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Research Article

Synthesis, characterization and antimicrobial, antifungal and antitubercular activity of some 3, 5- substituted isoxazoles, 4-[(3, 5-substituted, 1H-pyrazol-1-yl) carbonyl] pyridines and 4, 6-substituted pyrimidin-2-amines

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ABATRACT

In present study various chalcones were synthesized by the base catalyzed reaction between substituted aromatic ketones and substituted aromatic aldehydes. These chalcones were then subjected to the reaction with hydroxyl amine hydrochloride, guanidine hydrochloride and isoniazid to give 3,5- disubstituted isoxazoles, 4,6-disubstituted pyrimidine-2-amines and 3,5-disubstituted pyrazole derivatives respectively. All the synthesized compounds were characterized by IR, NMR and mass spectroscopy. Further these disubstituted compounds were evaluated for their antimicrobial, antifungal and antitubercular activity.

Keywords: Chalcones, substituted isoxazoles, antimicrobial, antifungal, antitubercular activity.

INTRODUCTION

Synthesis of isoxazole, pyrazole and pyrimidine derivatives has been a subject of consistent interest because of the wide applications of such heterocycles in pharmaceutical as well as agrochemical industry. Numerous compounds containing isoxazole, pyrazole and pyrimidine moiety have been reported as active hypoglycemic, antidiabetic[1-3], antipyretics, analgesics, anti-inflammatory[4-6], antiviral[7-9], antiallergic[10], anticancer agent[11-13], antidrepressant[14-15] and antimicrobial agents[16-19] which include antibacterial sulfonamides, semisynthetic penicillins and cephalosporines. These observations led us to attempt the synthesis of isoxazole, pyrazole and pyrimidine derivatives from the calchones made from aromatic aldehydes and aromatic ketones and to evaluate the synthesized compounds against

antimicrobial, antifungal and antitubercular activity.

MATERIALS AND METHODS

Melting points were determined in open capillaries and are uncorrected. The homogeneity of all compounds synthesized was checked by E-Merck precoated 0.2 mm silica gel 60 F 254 TLC plates and the spots were rendered visible by exposing to UV light or iodine vapours. The IR spectra were recorded on 8400F Shimazdhu FTIR spectrophotometer at University of Pune, using KBr disc pellet method. ^{1}HNMR spectra were recorded on AVANCE II 400 NMR spectrometer at Chandigadh University and Verian Mercury 300 at University of Pune and Jeol FT NMR AL-300 at National Institute of Sciences, Mumbai by using TMS as an internal standard and chemical shifts are expressed as δ ppm units. GC Mass spectra were recorded on GC-MS-QP-5050 Schimadzu at University of Pune. Antitubercular activity was evaluated by using the facilities at Maratha Mandals Dental Medical College & Research Centre, Belgaum, India.

Preparation of Various Chalcones (1)[20]

Appropriate acetophenone (0.01 mol) in ethanol and aromatic aldehyde (0.01 mol) in ethanol were mixed and 10 ml of 40% sodium hydroxide solution was added with stirring. The resulting solution was kept overnight at room temperature. The contents of mixture was then poured over crushed ice and acidified with dil. HCl. The solid obtained was filtered, dried and recrystallized from ethanol.

Preparation of substituted 3, 5-diphenyl isoxazole (2a – 2d)

Appropriate Chalcone (1) (0.01 mol) was dissolved in ethanol (10 ml) and a mixture of hydroxylamine hydrochloride (0.8 gm) in ethanol and water was added. Few drops of KOH (50%) were then added with stirring. The reaction mixture was refluxed for 9 hrs. The solid obtained was filtered off and recrystallized from ethanol. The physical data is presented in table 1 and spectroscopic data is presented in table 2.

Preparation of 4-[(substituted 3, 5-diphenyl-1H-pyrazol-1-yl) carbonyl] pyridine (3a – 3d)

Appropriate Chalcone (1) (0.01 mol) was dissolved in ethanol (10 ml). Isoniazid (0.02 mol) in ethanol (8ml) was added with stirring. Few drops of glacial acetic acid were then added. The reaction mixture was refluxed for 12 hrs. The solid formed was filtered off and recrystallized from ethanol.

Preparation of substituted 4,6-biphenylpyrimidin-2-amine (4a – 4h)

Appropriate Chalcone (1) (0.01mol) was dissolved in 1,4-dioxan (10 ml). Guanidine hydrochloride (0.01 mol) in 1,4-dioxan (5 ml) was added with stirring. Few drops of glacial acetic acid were then added. The reaction mixture was refluxed for 15 hrs on oil bath at 105-110^{0}C. The mixture obtained was concentrated in vacuum and solid obtained was separated dried and recrystallized from ethanol.

Antimicrobial activity

The anti-microbial activity was carried out by using Cup-plate method[21] by using microbial strains *Bacillus subtilis* (NCIM 2711), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aerugenosa* (ATCC 27853) and *Kleibsella pneumoniae* (ATCC 4352) with incubation period of 24 hrs at temperature 37^{0}C. Norfloxacin (500 µg ml^{-1}) was used as standard drug. All the test compounds were used at the concentration of 2000 and 4000 µg ml^{-1}. The zone inhibition reader was used to measure zone of inhibition. The results obtained are presented in table 3.

Antifungal activity

Anti-fungal activity was carried out using Cup-plate method by using fungal strains *Aspergillus niger* (NCIM 515), *Candida albicans* (ATCC 60193). The plates were incubated for 48 hrs at 28^{0}C. Griseofulvin (500 µg ml^{-1}) was used as standard drug. Test compounds were used at concentrations of 2000 & 4000 µg ml^{-1}.

Antitubercular activity (Antitubercular Sensitivity Test)[6]

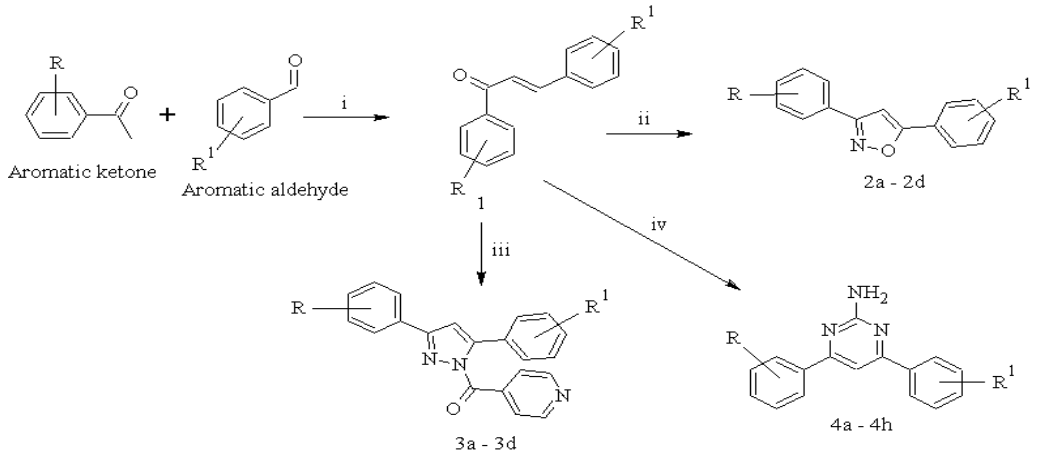
The anti-tubercular screening was carried out by Middle brook 7H-9 broth medium against *M. tuberculosis* H37Rv (ATCC 27294). The basal medium was prepared according to manufacturer's instructions (Hi-media) and sterilized by autoclaving. 4.5 ml of broth was poured into each one of the sterile bottles. To this, 0.5 ml of ADC supplement was added. This supplement contained catalase, dextrose, and bovine serum albumin fraction V. Then a stock solution (10 mg ml^{-1}) of the compound was prepared. Serial dilution was carried out so as to get the final concentrations of 10, 25 and 50 µg ml^{-1}. 10 µg suspension of *M. tuberculosis* H37Rv strain (100000 organisms/ml adjusted by McFarland's turbidity standard) was transferred to each of the tubes and incubated at 37°C. One growth control without test compounds and one drug control with isoniazid as standard was similarly prepared. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth. The growth was confirmed by making a smear from each bottle and by performing a Zeil-

nelson staining at the end of 4 weeks. The results obtained are presented in table 4.

RESULTS AND DISCUSSION

The results show that compounds 2c, 2d, 4a, 4c are active as antitubercular at 10 µg ml^{-1} concentration. Compounds 2c, 4a, 4b, 4e and 4g are active against *Bacillus subtilis* and *Staphylococcus aureus.* Compounds 2a, 4c, 4d and 4c are active against *Kleibsella pneumonia* and *Pseudomonas aerugenosa.* Interestingly from the results obtained it was found that all the synthesized compounds are active against *Candida albicans* and *Aspergillus niger* at 4000 µg ml^{-1} concentration. All other compounds are weakly active against test organisms.

Scheme of synthesis



i: Ethanol, 40% NaOH ii : $NH_2OH.HCl$, ethanol, few drops of 50% KOH, reflux for 9 hrs. iii: Isoniazid, few drops of glacial acetic acid, ehtanol, reflux for 12 hrs. iv: Guanidine hydrochloride, few drops of glacial acetic acid, 1,4-dioxane, reflux for 15 hrs

Table 1: Physical data of 3, 5- substituted isoxazoles, 4-[(3, 5-substituted, 1H-pyrazol-1-yl) carbonyl] pyridines and 4,6-substituted pyrimidin-2-amines

Comp	R	R^1	Molcular Formula	M.W.	M.P & Yield (%)	% Yield
2a	*p*-OH	*p*-H	$C_{15}H_{11}N\,O_2$	237.25	142^0C	55
2b	*p*-H	*m*-NO_2	$C_{15}H_{10}N_2O_3$	266.25	140^0C	50
2c	*p*-OH	*p*-Cl	$C_{15}H_{10}ClNO_2$	271.69	158-160^0C	60
2d	*p*-OH	*m*-NO_2	$C_{15}H_{10}N_2O_4$	282.25	186^0C	62
3a	H	*p*-Cl	$C_{21}H_{14}\,ClN_3\,O$	359.80	102^0C	62
3b	H	H	$C_{21}H_{15}N_3O$	325.36	96-98^0C	63
3c	*p*-OH	*p*-Cl	$C_{21}H_{14}ClN_3O_2$	375.80	158^0C	54
3d	*p*-OH	H	$C_{21}H_{15}N_3O_2$	341.36	130^0C	50
4a	*p*-OH	*o*-OH	$C_{16}H_{15}N_3O_2$	281.30	156-158^0C	60
4b	*p*-CH_3	*p*-Cl	$C_{17}H_{16}ClN_3$	297.78	136-140^0C	55
4c	*p*-CH_3	*m*-NO_2	$C_{17}H_{16}N_4O_2$	308.33	140^0C	50
4d	*p*-CH_3	*p*-N-(CH_3)	$C_{19}H_{22}N_4$	306.40	118^0C	62
4e	*p*-CH_3	*p*-OH	$C_{17}H_{17}N_3O$	279.33	115-120^0C	60
4f	*p*-CH_3	*o*-OH	$C_{17}H_{17}N_3O$	279.33	130^0C	57
4g	*p*-CH_3	*p*-OCH_3	$C_{18}H_{19}N_3O$	293.36	90^0C	52
4h	*p*-OH	*p*-OCH_3	$C_{17}H_{17}N_3O_2$	295.33	178^0C	60

Table 2: Analytical data

Comp	IR (cm^{-1})	^{1}H NMR (δ ppm) ,	GC-MS (m/z)
2a	829 (*p*-substituted Aromatic ring), 3053 (C-H str.)	^{1}HNMR (DMSO,400MHz): 6.75 (s, 1H, Isoxazole), 7.4-7.5 (m, 9H, Ar-H), 8.3 (s, 1H, -OH)	238 (M^+), 195, 152, 132.04, 105.03, 77, 42.
2b	829 (*p*-substituted Aromatic ring), 3068 (C-H str)	^{1}HNMR ($CDCl_3$,400MHz) 6.75 (s, 1H,Isoxazole), 7.4-7.8 (m, 6H, Ar-H), 8.2-8.65 (3H, Ar-H)	-
2c	829 (*p*-substituted Aromatic ring), 3068 (Ar.. CH str)	^{1}HNMR(DMSO,400MH) 6.75(s, 1H, Isoxazole), 6.9(m, 2H, Ar-H),7.5-7.8 (m, 6H, Ar-H), 8.2 (s, 1H, OH)	272 (M^+), 272.2 (Base.Peak), 178.01, 51.02, 67.01, 76.03.

2d	837 (*p*-substituted Aromatic ring), 3032 (Ar. CH str)	^{1}H NMR (DMSO, 400 MHz) 6.75 (s, 1H, Isoxazole), 7.0 (m, 2H, Ar-H), 7.9-8.6 (m, 3H, Ar-H), 7.5-7.8 (m, 3H, Ar-H), 8.3 (s, 1H, OH)	283 (M^+-1), 121.10 (Base. Peak), 221, 121, 93.03, 67.01, 51.
3a	1662(C=O str), 831(*p*-substituted Aromatic ring), 3049(Ar..CH str)	^{1}H NMR (DMSO ,400 MHz) 7.12 (d, 2H,Pyrazole), 7.4-7.6 (m, 5H, Ar-H), 8-8.9 (m, 4H,Phenyl), 8-8.9(m, 4H, Pyridine)	359.8 (M^+-1), 239.2 (Base Peak), 255, 218.08, 177, 106, 77.
3b	1662(C=O str), 831(*p*-substituted Aromatic ring), 3049(Ar..CH str)	^{1}H NMR ($CDCl_3$, 400 MHz) 7.12 (d, 2H,Pyrazole), 7.4-8(10H, Ar-H), 8-8.9 (m, 4H, Pyridine).	MS(m/e) 239.15(Base Peak),219,106,77
3c	1658(C=O str), 829 (*p*-substituted Aromatic ring), 3078(Ar. CH str)	^{1}H NMR (DMSO, 400 MHz) 7.12 (d, 2H,Pyrazole), 6.9-8 (8H, Ar-H), 8-8.9 (m, 4H, Pyridine), 5.0 (s, 1H,OH)	-
3d	1664 (C=Ostr), (*p*-substituted Aromatic ring), 3064(Ar. CH str)	^{1}H NMR (DMSO, 400MHz) 7.12(d, 2H, Pyrazole), 6.9-8 (m, 9H, Ar-H), 8-8.9 (m, 4H, Pyridine), 5.0 (s, 1H,OH).	-
4a	1597(-NH Bend), 833 (*p*-substituted Aromatic ring) 3074(Arm. CH str)	^{1}H NMR (DMSO, 300MHz) 6.8-8.26 (s,4H, Ar-H), 6.9(s,1H, NH_2), 7.85 (s,1H, Pyrimidine).	-
4b	1593(-NH Bend), 823 (*p*-substituted Aromatic ring), 3036(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300 MHz) 7.3-7.98 (s, 4H Ar-H), 7.2(s,1H, NH_2), 7.80 (s,1H, Pyrimidine), 2.44 (s, 3H,CH_3),	-
4c	1595(-NH Bend), 812 (*p*-substituted Aromatic ring), 3037(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300 MHz) 7.3-8.65 (s, 4H, Ar-H), 7.2(s,1H, NH_2), 7.86 (s,1H, Pyrimidine), 2.3(s, 3H,CH_3)	
4d	1600(-NH Bend), 815 (*p*-substituted Aromatic ring), 3034(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300 MHz) 7.3-7.7(s, 4H, Ar-H), 6.7(s,1H, NH_2), 7.81 (s,1H, Pyrimidine), 2.42(s, 3H, CH_3),3.08-3.04 (d, 6H, for each N-dimethyl).	304 (M^+-1), 265.3 (Base Peak), 291, 262, 236, 118, 91
4e	1600(-NH Bend), 839 (*p*-substituted Aromatic ring), 3028(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300MHz) 6.86-7.8(s, 4H, Ar-H), 6.89(s,1H, NH_2), 7.88(s,1H Pyrimidine), 2.4(s, 3H,CH_3).	277 (M^+-1), 44.10 (Base Peak), 237, 174, 146, 91, 40
4f	1595(-NH Bend), 821 (*p*-substituted Aromatic ring), 3047(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300MHz) 7.01-8.15(s, 4H, Ar-H), 6.89(s,1H, NH_2), 7.85 (s,1H, Pyrimidine),2.4 (s, 3H,CH_3).	277 (M^+-1), 221.2 (Base Peak), 260, 249, 236, 178, 186, 91
4g	1591(-NH Bend), 813 (*p*-substituted Aromatic ring), 3049(Ar. CH str)	^{1}H NMR ($CDCl_3$, 300MHz) 7.01-7.7(s, 4H Ar-H),6.95(s,1H, NH_2),7.91 (s,1H, Pyrimidine), 2.43(s, 3H,CH_3), 3.85(s, 3H,OCH_3).	-
4h	1597(-NH Bend), 817 (*p*-substituted Aromatic ring), 3082(Ar. CH str)	^{1}H NMR (DMSO, 300MHz) 6.8-7.6(s, 4H Ar-H), 6.97(s,1H, NH_2), 8.02 (s,1H Pyrimidine), 3.79 (s, 3H,OCH_3).	293 (M^+-1), 254.2 (Base Peak) 268, 252, 225, 107, 77, 93

Table 3: Antimicrobial activity

Comp.	Zone of Inhibition (In mm)											
	B.S.		S.A.		K.P.		P.A.		C.A.		A.N.	
Conc.	A	B	A	B	A	B	A	B	A	B	A	B
2a	07	09	07	10	10	12	03	06	07	09	08	11
2b	06	08	01	01	02	07	01	02	08	12	11	12
2c	09	12	08	10	06	12	07	10	07	11	09	11
2d	03	06	04	06	06	09	03	06	08	10	09	10
3a	05	06	07	09	07	09	06	09	10	12	09	12
3b	05	08	05	09	05	10	04	05	08	10	08	11
3c	05	08	06	08	05	06	05	07	10	12	07	12
3d	04	06	08	09	05	08	06	08	09	10	08	11
4a	08	11	07	10	09	13	05	08	07	11	08	10
4b	04	11	08	15	06	10	04	06	08	10	09	11
4c	04	07	08	09	11	12	07	09	08	09	07	12
4d	06	08	08	11	10	12	09	12	08	11	09	10
4e	07	10	10	12	05	10	09	11	08	11	07	08
4f	05	07	05	07	07	10	05	08	09	10	10	11
4g	09	12	11	12	05	13	08	10	08	10	10	11
4h	05	07	07	10	05	07	03	05	10	11	08	09
Norfloxacin 500 µg ml^{-1}					14 mm							
Griseofulvin 500 µg ml^{-1}					13 mm							

A = 2000 µg ml^{-1} B = 4000 µg ml^{-1} B.S.: *Bacillus subtilis* (NCIM 2711), S.A.: *Staphylococcus aureus* (NCIM 2079), K.P.: *Kleibsella pneumoniae* (ATCC 4352), P.A.: *Pseudomonas aerugenosa* (ATCC 27853), C. A.: *Candida albicans* (ATCC 60193), A.N.: *Aspergillus niger* (NCIM 515)

Table 4: Antitubercular activity

Compound	10 ug ml^{-1}	25 ug ml^{-1}	50 ug ml^{-1}
2a	R	R	R
2b	R	R	R
2c	S	S	S
2d	S	S	S
3a	R	S	S
3b	R	S	S
3c	R	R	R
3d	R	R	R
4a	S	S	S
4b	R	R	R
4c	S	S	S
4d	R	R	R
4e	R	S	S
4f	R	R	R
4g	R	R	R
4h	R	R	R
STM	S	S	S
INH	S	S	S

R=Resistant, S=Sensitive, STM: Streptomycin, INH: Isoniazid

CONCLUSION

From the obtained results, we conclude that the isoxazole derivatives substituted at 3 and 5 position with aromatic rings and pyrimidine derivatives substituted at 2 position with amino group with aromatic rings at 4 and 6 positions; possess promising antimicrobial, antifungal and antitubercular activity.

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