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Synthesis and Antioxidant Activity of S-[*bis*-2-(Chloroethyl)-amino]-4-substituted-6-phenyl Pyrimidine-2-yl-ethanethioate Derivatives with Nitrogen Mustards

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A series of new chalcones containing pyrimidine heterocyclic ring have been prepared by the condensation of various aldehydes with acetophenone followed by cyclization with thiourea in presence of alcoholic KOH. Chalcones thus prepared were subjected to acetylation with chloroacetyl chloride and the resultant S-chloroacetyl derivatives were subjected to a nucleophilic substitution reaction with diethanolamine by heating under reflux in pyridine. The compounds thus obtained were subjected to a chlorination reaction using phosphorous oxychloride to get title compounds. Compounds prepared were recrystallized from acetone, alcohol and diethyl formamide. All the synthesized compounds were screened for their antioxidant activity by 2,2'-diphenyl-1-picryl hydrazyl and 2,2'-azino *bis*- 3-ethylbenzothiazoline-6-sulphuric acid methods.

Key Words: Chalcones, Pyrimidines, Antioxidant activity.

INTRODUCTION

From the review of the literature, it is revealed that a number of heterocyclic compounds containing pyrimidine ring are associated with diverse pharmacological properties such as antimicrobial[1], antiviral[2], anticonvulsant[3], antidepressant and antitumour activity[4]. Literature review[5] suggested that oxidative damage is a common underlying mechanism in the progression of a large number of disorders such as atherosclerosis, carcinogenesis, arthritis, cataract and muscular degeneration. Therefore with a view to observe their effect on antioxidant activity, synthesis of some newer pyrimidine derivatives containing nitrogen mustards were attempted. The title compounds were synthesized as depicted in **Scheme-I**.

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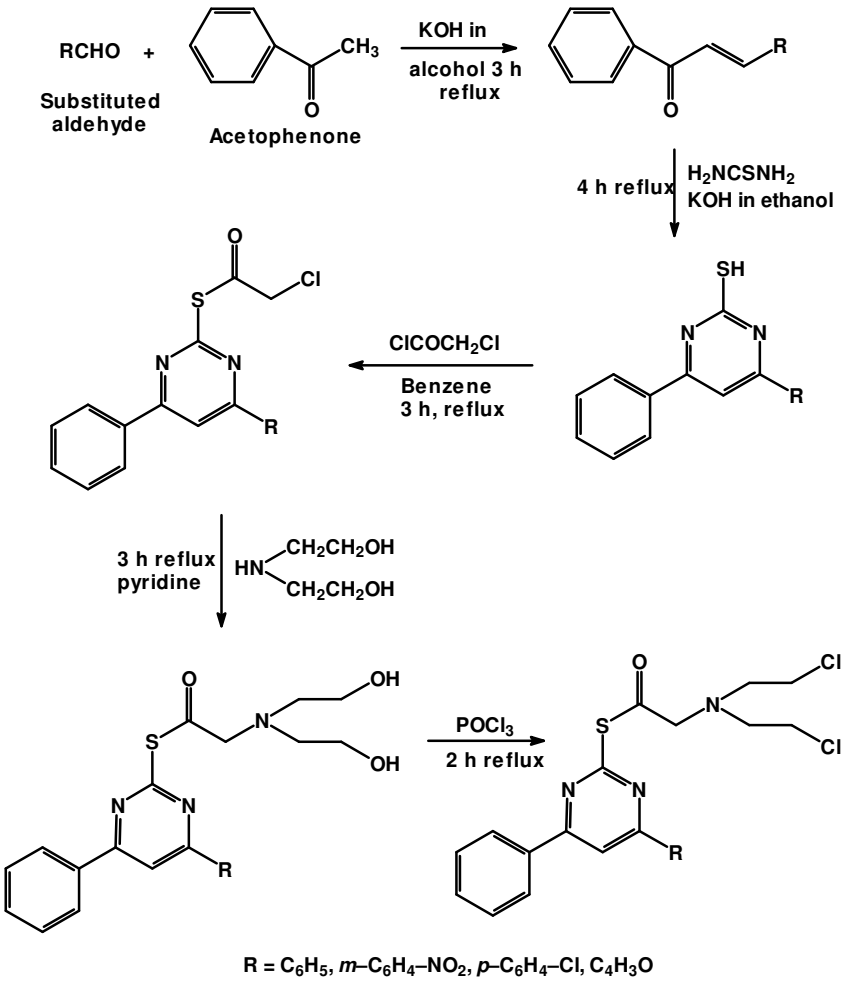

R = C_6H_5, *m*–C_6H_4–NO_2, *p*–C_6H_4–Cl, C_4H_3O

Scheme-I

EXPERIMENTAL

Melting points reported were determined in open capillaries using Veego VMP-1 melting point apparatus. The reactions were monitored by TLC (Precoated-merck) using methanol:ethyl acetate (1:1) and detected by UV and also using iodine as visualizing agent. The IR spectra of the compounds were recorded on Perkin-Elmer Infrared-283 spectrophotometer using KBr cm^{-1} and ^1H NMR spectra (DMSO-d_6) on EM 390 MHz spectrometer using TMS as internal standard (chemical shifts are expressed in δ ppm).

Synthesis of substituted chalcones: 3-Substituted-1-phenyl-propen-1-one[6]: To a solution of potassium hydroxide (0.55 mol) in alcohol (0.02 mol) was added acetophenone (0.43 mol) and benzaldehyde/substituted benzaldehydes (0.43 mol), the reaction mixture was stirred in an ice bath

for several hours until stirring was not possible owing to it's thickness. The flask was left overnight in a refrigerator, acidified with dil. HCl. The solid product was filtered and washed with ice cold water. The product was dried in air and recrystallized from acetone.

IR (KBr, ν_{max}, cm^{-1}): 3068 (Ar, C-H), 1656 (C=O), 1608 (CH=CH) and 1564 (C=C, aromatic). ^{1}H NMR (DMSO-d_6): δ 8.13-8.96 (12H, m, aromatic protons including chalcone protons CH=CH, R=C_6H_5).

Synthesis of 4-substiuted phenyl-6-phenyl pyrimidin-2-thiol[7]: A mixture of chalcone (0.05 mol), thiourea (0.05 mol) and KOH (0.01 mol) in ethanol (10 mL) was refluxed for 4 h. This was slowly poured into 400 mL of cold dil. HCl solution with continuous stirring. After 1 h, this was kept in a refrigerator for 1 d. The precipitated solid was washed with water, filtered, dried and recrystallized from petroleum ether. The purity was established by single spot on the TLC using ethyl acetate: petroleum ether (1:0.5) as solvent system.

IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar, C-H), 2924 (SH), 1676 (C=N) and 1569 (C=C,aromatic). ^{1}H NMR (DMSO-d_6): δ 3.30 (s, 1H, SH), 7.92-8.74 (m, 11H, aromatic R=C_6H_5).

Synthesis of S-[4-(substituted phenyl)-6-phenyl pyrimidin-2-yl]-chloroethane thioate[8]: To the phenyl pyrimidin-2-thiol derivatives (0.01 mol), chloroacetyl chloride (0.012 mol) was added and heated under reflux in dry benzene (20 mL) for 3 h on a steam bath. Benzene was distilled off to a possible extent and cooled. The product resulted was filtered, washed with small portions of petroleum ether and dried. The product was purified by recrystallization from alcohol to get a crystalline solid.

IR (KBr, ν_{max}, cm^{-1}): 3056 (Ar, C-H), 1656 (C=O), 1608 (C=N), 1560 (C=C, aromatic) and 734 (C=Cl). ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 2H, -CH_2Cl), 8.1-8.9 (m, 11 H, aromatic, R=C_6H_5).

Synthesis of S-[*bis*-2-(hydroxyethyl)-amino]-4-(substituted phenyl)-6-phenyl pyrimidin-2-yl-ethanethioate[9]: A mixture of chloroacetyl derivatives (0.01 mol) and diethanol amine (0.012 mol) in dry pyridine (20 mL) was heated under reflux for 3 h, then pyridine was distilled off as far as possible and the residue was poured into a little crushed ice containing few drops of hydrochloric acid, with stirring. It was kept aside for overnight and the product resulted was filtered and washed with small portion of cold water and dried. It was recrystallized from appropriate solvents to get pure compound.

IR (KBr, ν_{max}, cm^{-1}): 3464 (OH), 3024 (Ar, C-H), 1654 (C=O), 1617 (C=N) and 1570 (C=C, aromatic). ^{1}H NMR (DMSO-d_6): δ 3.12 (s, 2H, -CH_2N), 3.28 (t, 4H, OH-CH_2-CH_2-N-CH_2-CH_2OH), 3.56 (t, 4H, OH-CH_2-CH_2-N-CH_2-CH_2-OH), 5.42 (s, 2 × OH) and 7.82-8.89 (m, 11H, aromatic, R=C_6H_5).

Synthesis of S-[*bis*-2-(chloroethyl)amino]-4-substituted-6-phenyl pyrimidin-2-yl-ethanethioate[10]: A mixture of S-[*bis*-2-(hydroxyethyl)-amino]derivatives (0.01 mol) and $POCl_3$ (20 mL) was gently refluxed for 2 h. The excess $POCl_3$ was removed under vacuum, then poured into cold water to obtain the solid. The product was recrystallized from suitable solvents.

IR (KBr, ν_{max}, cm^{-1}): 3056 (Ar, C-H), 1687 (C=O), 1633 (C=N), 1576 (C=C, aromatic) and 737 (C=Cl). ^1H NMR (DMSO-d_6): δ 3.76 (s, 2H, $-CH_2N-$), 3.32 [t, 4H, $-N(CH_2-CH_2-Cl)_2$], 3.61 [t, 4H, $-N(CH_2-CH_2-Cl)_2$ and 7.94-8.76 (m, 11H, aromatic R=C_6H_5)].

TABLE-1
ANALYTICAL DATA OF S-[*bis*-2-(CHLOROETHYL)-AMINO]-4-SUBSTITUTED-6-PHENYL PYRIMIDIN-2-YL-ETHANETHIOATE DERIVATIVES

Compd. no.	Substituent R	m.f.	m.w.	m.p. (ºC)	Yield (%)	R_f value
I	C_6H_5	$C_{22}H_{21}N_3SOCl_2$	445	202-204	68	0.76
II	*m*-$C_6H_4NO_2$	$C_{22}H_{20}N_4SO_3Cl_2$	490	218-219	72	0.82
III	*p*-C_6H_4Cl	$C_{22}H_{20}N_3SOCl_3$	479	196-197	62	0.72
IV	C_4H_3O	$C_{20}H_{19}N_3SO_2Cl_2$	435	223-225	66	0.78

Antioxidant activity: The antioxidant activity of the samples was assessed on the basis of radical scavenging effect of the stable DPPH free radical. The assay was carried out in a 96 well microtitre plate (Tarsons Products (P) Ltd., Kolkata). To 2 mL of DPPH solution, 0.1 mL of each of the test sample was added separately in test tubes. The tubes were incubated at 37 ºC for 0.5 h and absorbance of each solution was measured at 490 nm against the corresponding test blanks. The remaining DPPH was calculated. IC_{50} value is the concentration of the sample required to scavenge 50 % DPPH free radical.

RESULTS AND DISCUSSION

All the above synthesized compounds were tested for their antioxidant activity, using *in vitro* DPPH and ABTS methods. Among the four compounds tested, compound-**III**, containing nitroderivative, exhibited potent antioxidant activity in the ABTS method. The IC_{50} value of the compound was found to be 25 µg/mL. The compound-**I** was found to be weakly active and the other two compounds were inactive.

In the DPPH method, all the four compounds exhibited moderate activity. Compound-IV was found to be more potent among the four compounds (IC_{50} = 180.00 ± 12.50) Compound-III was the least active (IC_{50} = 463.33 ± 21.09). However, the standards, ascorbic acid and Rutin showed very low IC_{50} values (2.69 ± 0.05 and 3.91 ± 0.10, respectively) indicating their potent nature (Table-2).

TABLE-2
in vitro ANTIOXIDANT ACTIVITY OF COMPOUNDS **I-IV**
BY DPPH AND ABTS METHODS

Substituent R	IC50 values ± SE (μg/mL)* by	
	DPPH	ABTS
C_6H_5 - **I**	263.00 ± 10.17	815
m-$C_6H_4NO_2$ - **II**	231.00 ± 6.29	> 1000
p-C_6H_4Cl - **III**	463.33 ± 21.09	25
C_4H_3O - **IV**	180.00 ±12.50	> 1000
Ascorbic acid	2.69 ± 0.05	11.25 ± 0.49
Rutin	3.91 ± 0.10	0.519 ± 0.01

*Average of three determinations.

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