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Synthesis, characterisation and antimicrobial activity of novel substituted pyrimidines

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ABSTRACT

Novel pyrimidine compounds were synthesised by condensation of substituted primary amine, ethyl acetoacetate and urea/ thiourea. This multicomponent reaction is of much importance due to excellent pharmacological properties of pyrimidines. On this account, we synthesized some substituted pyrimidines and their derivatives. Structures of these compounds were confirmed by 1H-NMR, 13C-NMR, FT-IR, and mass spectroscopy. The antibacterial activities of the synthesized compounds were screened against standard strains of Gram positive and Gram negative bacteria using the Agar plate diffusion technique. Most of the studied compounds showed promising activities against gram positive and gram negative bacteria.

Key words: Pyrimidines compounds, Nitroso and benzoyl derivatives, antimicrobial activity, Gram positive and Gram negative strains.

INTRODUCTION

Organic compounds play a vital role in modern society and possess various applications in different fields due to which research has been going on to synthesize new organic compounds including its derivative. In that heterocyclic compounds containing nitrogen, sulphur etc is an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes [1, 2]. Naturally occurring nucleic acids, plant alkaloids, some vitamins, proteins, hormones etc contains a pyrimidine base. Thus study of these compounds is the researcher's present view.

A large number of heterocyclic compounds containing pyrimidine rings are associated with diverse pharmacological properties such as antimicrobial, anticancer anticonvulsant, antiviral, anti-HlV, antifungal and antibacterial activities[3-7]. Pyrimidine are of great importance in fundamental metabolism for uracil, thiamine and cytosine, that are three bases found in the nucleotide and hence pyrimidine bases play significant role in vital biochemical processes for humans and animals.

Pyrimidine a heterocyclic compound having four carbon atoms and two nitrogen atoms in the six member ring. To this ring oxygen and sulphur are attached. Subsequently their derivatives are prepared. Due to N-C-S / N-C-O/N-C-N linkage it is used as antitubercular, antibacterial, antimicrobial, antitumor, insecticidal, fungicidal, herbicidal agents, tranquilizers and various dyes.

In the present study pyrimidines were synthesised by the reaction of 1, 3-diketones with primary aromatic amine (According to Paal- Knorr Synthesis). The Nitroso and Acetyl derivatives of these compounds are also prepared. Further characterisation and antimicrobial activity was performed[8,9].

MATERIALS AND METHODS

All chemicals used for the synthesis were of analytical grade. H NMR spectra were recorded on BRUKER AVANCE II 400 NMR Spectrometer. IR spectra were recorded by using AFFINITY-1 FTIR SPECTROPHOTOMETER. Melting points were determined by using INDO Melting Point M-AB-92 apparatus and were uncorrected. Purity of compounds and completion of reactions were checked by thin layer chromatography (TLC). The crude compounds were purified by recrystallization from ethanol. Mass spectra were also recorded.

Section A: Synthesis of 1-(N-phenyl-carboxamido)-propan-2-one:-
An equimolar (0.01 M) mixture of substituted primary amine and ethyl acetoacetate in ethanol (99% pure ethanol) was refluxed for 6-8 hrs. The reaction mixture was cooled and poured onto crushed ice while stirring continuously. Resultant solid was filtered, washed thoroughly with cold water, dried and purified by recrystalisation from ethanol to form **Ia**. Similarly **(Ib – If)** were synthesised by above method.

NMR:[Ia] Ar-H=δ(7.03 to 7.26ppm), NH=δ(9.21ppm), CH_2= δ(3.59ppm),-CH_3=δ(2.33ppm), OCH_3=δ(3.87ppm)

Section B: Synthesis of 4-[amino phenyl]-5-methyl pyrimidines :-
1-(N-phenyl-carboxamido)-propan-2-one (Ia) (0.01M) was then refluxed with urea/ thiourea (0.01M) in ethanol as a solvent for 2-3 hrs. After refluxing the reaction mixture was allowed to cool and poured it onto crushed ice. Shiny, white crystals of pyrimidines (IIa) were obtained. These were recrystallised with hot water. Similarly compounds **(IIb – IIf)** were synthesised.

Table 1: Physical data of synthesised pyrimidines (IIa to IIf)

SR. NO	1-N-phenyl-carboxamido)-propan-2-one	4-[amino phenyl]-6-methyl pyrimidines	MOL. FORMULA	MP(°C)	YIELD (%)	ELEMENTAL ANALYSIS obs (cal)				
						C	H	N	O	S
01	1-N [2-Methoxy Phenyl amido] propane-2-one **(Ia)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine **(IIa)**	$C_{12}H_{13}N_3O_2$	72	76	60.88 (62.33)	5.21 (5.62)	17.34 (18.18)	12.75 (13.85)	-----
02	1-N [3-Methoxy Phenyl amido] propane-2-one **(Ib)**	4-[3-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine **(IIb)**	$C_{12}H_{13}N_3O_2$	62	81	61.45 (62.33)	5.33 (5.62)	18.03 (18.18)	12.95 (13.85)	-----
03	1-N [4-Methoxy Phenyl amido] propane-2-one **(Ic)**	4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine **(IIc)**	$C_{12}H_{13}N_3O_2$	112	85	63.65 (62.33)	4.13 (5.62)	18.45 (18.18)	12.08 (13.85)	-----
04	1-N [2-Methoxy Phenyl amido] propane-2-one **(Ia)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine **(IId)**	$C_{11}H_{11}N_3OS$	92	68	54.08 (56.65)	5.14 (4.72)	17.45 (18.02)	6.17 (6.86)	17.16 (13.73)
05	1-N [3-Methoxy Phenyl amido] propane-2-one **(Ib)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine **(IIe)**	$C_{11}H_{11}N_3OS$	>300	85	55.16 (56.65)	4.44 (4.72)	17.56 (18.02)	5.87 (6.86)	16.97 (13.73)
06	1-N [4-Methoxy Phenyl amido] propane-2-one **(Ic)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine **(IIf)**	$C_{11}H_{11}N_3OS$	80	72	54.88 (56.65)	5.69 (4.72)	17.25 (18.02)	6.32 (6.86)	15.86 (13.73)

Analytical results (NMR & IR):
(1) 4-[2-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine (IIa) : Ar-H=δ(6.87 to 7.07ppm), NH=δ(9.43 ppm), -CH= δ(3.47ppm),-CH_3=δ(2.22ppm), -OCH_3=δ(3.47ppm) ; IR: Ar-OCH_3Str = 1035.84 cm^{-1}, N-H Str.=3075.63 cm^{-1}, C=N Str. =1514.19cm^{-1}, C-N Str. =1359.87 cm^{-1}, C=O Str. = 1712.86 cm^{-1}

(2) 4-[3-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine (IIb) Ar-H=δ(6.48 to 6.96 ppm), NH=δ(9.48ppm), -CH= δ(3.43ppm),-CH_3=δ(2.18ppm), -OCH_3=δ(3.49ppm) ; IR: Ar-OCH_3Str = 1065.04 cm^{-1}, N-H Str.=3078.21cm^{-1}, C=N Str. =1526.23cm^{-1}, C-N Str. =1350.17 cm^{-1}, C=O Str. = 1731.46 cm^{-1}

(3) 4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine (IIc) Ar-H=δ(7.04 to 7.07 ppm), NH=δ(9.33ppm), -CH= δ(3.34 ppm), -CH_3=δ(2.06 ppm), -OCH_3=δ(3.66 ppm) ; IR: Ar-OCH_3Str = 1053.32 cm^{-1}, N-H Str.=3065.68 cm^{-1}, C=N Str. =1518.10cm^{-1}, C-N Str. =1344.57 cm^{-1}, C=O Str. = 1720.93 cm^{-1}

(4) 4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine (IId) Ar-H=δ(6.96 to 7.48ppm), NH=δ(8.14ppm), -CH= δ(3.04ppm),-CH_3=δ(2.53ppm), -OCH_3= δ(3.55ppm) ; IR: Ar-OCH_3Str = 1073.43 cm^{-1}, N-H Str.=3072.74 cm^{-1}, C=N Str. =1686.82cm^{-1}, C-N Str. =1326.12 cm^{-1}

(5) 4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine (IIe) Ar-H=δ (7.47 to 7.50ppm), NH=δ (8.26), -CH= δ(3.13ppm),-CH_3=δ(2.08ppm), -OCH_3= δ(3.24ppm) ; IR: Ar-OCH_3Str = 1076.03 cm^{-1}, N-H Str.=3062.40 cm^{-1}, C=N Str. =1680.80 cm^{-1}, C-N Str. =1312.42 cm^{-1}

(6) 4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine (IIf) Ar-H=δ (7.04 to 7.08ppm), NH=δ(8.18 ppm), -CH= δ(3.18ppm),-CH_3=δ(2.15ppm), -OCH_3 = δ(3.62ppm) ; IR: Ar-OCH_3Str = 1067.34 cm^{-1}, N-H Str.=3034.72 cm^{-1}, C=N Str. =1676.30cm^{-1}, C-N Str. =1329.30 cm^{-1}

Section C: Derivatives of pyrimidines:

a) *Nitroso derivative of pyrimidiness*:-

IIa, IIb, IIc, IId, IIe, IIf (0.01 M) was made into solution with conc. HCl. Cool this solution at 0-5° C. To this acidic solution 5ml of 20% sodium nitrite was added with continuous stirring. The reaction mixture was allowed to stand for half an hrs for completion of reaction. It was filtered through Buchner funnel and washed with water [10 – 11]. Recrystalised with ethanol to form **IIIa –IIIf**. Physical data were shown in Table 2.

Table 2: Physical data of synthesised Nitroso derivatives of pyrimidines (III a to III f)

SR. NO	4-[amino phenyl]-6-methyl pyrimidines (II)	4-[Phynyl amino] 6-Methyl 2-N- [Oxa] , 3- Nitroso Pyrimidine(III)	MOL. FORMULA	MP (°C)	ELEMENTAL ANALYSIS obs (cal)				
					C	H	N	O	S
1	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine **(IIa)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] , 3- Nitroso Pyrimidine **(IIIa)**	$C_{12}H_{12}N_4O_3$	110	57.86 (55.38)	5.21 (4.61)	20.96 (21.53)	16.05 (18.46)	-----
2	4-[3-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine **(IIb)**	4-[3-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] 3- Nitroso Pyrimidine**(IIIb)**	$C_{12}H_{12}N_4O_3$	185	54.88 (55.38)	4.21 (4.61)	20.36 (21.53)	17.15 (18.46)	-----
3	4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine **(IIc)**	4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] 3- Nitroso Pyrimidine**(IIIc)**	$C_{12}H_{12}N_4O_3$	>300	54.33 (55.38)	4.93 (4.61)	20.04 (21.53)	16.95 (18.46)	-----
4	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine**(IId)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Nitroso Pyrimidine**(IIId)**	$C_{12}H_{12}N_4O_2S$	130	55.26 (52.17)	4.13 (4.34)	20.56 (20.28)	11.05 (11.59)	10.16 (11.59)
5	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine**(IIe)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Nitroso Pyrimidine**(IIIe)**	$C_{12}H_{12}N_4O_2S$	178	55.78 (52.17)	4.60 (4.34)	20.32 (20.28)	11.62 (11.59)	10.28 (11.59)
6	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine **(IIf)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Nitroso Pyrimidine**(IIIf)**	$C_{12}H_{12}N_4O_2S$	>300	55.67 (52.17)	4.19 (4.34)	20.06 (20.28)	12.55 (11.59)	11.30 (11.59)

Table 3: Physical data of synthesised Benzoyl derivatives of pyrimidines (IV a to IV f)

SR. NO	4-[amino phenyl]-6-methyl pyrimidines (II)	4-[Phenyl amino] 6-Methyl 2-N- [Oxa] , 3- Benzoyl Pyrimidine (IV)	MOL. FORMULA	MP (°C)	ELEMENTAL ANALYSIS obs (cal)				
					C	H	N	O	S
1	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine **(IIa)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] , 3- Benzoyl Pyrimidine **(IVa)**	$C_{19}H_{17}N_3O_3$	116	66.53 (68.05)	5.21 (5.07)	11.57 (12.53)	14.05 (14.32)	-----
2	4-[3-Methoxy Phenyl amino] 6-Methyl 2-N-[Oxa] Pyrimidine **(IIb)**	4-[3-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] 3- Benzoyl Pyrimidine **(IVb)**	$C_{19}H_{17}N_3O_3$	89	67.08 (68.05)	4.23 (5.07)	12.05 (12.53)	13.58 (14.32)	-----
3	4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] Pyrimidine **(IIc)**	4-[4-Methoxy Phenyl amino] 6-Methyl 2-N- [Oxa] 3- Benzoyl Pyrimidine **(IVc)**	$C_{19}H_{17}N_3O_3$	110	68.23 (68.05)	4.97 (5.07)	10.50 (12.53)	13.65 (14.32)	-----
4	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine**(IId)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Benzoyl Pyrimidine **(IVd)**	$C_{19}H_{17}N_3O_2S$	118	63.26 (64.95)	4.13 (4.84)	10.22 (11.96)	8.27 (9.11)	10.16 (9.11)
5	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine**(IIe)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Benzoyl Pyrimidine **(IVe)**	$C_{19}H_{17}N_3O_2S$	108	65.78 (64.95)	4.60 (4.84)	11.32 (11.96)	11.62 (9.11)	10.28 (9.11)
6	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] Pyrimidine **(IIf)**	4-[2-Methoxy Phenyl amino] 6-Methyl 2-N- [Thia] 3- Benzoyl Pyrimidine **(IVf)**	$C_{19}H_{17}N_3O_2S$	120	63.67 (64.95)	4.19 (4.84)	10.06 (11.96)	12.55 (9.11)	11.30 (9.11)

b) *Benzoyl derivative of pyrazoles*:
IIa, IIb, IIc, IId, IIe, IIf (0.01 M) of compound was mixed with NaOH solution .The reaction mixture was cooled on ice bath. Approximately 2ml Benzoyl Chloride was added drop wise and shake. Allow the reaction mixture to settle down. Filter the mixture [12-13]. Recrystallised with ethanol to form **IVa – IVf**. Physical data were shown in Table 3.

Section D: Antimicrobial Activity:
*Preparation of sample***:**
0.001 g/ 1 mg was taken and dissolved in 1 ml of DMSO.

Preparation of inoculums:
Stock cultures were maintained at 4^{o}C on slants of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated for 24 hrs at 37^{o}C .

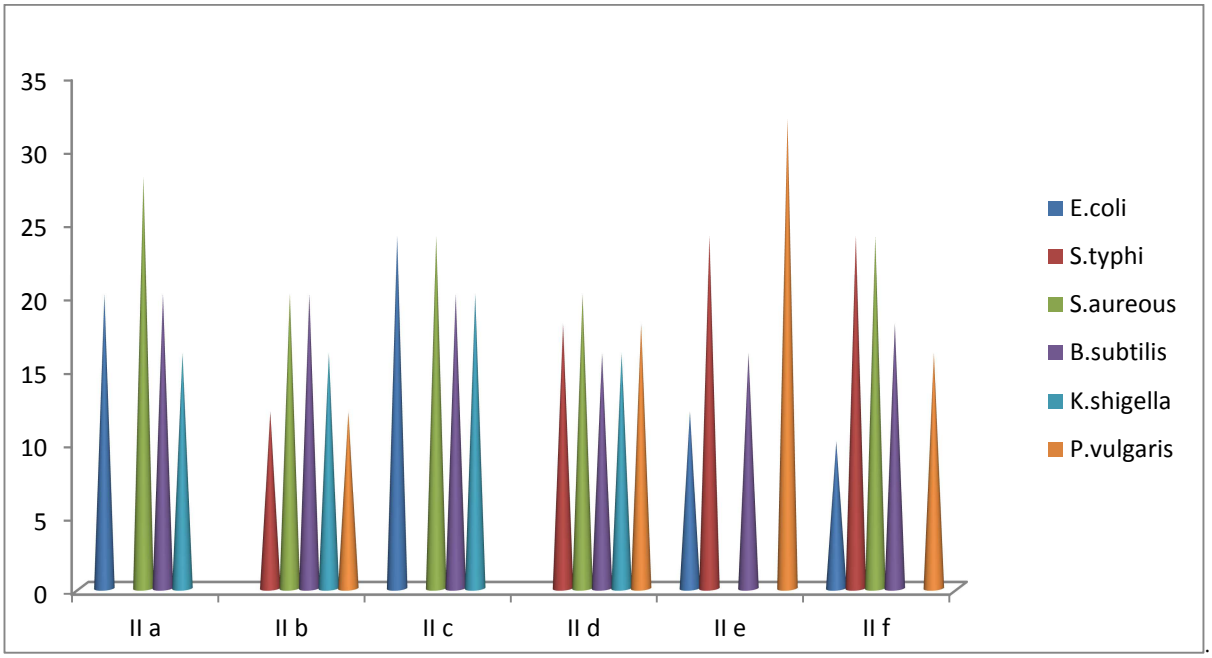
Screening of Bacteria:
The disk diffusion method was used for antimicrobial activity. The nutrient agar were poured in Petri plates and allowed it to solidify. The above prepared microbial cultures were spread uniformly on the surface of the agar. The diffused disks of each sample are placed on the agar. Plates were then incubated at 37°C for 24hrs [14-16]. Antimicrobial results were shown in Table 4.

Table 4: Results of Antimicrobial Testing for Synthesised compounds (II a to II f)

Name of Organism	Zone of Inhibition in mm					
	IIa	**IIb**	**IIc**	**IId**	**IIe**	**IIf**
Eichersia coli	20	-	24	-	12	10
Salmonella typhi	-	20	-	18	24	24
Staphylococcus aureous	28	20	24	20	-	24
Bacillus subtilis	20	20	20	16	16	18
Klebsiella shigella	16	16	20	16	-	-
Proteus vulgaris	-	12	-	18	32	16

Results of antimicrobial activity of synthesised pyrimidines were graphically represented as shown in figure 1.

Fig 1: Graphical representation of Antimicrobial Activity Results



RESULTS AND DISCUSSION

Novel pyrimidines were synthesised according to procedure explained above in experimental section. Structural conformations were carried out by H NMR, IR and Mass spectroscopic data. Simultaneously, Nitroso and benzoyl derivatives also prepared by simple techniques.

Antimicrobial activities of some synthesised compounds were determined by Agar Plate diffusion technique. Compounds were examined for antimicrobial activities against gram +ve and gram –ve strains. The diameter of inhibition zone around each disc was measured in mm. Results shows, various compounds gives moderate antimicrobial activity for selected gram +ve and gram –ve strains. In feature these pyrimidines and its derivatives are used as drugs. Similarly further studies were carried out for other strains of organisms.

CONCLUSION

Novel pyrimidines were successfully synthesised via multicomponent reaction starting from primary amines and ethyl acetoacetate. These compounds were structurally confirmed by analytical techniques. From antimicrobial results we can conclude that, compounds containing Nitrogen and Sulphur showed more antimicrobial activity as compared to that of compound containing only Nitrogen.

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