Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL 3-CHLORO-4-(SUBSTITUTED ARYL)-1,3DIPHENYL-1H-PYRAZOLE-4-YL-1-(6-METHYLPYRIDIN-2-YL) AZETIDIN-2-ONE DERIVATIVES

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ABSTRACT

Azetidin-2-one derivatives as novel antimicrobial agents were carried out. Literature survey Schiff base has good antimicrobial, antifungal activity and it can be prepared by the acid catalysed reaction of aldehyde or ketone and amines. Azetidin-2-one derivatives have been exhibited to possess biological properties like antimicrobial, antifungal, anti-inflammatory, antibiotic activities. These compounds are characterized by chemical and instrumental methods. Their important biological properties have been investigated.

Keywords: Schiff Base derivatives, Biological study, pyridine derivatives, Hydrazone derivatives, azetidin derivatives.

INRODUCTION

Hydrazones, possessing an azomethine –NHN=CH- proton, constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazones are synthesized by heating the appropriate substituted hydrazine/hydrazides with aldehydes and ketones in solvents like ethanol, methanol, butanol, glacial acetic acid, ethanol-glacial acetic acid. These are well known intermediates for the preparation of oxadiazolines, azetidinones, thiazolidinones and many other derivatives. Hydrazones exhibit a wide range of pharmacological activities like Anticancer[1], Antimalaria[2], and Antitubercular[3] etc.

A large number of substituted pyrazole derivatives are prepared and tested for variety of biological activities like Anti HIV[4], Antiviral[5], Antiparasitic[6] etc.

As part of interest in hetrocycles that have been explored for developing pharmaceutically important molecules, 2-azetidinones have played an important role in medicinal chemistry. Moreover they have been studied extensively because of their ready accessibility, diverse chemical reactivity and broad spectrum of biological activity.

MATERIALS AND METHODS

The compounds 3-chloro-4(1, 3-diphenyl-1H-pyrazol-4-yl)-1-(6-methylpyridin-2-yl) azetidin-2-one (I_{a-m}) were obtained by following preparation method (I-a) (figure-1).

[A] Synthesis of N-Phenyl amino-α-methyl-phenyl azomethine

A mixture of phenyl hydrazine (1.08gm, 0.01M) and aceptophene (1.20gm, 0.01M) in absolute ethanol was refluxed in water bath for 4 hrs in presence of 1ml of glacial acetic acid. Product obtained after cooling with crystallized from absolute ethanol (Ejima Akio more *et.al*[7]). Yield, 1.8gm (90%), M.P.: 64°C. ($C_{14}H_{14}N_2$; Calculated: C, 80.00; H, 6.66; N, 13.37%; Found: C, 79.92; H, 6.64; N, 13.34%).

This typical experimental procedure was followed to prepare other analogs of this series.

[B] Synthesis of 1, 3 –diphenyl- 1H-pyrazole- 4-carbaldehyde

N-Phenyl amino-α-methyl-phenyl azomethine (0.84gm, 0.004M) was added in a mixture of Vilsmeier – Haack reagent (prepared by drop wise addition of 1.2ml $POCl_3$ ice cooled 10ml DMF) and refluxed for 6hrs. The reaction mixture was poured into crushed ice followed by neutralization using sodium bicarbonate. Crude product was isolated and crystallized from methanol (Ejima Akio more *et.al*[7]). Yield, 2.16gm (87%), M.P.: 120°C. ($C_{16}H_{12}N_2O$; Calculated: C, 77.42; H, 4.48; N, 11.29%; Found: C, 77.39; H, 4.80; N, 11.28%).

Exactly similar experimental procedure was followed to prepare other analogs of this series.

[C] (E)-N-((1, 3-diphenyl-1H-pyrozol-4-yl) methylene)-6-methyl pyridine-2-amine

A mixture of 1, 3- diphenyl-1H- pyrazole-4- carbaladehyde (2.48gm, 0.01M) and 2-Amino,6-methyl pyridine (1.08gm, 0.01M) was taken in absolute ethanol and few drops of glacial acetic acid was added. Then the mixture was refluxed for 6h on water bath. The separated solid was filtered, washed and recrystallized from ethanol (S.A.Patil more *et.al*[8]). M.P. 142°C, Yield 89%, and $C_{22}H_{18}N_4$; Calculated: C, 78.07; H, 5.36 N, 16.56; Found: C, 78.01; H, 5.38; N, 16.51%)

The same experimental procedure was utilized to prepare other analogs of this series.

[D] 3-chloro-4(1,3-diphenyl-1H-pyrazol-4-yl)-1-(6-methyl pyridin-2-yl) Azetidin-2-one

To (E)-N-((1, 3-diphenyl - 1H – pyrozol – 4 – yl) methylene) -6-methyl pyridine-2-amine (0.01 mol) in dioxane (50 ml) were added chloroacetyl chloride (0.01 mol) and Et_3N (0.1 mol) at 0° with stirring. The mixture was left at room temperature for 3 hours, and then refluxed for 10 hours; excess of solvent distilled off and the residue was poured over crushed ice. The resulting solid was crystallized from proper solvent. Yield, 2.52gm (80 %), M.P: 180°C ($C_{24}H_{19}N_4OCl$) Calculated: C, 69.48; H, 4.62; N, 13.50% Found: C,67.90., H,4.11; N,12.15%).

The same experimental procedure was utilized to prepare other analogs of this series (I a-m). Their physical constant data are given in Table 1. The purity of synthesized compounds was checked by thin layer chromatography (TLC) on silica gel plate using ethyl acetate: cyclohexane (50:50). Melting points were determined by open capillary method and are uncorrected. IR spectra are recorded on FT-IR Perkin-Elmer spectrophotometer RXI using KBr disc. ^1H NMR spectra are recorded on in $CDCL_3$ ON a Bruker DRX-400 MHz using TMS as inter standard. The chemical shifts are reported as parts per million (ppm) and ESI MS were determined on Discovery Make Thermo Spectrometer.

The characterization data of compounds (Ia-m) are described in Table 1 and antimicrobial data are described in Tables 2, 3, 4 and 5.

RESULTS AND DISCUSSION

The synthesis of 3-chloro-4(1, 3-diphenyl-1H-pyrazol-4-yl)-1-(6-methylpyridin-2-yl) azetidin-2-one derivatives (Ia-m) involved the reaction between (E)-N-((1, 3-diphenyl - 1H – pyrozol – 4 – yl) methylene) -6-methyl pyridine-2-amine (0.01 mol) in dioxane (50 ml) were added chloroacetyl chloride (0.01 mol) and Et_3N (0.1 mol) at 0° with stirring appropriate as described in the general procedure.

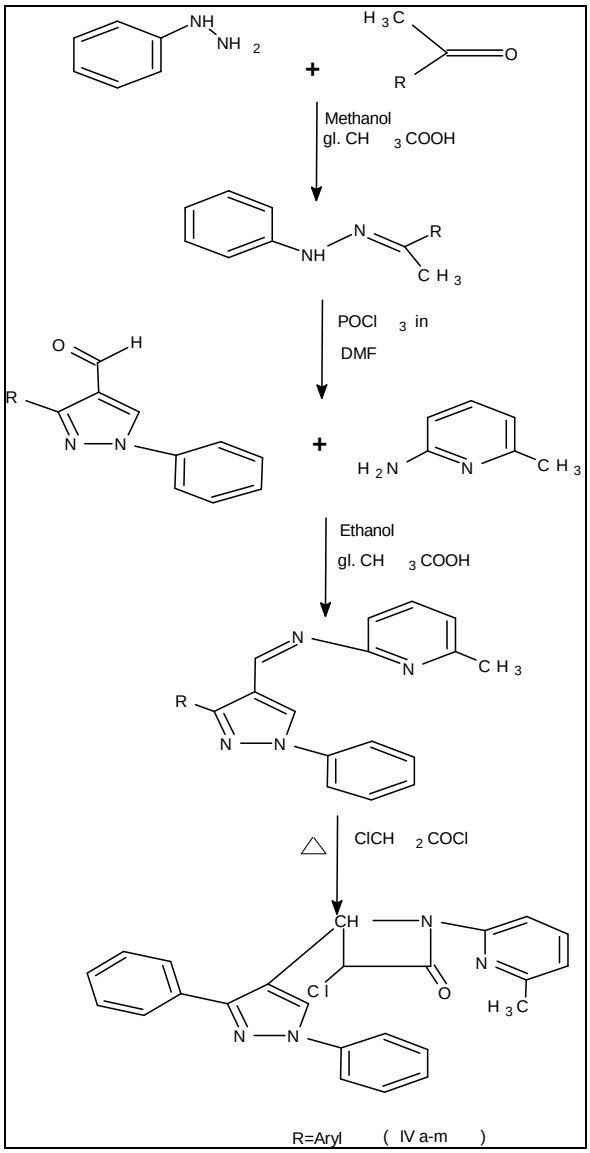

Figure 1

Table 1: Physical and chemical characteristics

Compound No.	R	Molecular Formula	Formula Weight	Solvent for crystallization (Final Step)	% yield/ Colour	M.P (°C)/ R.F.	% Carbon Found/ (Calcu.)	% Hydrogen Found/ (Calcu.)	% Nitrogen Found/ (Calcu.)
IV-a	$-C_6H_5$	$C_{24}H_{19}N_4OCl$	414.12	Ethanol	81/w	180/0.64	67.90/69.48	4.11/4.62	12.15/13.50
IV -b	4-Cl-C_6H_4	$C_{24}H_{18}N_4OCl_2$	448.08	Ethanol	89/w	130/0.55	62.32/64.15	3.76/4.03	11.99/12.46
IV -c	2-OH-C_6H_4	$C_{24}H_{19}N_4O_2Cl$	430.87	Ethanol	80/w	205/0.65	65.40/66.90	4.11/4.44	12.04/12.99
IV -d	4-OH-C_6H_4	$C_{24}H_{19}N_4O_2Cl$	430.87	Ethanol	84/w	105/0.70	66.11/66.90	3.91/4.44	11.87/12.99
IV -e	3-OH-C_6H_4	$C_{24}H_{19}N_4O_2Cl$	430.87	Ethanol	88/w	224/0.68	65.95/66.90	3.66/4.44	11.65/12.99
IV -f	4-NO_2-C_6H_4	$C_{24}H_{18}N_5O_3Cl$	459.85	Ethanol	80/y	118/0.72	61.70/62.68	3.28/3.94	18.15/18.22
IV -g	3-NO_2-C_6H_4	$C_{24}H_{18}N_5O_3Cl$	459.85	Ethanol	82/y	115/0.64	61.14/62.68	3.17/3.94	18.13/18.22
IV -h	4-Br-C_6H_4	$C_{24}H_{18}N_4OBrCl$	492.035	Ethanol	89/y	140/0.67	57.24/58.38	3.21/3.67	10.79/11.34
IV -i	4-CH_3SO_2-C_6H_4	$C_{25}H_{21}N_4O_3ClS$	492.10	Ethanol	84/w	185/0.72	59.36/60.91	3.63/4.29	10.57/11.36
IV -j	2,4diOH-C_6H_3	$C_{24}H_{19}N_4O_3Cl$	446.86	Ethanol	86/w	160/0.69	63.85/64.50	3.87/4.28	11.97/12.53
IV -k	2,4-diCl-C_6H_3	$C_{24}H_{17}N_4OCl_3$	482.04	Ethanol	83/w	152/0.61	58.60/59.59	3.14/3.54	11.01/11.57
IV -l	4-OCH_3-C_6H_4	$C_{25}H_{21}N_4O_2Cl$	444.86	Ethanol	80/w	192/0.62	66.29/67.49	4.27/4.75	11.62/12.58
IV -m	4-$CH_3C_6H_4$	$C_{25}H_{21}N_4OCl$	428.87	Ethanol	84/w	100/0.60	68.95/70.01	4.12/4.93	12.36/13.05

w = white; y = yellow

Table 2: Antibacterial activity of test compounds

COMD. No.	Substituent group (R) ↓	*E. COLI* (MTCC 443)					*P.AERUGINOSA* (MTCC 424)					*S.AUREUS* (MTCC 96)					*S.PYOGENES* (MTCC 442)				
		Zone of Inhibition in mm																			
	Conc (µg/ml) →	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
IV-a	2,4di-OH-C_6H_3	-	13	18	18	19	-	12	13	15	17	-	11	12	15	17	-	12	14	18	20
IV-b	4-NO_2-C_6H_4	-	15	13	17	20	-	10	11	13	14	-	12	13	14	18	-	11	15	17	20
IV-c	4-Cl-C_6H_4	-	12	11	14	15	-	14	17	18	21	-	11	14	15	16	-	12	13	14	17
IV-d	C_6H_5	-	13	15	18	26	-	12	15	20	22	-	13	15	16	17	-	12	14	15	21
IV-e	4-CH_3 –C_6H_4	-	15	13	17	17	-	11	12	12	14	-	10	12	15	18	-	12	15	17	18
IV-f	4-CH_3SO_2-C_6H_4	-	12	15	15	18	-	10	11	12	15	-	12	14	17	19	-	12	13	14	17
IV-g	4-Br-C_6H_4	-	11	13	16	17	-	12	14	17	19	-	12	14	15	16	-	11	13	14	16
IV-h	4-OH-C_6H_4	-	12	15	16	17	-	10	14	15	18	-	13	15	17	18	-	12	13	15	20
IV-i	2-OH-C_6H_4	-	14	17	20	25	-	12	18	20	21	-	14	15	18	19	-	11	12	15	18
IV-j	2,4di-Cl-C_6H_3	-	12	17	19	22	-	11	15	18	19	-	14	16	18	20	-	12	13	15	17
IV-k	3-NO_2-C_6H_4	-	14	14	16	21	-	11	12	14	16	-	10	14	15	19	-	11	14	16	20
IV-l	3-OH-C_6H_4	-	12	14	17	18	-	11	14	16	18	-	12	14	16	19	-	10	14	16	19
IV-m	4-OCH_3	-	14	17	18	21	-	12	17	20	22	-	12	16	18	19	-	12	14	17	20

Table 3: Antibacterial activity of standard drugs

Standard Drug ↓	*E. COLI* (MTCC 443)					*P.AERUGINOSA* (MTCC 424)					*S.AUREUS* (MTCC 96)					*S.PYOGENES* (MTCC 442)				
	Zone of Inhibition in mm																			
Conc (µg/ml) →	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Ampicillin	14	15	16	19	20	14	15	15	18	20	10	13	14	16	18	11	14	16	18	19
Chloramphenicol	14	17	23	23	23	14	17	18	19	21	12	14	19	20	21	10	13	19	20	20
Ciprofloxacin	20	23	28	28	28	20	23	24	26	27	17	19	21	22	22	16	19	21	21	22
Norfloxacin	22	25	26	27	29	18	19	21	23	23	19	22	25	26	28	18	19	20	21	21

Table 4: Antifungal activity of test compounds

Compound No.	Substituent group (R) ↓	*A. NIGER* (MTCC 282)					*C. ALBICANS* (MTCC 227)				
		Zone of Inhibition in mm									
	Conc (µg/ml) →	5	25	50	100	250	5	25	50	100	250
IV-a	2,4di-OH-C_6H_3	-	18	20	21	22	-	20	20	22	22
IV-b	4-NO_2-C_6H_4	-	21	21	23	24	-	19	21	24	25
IV-c	4-Cl-C_6H_4	-	18	18	19	22	-	18	20	23	25
IV-d	C_6H_5	-	18	19	21	22	-	20	22	23	25
IV-e	4-CH_3 –C_6H_4	-	18	20	22	22	-	20	22	23	25
IV-f	4-CH_3SO_2-C_6H_4	-	21	22	23	25	-	20	22	25	27
IV-g	4-Br-C_6H_4	-	19	21	22	25	-	22	25	25	25
IV-h	4-OH-C_6H_4	-	21	23	25	27	-	18	20	22	23
IV-i	2-OH-C_6H_4	-	23	23	25	25	-	18	19	22	25
IV-j	2,4di-Cl-C_6H_3	-	20	20	22	22	-	21	22	23	25
IV-k	3-NO_2-C_6H_4	-	20	21	24	20	-	19	22	24	26
IV-l	3-OH-C_6H_4	-	19	21	24	25	-	20	24	25	26
IV-m	4-OCH_3	-	21	23	24	26	-	22	24	24	25

Table 5: Antifungal activity of standard drugs

Standard Drug ↓	*A. NIGER* (MTCC 282)					*C. ALBICANS* (MTCC 227)				
	Zone of Inhibition in mm									
Conc (µg/ml) →	5	25	50	100	250	5	25	50	100	250
Griseofulvin	19	23	25	25	28	18	21	22	22	24
Nystatin	18	19	24	29	29	18	21	24	25	26

IR spectra showed the Azetidin ring C=O stretching vibration peak at 1598.71 cm^{-1} and the pyridine moiety also confirmed by an intense band of 1017.45.45cm^{-1}. The other peaks of IR spectra also prove the structure of pyrazol derivatives. The nuclear magnetic resonance spectra (^{1}H NMR) showed the (N-CH) at 8.4684 ppm, (C-H) at 5.2346-5.5624 azetidin and the mass spectrum of comp. (I-a) shows the $[M+1]^+$ molecular ion (m/z = 415) a base peak. Many times, due to collision of secondary ion with sample molecular ion, $[M+1]^+$ or $[m+2]^+$ is formed and is sometimes prominent base peak, which undergoes less fragmentation. As per the nitrogen rule, it must have even molecular weight, which is 414.125 (isotopic mass). 416 peak is 25.61 % of 415 $[M+1]^+$ peak indicating the presence of 24 carbon atoms (confirmed by the rule of thirteen). Fragments showed peaks at m/z 245.008 [(base peak), 337.108] and m/z etc.

Antimicrobial activity

Antimicrobial activity testing was carried out by using Agar cup method. Each purified compound was dissolved in dimethyl sulfoxide (DMSO), sterilized by filtration using sintered glass filter and stored at 4°C. All the synthesized compounds were screened for their antibacterial and antifungal activities against the *E. coli*, *P. auregenosa*, *S. aures*, *S. pyogenus* and the fungi *C. albicans*, *A. niger*, and *C.albicans*. The compounds were tested at 250, 100, 50 and 25 concentration using nutrient agar tubes. The highest dilution showing at least 99 % inhibition is taken as MBC (minimal bactericidal concentration). Control experiments were carried out under similar condition by using gentamycine, ampicillin and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs.

E. Coli: Compounds no IV-b, IV-d, IV-i, IV-j, IV-k and IV-m possess higher or equal antibacterial activity against *E.Coli* compared to standard antibiotic ampicillin at low concentration. The compounds IV-d, IV-i, possess higher or equal antibacterial activity also compared to chloramphenicol.

P. Aeruginosa: Compounds IV-c, IV-d, IV-i, IV-m exhibit equal or greater antibacterial activity compared to antibiotic ampicillin at 250µg/ml concentration and also compared to chloramphenicol at 250µg/ml

S. Aureus: The compounds IV-b, IV-e, IV-f, IV-h, IV-i, IV-j, IV-k, IV-l and IV-m showed equal or higher antibacterial activity compared to ampicillin at 250µg/ml concentration.

S. Pyogenes: In comparison to the standard drug ampicillin, compounds IV-a, IV-b, IV-d, IV-h, IV-k, IV-l and IV-m showed equal antibacterial activity and the compounds IV-a, IV-b, IV-d, IV-h, IV-k and IV-m exhibited equal antibacterial activity also compared to chloramphenicol.

Antifungal activity: The antifungal study was carried out with *A.niger* no compound showed equal antifungal activity compared to griseofulvin. Against *C.albicans*, the compounds IV-b, IV-c, IV-d, IV-e, IV-f, IV-g, IV-i, IV-j, IV-k, IV-l, IV-m possess equal or higher antifungal activity compared to the standard antibiotic griseofulvin.

Spectral study of 4-((1,3–diphenyl-1H-pyrazol-4-yl) methyleneamino)-1,5-dimethyl-2-phenylpyrazolildin-3-one (i-a) [isotopic weight = 435.12 g].

IR (KBr) cm^{-1}: 1598.71 (C=O Stretching of Azetidine), 2918.57 (C-H Str. Asym.), 1368.20 (C-H def. sym.), 3124.87 (Ar C-H Stretching), 1569.80 (C=N Str. Of pyrazole ring), 1017.45 (Ring breathing Str pyridine moiety).

H NMR ($CDCl_3$) δ (ppm): 5.2346-5.5624(2 H, Azetidine ring), 8.4684 (1H, pyrazol ring), 7.1793-7.7602 (13 H, Ar-H), 1.1748(3H, C-CH3).

Mass Spectra (m/z) = 415 $(M+1)^+$, 416$(M+2)^+$, 245.008, 337.108.

CONCLUSION

Some of the compounds synthesized show promising antimicrobial activity, in particular the compound IV-m showed good antibacterial and antifungal activity. The modifications of this compound may result in the compounds with potent antimicrobial and subsequently antibiotic action.

However, certain structural alterations did not increase antimicrobial activity and working ahead in that direction may give quite promising results.

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