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Short communication

Synthesis and pharmacological properties of pyrazolotriazolopyrimidine derivatives*

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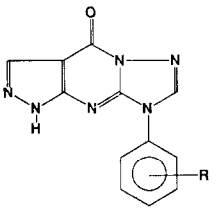
Summary — As a part of research on anti-inflammatory-analgesic compounds, pyrazolotriazolopyrimidines were prepared by cycling the corresponding 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one derivatives **3a–h** with triethylorthoformate, in the presence of *p*-toluenesulfonic acid. The results of the pharmacological screening indicate that some of the derivatives which were tested, especially **3c** and **6e**, showed a good anti-inflammatory activity associated with non-narcotic analgesic properties and a remarkable systemic and gastric tolerance.

pyrazolotriazolopyrimidine derivatives / anti-inflammatory / analgesic activity

Introduction

In previous papers we have described the preparation of heterocyclic compounds with condensed nuclei containing the pyrimidine system [1, 2] and lack of acid functions. Some of these showed analgesic/anti-inflammatory activity and low toxicity, while there was no ulcerogenic activity. On the basis of these results we synthesized another series of compounds having a general formula (scheme 1), which presents the pyrazole nucleus of biological interest, with the aim of obtaining new effective molecules, which lack ulcerogenic effects.

The introduction on condensed triazolopyrimidine ring of pyrazole nucleus, previously tested showing good outcome [1], is linked to the importance that it holds in the field of analgesic/anti-inflammatory agents. The afore-mentioned reason makes it a current subject for research, to find more new substances as equally effective as the well known drugs less detrimental to health. The structure of the triazole ring and its pharmacophoric properties in the field of inflammation are also confirmed by a recent paper, which reports 1, 2, 4, triazoles as antiarthritic agents [3].



Scheme 1.

We chose the phenyl ring, variously substituted as radical, on the triazole nucleus, with particular attention to the role that the extension of the aromatic-lipophilic parts of the molecule plays and to the different effects which the substitutions play on biological activity. To obtain more detailed information

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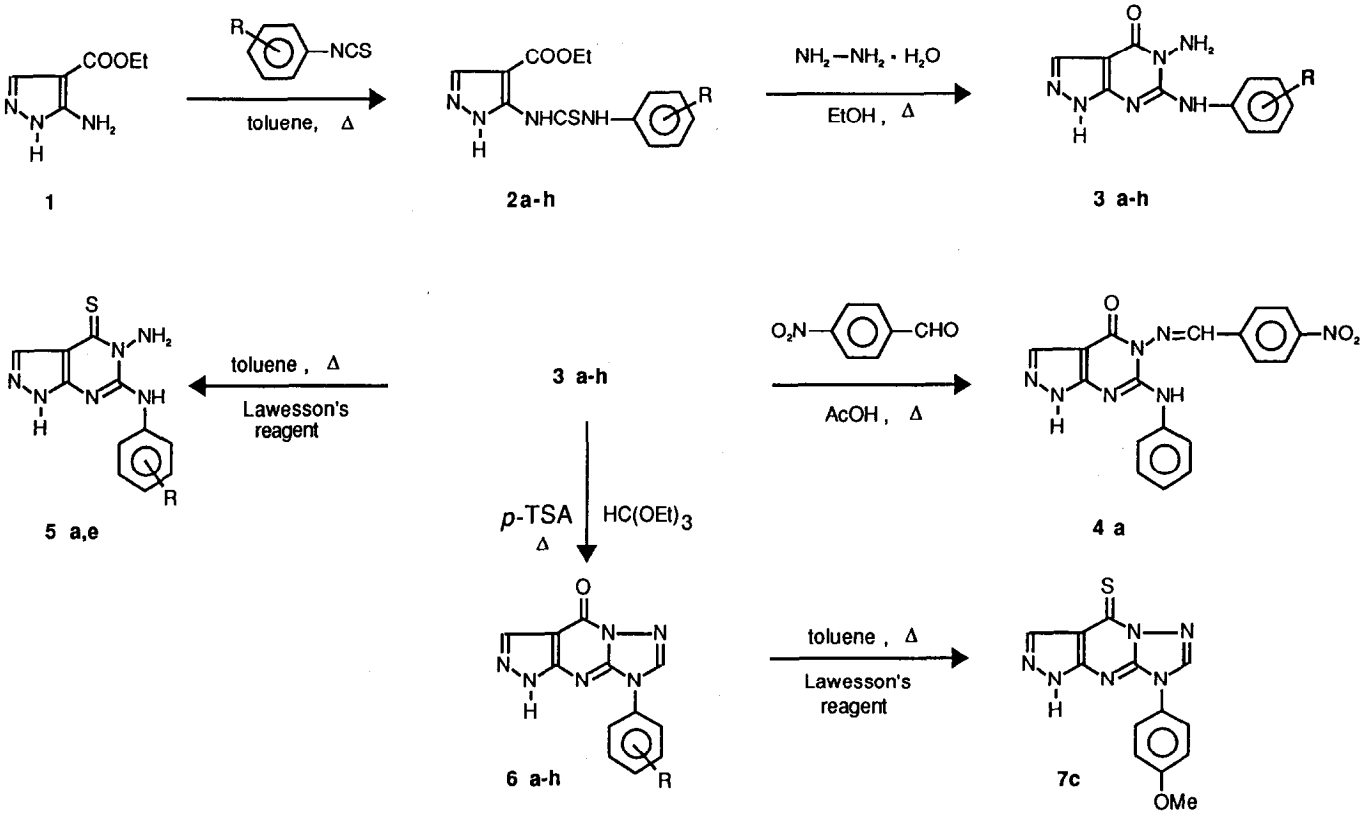
on the structure–activity relationship we transformed compounds **3a**, **e** and tricyclic derivative **6c** to the corresponding 4-thiones. Further speculations are possible about the structure of these molecules and their characteristic of forming copper complexes, considering a possible connection between this property and anti-inflammatory activity [4, 5]. In particular, copper [4, 6] and gold [7, 8] complexes have shown anti-inflammatory effects and several reports have claimed that copper complexes behave more effectively as anti-inflammatory and less as ulcerogenic drugs, compared to their parent 'ligand' [9]. The pharmacological properties of pyrazolotriazolopyrimidine derivatives **6a–h** and those of their parent 'open models' N-amino derivatives **3a–h** (table VI) will be studied in order to have a clearer idea of the structure–activity relationship.

Chemistry

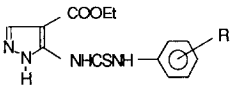
Synthesis of 1H-pyrazolo[3,4-d][1,2,4]8H-triazolo-[2,3-a]4H-pyrimidin-4-ones ***6a–h***

Pyrazolotriazolopyrimidine derivatives **6a–h** were prepared by cycling the corresponding 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one derivatives **3a–h** with triethylorthoformate, in the presence of *p*-toluensulfonic acid (table III). The starting compounds **3a–h** (table II) were synthesized from the reaction of hydrazine hydrate with appropriate N-(4-carboethoxypyrazol-3-yl)N'-arylthiourea **2a–h** (table I), obtained by aminoester **1**, commercially available, with phenylisothiocyanates. The presence of the amino group in position 3 of the pyrimidine ring was verified by the corresponding Schiff's base **4a** with *p*-nitrobenzaldehyde. Compound **4a** exhibits absorption in the region of 3200 cm^{-1} attributed to N-H stretching



Scheme 2.

Table I. Compounds **2a–g** were recrystallized from toluene/EtOH (70:30, v/v), compound **2h** from toluene.

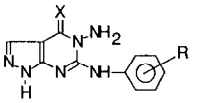


Comp	*R*	*mp (°C)*	*Yield (%)*	*Formula*	*IR (KBr, cm^{-1})*	
					C=O	*N–H*
2a*	H	159–61	60	$C_{13}H_{14}N_4O_2S$	1695	3380; 3240
2b	*p*-Me	176–77	50	$C_{14}H_{16}N_4O_2S$	1665	3380; 3300
2c	*p*-OMe	188–89	65	$C_{14}H_{16}N_4O_3S$	1665	3400; 3310
2d	*p*-Br	202–03	50	$C_{13}H_{13}BrN_4O_2S$	1670	3380; 3240
2e*	*p*-Cl	199–200	54	$C_{13}H_{13}ClN_4O_2S$	1695	3380; 3220
2f	*o*-Cl	186–88	50	$C_{13}H_{13}ClN_4O_2S$	1660	3390; 3300
2g	*p*-F	154–55	65	$C_{13}H_{13}FN_4O_2S$	1660	3390; 3320
2h	*m*-F	172–74	50	$C_{13}H_{13}FN_4O_2S$	1660	3380; 3290

*Noted compounds [10].

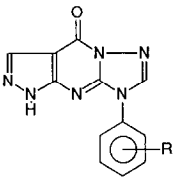
and a band at 1690 cm^{-1} due to the carbonyl group. Mass spectrum shows the molecular peak at m/z 375. We assume that this reaction is common to the other 3-amino compounds **3a–h**. Compounds **5a, e** and **7c** were prepared by treating corresponding 4-oxo compounds **3a, e** and **6c** with Lawesson's reagent. The above reactions are reported in scheme 2. The elemental analyses and IR spectra data of the synthesized compounds were consistent with the assigned structures. The structures of the compounds were also ascertained by the ^{1}H NMR, ^{13}C NMR (assigned bands) (table IV) and MS measurements (table V). All data are also consistent with already noted compounds **2a, e** [10]. Mass spectra of tricyclic compounds 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. Mass spectrometric measurements of crude tricyclic compounds manifested a rather intense peak at M + 28, besides characteristic fragmentation of pure pyrazolotriazolopyrimidines **6a–h**. We suppose this peak is due to the *N*-formyl pyrazole derivative, formed as subproduct of the reaction by an initial condensation of pirazole NH with triethylortoformate and subsequent decomposition of the non-stable intermediate. This compound is, however, easily converted in the corresponding 1H-pyrazolotriazolopyrimidine derivatives **6a–h** by heating during recrystallization or by standing solution (DMF) at room temperature for several days.

Table II. Compounds **3a–g** and **5a, e** were recrystallized from EtOH, compound **3h** from EtOH/water.



Comp	*R*	*X*	*mp (°C)*	*Yield (%)*	*Formula*	*IR (KBr, cm^{-1})*	
						C=X	*N–H*
3a	H	O	271–72	40	$C_{11}H_{10}N_6O$	1710	3325; 3200
3b	*p*-Me	O	284–85	60	$C_{12}H_{12}N_60$	1670	3340; 3220
3c	*p*-OMe	O	264–65	70	$C_{12}H_{12}N_6O_2$	1685	3350; 3215
3d	*p*-Br	O	293–94	45	$C_{11}H_9BrN_6O$	1675	3360; 3245
3e	*p*-Cl	O	282–83	55	$C_{11}H_9ClN_6O$	1685	3300; 3200
3f	*o*-Cl	O	265–67	90	$C_{11}H_9ClN_6O$	1660	3390; 3300
3g	*p*-F	O	296–97	48	$C_{11}H_9FN_6O$	1710	3330; 3200
3h	*m*-F	O	254–56	85	$C_{11}H_9FN_6O$	1680	3330; 3200
5a	H	S	243–44	60	$C_{11}H_{10}N_6S$	1205	3340; 3200
5e	*p*-Cl	S	258–60	50	$C_{11}H_9ClN_6S$	1210	3300; 3220

Table III. Compounds **6a–h** were recrystallized from EtOH/DMF.

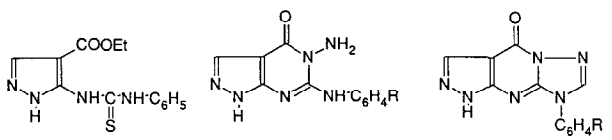


Comp	R	mp (°C)	Yield (%)	Formula	IR (KBr, cm^{-1})	
					C=O	N–H
6a	H	290–91	75	$C_{12}H_8N_6O$	1735	3530
6b	*p*-Me	294–95	45	$C_{13}H_{10}N_6O$	1710	3200
6c	*p*-OMe	290–92	50	$C_{13}H_{10}N_6O_2$	1710	3200
6d	*p*-Br	296–97	40	$C_{12}H_7BrN_6O$	1685	3300
6e	*p*-Cl	> 300	50	$C_{12}H_7ClN_6O$	1735	3315
6f	*o*-Cl	> 300	30	$C_{12}H_7ClN_6O$	1700	3280
6g	*p*-F	> 300	65	$C_{12}H_7FN_6O$	1705	3560
6h	*m*-F	> 300	70	$C_{12}H_7FN_60$	1700	3180

Table IV.

Compd	^1H NMR (ppm)	^{13}C NMR (ppm, selected lines)
2b	1.31 (t, 3H, CH_3), 2.32 (m, 3H, Ar-CH_3), 4.30 (q, 2H, -CH_2-), 7.22–7.51 (m, 4H, ArH), 8.45 (s, 1H, pyrazole), 9.48 (s, 1H, NH), 11.44 (br s, 1H, NH)	
2e	1.31 (t, 3H, CH_3), 4.30 (q, 2H, -CH_2-), 7.47–7.69 (m, 4H, ArH), 8.45 (s, 1H, pyrazole), 9.56 (s, 1H, NH), 11.45 (br s, 1H, NH)	
2f	1.31 (t, 3H, CH_3), 4.30 (q, 2H, -CH_2-), 7.32–7.41 (2t, 2H, ArH), 7.58 (d, 1H, ArH), 8.04 (d, 1H, ArH), 8.46 (s, 1H, pyrazole), 9.64 (s, 1H, NH), 11.50 (s, 1H, NH), 13.61 (br s, 1H, pyrazole NH)	14.2 (CH_3), 60.6 (CH_2), 99.4 (pyrazole C_4), 127.7–128.4–128.8–129.8 (ArH $C_{3,4,5,6}$), 133.3 (pyrazole, C_3), 128.5–136.1–149.3 (ArH $C_{1,2}$, pyrazole C_5), 163.9 (CO), 177.6 (CS)
2h	1.30 (t, 3H, CH_3), 4.29 (q, 2H, -CH_2-), 7.10–7.42–7.79 (m, 4H, ArH), 8.46 (s, 1H, pyrazole), 9.57 (s, 1H, NH), 11.55 (s, 1H, NH), 13.56 (s, 1H, pyrazole NH)	14.2 (CH_3), 60.6 (CH_2), 99.7 (pyrazole C_4), 111.3 (d, ArH C_4, J = 26 Hz), 112.9 (d, ArH C_2, J = 22 Hz), 120.3 (ArH, C_6), 130.8 (d, ArH, C_5, J = 10 Hz), 133.4 (pyrazole C_3), 140.6 (d, ArH C_1, J = 10 Hz), 149.4 (pyrazole C_5), 162.3 (d, ArH, C_3, J = 243 Hz), 163.8 (CO), 176.9 (CS)
3e	5.58 (s, 2H, NH_2), 7.42–7.86 (m, 4H, ArH), 7.94 (br s, 1H, pyrazole), 9.63 (br s, 1H, NH), 13.18 (br s, 1H, pyrazole NH)	100.2 (C_{3a}), 123.3 (2C)–128.8 (2C) (ArH, $C_{2,3,5,6}$), 127.5 (ArH, C_4), 135.7 (C_3), 137.8–151.2–152.5 (C_6, C_{7a}, ArH, Cl), 157.7 (CO)
5e	6.16 (s, 2H, NH_2), 7.44–7.77 (d, 4H, ArH), 7.96 (s, 1H, pyrazole), 9.90 (br s, 1H, NH), 13.33 (br s, 1H, pyrazole NH)	112.4 (C_{3a}), 124.3 (2C)–128.9 (2C) (ArH $C_{2,3,5,6}$), 138.3(C_3), 128.3–137.4–147.0–150.3 (ArH, $C_{1,4}$, C_6, C_7), 179.7 (CS)
6a	7.53 (t, 1H, ArH, H_4), 7.64 (t, 2H, ArH $H_{3,5}$), 7.87 (d, 2H, ArH $H_{2,6}$), 8.18 (s, 1H, pyrazolo), 9.24 (s, 1H, H_7), 13.70 (v br s, 1H, pyrazolo NH)	101.2 (pyrazole C_{3a}), 124.4 (2C)–129.0-129.9 (2C) (ArH, $C_{2,3,5,6}$), 133.3–135.6–142.0–148.4–152.1 (ArH, C_1, C_3, C_7, C_{8a}, C_{9a}), 152.1 (CO)
6e	7.73–7.93 (d, 4H, ArH), 8.19 (s, 1H, pyrazole), 9.24 (s, 1H, H_7), 13.56 (v br s, 1H, pyrazole NH)	101.3 (pyrazole C_{3a}), 126.0 (2C)–129.9 (2C) (ArH, $C_{2,3,5,6}$), 132.2–133.4 (ArH $C_{1,4}$), 135.6 (C_3), 141.9–148.3–152.1 (C_7, C_{8a}, C_{9a}), 154.5 (CO)

Table V. EI mass spectra data of **2a**, **3a**, **d**, **e**, **g**, **6c**, **e**.



2a **3a,d,e,g** **6c,e**

Compd	*R*	*m/z (relative abundance %)*
2a	H	290 ($M^{+\cdot}$, 54), 243 (13), 211 (22), 155 (73), 152 (39), 136 (24), 135 (18), 110 (22), 109 (70), 93 (100), 77 (44)
3a	H	242 ($M^{+\cdot}$, 100), 225 (12), 211 (60), 150 (15), 110 (50), 93 (80), 77 (98)
3d	*p*-Br	320 ($M^{+\cdot}$, 100), 322 (M+2, 98), 303–305 (20), 241 (15), 289–291 (55), 170– 172 (50), 150 (97), 135 (32), 121 (45), 110 (65)
3e	*p*-Cl	276 ($M^{+\cdot}$, 100), 278 (M+2, 34), 261 (8), 259 (22), 247 (22), 245 (62), 150 (29), 127 (14), 126 (21), 111 (14), 110 (17), 75 (13)
3g	*p*-F	260 ($M^{+\cdot}$, 100), 243 (21), 229 (68), 150 (23), 110 (33)
6c	*p*-OCH_3	282 ($M^{+\cdot}$, 100), 267 (35), 239 (14), 133–92 (7), 77 (8), 64 (6)
6e	*p*-Cl	286 ($M^{+\cdot}$, 100), 288 (M+2, 34), 152 (9), 135 (8), 111 (22), 75 (12), 57 (10), 43 (10)

Results

Behavioural effects and acute toxicity in mice

In mice the test compounds did not show any significant gross behavioural and toxicological effects at doses of 500 mg/kg po and 150 mg/kg ip. With doses higher than 700 mg/kg po and 300 mg/kg ip they produced dose-related sedation, motor incoordination hypotonia and bradypnoea. As table VI shows, the approximate LD_{50} values were about 1000 mg/kg po and 800 mg/kg ip. At these doses, death generally occurred at 6–12 h postdrug in 20–60% of animals.

Phenylquinone writhing test

In the mouse phenylquinone-induced writhing test, drugs **3b**, **c**, **d** and **6e**, **g** showed a significant dose-dependent analgesic-action at 10 mg/kg po, being more or comparably active than phenylbutazone and mefenamic acid at the same dosage. The most active derivatives were **3c** and **6e**.

Anti-inflammatory activity

In the acetic acid peritonitis assay at the dose of 10 mg/kg po some compounds exhibited fair anti-exudate activity. The most active derivatives were **3c**, **6e** and **6g** who afforded a protection of 43, 34, and 32% respectively. Among the reference drugs, at the same dosage, phenylbutazone and mephenamic acid were ineffective. In the rat paw oedema test compounds, **3c** and **6e** showed remarkable activity; their potency was comparable to PBZ and higher than MFA. However, all compounds reported in table VI showed a fair anti-inflammatory activity.

Table VI. Oral administration for all tests; substances partially solubilized in CMC 0.5%.

Comp	*LD_{50}*		*Anti-inflammatory activity*		*Analgesic activity*	*Ulcerogenic index* [b]
	os	*ip*	*Carrageenan paw oedema* [a] *100 mg/kg*	*Acetic acid peritonitis* [a] *10 mg/kg*	*Phenylquinone Writhing-test* [a] *10 mg/kg*	*400 mg/kg x 2*
3a	> 1000	800	19	15	11	0
3b	> 1000	700	33*	25*	32**	0
3c	> 1000	700	50**	43**	40**	0
3d	> 1000	800	27*	23	22**	0
3e	> 1000	> 800	9	3	12	–
3f	> 1000	> 800	7	0	0	–
3g	> 1000	700	20	17	13	0
3h	> 1000	800	9	4	10	–
5a	> 1000	700	7	2	8	–
5e	> 1000	700	5	0	0	–
6a	> 1000	800	30*	24*	24**	0
6b	> 1000	> 800	5	0	0	–
6c	> 1000	800	0	0	0	–
6d	> 1000	700	26*	22	20	0
6e	> 1000	800	43**	34**	38**	0
6f	> 1000	800	9	4	10	–
6g	> 1000	700	39**	32**	35**	0
6h	> 1000	800	8	2	12	–
7c	> 1000	800	0	0	12	–
PBZ	≈ 700	300	57**	6	26**	4 [c]
MFA	≈ 2000	600	35**	5	37**	4

[a]Values are percent of the controls. [b]More active compounds were tested for their ulcerogenic activity. [c]PBZ 100 mg/kg x 2; *$P < 0.05$; **$P < 0.01$; Student's *t*-test *vs* controls.

Ulcerogenic activity

No compound showed any ulcerogenic effects or hyperhemia and mucus effusion in the gastric mucosa as the total dose of 400 mg/kg po administered twice at 2-h intervals in fasted rats, whereas phenylbutazone and mefenamic acid (2 x 400 mg/kg) caused gastric ulcers in all animals.

Conclusion

The results obtained indicate that some of the tested derivatives, especially compounds **3c** and **6e** (table VI) showed good anti-inflammatory activity associated with non-narcotic analgesic properties and a remarkable systemic and gastric tolerance. With regard to previous synthesized compounds containing triazolopyrimidine moiety [1], the effect of the pyrazole ring, on the anti-inflammatory activity is considerable, while there are no important differences in analgesic activity. The (p) substitution, especially with chlorine and fluorine, on the benzene ring produced a considerable increase in both anti-inflammatory and analgesic activities compared to the unsubstituted compounds. The (p) substituted compounds are always more effective than compounds (o) and (m) substituted on the benzene ring. 'Open models' N-amino derivatives **3a–h** are less or equally active as anti-inflammatory agents, compared to the corresponding tricyclic **6a–h** derivatives except for **3c** (the most effective of the tested compounds, with regard to **6c**), while we did not see any important differences as analgesic agents between the two classes of molecules. We did notice a negative change in both activities in the 4-thioderivatives **5a**, **e** and **7c** compared to 4-oxo compounds **3a**, **e** and **6c**, in themselves little active. To our knowledge these molecules can act as ligands towards various metal ions. Lippard *et al* reported the X-ray structure of a platinum (II) complexes with 6-mercaptopurine in which this molecule is bound to platinum (II) through the sulfur and N7 atoms giving a distorted chelate complex [16]. 9-Methylhypoxanthine, an oxygen analog of the previous molecule, forms stable complexes with copper (II), in which the metal ion is bound through N7 atom [17]. Preliminary spectrophotometric tests have shown that title compounds react with various metal ions. In particular, we have tested platinum (II) and copper (II). The binding site(s) and the structure of these complexes are being investigated.

Experimental protocols

Chemical methods

All melting points were taken in open capillaries using a Gallenkamp melting point apparatus with digital thermometer MFB-595 and are uncorrected. The IR spectra were recorded with a Perkin-Elmer 281 spectrometer on KBr disks. Elemental analyses for C, H and N were obtained on a Carlo Erba Mod 1106 instrument and were within ± 0.4% of the theoretical values. The ^1H and ^{13}C NMR spectra were recorded in DMSO–d_6 on a Bruker MSL 200 operating at a frequency of 200 and 50.3 MHz respectively. NMR data are reported in ppm from TMS as an internal reference and are given in units δ; coupling constants are reported in Hertz. Carbon assignment was done by a standard APT pulse sequence. In some spectra the resonance relative to the pyrazole NH is undetectable due to the coupling with the quadrupolar ^{14}N nucleus and for the rapid exchange with the small amount of water present in the solvent. Mass spectrometric measurements were performed on a VG 70-70E instrument operating in electron impact (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°C. The purity of the synthesized compounds was checked by thin-layer chromatography (TLC) on aluminium sheets silica gel 60 F254 (Merck); system: ethyl acetate/cyclohexane (80:20, **2a–h**); ethyl acetate (**6a–h**); detection: UV (λ = 254 and 366 mm). The solvents and reagents were purified in the usual way.

*Synthesis of N-(4-carboethoxypyrazol-3-yl)N'-aryl-thioureas **2a–h***

General procedure. A solution of **1** (0.02 mol) and appropriate phenylisothiocyanate (0.02 mol) in 20 ml of toluene was heated under reflux for 6 h. The product was collected and, after drying, recrystallized from toluene-ethanol (7:3). Yields, melting points, crystallization solvents and IR data of the compounds are reported in table I. ^1H, ^{13}C NMR (table IV) and MS (table V) data respectively of the samples **2a** [10], **b**, **f**, **g**, **h** are reported.

*Synthesis of 2-phenylamino-3-aminopyrazolo[3,4-d]pyrimidin-4-one derivatives **3a–h***

General procedure. 1) To a solution of *N*-(4-carboethoxy-pyrazol-3-yl)*N'*-arylthiourea **2a** (0.02 mol) in 45 ml of ethanol, hydrazine hydrate (6 ml) was added slowly under stirring and was heated under reflux for 8 h. The product that crystallized on cooling, was collected, washed with water, dried and recrystallized from ethanol.

2) A suspension of **2a** (0.02 mol) and 20 ml (0.4 mol) of hydrazine hydrate was stirred for 24 h at room temperature. The solid material was collected, washed with water, dried and recrystallized from ethanol. In the same way the compounds **3b–h** were synthesized.

Yields (approximately equal for both methods), melting points, crystallization solvents and IR data of the compounds are reported in table II. ^1H, ^{13}C NMR (table IV) and MS (table V) data respectively of the samples **3a**, **d**, **e**, **g** are reported.

*Synthesis of 2-phenylamino-3-(p-nitrobenzylidenamino)-pyrazolo[3,4-d]pyrimidin-4-one **4a***

A solution of 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **3a** (0.004 mol) in 12 ml of acetic acid was heated under reflux with *p*-nitrobenzaldehyde (0.004 mol) for 4 h. The resulting solution after filtration was concentrated to dryness under reduced pressure and the yellow residue was washed with ethanol, collected and recrystallized from acetic acid/water. Yield 70%; mp = 272–273°C; IR (cm^{-1}) 3320 (N-H), 1690 (C=O), 1530 (NO_2); Anal $C_{18}H_{13}N_7O_3$ (C, H, N); MS: $M^{+\cdot}$ 375; TLC system: ethyl acetate.

*Synthesis of 2-phenylamino-3-aminopyrazolo[3,4-d]pyrimidin-4-thione-derivatives **5a, e***

General procedure. A stirred mixture of 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **3a** (0.02 mol) and *p*-methoxyphenylthiophosphine sulfide dimer (Lawesson's reagent) (0.09 mol) in 120 ml of dry toluene was heated under reflux for 10 h. The yellow solid material was collected and recrystallized from ethanol. Compound **5e** was similarly prepared. Yields, melting points, crystallization solvents and IR data of the compounds are reported in table II. ^1H, ^{13}C NMR data of the sample **5e** are reported in table IV.

*Synthesis of 1H-pyrazolo[3,4-d][1,2,4]8H-triazolo[2,3-a]4H-pyrimidin-4-one derivatives **6a–h***

General procedure. A mixture of 2-phenylamino-3-aminopyrazolo[3,4-*d*]pyrimidin-4-one **3a** (0.012 mol), *p*-toluenesulphonic acid (0.016 mol), and 45 ml of triethylorthoformate was heated under reflux while stirring for 18 h. The solid material was collected by filtration, washed with water, dried and recrystallized from ethanol–DMF. In the same way, the compounds **6b–h** were synthesized. Yields, melting points, crystallization solvents and IR data of the compounds are reported in table III. ^1H, ^{13}C NMR (table IV) and MS (table V) data respectively of the samples **6c**, **e** are reported.

*Synthesis of 1H-pyrazolo[3,4-d][1,2,4]8H-triazolo[2,3-a]4H-pyrimidin-4-thione **7c***

A stirred mixture of pyrazolotriazolopyrimidine **6c** (0.01 mol) and *p*-methoxyphenylthiophosphine sulfide dimer (Lawesson's reagent) (0.09 mol) in 160 ml of dry toluene, was heated under reflux for 18 h. The yellow solid material was collected and crystallized from ethanol. Yield 90%; mp = 300°C; IR (cm^{-1}) 3180 (N–H), 1260 (C=S); ^1H NMR (DMSO–d_6): 3.88 (s, OCH_3, 3H), 7.19–7.74 (dd, 4H aromatic), 8.24 (s, 1H pyrazole), 9.35 (s, 1H triazole), 13.65 (s, pyrazole NH); ^{13}C NMR (DMSO–d_6): 55.82 (OCH_3, 1C), 115.03–125.65–126.86–160.11 (phenyl ring 2C + 1C + 2C + 1C), 138.01 (pyrazole-C3, 1C), 144.02–146.74–148.90 (3C), 171.74 (C=S, 1C). Anal $C_{13}H_{10}N_6OS$ (C, H, N); MS: $M^{+\bullet}$ 298; TLC system: ethyl acetate.

Pharmacological evaluation

Experimental

The compounds described in this paper were screened for their analgesic, anti-exudative and anti-inflammatory activities, as well as for their gross behavioural effects and acute toxicity. Phenylbutazone and mefenamic acid were used as reference drugs. Results of tested compounds are summarized in table VI.

Pharmacology

Experiments were carried out on male albino Swiss mice (24–26 g) and Sprague–Dawley rats (140–160 g). The test compounds were administered orally or ip in 0.5% methylcellulose suspension. Statistically analysis was made using the Student's *t*-test *versus* controls. The level of significance was set at $P < 0.05$.

Behavioural effects and acute toxicity in mice

The Irwin's screening-evaluative procedure [11] was used on groups of 5 animals. The compounds were administered orally at three levels of dose (500, 700, 1000 mg/kg) and ip (150, 300, 500 mg/kg). The animals were kept under observation for 6 h and the symptomatology was checked again 24 h later. The approximate LD_{50} was obtained from the mortality 7 days later.

Analgesic activity (phenylquinone writhing test)

The test was performed using Berkowitz's *et al* [12] technique. Groups of 5 mice were injected ip with 0.25 ml of a 0.02% solution of phenylquinone 60 min after the oral administration of test drugs. The writhing response frequency was counted in each animal for 5 min (between the 5th and 10th min) after injection of the irritant. The analgesic effect was expressed as a percentage of protection in comparison with controls.

Anti-inflammatory activity

Anti-exudative activity

The acetic acid peritonitis method [13] was used. Groups of 5 rats were given 10 ml/kg ip of a 0.5% CH_3COOH solution 1 h after the oral administration of the test compounds. 30 min later the rats were killed with $(C_2H_5)_2O$ and peritoneal exudate was collected and measured. The anti-exudative response was expressed as the percentage of the exudate volume reduction compared with controls.

Carrageenan-induced rat paw-oedema

The test was performed using Winter *et al*'s technique [14] on groups of 5 rats. The test compounds were administered orally and 60 min later 0.1 ml of 1% carrageenan solution was injected into the plantar aponeurosis of the rat hind paw. The volume of the paw was measured by a mercury plethysmometer prior to the injection of carrageenan and 3 h later. The anti-inflammatory activity was given as the percentage of inhibition of oedema of treated groups, compared with those of the controls.

Ulcerogenic activity

The experiment was carried out in rats according to Domenjoz [15]. The compounds were given orally to groups of 4 rats fasted for 24 h and after 2 h the treatment was repeated. The animals were sacrificed 6 h after the first dose with $(C_2H_5)_2O$ inhalation, their stomachs removed and examined with a dissecting microscope. The severity of mucosal damage (ulcerogenic index) was graduated by means of scores from 0 (no lesion) to 4 (exceptionally severe lesions).

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