AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW (*E*)-4-CHLORO-2-((3-(SUBSTITUTED ARYL)-1-PHENYL-1*H*-PYRAZOLE-4-YL) METHYLENE AMINO)-6-METHOXY PYRIMIDINE-5-CARBALDEHYDE DERIVATIVES

Jabali J. Vora[1], Akash R. Patel[1*], Dinesh R. Patel[2], Sandip Dholakiya[3]

1. Department of Chemistry, Hemchandracharya North Gujarat University,Patan,Gujarat, India.
2. The Urban Science College, Mehsana, Gujarat, India.
3. Shankersinh vaghelabapu institute of pharmacy, Gandhinagar

ABSTRACT

Schiff Base derivatives are important class of compounds. They possess different types of Biological activities like antibacterial, antiviral, anti HIV, antifungal etc. Schiff base derivatives are prepared by the condensation of aldehyde and amine and these compounds are characterized by chemical and instrumental methods. Their important biological properties have been investigated.

Key words: Schiff Base derivatives, Biological study, pyrimidine derivatives, Hydrazone derivatives

INTRODUCTION

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,

*Corresponding Author Email: akpatel_79@yahoo.co.in

Received 18 September 2011, Accepted 23 September 2011

Please cite this article in press as: Patel AR *et al.*, Synthesis and Antimicrobial activity of some new (*E*)-4-Chloro-2-((3-(Substituted Aryl)-1-Phenyl-1*h*-Pyrazole-4-Yl) methylene amino) -6- methoxy pyrimidine -5-carbaldehyde derivatives. American Journal of PharmTech Research 2011.

thiazolidinones and many other derivatives. Hydrazones exhibit a wide range of pharmacological activities like Anticancer [1] Ant malaria [2] and Ant tubercular [3]etc.

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

Schiff base of have a variety of application 2-amino-4-chloro-6-methoxy pyrimidine-5-carbaldehyde in biological, clinical, analytical and pharmacological areas. Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists. Earlier work reported that some drugs showed increased activity.Deoxyriboncleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biologists.

MATERIAL AND METHOD

All Raw materials was purchase from S. D. Fine Chemicals.

The compounds (Z)-4-chloro-2- ((1- (4-chlorophenyl)- 3-phenyl- 1H-pyrazol-4-yl) methyl eneamino) 4-chloro-6-methoxypyrimidine -5 -carbaldehyde. (I_{a-m}) were obtained by following preparation method (I-a) (Figure-1).

Synthesis of N-Phenylamino-α-methyl-chloro phenyl azomethine

A mixture of phenyl hydrazine (1.08gm, 0.01M) and 4-chloro aceptophene (1.20gm, 0.01M) in absolute ethanol was refluxed in water bath for 4 h. In presence of 1ml glacial acetic acid. Product obtained after cooling was crystallized from absolute ethanol (Ejima Akio more, et.al[7]). Yield, 1.8gm (90%), M.P.: 64°C. ($C_{14}H_{13}N_2Cl$, Calculated: C, 68.57; H, 5.30; N, 11.42 Found: C, 68.10; H, 5.15; N, 11.20).

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

Synthesis of [3-(4-Chlorophenyl) -1-Phenyl] -1H-pyrazole -4- carbaldehyde

N-Phenylamino-α-methyl-chloro phenyl azomethine (0.98gm, 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 6h. 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.: 110°C. ($C_{16}H_{11}N_2OCl$; Calculated: C, 67.84 H, 3.88; N, 9.89 Found: C, 67.60; H, 3.80; N, 9.80).

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

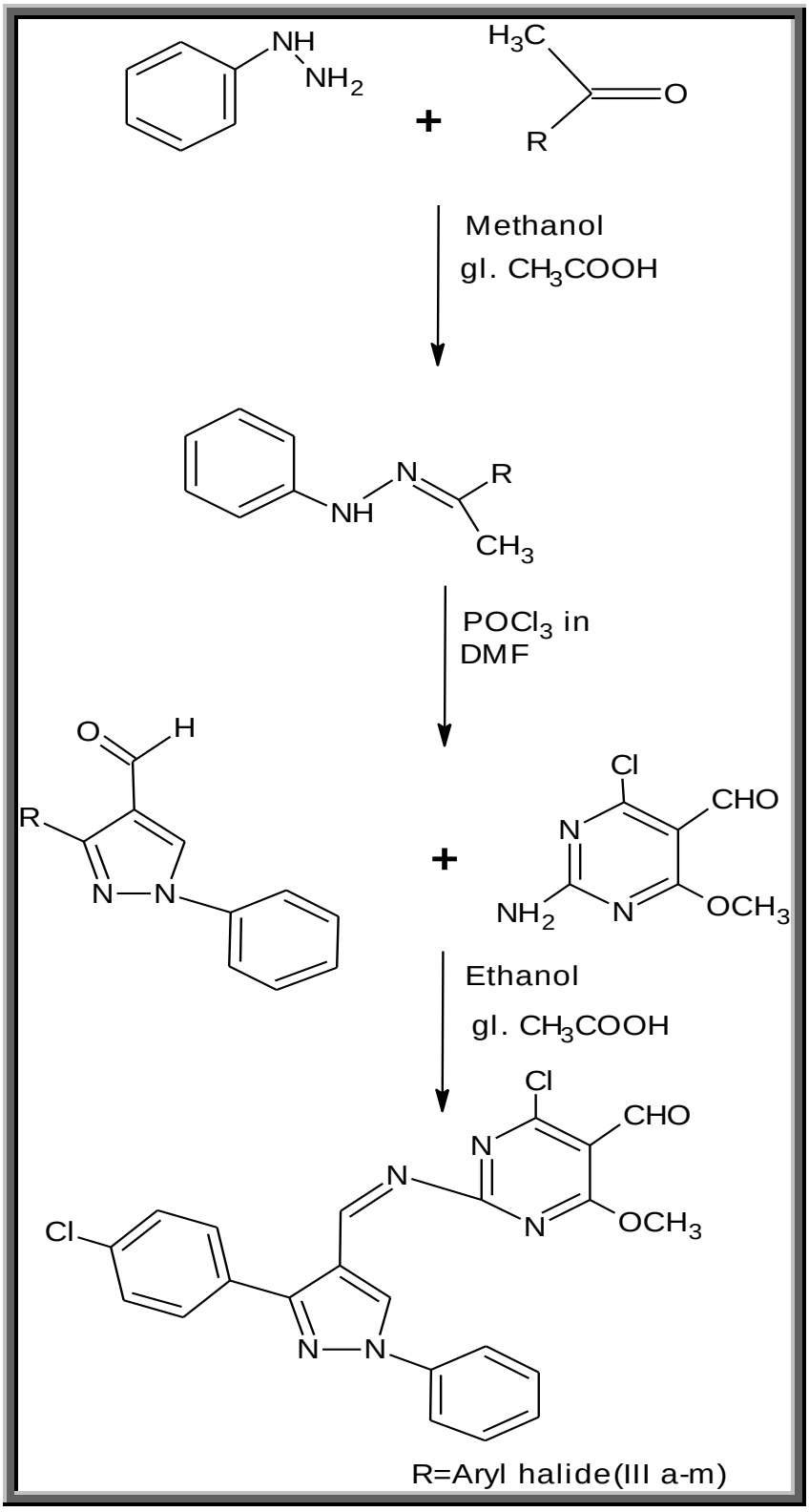

Figure 1 Synthetic scheme for predicted compounds

Synthesis (Z)-4- chloro- 2-((1-(4- chlorophenyl) -3-phenyl- 1H- pyrazol- 4-yl) methyleneamino) 4- chloro-6-methoxypyrimidine-5-carbaldehyde.

A mixture of (3-(4-chloro phenyl)-1-phenyl)-1H- pyrazole -4- carbaldehyde (2.83gm,0.01M) and 2-amino-4-chloro-6-methoxy pyrimidine-5-carbaldehyde (1.88gm, 0.01M) was taken in absolute ethanol and few drops of glacial acetic acid were added. Then the mixture was refluxed for 6h on water bath. The separated solid was filtered, washed and recrystalized from ethanol[8]

M.P. 125°C, Yield 88%, and $C_{22}H_{15}N_5O_2Cl_2$; Calculated: C, 58.52; H, 3.35; N, 15.52; Found C, 58.45; H, 3.30; N, 15.45 ;).

The same experimental procedure was utilized to prepare other analogs of this serieI (I a-m). Their physical constant data are given in Table 1.

The same experimental procedure was utilized to prepare other analogs of this series (Ia-m). The purity of synthesized compounds was checked by thin layer chromatography (TLC) on silica gel plate using ethyl acetate: cyclohexane (80:20). 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. ^{1}H 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 Table 2, 3, 4 and 5.

RESULT AND DISCUSSION

The synthesis of (Z)-4-chloro-2-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methylene amino) 4-chloro-6-methoxypyrimidine-5-carbaldehyde Derivatives (Ia-m) involved the reaction between appropriate 1, 3 –diphenyl- 1H-pyrazole- 4- carbaldehyde (I_{a-m}**)** and 2-amino-4-chloro-6-methoxy pyrimidine-5-carbaldehyde as described in the general procedure.

IR spectra showed the C-Cl stretching vibration peak at 0751.95 cm^{-1} and The Schiff base also confirmed by an intense band of C=N around 1535.50cm^{-1}. The other peaks of IR spectra also prove the structure of hydrazones derivatives. The nuclear magnetic resonance spectra (^{1}H NMR) showed the amine proton (N=C-H) at 8.4684 ppm and (N-CH) at 7.7796 ppm and the mass spectrum of comp. (I-a) shows the $[M]^+$ molecular ion (m/z = 451) a base peak. Many times, due to collision of secondary ion with sample molecular ion, $[M]^+$ or $[m+1]^+$ 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 451.06(isotopic mass). 452 peak is 25 % of

Table 1 Physical and Chemical Characteristics of (Z)-4-chloro-2-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methyleneamino) 4-chloro-6-methoxypyrimidine-5-carbaldehyde. y= yellow, w= white

Comp. No.	R	Molecular formula	Formula weight	Solvent for crystallization on (Final Step)	% yield Color	M.P°C R.F.	% Carbon Found Calcu.	% Hydrogen Found Calcu.	% Nitrogen Found Calcu.
III-a	-C_6H_5	$C_{22}H_{16}N_5O_2Cl$	414.96	Ethanol	83/w	140/0.62	63.70/(63.75)	3.91/(3.89)	16.85/(16.90)
III-b	4-Cl-C_6H_4	$C_{22}H_{15}N_5O_2Cl_2$	451.06	Ethanol	87/w	125/0.57	58.45/(58.52)	3.30/(3.35)	15.45/(15.52)
III-c	2-OH-C_6H_4	$C_{22}H_{16}N_5O_3Cl$	435.12	Ethanol	89/y	187/0.60	60.60/(60.67)	3.65/(3.70)	16.02/(16.09)
III-d	4-OH-C_6H_4	$C_{22}H_{16}N_5O_3Cl$	435.12	Ethanol	85/y	108/0.61	60.62/(60.67)	3.71/(3.70)	16.05/(16.09)
III-e	3-OH-C_6H_4	$C_{22}H_{16}N_5O_3Cl$	435.12	Ethanol	80/y	180/0.63	60.60/(60.67)	3.68/(3.70)	16.01/(16.09)
III-f	4-NO_2-C_6H_4	$C_{22}H_{15}N_6O_4Cl$	459.04	Ethanol	83/y	090/0.72	57.45/(57.51)	3.20/(3.29)	18.20/(18.30)
III-g	3-NO_2-C_6H_4	$C_{22}H_{15}N_6O_4Cl$	459.04	Ethanol	84/y	142/0.64	57.48/(57.51)	3.25/(3.29)	18.24/(18.30)
III-h	4-Br-C_6H_4	$C_{22}H_{15}N_5O_2BrCl$	492.85	Ethanol	80/y	125/0.66	53.71/(53.65)	3.15/(3.07)	14.32/(14.40)
III-i	4-CH_3SO_2-C_6H_4	$C_{23}H_{18}N_5O_4SCl$	497.13	Ethanol	81/w	145/0.64	55.45/(55.51)	3.55/(3.64)	14.01/(14.08)
III-j	2,4diOH-C_6H_3	$C_{22}H_{16}N_5O_4Cl$	451.91	Ethanol	82/y	155/0.61	58.41/(58.52)	3.51/(3.57)	15.45/(15.52)
III-k	2,4-diCl-C_6H_3	$C_{22}H_{14}N_5O_2Cl_3$	487.05	Ethanol	85/w	110/0.59	54.14/(54.20)	2.81/(2.89)	14.31/(14.37)
III-l	4-OCH_3-C_6H_4	$C_{23}H_{18}N_5O_3Cl$	449.14	Ethanol	89/w	190/0.63	61.45/(61.50)	4.10/(4.03)	15.59/(15.51)
III-m	4-$CH_3C_6H_4$	$C_{23}H_{18}N_5O_2Cl$	431.16	Ethanol	83/w	110/0.60	64.25/(64.31)	4.15/(4.22)	16.25/(16.31)

Table:2 Antibacterial Activity of (Z)-4-chloro-2-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methyleneamino) 4-chloro-6-methoxypyrimidine-5-carbaldehyde.

Comp. No.	R	E. COLI MTCC 443					P.AERUGINOSA MTCC 424					S AUREUS MTCC 96					S.PYOGENES MTCC 442				
III-a	$-C_6H_5$	-	15	16	19	21	-	10	15	16	17	-	14	16	17	18	-	10	13	15	20
III-b	$4\text{-Cl-}C_6H_4$	-	12	13	15	16	-	11	13	15	18	-	11	13	15	17	-	11	12	15	18
III-c	$2\text{-OH-}C_6H_4$	-	11	13	16	16	-	10	12	14	16	-	14	17	18	19	-	12	15	17	20
III-d	$4\text{-OH-}C_6H_4$	-	12	13	15	15	-	11	12	13	15	-	15	16	17	18	-	12	15	16	21
III-e	$3\text{-OH-}C_6H_4$	-	13	14	15	17	-	10	11	14	17	-	10	12	14	15	-	12	14	15	19
III-f	$4\text{-NO}_2\text{-}C_6H_4$	-	12	13	15	18	-	13	14	16	17	-	10	14	17	18	-	12	13	15	17
III-g	$3\text{-NO}_2\text{-}C_6H_4$	-	13	15	18	20	-	10	11	14	15	-	12	14	15	18	-	10	12	15	21
III-h	$4\text{-Br-}C_6H_4$	-	15	17	18	20	-	8	10	12	17	-	14	16	18	21	-	12	13	15	17
III-i	$4\text{-CH}_3\text{SO}_2\text{-}C_6H_4$	-	14	15	17	22	-	13	14	17	23	-	12	15	17	20	-	11	12	16	17
III-j	$2,4\text{diOH-}C_6H_3$	-	12	15	17	19	-	14	16	18	21	-	13	15	17	18	-	12	14	15	17
III-k	$2,4\text{-diCl-}C_6H_3$	-	14	17	18	19	-	10	12	16	17	-	12	14	16	18	-	12	14	15	19
III-l	$4\text{-OCH}_3\text{-}C_6H_4$	-	13	15	17	19	-	12	12	13	14	-	12	14	15	16	-	11	13	14	17
III-m	$4\text{-CH}_3C_6H_4$	-	10	14	15	17	-	11	13	14	17	-	12	13	15	17	-	10	15	17	18

Zone of Inhibition in milli meter, µg/ml =micro gram/ml, 5, 25, 50,100,250 = various concentrations

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				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Amplicilline	11	14	16	18	19	10	13	14	16	18	14	15	16	19	20	14	15	15	18	20
Chloramphenicol	10	13	19	20	20	12	14	19	20	21	14	17	23	23	23	14	17	18	19	21
Ciprofloxacin	16	19	21	21	22	17	19	21	22	22	20	23	28	28	28	20	23	24	26	27
Norfloxacin	18	19	20	21	21	19	22	25	26	28	22	25	26	27	29	18	19	21	23	23

Zone of Inhibition in milli meter, µg/ml =micro gram/ml , 5, 25, 50,100,250 = various concentrations

Table 4 AntifungalActivity of (Z)-4-chloro-2-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methyleneamino) 4-chloro-6-methoxypyrimidine-5-carbaldehyde.

Comp. No.	R	A. NIGER MTCC 282					C. ALBICANS				
		5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
III-a	3-NO_2- C_6H_4	-	21	22	23	25	-	21	21	23	24
III-b	4-NO_2- C_6H_4	-	19	21	22	25	-	18	18	19	22
III-c	4-OCH_3- C_6H_4	-	18	20	22	23	-	18	19	21	22
III-d	C_6H_5	-	18	19	22	25	-	18	20	21	22
III-e	4-Cl- C_6H_4	-	21	22	23	25	-	21	21	23	24
III-f	4-Br- C_6H_4	-	19	22	25	25	-	18	18	19	22
III-g	4-CH_3SO_2- C_6H_4	-	18	18	21	22	-	18	19	21	22
III-h	3-OH- C_6H_4	-	22	22	24	24	-	18	20	22	22
III-i	4-OH- C_6H_4	-	18	20	21	22	-	21	22	23	25
III-j	2,4di-Cl- C_6H_3	-	21	21	23	24	-	19	21	22	25
III-k	4-CH_3- C_6H_4	-	18	18	19	22	-	18	20	22	23
III-l	2,4diOH- C_6H_3	-	18	19	21	22	-	21	23	25	27
III-m	2-OH- C_6H_4	-	22	20	22	25	-	19	21	23	26

Zone of Inhibition in milli meter, µg/ml =micro gram/ml,
5, 25, 50,100,250 = various concentration

Table No. 5 Antifungal activity of standard drugs

Standard Drug	A. Niger MTCC 282					C. Albicans MTCC 227				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Greseofulvin	19	23	25	25	28	18	21	22	22	24
Nystatin	18	19	24	29	29	18	21	24	25	26

Zone of Inhibition in milli meter, µg/ml =micro gram/ml,
5, 25, 50,100,250 = various concentration

451 $[M]^+$ peak indicating the presence of 22 carbon atoms (confirmed by the rule of thirteen) and the intensity of M + 2 is around 70% of M when 2 chlorine atoms are present in a molecule the ratio of M, M + 2 and M + 4 is 9:6:1 as per the natural abundance. The observed value is 70%. The intensity of M + 4 is 16% of M thus it confirms the 9:6:1 ratio hence presence of two chlorine atoms in the molecule is confirmed. Fragments showed peaks at m/z 162.008 [(base peak), 163.115] 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 4^0C. 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 greseofulvin for antifungal activity as standard drugs.

E.Coli-: The compounds No III-a,III-g,III-h,III-i, III-j, III-k and III-l possess higher or equal antibacterial activity compared to standard antibiotic amplicilline at 250 µg/ml concentration. The compound III-a and III-h possess equal antibacterial activity also compared to chloramphenicol.

P.Aeruginosa-: Compounds III-b, III-i, III-j showed equal or greater activity compared to antibacterial amplicilline at 250µg/ml concentration.Compound III-I and III-j had equal antibacterial activity also compared to chloramphenicol at 250µg/ml concentration.

S.Aureus-: Compound III-a,III-c,III-d,III-g,III-h,III-i, exhibited equal or higher activity compared standard antibiotic amplicilline.

S.Pyogenes-: In comparision to the the standard amplicilline, compounds III-a, III-c and III-d showed equal antibacterial activity at 250µg/ml coincentrations.

Antifungal activity-: Standard antibiotics in general possess antifungal activity even at low concentrations; it was observed the thirteen compounds synthesized in this series did not show much activity at low concentrations.

A.Niger-: Out of the thirteen compounds, none of them showed equal antifungal activity compared to nystatin or greseofulvin at lower or higher concentractions

C. Albicans-: The compounds III-a,III-e,III-i,III-j,III-l and III-m show equal antifungal activity as greseofulvin and the compounds III-I and III-m show equal antifungal activity as nystatin.

Spectral study of (Z)-4-chloro-2-((1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl) methylene amino) 4-chloro-6-methoxypyrimidine-5-carbaldehyde (i-a) [isotopic weight = 451.06 g].

IR (KBr) cm^{-1}: 1535.50 (C=N Stretching of Schiff base), 2918.57(C-H Str. Asym.), 1367.20 (C-H def. sym.), 3124.87 (Ar C-H Stretching), 1598.71 (C=N Str. Of pyrazole ring), 0751.95 (C-Cl Str pyrimidine moiuety) and 1569.80 (C=N Str pyrimidine moiety).

H NMR ($CDCI_3$) δ (ppm): 8.4684(1 H, –CH=N-), 7.7796 (1H, pyrazol ring), 9.9690(1H, CHO pyrimidine ring) 7.1906-7.7734 (9 H, Ar-H), 3.8133(3H, C-CH3).

Mass Spectra (m/z) = 451 $(M)^+$, 452(M+2), 162,163.

CONCLUSION

Some of the compounds synthesized showed promising antimicrobial activities. These compounds may result in the compounds with potent antimicrobial action. However, certain structural alterations did not increase antimicrobial activity and working ahead in that direction may give quite promising results

ACKNOWLEDGEMENT

The authors are thankful to the Director, SAIF, Punjab University, Chandhigarh for providing the IR, ^{1}H NMR, MASS spectral data and are also thankful to Mr. Dhansukh Rajani (Microbiologist), Micro care Laboratory, Surat for antimicrobial screening. We are also thankfull to trusty shri shankarsinh vaghela bapu, Jayendrakumariba, Shri C. J. Josh and my principal Shri Milan Satia, Shankarsinh Vaghela Bapu Institute of Pharmacy, Gandhinagar

REFERENCES

1. Haskell C. Cancer. Treatment, Philadelphia Saunders. 1985, 2, 43-106.
2. Http: / / www.answers.com /drug& r-67.
3. Wolff ME. Bueger's. Medicinal Chemistry. Wiley Interscience. New York. 1979.
4. Selvam P, Chandramohan M, DeClercq E, Witvrouw M, Pannecouque C. Synthesis and anti –HIV activity of 4-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N (4, 6-dimethyl-2-pyrimidiny)-benzene sulfonamide and its derivatives. Eur J Pharm Sci, 2001, 14(4): 313-316.
5. Collla L, Manfredint P, Simoni S. Pyrazole- Related Derivatives Endowed with antitumor and antiviral activities, Procedures for their preparation, Pharmaceutical Formulations containing them. Chem Ber 1885; 18(1): 311-317.
6. Rathelot P, Azas N, Hussein El-Kashef F, Delmas. 1, 3- Diphenylpyrazoles: synthesis and antiparsitic activities of azolmethine derivatives. Eur J Med Chem. 2002, 37(8): 671-679.
7. Akio E, Satoru O. Pyrazole Derivatives. PC T Int. Appl., (wo/1998/032739).
8. Sharma VK, Srivastava S. Synthesis and characterization of trivalent chromium, manganese, iron and cobalt complexes with Schiff bases deried from 4-amino-5-mercapto-1, 2, 4-triazoles. Ind J Chem 2006; 45A: 1368-1374