Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 2 [6] December 2014: 54-59
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]

Website: www.aelsindia.com/rjces.htm

RJCES

ORIGINAL ARTICLE

Antimicrobial Activity of Some Synthesized Piperazinyl Derivatives

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ABSTRACT
Some newly synthesized Schiff bases of N-methyl piperazine have been synthesized to study their antimicrobial potential. Thiazole substituted Schiff base namely 1'-[(N-methyl piperazino]-3"nitro anilino]-2- benzilidine substituted 1,3-thiazole and oxazole substituted Schiff bases 1'-[(N-methyl piperazino]-3"nitro anilino]-2- benzilidine substituted 1,3- oxazole were prepared by treating N-methyl piperazine with 3-chloro-2-nitro aniline leading to the formation of 3-nitro anilino-N-methyl piperazine. Chloroacetylation under anhydrous conditions gave the acetyl derivative which when further treated with thiourea and urea respectively led to the formation of piperazine substituted 2-amino thiazoles and 2-amino oxazoles by indirect cyclization.Subsequent reaction with aromatic aldehydes gave the title comounds respectively. The compounds have responded to E.coli, S. aureus and C. albicans (in vitro) at 125 µg/disc and 250 µg/disc respectively.
***Keywords**: N-methyl piperazine, thiazole, oxazole, antimicrobial activity.*

Received 19.08.2014 Accepted 18.10.2014

INTRODUCTION

Many currently notable drugs contain piperazine ring as part of their molecular structure. Piperazine itself behaves as an anthelminthic drug [1]. Diverse chemical reactivity and biological activity such as anticancer2, anti-inflammatory [3], antipsychotics [4-5] , antimicrobial, anti TMV [6] etc. have been reported by various piperazinyl derivatives. Substituted 1,3- oxazoles and thiazoles have also displayed diverse biological profile i.e. antituberculosis, fungicidal, as Brain-Derived Neurotrophic Factor Inducers, antiinflammatory, antimicrobial [7-11] etc.

MATERIALS AND METHODS

1-(4-chloro-2-nitro anilino)-N-methyl piperazine(I)

Equimolar (0.01) mole of N-methyl piperazine and 4-chloro-2-nitro aniline were refluxed for 5-6 hours in presence of dimethyl formamide (10 ml). After cooling, the solution was poured into crushed ice and left over night. A solid separated out which was filtered, dried and recrystallised using ethanol water system (4:1).

Yield : 80%

M.P. :110^{0}C

M. F. : $C_{11}H_{16}N_4O_2$

	C	H	N
% Calculated	55.93	6.78	23.06
% Found	56.04	6.65	23.17

IR (KBr) : 1631.55 cm^{-1} (NO_2), 2349.3 cm^{-1} (Ar-H), 3567 cm^{-1} (-NH_2)

^{1}H NMR (TMS) δ ppm: δ 1.56 (s, 3H-N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 4.38 (s,2H -NH_2), δ 7.62-7.99 (m,3H Ar-H)

1'-(N-methyl piperazino)-3'nitro N-chloro acetyl aniline (II)

0.001 mole of compound I was taken in a flask and magnetically stirred in presence of 0.001 mole of chloro acetyl chloride which was added drop wise during the first hour. Reaction mixture was further stirred for one hour and then refluxed for another one hour. After cooling, the contents were poured into crushed ice and left overnight. A solid was obtained which was filtered, dried and recrystallised using ethanol.

Yield : 78%

M.P. : 125^{0}C
M.F. : $C_{13}H_{17}N_3O_3Cl$

	C	H	N
% Calculated	52.26	5.69	14.09
% Found	52.13	5.55	13.97

IR (KBr) : 1324 cm^{-1} (-Cl),1631.55 cm^{-1} (NO_2), 1686.51 cm^{-1} (C=O), 3349 cm-1(-NH)
^{1}H NMR (TMS) δ ppm: δ 1.56 (s, 3H -N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 2.89 (s, 2H –CH_2Cl)δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 7.62-8.01 (m,3H Ar-H)

1'-[(N-methyl piperazino)-3'nitro anilino]-2-amino 1,3 thiazole (III_a)

0.002 mole of compound II was refluxed for 12 hours with 0.025 mole thiourea in presence of ethanol(10 ml). Excess solvent was distilled off and the contents after cooling were poured into ice. Obtained solid was filtered, washed with 2% $NaHCO_3$ solution followed by water. It was dried and recrystallised from ethanol.
Yield : 82%
M.P. : 145^{0}C
M.F. : $C_{14}H_{15}N_6SO_2$

	C	H	N	S
% Calculated	50.75	4.32	25.12	10.78
% Found	50.81	4.35	25.07	10.89

IR (KBr) :1350 cm^{-1}(-N=C-S), 1631.55 cm^{-1} (NO_2), 2389.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH), 3567 cm-1(-NH_2)
^{1}H NMR (TMS) δ ppm: δ 1.56 (s,3H -N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ6.09 (s,2H –NH_2), δ 7.26 (s,1H –C_5-thiazole), δ7.62-7.96 (m,3H Ar-H) ,δ 10.16 (s,1H –NH)

1'-[(N-methyl piperazino)-3'nitro anilino]-2-amino 1,3 oxazole(III_b)

This was synthesized following the same procedure as given for compound III_a.
R=O
Yield : 89%
M.P. : 135^{0}C
M.F. : $C_{14}H_{15}N_6O_3$

	C	H	N
% Calculated	53.33	4.76	15.24
% Found	53.31	4.81	15.29

IR (KBr) : 1631.55 cm^{-1} (NO_2), 2156 cm^{-1}(-N=C-O)2389.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH), 3567 cm^{-1} (-NH_2)
^{1}H NMR (TMS) δ ppm: δ 1.56 (s, 3H-N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 6.09 (s,1H -NH), δ 7.26 (s,1H –C_5-oxazole), δ7.62-7.96 (m,3H Ar-H), δ11.31 (s, 2H –NH_2)

1'-[{(N-methyl piperazino)-3''-nitro anilino}-4'''-nitro benzilidene]-1,3-thiazole (IV_a)

Equimolar ratio of compound III_a and p-nitro benzaldehyde were refluxed for 4-5 hour using absolute alcohol (12 ml) as the solvent in presence of 4-5 drops of glacial acetic acid. Excess solvent was distilled off and the contents were then poured into crushed ice and left overnight. A solid separated out which was filtered, dried and recrystallised using ethanol.
Yield : 72%
M.P. : 165^{0}C
M.F. : $C_{21}H_{21}N_7SO_4$

	C	H	N	S
% Calculated	53.96	4.49	20.98	6.85
% Found	54.05	4.39	20.88	6.89

IR (KBr) : 1350 cm^{-1}(-N=C-S), 1615 cm^{-1}(-N=CH), 1631.55 cm^{-1} (NO_2), 2349.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH),
^{1}H NMR (TMS) δ ppm: δ 1.56 (s,3H -N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 4.21 (s, 1H =CH), δ 7.26 (s,1H –C_5-thiazole), δ7.62-8.46 (m,7H Ar-H)
Similarly 1'-[{(N-methyl piperazino)-3''-nitro anilino}-4'''-hydroxy benzilidene]-1,3-thiazole (IV_b) was synthesized using 4-hydroxy benzaldehyde.
R=S; R_1=OH; R_2=H
Yield : 72%
M.P. : 165^{0}C
M.F. : $C_{21}H_{22}N_6SO_4$

	C	H	N	S
% Calculated	57.53	5.02	19.17	7.30
% Found	57.50	5.11	19.08	7.39

IR (KBr) : 1350 cm^{-1}(-N=C-S), 1615 cm^{-1}(-N=CH), 1631.55 cm^{-1} (NO_2), 2349.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH), 3749 cm^{-1} (-OH)

^{1}H NMR (TMS) δ ppm: δ 1.56 (s,3H -N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 4.21 (s,1H =CH), δ 7.26 (s,1H –C_5-thiazole), δ7.62-8.76 (m,7H Ar-H)

1'-[{(N-methyl piperazino)-3''-nitro anilino}-4'''-nitro benzilidene]-1,3-oxazole (V_a)

1'-[(N-methyl piperazino]-3'nitro anilino]-2-amino 1,3 oxazole and p-nitro benzaldehyde were refluxed for 4-5 hours in presence of absolute alcohol (12 ml) containing 4-5 drops of glacial acetic acid. After removing excess ethanol, the solution was poured into crushed ice and left overnight. A solid which separated out was filtered, dried and recrysatalized with ethanol.

Yield : 83%

M.P. : 150^{0}C

M.F. : $C_{21}H_{21}N_7O_5$

	C	**H**	**N**
% Calculated	55.87	4.65	21.73
% Found	55.81	4.58	21.67

IR (KBr) : 1615 cm^{-1}(-N=CH), 1631.55 cm^{-1} (NO_2), 2156 cm^{-1}(-N=C-O) 2389.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH)

^{1}H NMR (TMS) δ ppm: δ 1.56 (s,3H -N-CH_3) δ 2.20 (t, 4H –CH_2-N-CH-CH_3) and δ 3.23 (t,4H -CH_2-N-CH-C_6H_5) δ 4.21 (s,1H =CH) δ 7.26 (s,1H –C_5-oxazole) δ7.62-8.76 (m,7H Ar-H)

1'-[{(N-methyl piperazino)-3''-nitro aniline}-3'''-methoxy-4'''-hydroxy benzilidene]-1,3-oxazole (V_b) was prepared by following the above procedure with 3-methoxy-4-hydroxy benzaldehyde.

R=O; R_1= OH; R_2=OCH_3

Yield : 79%

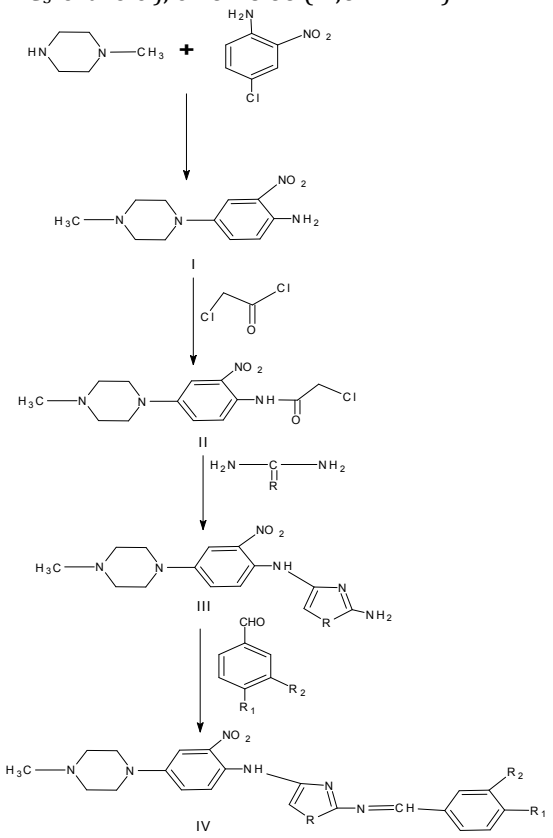
M.P. : 160^{0}C

M.F. : $C_{22}H_{25}N_6O_5$

	C	H	N
% Calculated	58.40	5.53	21.68
% Found	58.38	5.51	21.73

IR (KBr) : 1134 cm^{-1}(-OCH_3), 1615 cm^{-1}(-N=CH),1631.55 cm^{-1} (NO_2),2156 cm^{-1}(-N=C-O) 2389.3 cm^{-1} (Ar-H), 3349 cm^{-1} (-NH),3750 (-OH)

^{1}H NMR (TMS) δ ppm: δ 1.56 (s,3H -N-CH_3), δ 2.20 (t, 4H –CH_2-N-CH-CH_3), δ 3.23 (t,4H -CH_2-N-CH-C_6H_5), δ 4.21 (s,1H =CH), δ 7.26 (s,1H –C_5-oxazole), δ7.62-8.66 (m,6H Ar-H)



III_a R=S ; III_b= R=O
IV_a R=S, R_1=NO_2 R_2=H; IV_b R=S. R_1=OH, R_2=H
V_a R=O, R_1=NO_2 R_2=H; V_b R=O, R_1=OH, R_2=OCH_3

SCHEME

Antimicrobial activity:- The antimicrobial activity was measured by disc diffusion technique[12-13] at 125 and 250 µg/disc against pathogenic strains E. coli and S.aureus while antifungal activity was determined against Candida albicans.

The petri dishes were thoroughly washed, dried at 35-37^{0}C for about 30 minutes. Prepared sterilized medium was then poured into 90mm diameter sterile petri dishes to a depth of 4 mm (about 25ml per plate). The plated petri dishes were kept on a plane surface to avoid nonuniform solidification of medium. All these operations were performed in a sterile room fitted with laminar flow. The petri dishes were dried at 35-37^{0}C in an incubator for about 30 minutes.Sterile loops of about 4mm diameter were used to apply a loopfull of the test organism and the suspension was placed at the center of the petri dishes. A sterile dry cotton wool swab was used to spread the inoculum evenly on the dishes, which were then incubated for 15 min. Discs of 6.35mm in diameter were punched from a sheet of Whatman Filter Paper No. 1 and placed in petri dish, allowing a distance of 2-4 mm between each disc and sterilized in a hot air oven at 160^{o}C for 1 hrs. Sterilized discs were then impregnated with the prepared stock solution of the test compound. These discs were then dried in an oven at 25^{0}C. Antimicrobic discs were applied to the surface of the plates with sterile forceps. The spatial arrangement of the discs was such that they were not closer than 15 mm from the edges of the dishes to prevent overlapping of the zones of inhibition. The discs were not moved once they came in contact with agar surface. Each test compound was applied in triplicate and the zone of inhibition was determined by taking its average. Simultaneous discs were prepared for the control and the standard drugs. The petri discs were incubated at 37^{0}C for 24 hrs in case of bacteria and at 30^{0}C for 72 hrs for the fungus. Zone of inhibition was measured from the edge of the disc to the edge of the zone by a multimeter scale. Chloramphenicol and Amphotericin B were used as the standard drugs for bacteria and fungus respectively(10 µg/disc).

Table 2

Compound No.	Concentration µg/disc	Antibacterial S.aureus	Antibacterial E.coli	Antifungal C. albicans
III_a	125	++	++	+
	250	++	++	+
III_b	125	+	-	-
	250	+	-	-
IV_a	125	±	++	++
	250	±	++	++
IV_b	125	-	-	+
	250	-	-	+
V_a	125	-	+ +	-
	250	-	+ +	-
V_b	125	+	++	++
	250	+	+ +	++

Disc size 6.35
Duration : 24 hrs
Control : DMF
Standard Drug: Chloramphenicol

Duration 72 hrs
Control : DMF
Standard Drug :Amphotericine B

- Inactive
± Moderately active (8-12 mm)
+ Active (15-19mm)
++ Highly active (20-24mm)

- Inactive (heavy fungal colony)
± Moderately active (two three fungal colony)
+ Active (one fungal colony)
++ Highly active(no fungal colony)

RESULT AND DISCUSSION

The IR spectra of 1-(4-chloro-2-nitro anilino)-N-methyl piperazine (I) showed a peak at 1631.55 cm^{-1} and another at 3567 cm^{-1} which were identified as that of a nitro and amino group. Chloroacetylation gave compound II whose IR spectra showed three new frequencies at 1324 cm^{-1},1686 cm^{-1} and 3349 cm^{-1}. These signals indicated the presence of a halogen, carbonyl and NH in the nucleus thus supporting the

structure. Reaction with thiourea led to the formation of compound III_a. New frequencies at 1350 cm^{-1} were identified for N=C-S linkage part of the 1,3-thiazole ring. Similarly reaction with urea gave compound III_b . This derivative showed a new frequency at 2389cm^{-1} which indicated the presence of –N=C-O linkage. Subsequent reaction with p-nitro benzaldehyde, p-hydroxy benzaldehyde and 3-methoxy-4-hydroxy benzaldehyde gave the Schiff bases IV_a,IV_b,V_a and V_b . A new peak at 1615 cm^{-1} characterized as –N=CH group was visible in all derivatives.

The NMR spectra of compound I showed a signal at δ 1.56 which integrated for three protons indicating the presence of methyl group.Triplets at δ2.20 and δ 3.23 were identified as the protons part of the piperazine. The higher value were those protons attached to the benzene ring. Singlet at δ4.38 was also visible identified as the amino protons hence indicating the joining of 4-chloro-2-nitro aniline to the piperazine moiety.Multiplet for the aromatic protons were visible between δ7.62-7.99. Chloroacetylation gave compound II showed a new peak as a singlet at δ 2.89 identified as the methylene protons.Further reaction with thiourea and urea gave compounds III_a and III_b. The PMR spectra showed a signal as a singlet δ 7.26 ppm in both these derivatives. This was characterized as the proton attached to C_5 carbon of thiazole and oxazole ring. Subsequent reaction with p-nitro benzaldehyde,p-hydroxy benzaldehyde and vanilline gave the Schiff bases IV_a,IV_b,V_a and V_b. In all these derivatives a new peak at δ 4.21 ppm integrating for one proton was visible. This was the methine proton hence indicating the formation of the Schiff bases.

Antibacterial activity:

Maximum inhibition was shown by only one compound III_a against the gram positive bacteria S. aureus, while four compounds namely III_a, IV_a, V_a, and V_b were also highly active (20-24mm) against gram negative bacteria E. coli at both the concentration levels. Two derivatives III_b and V_b were also active with a zone size of 15-19 mm against S. aureus while one derivative IV_a inhibited the growth of these strains but with lesser radii.

However IV_b,and V_a were inactive against the gram positive bacteria S. aureus while compounds III_b and IV_b also did not inhibit the growth of E.coli.

Antifungal activity :

Among the six piperazinyl derivatives tested for the antifungal action, two compounds IV_a and V_b showed maximum activity against C. albicans with development of no fungal colony after 72 hours. III_a and IV_b were also active and showed only one fungal colony after the same time period. Other derivatives i.e. III_b, and V_b were inactive.

ACKNOWLEDGEMENTS

One of the authors Reena Rathore is thankful to CSIR for the award of JRF. Thanks are also to the Head, Department of Chemistry for providing the laboratory facilities and to Head, Department of Microbiology MDM Hospital Jodhpur for the antimicrobial evaluation. The authors are also thankful to SAIF Chandigarh for spectral data.

REFERENCES

1. Modell W, Schild H O,Wilson A (1976). Applied Pharmacology. American Edition, pp 711.
2. Mallams A K ,Das M B, Neusyadt B R, Demma M, Vaccaro H A (2007). Preparation of piperazinomethyl substituted quinazolines useful in cancer treatment.PCT Int. Appl. WO. Chemical Abstract. 146:1082.
3. Brown M F, Blumberg L C, Poss C S, Hayward M M, (2002). Preparation of N(phenoxyacetyl)piperazine derivatives for treatment of inflammation. Braz. Pedido PI BR. Chemical Abstract. 147:846.
4. Chen Y, Wang S, Xiangqingu X, Liu X, Minquan Y, Song Z, Liu S, Qiu Y, Zhang T, Liu B F,Zhang G(2013).Synthesis and biological investigation of coumarin piperazine (piperidine) derivatives as potential multireceptor atypical antipsychotics. *J. Med. Chem.* 56:4671–4690.
5. Yevich J P ,New J S, Smith D W (1986).Synthesis and biological evaluation of 1-(1,2-benzisothiazol-3-yl)- and (1,2-benzisoxazol-3-yl)piperazine derivatives as potential antipsychotic agents. J. Med. Chem. 29: 359–69.
6. Chenn R,Reddy K,Rasheed S, Rao S D, Adam S, Reddy R V Y, Raju N C (2013). New urea and thiourea derivatives of piperazine doped with febuxostat: synthesis and evaluation of anti-TMV and antimicrobial activities. The Sci. World J. 2013:10.
7. Tsuyoshi M, Nozomu S, Hiroyuki T, Katsuhito M, Masatoshi H,Yasuo S, Yu M (2003). Synthesis and biological activity of novel 5-(ω-Aryloxyalkyl)oxazole derivatives as brain-derived neurotrophic factor inducers. Chem. Phar. Bulletein. 51:565-573.
8. Hirofumi S H, Motohiro I, Hideaki K., Hiroyuki H, Tatsuo T, Masanori K, Makoto M, Makoto K, Hidetsugu T (2006). Synthesis and antituberculosis activity of a novel series of optically active 6-nitro-2,3-dihydroimidazo[2,1-b]oxazoles. J. Med. Chem. 49:7854-7860.
9. Malik D, Yadav P, Kumar S, Malik V (2014). Synthesis and fungicidal activities of transition metal complex of ligand 2 - amino -4- (p-hydroxyphenol) oxazole. IJREAS. 4:2.

10. Paul C U, David T C, Wiaczeslaw A C, Roderick J S, Catherine R K , Jagadish C S, Clifford D W, Denis J S, Richard D D,(1994). Synthesis and biological evaluation of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl] methylene] oxazoles-thiazoles and -imidazoles: novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity. J. Med. Chem. 37: 322–328.
11. Helal M H M, Salem M A E, Gaby M S A Aliahdali M (2013). Synthesis and biological evaluation of some novel thiazole compounds as potential anti-inflammatory agents.Euro. J. Med. Chem.62:270-279.
12. Berry A L(1977). The antimicrobial susceptibility test. Principles and practice. Chemical Abstract. 64:25183.
13. Bauer A W, Kirby W M, Sherris J C, Turk M (1996). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol. 44:493-496.

CITE THIS ARTICLE
Reena R, Pramilla S. Antimicrobial Activity of Some Synthesized Piperazinyl Derivatives. Res. J. Chem. Env. Sci. Vol 2 [6] December 2014. 54-59