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MICROWAVE ASSISTED SYNTHESIS AND ANTIBACTERIAL STUDIES OF OXADIAZOLE SUBSTITUTED PYRIMIDINE COMPOUNDS

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ABSTRACT

A series of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one derivatives have been synthesized, by changing various substituted benzaldehyde. Simple synthetic methods of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one (3a-e) are described. Compound 1 is converted to hydrazine carbothiamide2 by reacting compound 1 with thiosemicarbazide in catalytic amount of acetone is irradiated with help of domestic microwave oven (200W) for 2 minutes. Compound 2is an intermediate for the final compounds. The compound 2 is converted to corresponding oxadiazole3 by treatment with NaOH follow by KI. Structural elucidation is accomplished by IR, ^{1}H and ^{13}CNMR, Elemental analysis and GC-Mass spectral data of the synthesized compounds. Few of these Pyrimidine derivatives have been evaluated for their possible antibacterial activity. Most of the tested compounds show significant antibacterial activity.

KEYWORDS

Pyrimidine, Oxadiazole, Carbothiamide, Antibacterial activity and Microwave.

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INTRODUCTON

Pyrimidine derivatives and antimicrobial agent[1] were found to be associated with variety of biological activities such as insecticidal, antimicrobial, antiviral etc., pyrimidine derivatives[2-8] with powerful C-C bond formation have broad applications over the preparation of diverse aminoalkyl derivatives. It involves the condensation of compound with the ability of supplying one or more active hydrogen atom to aldehyde and primary or secondary amine. Mannich bases are physiologically reactive because of the molecule

soluble in aqueous solvent when it is transformed into ammonium salt. Medicinal uses of several Mannich bases have been reviewed by Tromontini and Angiolini[9]. Besides this, considerable work has been reported on synthesis and pharmacological activities of various Mannich bases for antiviral, analogies, antispasmodic, anesthetic and antimalarial as well as intermediates in drug synthesis. Antiviral properties of certain urea and thiourea derivatives have been reported, in which the antiviral effect is attributed due to the presence of an intact NH-(C=S)-NH and NH-(C=O)-NH grouping[10]. In this direction the synthesis and pharmacological study of Mannich bases of 3-and 5-mercapto derivatives of 1, 3, 4-oxadiazole have been reported in literature[11-16]. Several, pyrimidines, fused heterocyclic pyrimidine derivatives and dihydropyrimidones are known for their biological activity such as antiviral, antitumor, antimicrobial fungicide, algaecide and as antibiotics [17-26]. Moreover, the presences of different functional groups show their great synthetic potential. In continuation of this work, herein is reported that the synthesis and *in vitro* study of antibacterial activity of heterocyclic N-Mannich bases of 5-(5-amino-1,3,4-oxadiazol-2yl)-3,4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one (3a-e) against *Streptococcus faecalis (Gram +ve),Bacillus sps (Gram +ve) and Escherichia coli (Gram –ve)*and Ciprofloxacinis used as standard drug. For this purpose, heterocyclic precursors DHPMs (1a-e) are synthesized by microwave irradiation of aromatic aldehydes, ethylacetoacetate and thiourea according to the literature procedure[27,28] and these DHPMs are used to synthesis compounds (2a-e) and characterized by using elemental analysis, mass spectra, ^{1}H and ^{13}CNMR spectral studies.

Experimental section

Melting points are determined using open capillary method and are uncorrected. The compounds are checked for homogeneity by TLC on silica gel-G. The FT-IR Thermo Nicolet Avatar 370 spectrophotometer is used to record IR spectra using KBr disc method. The ^{1}H and ^{13}CNMR are recorded on Bruker Avance-III 400MHz FTNMR spectrometer using DMSO-d_6 and mass spectrums are recorded on Joel GC-mate spectrometer. Elemental analysis was recorded on Elemental Vario EL-III instrument. All compounds given satisfactory micro analytical results. Pyrimidine (1) is prepared by reported method[27].

RESULTS AND DISCUSSION

Compounds (3a-e) were synthesized according to the scheme 1and 2. The compound 3a is prepared by reacting hydrazine carbothioamide compound 2 a with NaOH follow by KI. Hydrazine carbothioamide compound 2a is synthesized by reacting pyrimidine ethyl ester 1 with thiosemicarbazide is irradiated in a domestic microwave oven (200W) for 2 minutes[29] and cooled to obtain the product which is recrystallized from ethanol.

The pyrimidine ethyl ester compound 1a prepared by a mixture of aromatic aldehyde (0.01mol), ethylacetoacetate (0.01mol) and urea (0.01mol) is mixed thoroughly with 0.15 mole of tin (II) chloride as catalyst in a conical flask. The content of the flask is irradiated (400W) in a domestic microwave oven for 6 minutes. The completion of the reaction is monitored by TLC. The synthesized compounds are confirmed by IR, ^{1}H and ^{13}C-NMR, GC-MS and CHN analysis. Formation of compound 2a is confirmed by the N-H stretching peaks at 3365, 3241 cm^{-1} and 3116 cm^{-1} and C=O stretching peaks at 1724 cm^{-1} in IR and singlet at δ6.50 for NH_2 group in ^{1}HNMR spectra.

Treatment of compound 2a with NaOH follow by KI, furnished 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one (3a-e). The structure of 3a is elucidated on the basis of C-O-C linkage in the oxadiazole ring, which causes a sharp absorption band at 1020 cm^{-1} in its IR spectrum. ^{1}HNMR spectrum showed a singlet at δ 3.98 due to NH_2 functionality confirmations of the structure 3a.

The IR and ^{1}HNMR spectral data revealed carbonyl absorption band at 1699 cm^{-1} of NH-CO-NH group, N-N stretching band at1089 cm^{-1} aliphatic C-H and aromatic C-H stretching at 2978 cm^{-1}and 3033cm^{-1} for pyrimidine moiety 3. Mass spectrum also

supports the proposed structure by viewing molecular ion peak at*m/z* 271 M^+.

General Procedure

Synthesis of 5-(hydrazine carbothioamide)-6-methyl-4-phenyl-3, 4-dihydropyrimidine -2(1*H*)-one (2a)

An equimolar mixture of compound**1** (0.01mol) and thiosemicarbazide (0.01mol) with catalytic amount of acetone is irradiated in a domestic microwave oven (200W) for 2 minutes and cooled to obtain the product which is recrystallized from ethanol. The compounds prepared in this manner (2a-e) are listed in Table**1**. Melting point of the compound is 140^0C yield 85%. ^{1}HNMR (400MHz, DMSO-d_6) δ 2.251 (s, 3H), 5.152 (d, *J* = 3.2Hz,1H), 6.501 (s, 2H), 7.213–7.336 (m, 5H), 7.702 (d, *J*= 2.8Hz, 1H), 8.175 (d, *J*= 6.4Hz, 2H,), 9.149 (s, 1H); ^{13}CNMR (400MHz, DMSO-d_6) δ 17.72, 59.17, 99.33, 126.21, 127.23, 128.34, 148.25, 151.71, 152.16, 165.33, 178.40; FT-IR (KBr) 3365, 3241, 3116 (NH), 3079 (Ar-H), 2978 (CH), 1724 (C=O), 1385 (C-N), 1219 (C=S), 1089 (N-N) cm^{-1}; GCMS: *m/z* 305 [M^+]. Elemental Anal. (%) ($C_{13}H_{15}O_2N_5S$), Calculated; C 51.17, H 4.94, N 22.50, S 10.47. Found; C 51.10, H 4.85, N 22.24, S 10.94.

General procedure for Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one (3a)

General procedure for Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-phenylpyrimidin-2(1*H*)-one, (3a), for the compounds (3a-e) are listed in Table No.2, carbothioamide2 (0.01 mol) is added into 10% NaOH with cooling and shaking. Then Iodine solution in KI is added gradually and shaking until the Iodine color persisted. This reaction mixture is heated continuously for 5hr and it is concentrated the residue, its cooled and poured onto ice cold water. This solution is filtered and acidified with 10% HCl to isolate the product. It is filtered and washed with cold water and little amount of $CS_2$is added. The product is purified by recrystallization from alcohol. m.p.175-177^0C, Yield 78%. ^{1}HNMR (DMSO-d_6): δ 2.264 (s,3H,CH_3), 3.987 (s, 2H, NH_2), 5.167 (*J*=3.2Hz, d, 1H, CH), 7.245-7.347 (m, 5H, Ar-H), 7.717 (*J*=1.6Hz, d, 1H, NH), 9.165 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ17.73, 59.14, 99.29, 126.22, 127.21, 128.33, 142.03, 144.83, 148.28, 152.14, 165.31; FT-IR (KBr): 3375, 3242, 3115(NH), 3033 (Ar-H), 2978(CH), 1699(C=O), 1598 (C=N), 1340 (C-N), 1089 (N-N), 1020cm^{-1}(C-O); GCMS: *m/z* [271 M^+].

Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-4-(4-chlorophenyl)-3, 4-dihydro-6-methylpyrimidin-2(1*H*)-one (3b): **1**HNMR (DMSO-d_6): δ2.255 (s, 3H, CH_3), 3.997 (s,2H, NH_2), 5.152 (*J*=3.6Hz, d,1H, CH), 7.243-7.299 (m, 4H, Ar-H), 7.735 (*J*=2.8Hz, d, 1H, NH), 9.207 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ17.76, 59.21, 98.85, 128.15, 128.34, 131.77, 143.76, 148.65, 151.94, 165.17; FT-IR (KBr): 3376, 3244, 3116(NH), 2980 (Ar-H), 2956 (CH), 1723 (C=O), 1534 (C=N), 1367 (C-N), 1170 (C-O), 1087cm^{-1}(N-N); GCMS: *m/z* [305 M^+].

Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-4-(4-hydroxyphenyl)-6-methylpyrimidin-2(1*H*)-one (3c):^{1}HNMR(DMSO-d_6): δ2.286 (s, 3H, CH_3), 4.046 (s, 2H, NH_2), 5.100 (*J*=3.6Hz, d, 1H, CH), 6.765-7.793 (q, 2H, Ar-H), 7.013-7.033 (q, 2H, Ar-H), 9.187 (*J*=1.2Hz, d, 1H, NH), 9.347 (s, 1H, NH), 10.296 (s, 1H, OH); ^{13}CNMR (DMSO-d_6): δ17.69, 59.07, 99.78, 114.96, 127.36, 135.41, 136.72, 148.10, 151.98, 152.17, 165.25; FT-IR(KBr): 3291 (OH), 3218,3121 (NH), 3021 (Ar-H), 2981 (CH), 1692 (C=O), 1514 (C=N), 1316 (C-N), 1101 (N-N), 1023cm^{-1} (C-O); GCMS: *m/z* [287 M^+].

Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-4-(4-(dimethylamino) phenyl)-3, 4-dihydro-6-methylpyrimidin-2(1*H*)-one (3d):**1**HNMR (DMSO-d_6): δ2.227 (s, 3H, CH_3), 2.848 (s, 6H, $N(CH_3)_2$), 3.986 (s, 2H, NH_2), 5.038 (*J*=4.4Hz, d, 1H, CH), 6.658 (*J*=8.8Hz, d, 2H, Ar-H), 7.022-7.058 (m, 2H, Ar-H), 7.539 (*J*=2.8Hz, d, 1H, NH), 9.041(s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ17.68, 53.28, 59.05, 99.89, 112.29, 126.86, 132.76, 134.25, 147.50, 149.65, 152.25, 165.46. FT-IR (KBr): 3380, 3245, 3114 (NH), 2953 (Ar-H), 2811 (CH), 1719 (C=O), 1560 (C=N), 1384 (C-N), 1091 (N-N), 1025cm^{-1}(C-O); GCMS: *m/z* [314 M^+]. Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl-4-(3-nitrophenyl) pyrimidin-2(1*H*)-one (3e): ^{1}HNMR (DMSO-d_6): δ2.283 (s, 3H, CH_3), 4.039 (s, 2H,

NH_2), 5.314 (*J*=3.6Hz,d, 1H, CH), 7.699-8.142 (m, 4H, Ar-H), 8.722 (*J*=10.4Hz, d, 1H, NH), 9.310 (s, 1H, NH); ^{13}CNMR (DMSO-d_6): δ17.81, 59.35, 98.35, 122.26, 130.10, 130.14, 132.94, 146.96, 147.70, 148.30, 149.35, 151.77, 165.02; FT-IR (KBr): 3566, 3440, 3335 (NH), 3090 (Ar-H), 2966 (CH), 1707 (C=O), 1526 (C=N), 1316 (C-N), 1088 (N-N), 1007cm^{-1} (C-O); GCMS: *m/z* [316 M^+].

Antibacterial studies

Among the newly synthesized pyrimidine derivatives are screened for their antibacterial activity *in vitro* against the species of *Streptococcus faecalis, Bacillus sps and Escherichia coli,* using agar well disk diffusion method.

The test compounds are dissolved in DMSO to get a solution of 10µg/mL concentration. The inhibition zones are measured in millimeters at the end of an incubation period of 18hrs at 37^{0}C. Ciprofloxacin is used as a standard and the results are shown in Table No.3. Most of the tested compounds show moderate to good inhibition.

Table No.1: Physical and analytical data of compounds (2a-e)

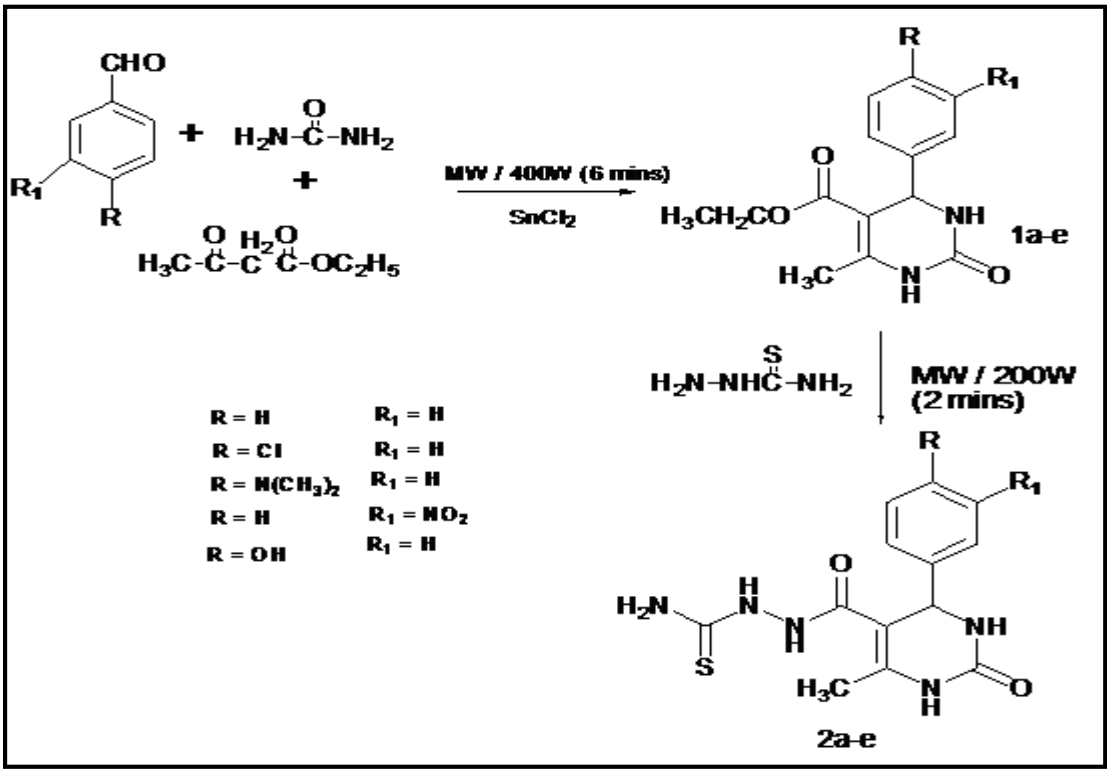
Compd.	Mol. Formula	R	R_1	X	Mol.Wt	Yield (%)	m.p (^{0}C)	Calcd. /Found (%)			
								C	N	H	S
2a	$C_{13}H_{15}O_2N_5S$	H	H	O	305	85	140	51.17 (51.94	22.50 22.24	4.94 4.85	10.47 10.94)
2b	$C_{13}H_{14}O_2N_5SCl$	Cl	H	O	339	70	145	46.05 (46.30	20.65 20.94	4.15 4.60	9.42 9.49)
2c	$C_{13}H_{15}O_3N_5S$	OH	H	O	321	83	160	48.62 (48.75	21.18 21.19	4.70 4.32	9.95 9.36)
2d	$C_{15}H_{20}O_2N_6S$	$N(CH_3)_2$	H	O	348	78	170	52.35 (52.79	24.42 24.77	5.84 5.83	9.28 9.85)
2e	$C_{13}H_{14}O_4N_6S$	H	NO_2	O	350	81	132	44.60 (44.06	24.00 24.07	4.02 4.43	9.13 9.22)

Table No.2: Physical and analytical data of compounds (3a-e)

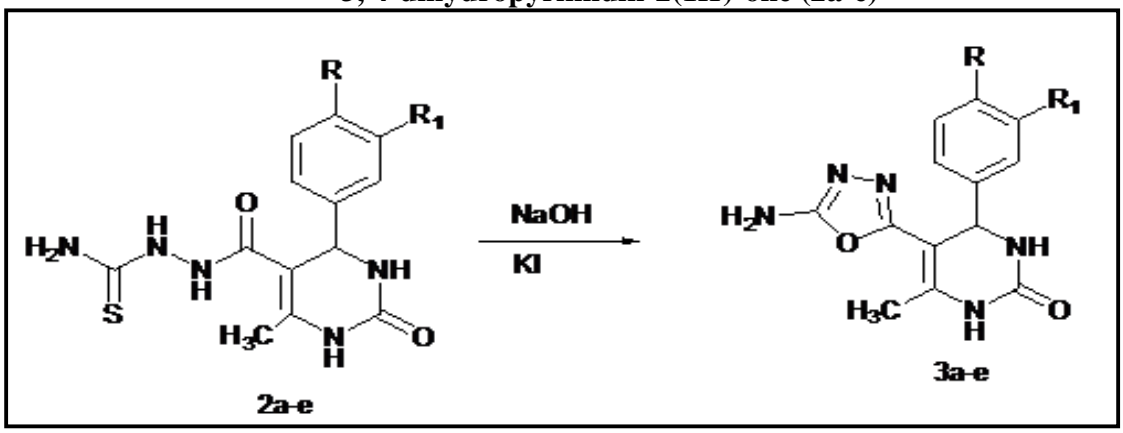
Compd	Mol. Formula	R	R_1	X	Mol.Wt	Yield (%)	m.p (^{0}C)	Calcld. /Found (%)			
								C	N	H	S
3a	$C_{13}H_{13}O_2N_5$	H	H	O	271	78	175-177	57.52 (57.59	25.80 25.82	4.07 4.83	0.00 0.00)
3b	$C_{13}H_{12}O_2N_5Cl$	Cl	H	O	305	72	197-199	51.28 (51.07	22.76 22.95	3.64 3.96	0.00 0.00)
3c	$C_{13}H_{13}O_3N_5$	OH	H	O	287	85	124-126	54.10 (54.38	24.95 24.38	4.57 4.56	0.00 0.00)
3d	$C_{15}H_{18}O_2N_6$	$N(CH_3)_2$	H	O	314	88	204-206	57.51 (57.34	26.71 26.75	5.25 5.77	0.00 0.00)
3e	$C_{13}H_{12}O_4N_6$	H	NO_2	O	316	69	135-137	49.89 (49.39	26.46 26.82	3.86 3.82	0.00 0.00)

Table No.3: Antibacterial activities of compounds (3a-e)

S.No	Compound	Streptococcus faecalis(+ve)	Bacillus sps (+ve)	Escherichia coli (-ve)
1	Control	0	0	0
2	3a	7	8	-
3	3b	9	12	-
4	3c	12	6	9
5	3d	7	9	-
6	3e	9	4	6



Scheme No.1: Synthesis of 5-(hydrazine carbothioamide)-6-methyl-4-phenyl-3, 4-dihydropyrimidin-2(1*H*)-one (2a-e)



Scheme No.2: Synthesis of 5-(5-amino-1, 3, 4-oxadiazol-2-yl)-3, 4-dihydro-6-methyl 4-Phenylpyrimidin-2(1*H*)-one (3a-e)

CONCLUSION

The investigation of antibacterial screening data reveals that, all the tested compounds show moderate to good inhibition at 10µg/ml concentration. Especially, the compound 3b and 3c shows very good activity than the others and also the compound3e show moderate inhibition against all the three species.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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