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Design, Synthesis, and Antimicrobial Activity of Some New Pyrazolo[3,4-*d*]pyrimidines

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ABSTRACT: *2-Benzyl- and 2-aryloxymethyl-3-amino-1-phenyl-pyrazolo[3,4-*d*]pyrimidine-4-ones* **5a–f** *have been synthesized by reacting the corresponding arylacetylamino derivatives* **3a–f** *with hydrazine hydrate. Thionation of compounds* **5d–f** *by action of P_2S_5 in pyridine yielded 2-aryloxy-methyl-3-amino-1-pheny-lpyrazolo[3,4-*d*]pyrimidin-4-thions* **6a–c**. *2,5-Diphenyl-2,3-dihydro-1*H*-pyrazolo[5′,1′:4:5]-pyrazolo[3,4-*d*]pyrimidine-8-one (***8***) was also obtained via reaction of ethyl-2-cinnamoylamino-1-phenyl-pyrazole-4-car-boxylate (***7***) with hydrazine hydrate. The prepared compounds were screened in vitro for their antimicrobial activity. Some of the tested compounds were found to be active at 100 μg/ml compared with reference compounds (Ampicillin and Trivid) as antibacterial agents and claforan as antifungal agent.* © 2003 Wiley Periodicals, Inc. Heteroatom Chem 14:530–534, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10187

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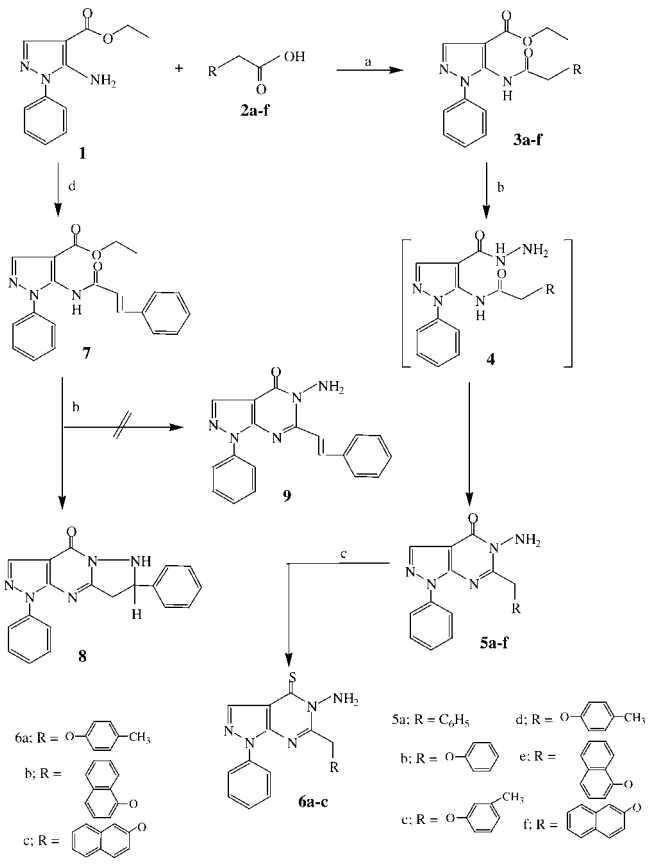
INTRODUCTION

Several 3-substituted pyrazolo[3,4-*d*]pyrimidine derivatives have shown pharmacological activity like allopurinol, which is the inhibitor of xanthine oxidase [1,2]. Also, some substituted pyrazolopyrimidines have been documented as adenosine antagonists [3–5] and to possess antibacterial [6], antifungal [7], and antitumor activity [8,9]. As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds [10–14], we now wish to report the synthesis of some new pyrazolo[3,4-*d*]pyrimidine derivatives as purine isosteres to evaluate their antimicrobial activity.

CHEMISTRY

The starting material ethyl-5-amino-1-phenylpyrazole-4-carboxylate (**1**) was prepared as reported [15] from ethyl-2-cyano-3-ethoxyacrylate and phenyl hydrazine. The synthesis of the target compounds 2-benzyl- or 2-aryloxymethyl-3-amino-1-phenyl-pyrazolo[3,4-*d*]pyrimidine-4-ones **5a–f** and 2,5-dipenyl-2,3-dihydro-1*H*-pyrazolo[5,1:4:5]pyrazolo[3,4-*d*]-pyrimidine-8-one (**8**) was achieved by the route depicted in Scheme 1.

Compound **1** was acylated with various benzyl or aryloxyacetic acids **2a–f** in presence of PCl_3



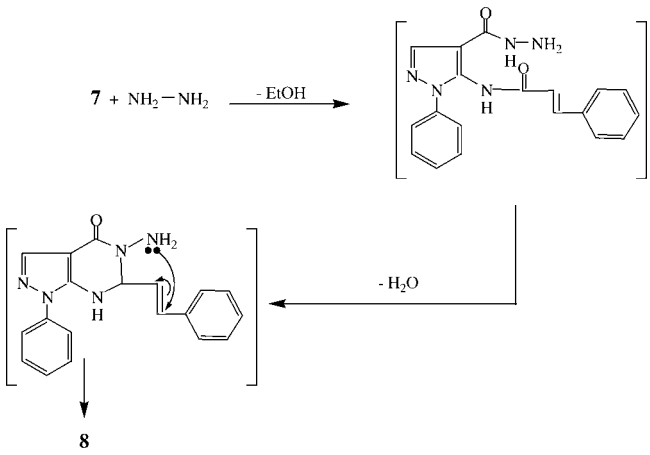
SCHEME 1 Synthesis of pyrazolopyrimidine derivatives (**5a–f, 6a–c**, and **8**). a: PCl_3/xylene, reflux, 5 h; b: $N_2N_4{\cdot}H_2O$, *n*-BuOH, reflux 6 h; c: P_2S_5/pyridine, reflux 16 h; d: cinnamic acid/PCl_3/xylene, reflux, 5 h.

to obtain ethyl-5-(phenyl or aryloxy)acetylamino-1-phenyl-pyrazole-4-carboxylate **3a–f** in good yield.

Cyclocondensation of **3a–f** with hydrazine hydrate afforded **5a–f**. The reaction proceeds via the intermediate **4**, which undergoes a nucleophilic addition to the carbonyl of the side chain followed by the loss of 1 mol of water.

The thione derivatives **6a–c** were synthesized via reaction of **5d–f** with P_2S_5 in pyridine.

The ethyl-5-(cinnamoylamino)-1-phenyl-pyrazole-4-carboxylate (**7**) was obtained in good yield via reaction of **1** with cinnamic acid in presence of PCl_3. Refluxing the amide **7** with hydrazine hydrate in *n*-butanol for 6 h effected double cyclization to give 2,5-diphenyl-2,3-dihydro-1*H*-pyrazolo[5′,1′:4:5]-pyrazolo[3,4-*d*]pyrimidine-8-one (**8**) (Scheme 2) instead of the expected *N*-amino derivative **9**, which was eliminated from consideration on the basis of elemental analyses and ^{1}H NMR spectrum which showed the absence of an amino group.



SCHEME 2 Rationalized formation of compound **8**.

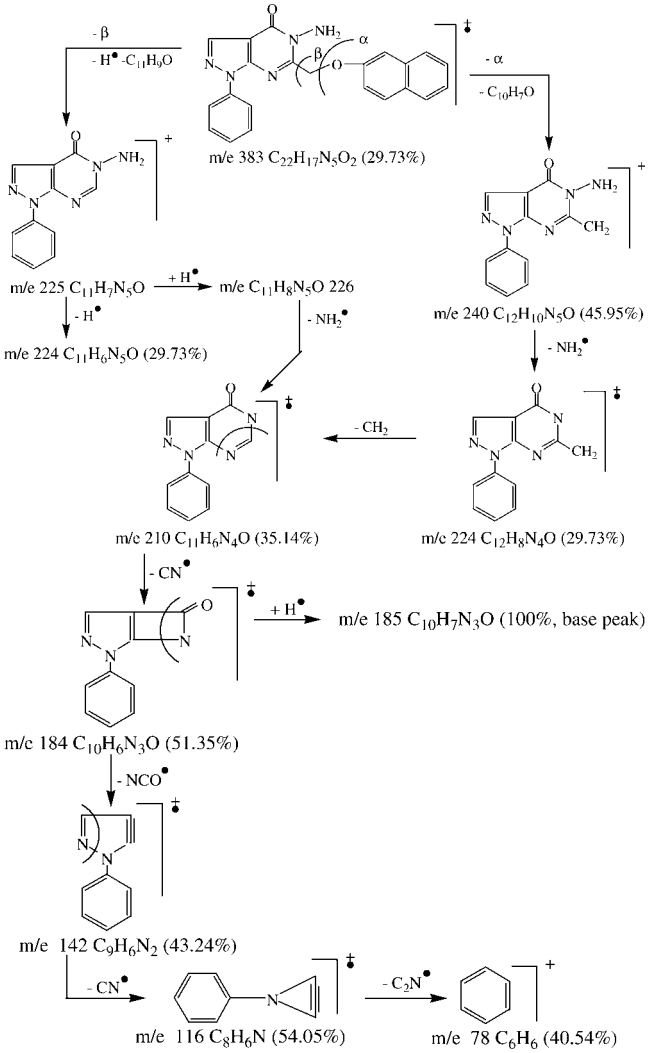
SPECTRA

The synthesized compounds were characterized by elemental analyses and spectral and physical data. IR spectra of compounds **3a–f** show the sharp peak around 3200–3147 cm^{-1} (NH), 1716–1700 (C=O) ester. The IR spectra of compounds **5a–f** showed the distinct peaks for amino group (NH_2), and one carbonyl group (C=O). Their ^{1}H NMR spectra revealed the presence of primary amine protons at $\delta = 5.8$–6.3 ppm that were D_2O exchangeable. The methylene protons in the side chain were found at $\delta = 4.8$–4.2 ppm. The mass spectra of compounds **5b, 5e**, and **5f** showed the molecular ion peak and a prominent M^+-16 peak indicating the early loss of the free amino function at position N^3. Further fragmentation of the molecule was found to be satisfactory (Scheme 3).

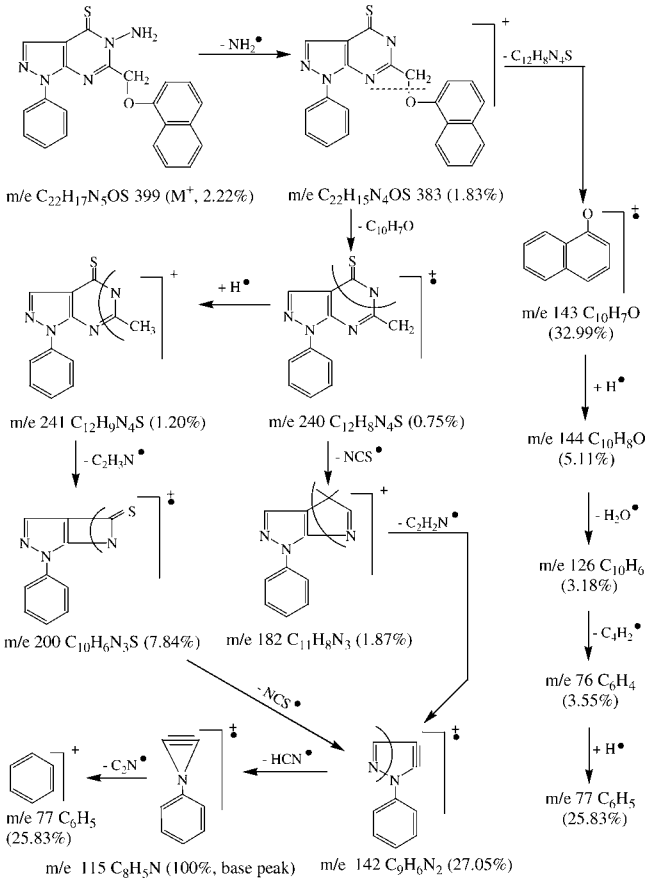
The IR spectra of compounds **6a–c** revealed the absence of C=O and presence of C=S band and NH band. ^{1}H NMR spectrum of **6a** showed signal at $\delta = 5.7$ ppm (NH_2), exchangeable with D_2O. Mass spectrum of compound **6b** showed a molecular ion peak *m/z* 399 (M^+, 2.22%) with a base peak at 115 (Scheme 4).

BIOLOGICAL ACTIVITY

The antimicrobial screening of the synthesized compounds was undertaken using the agar diffusion assay [16]. Table 1 lists the screening results of the tested compounds against the Gram-negative bacteria *Serratia marcescens* and *Proteus merabities*, and the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*, in addition to the pathogenic fungi *Aspergillus oschraceus* wilhelm.



SCHEME 3 Mass fragmentation pattern of compound **5f**.



SCHEME 4 Mass fragmentation pattern of compound **6b**.

It was found that the pyrazole derivatives **3a, 3b**, and **3d** having both acetamide and ester moieties were found to be the most active compounds against Gram-negative bacteria *S. marcescens* and *P. merabities* with MICs (100 μg/ml) compared with the reference compound. Also, the amide derivatives **3e** and compound **5a** having both 5-amino and 6-benzyl moieties were found the most active compounds against *S. marcescens*. In addition, the amide derivatives **3c, 3f**, and **5f** (*N*-aminopyrazolopyrimidine) showed higher activity against *P. merabities* (MICs of 100 μg/ml) compared with antibiotics Ampicillin and Trivid. On the other hand, the amide derivatives **3b, 3c**, and **3d** were found to be the most active compounds against Gram-positive bacteria *S. aureus* and *B. cereus* (with MICs of 100 μg/ml).

Most of the synthesized compounds showed a remarkable activity against *A. oschraceus* wilhelm and less active than fungicide claforan.

From these results it can be concluded that the biologically active compounds **3a, 3b, 3d, 3f, 5a**, and **5f** (MIC values were 100 μg/ml) are nearly as active as standard antibiotics Ampicillin and Trivid, and less active than the fungicide claforan.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded in potassium bromide on a Perkin-Elmer 841 grating spectrophotometer (Perkin-Elmer, USA). ^{1}H NMR spectra were recorded on a Varian EM 360 (240 MHz) instrument using TMS as an internal standard (chemical shift in δ ppm). Microanalytical data (C, H, N) were in agreement with the proposed structure within +0.4% of the theoretical values determined at the Microanalytical Centre, Cairo University, Egypt. Mass spectra were run using HP Model MS-5988.

The 5-amino-4-ethoxycarbonyl-1-phenylpyrazole **1** was synthesized according to the literature method [17]. All the other reagents were of reagent grade.

TABLE 1 Antimicrobial Activity of the Synthesized Compounds

	Inhibition Zones[a] (mm)				
Compound	Serratia marcesens *(IMRU-70)*	Proteus merabities *(NTC-289)*	Staphylococcus aureus *(NCTC-7447)*	Bacillus cereus *(ATCC-14579)*	Aspergillus oschraceus *Wilhelm (AUCC-230)*
3a	24 (100)	26 (100)	10	10	20
3b	26 (100)	26 (100)	20 (100)	20 (100)	20
3c	18	24 (100)	20 (100)	26 (100)	10
3d	26 (100)	24 (100)	20 (100)	20 (100)	10
3e	24 (100)	14	10	10	20
3f	14	24 (100)	10	10	20
5a	26 (100)	14	10	10	20
5b	14	14	10	10	20
5c	14	14	10	18	20
5d	14	14	10	18	20
5e	14	14	10	10	20
5f	14	26 (100)	10	10	20
7	14	14	10	18	10
8	14	14	10	18	20
Ampicillin	30	29	28	30	–
Trivid	32	30	28	28	–
Claforan	–	–	–	–	29

[a]Slight activity: 10–14 mm (+); moderate activity: 14–18 mm (++); high activity: 20–26 mm (+++); very high activity ≥26 mm (++++).

*Synthesis of the Ethyl-5-(phenyl or aryloxy)acetylamino-1-phenylpyrazole-4-carboxylate (**3a–f**)*

To a solution of **2a–f** (0.01 mol) and **1** (2.31 g, 0.01 mol) in xylene (50 ml), phosphorus trichloride (3 ml) was added. The reaction mixture was heated under reflux for 3–4 h. The crude product was recrystallized from ethanol to yield 76% of **3a**, m.p. 131–133°C. IR (KBr, cm^{-1}) 3166, 2977, 1716, 1662; ^{1}H NMR δ 1.1 (t) 3H, CH_3 ethyl; δ 4.0 (q) 2H, CH_2 ethyl; δ 4.6 (s) 2H, CH_2CO; δ 7.2–8.0 (m) 10H, Ar—H; δ 10.1 (s) 1H, NH.

3b: Yield, 68% m.p. 123–125°C, IR (KBr, cm^{-1}) 3147, 2931, 1701, 1690; ^{1}H NMR δ 1.3 (t) 3H, CH_3 ethyl; δ 4.3 (q) 2H, CH_2 ethyl; δ 4.6 (s) 2H, CH_2CO; δ 7.0–7.8 (m) 10H, Ar—H; δ 8.2 (s) 1H, CH pyrazole; δ 10.4 (s) 1H, NH.

3c: Yield, 62%, m.p. 129–131°C; IR (KBr, cm^{-1}) 3201, 2920, 1690, 1670.

3d: Yield, 81%, m.p. 121–123°C; IR (KBr, cm^{-1}) 3163, 2927, 1716, 1697; ^{1}H NMR δ 1.2 (t) 3H, CH_3 ethyl; δ 2.2 (s) 3H, CH_3; δ; 4.4 (q) 2H, CH_2 ethyl; δ 5.3 (s) 2H, CH_2CO; δ 6.8, 7.1 (2d) 4H, Ar—H, AB system; δ 7.4–7.8 (m) 5H, Ar—H; δ 8.1 (s) 1H, CH; δ 10.3 (s) 1H, NH.

3e: Yield, 68%, m.p. 127–129°C; IR (KBr, cm^{-1}) 3201, 2930, 1710, 1700, 1630; ^{1}H NMR δ 1.2 (t) 3H, CH_3 ethyl; δ 4.2 (q) 2H, CH_2 ethyl; δ 4.8 (s) 2H, CH_2CO; δ, 6.9–8.0 (m) 12H, Ar—H; δ 8.3 (s) 1H, CH; δ 10.4 (s) 1H, NH.

3f: Yield, 92%, m.p. 135–137°C; IR (KBr, cm^{-1}) 3180, 2920, 1720, 1690; ^{1}H NMR δ 1.3 (t) 3H, CH_3 ethyl; δ 4.4 (q) 2H, CH_2 ethyl; δ 4.9 (s) 2H, CH_2CO; δ 7.0–8.1 (m) 12H, Ar—H; δ 8.4 (s) 1H, CH; δ 10.3 (s) 1H, NH.

*Synthesis of 2-Benzyl- or 2-Aryloxymethyl-3-amino-1-phenyl-pyrazolo[3,4-d]pyrimidine-4-one (**5a–f**)*

A mixture of **3a–f** (0.01 mol) and hydrazine hydrate (95%) (0.05 mol) were dissolved in *n*-butanol (30 ml) and refluxed for 3–5 h. The solvent was concentrated and the residue was recrystallized from ethanol to give **5a–f**.

5a: Yield, 62%, m.p. 165–167°C; IR (KBr, cm^{-1}) 3380, 3250, 2924, 1697, 1616; ^{1}H NMR δ 4.4 (s) 2H, CH_2; δ 5.7 (s) 2H, NH_2 (exchangeable D_2O); δ 7.1–8.0 (m) 10H, Ar—H; δ 8.5 (s) 1H, CH.

5b: Yield, 81%, m.p. 131–133°C; IR (KBr, cm^{-1}) 3313, 3201, 2910, 1666, 1612; MS (*m/z*): 317 ($M^+ - NH_2$), 300, 235, 181, 116, 91, 77.

5c: Yield, 84%, m.p. 190–192°C; IR (KBr, cm^{-1}) 3300, 3240, 2930, 1670, 1610.

5d: Yield, 86%, m.p. 141–143°C; IR (KBr, cm^{-1}) 3394, 3313, 2908, 1682, 1620; ^{1}H NMR δ 2.2 (s) 3H, CH_3; δ 4.5 (s) 2H, CH_2O; δ 6.3 (s) 2H, NH_2 (exchangeable D_2O); δ 6.8, 7.2 (2d) 4H, Ar—H, AB system; δ 7.5–7.7 (m) 5H, Ar—H; δ 8.0 (s) 1H, CH.

5e: Yield, 88%, m.p. 154–156°C; IR (KBr, cm^{-1}) 3294, 3147, 2924, 1658; MS (*m/z*): 383 (M^+), 382 (M − 1), 351, 269, 246, 174, 143, 87, 77.

5f: Yield, 93%, m.p. 170–172°C; IR (KBr, cm^{-1}) 3309, 3201, 2930, 1680, 1627; MS (*m/z*): 383 (M^+), 240, 224, 210, 185, 142, 116, 78 (Scheme 4).

*Synthesis of 2-Substituted-3-amino-1-phenyl-pyrazolo[3,4-d]pyri-midine-4-thione (**6a–c**)*

A mixture of **5d–f** (0.01 mol) and phosphorus penta sulfide (0.015 mol) in pyridine (50 ml) was refluxed for 16 h. The reaction mixture was cooled and poured onto ice water/HCl. The obtained product was recrystallized from dioxan to give **6a–c**.

6a: Yield, 67%, m.p. 122–124°C; IR (KBr, cm^{-1}) 3440, 2924, 1620, 1234; ^{1}H NMR δ 2.3 (s) 3H, CH_3; δ 4.1 (s) 2H, CH_2; δ 5.7 (s) 2H, NH_2; δ 7.1–8.1 (m) 9H, Ar—H; δ 8.2 (s) 1H, CH.

6b: Yield, 59%, m.p. 115–117°C; IR (KBr, cm^{-1}) 3417, 2923, 1628, 1396; MS (*m/z*): 399 (M^+), 383, 240, 241, 200, 182, 142, 115, 77.

6c: Yield, 63%, m.p. 127–129°C; IR (KBr, cm^{-1}) 3380, 2920, 1610, 1320.

*Synthesis of Ethyl-5-(cinnamoylamino)-1-phenylpyrazole-4-carboxylate (**7**)*

A mixture of cinnamic acid (1.48 g, 0.01 mol) and **1** (2.31 g, 0.01 mol) in xylene (50 ml) containing phosphorus trichloride (3 ml) was refluxed for 5 h. The obtained product was recrystallized from ethanol to give **7**. Yield, 77%, m.p. 122–124°C; IR (KBr, cm^{-1}) 3150, 2920, 1697, 1680, 1627; ^{1}H NMR (δ, ppm) δ 1.3 (t) 3H, CH_3; δ 4.3 (q) 2H, CH_2; δ 6.4, 6.6 (2s) 2H, CH=CH; δ 7.3–8.0 (m) 10H, Ar—H; δ 8.4 (s) 1H, CH, 10.2 (s) 1H, NH.

*Synthesis of 2,5-Diphenyl-2,3-dihydro-1H-pyrazolo[5′,1′,4,5]-pyrazolo[3,4-d]-pyrimidine-8-one (**8**)*

A mixture of **7** (3.6 g, 0.01 mol) and hydrazine hydrate (95%) (0.05 mol) were dissolved in *n*-butanol (30 ml) and refluxed for 4 h. The obtained product was recrystallized from ethanol to give **8**. Yield: 81%, m.p. 187–189°C; IR (KBr, cm^{-1}) 3325, 2924, 1690, 1612; ^{1}H NMR δ 4.1 (s) 2H, CH_2; δ 6.3 (s) 1H, CH; δ 7.2–8.1 (m) 10H, Ar—H; δ 9.0 (s) 1H, CH pyrazole.

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