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Synthesis of Certain Mercapto-and Aminopyrimidine Derivatives as Potential Antimicrobial Agents

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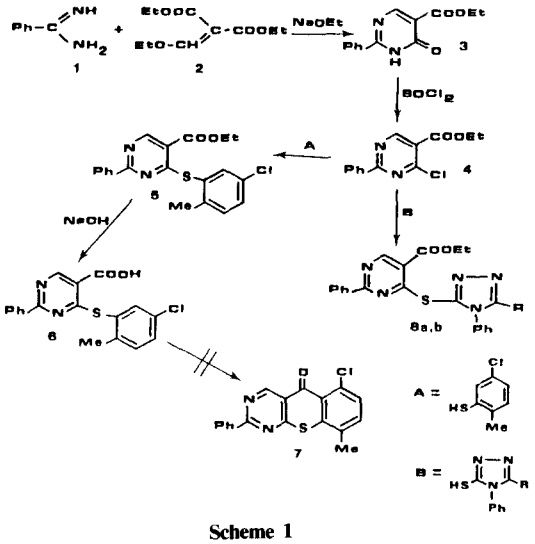
Abstract□Reaction of ethyl 4-chloro-2-phenylpyrimidine-4-carboxylate (**4**) with 5-chloro-2-methylthiophenol or 3-aryl-4-phenyl-1,2,4-triazole-5-thiol yielded the corresponding thioethers (**5**) and (**8a, b**), respectively. Careful alkaline hydrolysis of (**5**) yielded the corresponding carboxylic acid (**6**). Reaction of (**4**) with *p*-aminoacetophenone yielded compound (**10**) which was reacted with certain aromatic aldehydes to afford the α, β-unsaturated ketones (**11a-d**). Condensation of (**11a-d**) with malononitrile or phenylhydrazine yielded the 2-amino-3-cyanopyridines (**12a-f**) or the 2-pyrazolines (**13a, b**), respectively. Seven representative compounds were tested for their *in vitro* antimicrobial activity against some pathogenic micro-organisms, some of them were proved to be active.

Keywords□Synthesis, antimicrobial activity, pyrimidines, 1,2,4-triazoles, pyridines, 2-pyrazolines.

Pyrimidine nucleus constitutes the main part of several medicinally useful drugs. Due to the interference of some pyrimidine derivatives with DNA biosynthesis, certain pyrimidines were reported to possess bacterial[1-3], fungicidal[4, 5], antiviral[6-10], antineoplastic[8-13] and antiparasitic[14, 15] activities. In connection to a previous work[16], we wish to report the synthesis of certain pyrimidine derivatives carrying different arylmercapto or arylamino substituents as potential antimicrobial agents.

RESULTS AND DISCUSSION

The starting material, ethyl 4-chloro-2-phenylpyrimidine-5-carboxylate (**4**), was prepared by intertaction of benzamidine (**1**) with ethyl ethoxymethylenemalonate (**2**) in presence of sodium ethoxide to yield ethyl 4-oxo-2-phenyl-3,4-dihydropyrimidine-5-carboxylate (**3**)[17], which was then reacted with thionyl chloride to afford (**4**)[18]. Reaction of (**4**) with 5-chloro-2-methylthiophenol or 3-(4-pyridyl or 2-thienyl)-4-phenyl-1,2,4-triazole-5-thiol in dry acetone, in presence of anhydrous potassum carbonate furnished the thioethers (**5**) and (**8a, d**), respectively. Careful hydrolysis of compound (**5**) using 1% sodium hydroxide at room temperature yielded the corresponding carboxylic acid (**6**). Attempted cyclization of compound (**6**) to compound (**7**), by prolonged heating with polyphosphoric acid (PPA) or polyphosphoric acid ethyl ester (PPE), was unsuccessful and compound (**6**) was recovered unchanged. The action of cold sulpuric acid led to the same result, whereas heating with sulphuric acid at 50-60°C resulted in the formation of unidentified tarry matter (Scheme 1).



Scheme 1

Ethyl 4-(4-acetylphenylamino)-2-phenylpyrimidine-5-carboxylate (**10**), was prepared by heating under

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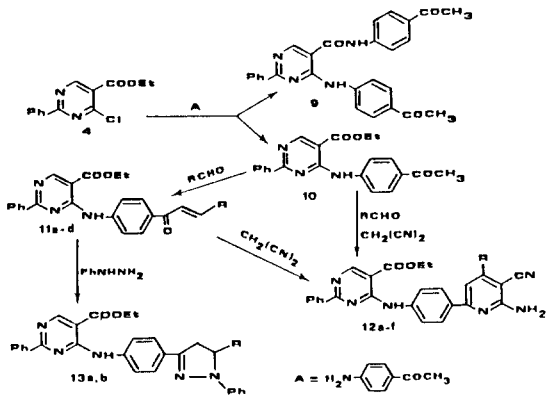

Scheme 2

reflux a mixture of equimolar amounts of (**4**) and *p*-aminoacetophenone in ethanol containing dilute hydrochloric acid for 30 minutes. The structural assignment of (**10**) was based on elemental analysis and IR data. Under this conditions, selective displacement of the chlorine atom took place leaving the ester moiety intact. Increasing the reaction time up to 3 hours resulted in the isolation of compound (**9**) in addition to (**10**) in poor yields. Compound (**9**) was also obtained in good yield (90%) by treating compound (**4**) with two equivalents of *p*-aminoacetophenone. Reaction of compound (**10**) with certain aromatic aldehydes in alcohlic sodium hydroxide solution yielded the required α , β-unsatured ketones (**11a-d**), which were condensed with malononitrile in presence of excess ammonium acetate, in acetic acid to yield the corresponding 2-amino-3-cyanopyridines (**12a-c**). Compounds (**12d-f**) were directly prepared by reaction of compound (**10**), the appropriate aldehyde, malononitrile and ammonium acetate in benzene. Reaction of compounds (**11a,b**) with phenylhydrazine in boiling acetic acid afforded the corresponding 2-pyrazolines (**13a, b**), under this condition cyclization took place without nucleophilic displacement of the ester moiety at position 6, as evidenced by elemental analysis and ^{1}H-NMR data.

The cup-plate method[19] was adopted for measuring the preliminary antimicrobial activity of compounds **6, 8a, 8b, 11b, 11d, 12a** and **12b**, against the micro-organisms, *Staphylococcus aureus* (S.A), *Pseudomonas aeruginosa* (P.A.) and *Candida albicans* (C.A.). Cups were made in agar plates that have been previously seeded with the micro-organisms. The cups were filled with a solution of the compound in dimethylformamide in a concentration of 1 mg/m*l* and the plates were incubated at 37°C for 24 hours. The diameter of the growth inhibition zones around the cups were measured to the nearest mm (Table I).

Table I. Results of antimicrobial activity of compounds 6, 8a, 8b, 11b, 11d, 12a and 12b

Comp. No.	S.A.	P.A.	C.A.
Control*	—	—	–
6	10	8	–
8a	12	10	–
8b	17	12	–
11b	18	10	–
11d	20	–	–
12a	12	18	–
12b	10	16	–
P.G.	21	#	#

*Cup containing dimethylformamide.
(–) inactive, no inhibition zone.
(#) not tested.

The results revealed that the tested compounds were found to be active against S.A. and P.S. rather than C.A., compounds **8b, 11b** and **11d** were the most active against S.A., while compounds **12a** and **12b** were the most active against P.A. None of the tested compounds exhibited superior activity over the antibiotic Penicillin G (P.G.) in a concentration of 100 i.u./m*l*.

EXPERIMENTAL

Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were obtained on a Pye Unicam SP 1000 spectrophotometer in KBr discs (ν , in cm^{-1}). ^{1}H-NMR spectra were obtained using an IBM FT-200 spectrometer (Chemical shift in δ , ppm). Crystallization solvents, melting points, yield percentages and molecular formulae of the newly synthesized compounds are shown in Table II.

Ethyl 4-[(5-chloro-2-methylphenyl) thio]-2-phenylpyrimidine-5-carboxylate (5)

A mixture of compound **4** (13.1g, 0.05 mole), 5-chloro-2-methylthiophenol (7.9g, 0.05 mole), anhydrous potassium carbonate (6.9g, 0.05 mole), in dry acetone (100 m*l*), was heated under reflux for 4 hours and the solvent was then removed *in vacuo*. The obtained residue was washed with water, filtered, dried and crystallized (Table II). ^{1}H-NMR (DMSO-d_6): 1.2-1.4 (*t*, 3H, $CH_2\underline{CH_3}$), 2.3 (*s*, 3H, CH_3), 4.2-4.4 (*q*, 2H, $\underline{CH_2}CH_3$), 7.3-7.9 (*m*, 8H, Ar-H) and 9.0 (*s*, 1H, pyrimidyl-H). IR: 1710 (C=O).

5-[(5-chloro-2-methylphenyl) thio]-2-phenyl-

Table II. Crystallization solvents, melting points, yield percentages and molecular formulae of the newly synthesized compounds

Comp. No.	R	Cryst Solv.	M.p. °C	Yield %	Molecular formula*
5	–	EtOH	125	70	$C_{20}H_{17}ClN_2O_2S$
6	–	EtOH	278	60	$C_{18}H_{13}ClN_2O_2S$
8a	4-Pyridyl	EtOH	>300	60	$C_{26}H_{20}N_6O_2S$
8b	2-Thienyl	EtOH	>300	65	$C_{25}H_{19}N_5O_2S_2$
9	–	EtOH	258	90	$C_{27}H_{22}N_4O_3$
10	–	EtOH	148	80	$C_{21}H_{19}N_3O_3$
11a	2-ClC_6H_4	EtOH	>300	60	$C_{28}H_{22}ClN_3O_3$
11b	4-ClC_6H_4	Acetone	>300	63	$C_{28}H_{22}ClN_3O_3$
11c	3-BrC_6H_4	H_2O/EtOH	>300	58	$C_{28}H_{22}BrN_3O_3$
11d	4-BrC_6H_4	EtOH	>300	60	$C_{28}H_{22}BrN_3O_3$
12a	2-ClC_6H_4	H_2O/EtOH	210	55	$C_{31}H_{23}ClN_6O_2$
12b	4-ClC_6H_4	H_2O/Acetone	185	60	$C_{31}H_{23}ClN_6O_2$
12c	4-BrC_6H_4	EtOH	230	55	$C_{31}H_{23}BrN_6O_2$
12d	4-$NO_2C_6H_4$	EtOH	155	60	$C_{31}H_{23}N_7O_4$
12e	4-Pyridyl	EtOH	285	55	$C_{30}H_{23}N_7O_2$
12f	2-Thienyl	EtOH	160	55	$C_{29}H_{22}N_6O_2S$
13a	2-ClC_6H_4	Acetone	200	50	$C_{34}H_{27}ClN_5O_2$
13b	4-BrC_6H_4	EtOH	185	55	$C_{34}H_{27}BrN_5O_2$

*All compounds gave satisfactory analysis for C, H, S or N within ±0.4% of the theoretical values.

pyrimidine-5-carboxylic acid (6)

A suspension of compound **5** (0.4g, 0.001 mole), in 1% sodium hydroxide solution (30 m*l*), was stirred for 24 hours at ambient temperature. The mixture was then heated on a water bath for 2 hours. On cooling, the mixture was acidified with dilute hydrochloric acid and the separated solid was filtered, washed with water, dried and crystallized (Table II). IR: 1680 (C=O) and 3100 (NH).

Ethyl 4-[(4-phenyl-5-aryl-1,2,4-triazol-3-yl) thio]-2-phenyl-pyrimidine-5-carboxylates (8a,b)

A mixture of compound **4** (2.6g, 0.01 mole), 4-phenyl-3-(4-pyridyl or 2-thienyl)-1,2,4-triazole-5-thiol (0.01 mole), anhydrous potassium carbonate (1.0g), in dry acetone (20 m*l*), was heated under reflux for 6 hours, then filtered hot and allowed to stand overnight. The separated solid was filtered, dried and crystallized (Table II). ^{1}H-NMR, **8b** (DMSO-d_6): 1.25-1.35 (*t*, 3H, $CH_2\underline{CH_3}$), 4.3-4.45 (*q*, 2H, $\underline{CH_2}CH_3$), 7.3-8.1 (*m*, 13H, Ar-H) and 8.9 (*s*, 1H, Pyrimidyl-H).

Ethyl 4-(4-acetylphenylamino)-2-phenylpyrimidine-5-carboxylate (10)

A mixture of compound **4** (2.6g, 0.01 mole), *p*-aminoacetophenone (1.35g, 0.01 mole), 10% hydrochloric acid (10 m*l*), in ethanol (10 m*l*), was heated under reflux for 30 minutes. On cooling, the reaction mixture was rendered alkaline with dilute ammonium hydroxide solution and the separated solid was filtered, dried and crystallized (Table II). IR: 1680 (C=O) and 3100 (NH).

4-(4-Acetylphenylamino)-5-(4-acetylphenylaminocarbonyl)-2-phenyl-pyrimidine (9)

A mixture of compound **4** (2.6g, 0.01 mole), *p*-aminoacetophenone (2.7g, 0.02 mole), 10% hydrochloric acid (10 m*l*), in ethanol (10 m*l*), was heated under reflux for 3 hours and continued as mentioned under compound **10** (Table II). IR: 1680 (C=O), 1760 (CONH) and 3100 (NH).

Ethyl 4-[4-(3-aryl-1-oxo-2-propen-1-yl) phenylamino]-2-phenyl-pyrimidines (11a-d)

The appropriate aldehyde (0.01 mole), was added to a solution of compound **10** (3.6g, 0.01 mole) in 2.5% ethanolic sodium hydroxide solution (10 m*l*) and the mixture was stirred at ambient temperature for 3 hours. The separated solid was then filtered, dried and crystallized (Table II). IR (**11b**): 1660 & 1680 C=O) and 3280 (NH).

Ethyl 4-[4-(2-amino-4-aryl-3-cyanopyridin-6-yl) phenylamino]-2-phenylpyrimidine-5-carboxylates (12a-f)

Method A (compounds 12a-c): A mixture of compound **11a-c** (0.005 mole), malononitrile (0.33g, 0.005 mole) and ammonium acetate (3.1g, 0.04 mole), in ethanol (30 m*l*), was heated under reflux for 8 hours. The reaction mixture was heated under reflux for 8 hours. The reaction mixture was then concentrated to half of its original volume. On cooling, the separated solid was filtered, dried and crystallized (Table II). IR (**12b**): 1700 (C=O), 2220 (CN) and 3100, 3320 & 3400 (NH & NH_2).

Method B (compounds 12a-f): A mixture of compound **10** (3.6g, 0.001 mole), malononitrile (0.66g, 0.01 mole), the appropriate aldehyde (0.01 mole) and ammonium acetate (1.5g, 0.02 mole), in benzene (5 m*l*), was heated under reflux for 4 hours using water trap. The solvent was then removed *in vacuo* and the obtained residue was treated with ethanol (5 m*l*) and left overnight. The obtained solid was filtered, washed with water, dried and crystallized (Table II). ^{1}H-NMR, **12e** (DMSO-d_6): 1.35-1.55 (*t*, 3H, $CH_2\underline{CH_3}$), 4.3-4.45 (*q*, 2H, $\underline{CH_2}CH_3$), 6.7-8.1 (*m*, 16H, Ar-H & NH_2), 9.1 (*s*, 1H, pyrimidyl-H) and 11.5 (*s*, 1H, NH).

Ethyl 4-[4-(4,5-dihydro-5-aryl-1-phenyl-1H-pyrazol-3-yl) phenylamino]-2-phenylpyrimidine-5-carboxylates (13a,b)

Phenylhydrazine (1.1g, 0.01 mole), was added to a solution of compound **11a, b** (0.01 mole), in acetic acid (20 m*l*), and the mixture was heated under reflux for 5 hours. On cooling, the separated solid was filtered, dried and crystallized (Table II). ^{1}H-NMR, **13a** (DMSO-d_6): 1.3-1.45 (*t*, 3H, $CH_2\underline{CH_3}$), 4.35-4.5 (q, 2H, $\underline{CH_2}CH_3$) 4.4-4.5 (*m*, 2H, pyrazolinyl-CH_2 at 4-), 5.5-5.7 (*m*, 1H, pyrazolinyl-H at 5-), 6.7-8.6 (*m*, 19H, Ar-H & NH) and 8.9 (*s*, 1H, pyrimidyl-H).

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