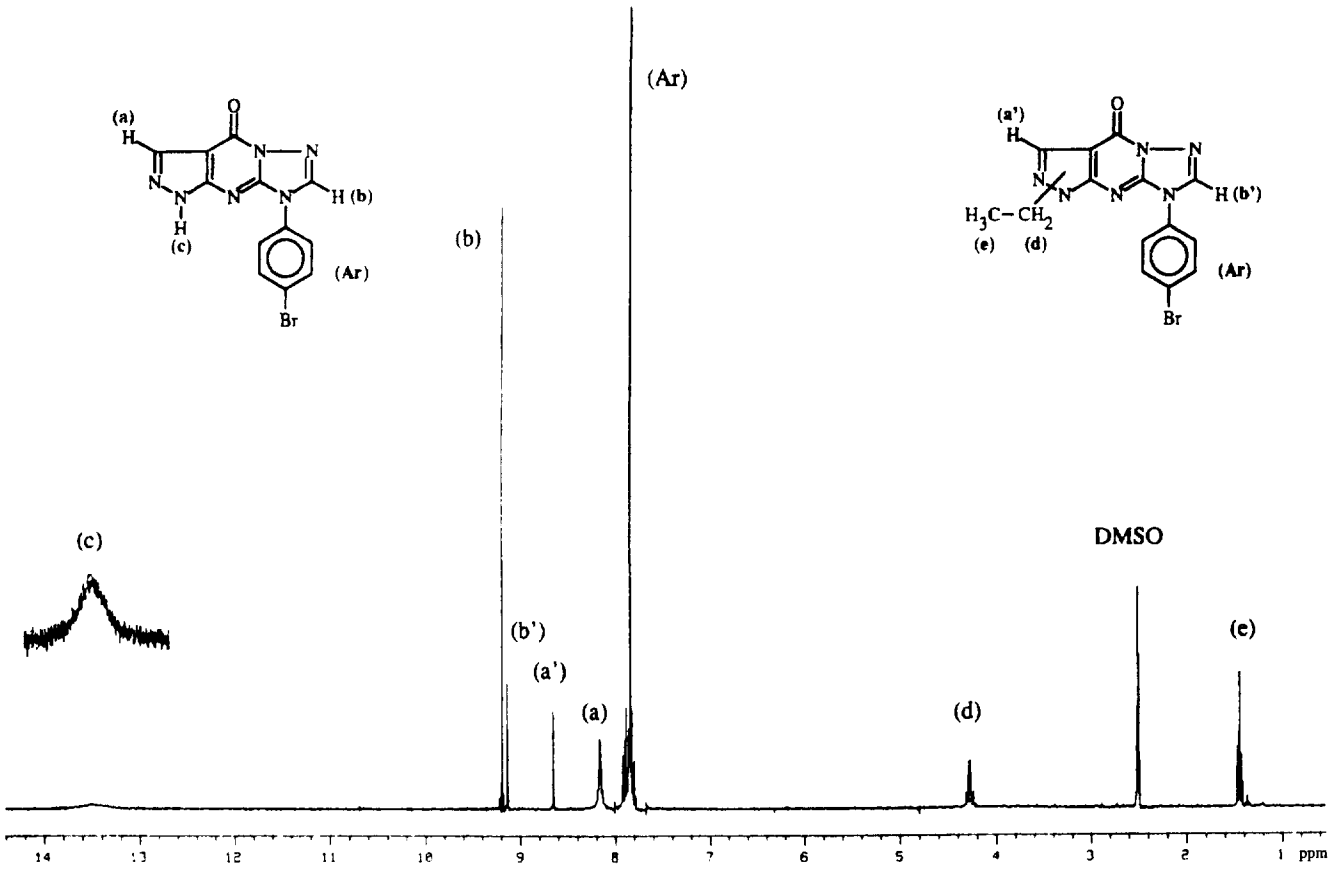


Fig 3. ^{1}H-NMR spectrum of the crude reaction product.

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. Anal $C_{14}H_{11}BrN_6O$ (C, H, N). MS (relative abundance): 358 ($M^{+\bullet}$, 100), 360 (M + 2, 99), 345 (7), 343 (7), 332 (32), 330 (33), 280 (8), 157 (10), 155 (11).

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