HL
http://heteroletters.org

ISSN: 2231 – 3087(print)
ISSN: 2230 – 9632 (Online)
Coden HLEEAI
Vol. 5: (1), 2015, 79-84

ANTIMICROBIAL EVALUATION OF NOVEL SCHIFF'S BASES

Purvesh J. Shah

Chemistry Department, K.K.Shah Jarodawala Maninagar Science College, Maninagar, Ahmedabad, Gujarat (India).
**E-mail: purvesh23184@gmail.com*

Abstract: Mannich reaction of benzotriazole **(I)**, ethyl-p-amino benzoate **(II)** and formaldehyde afforded 4-(1H)-benzotriazoyl methyl amino benzoate **(III)**, which react with hydrazine hydrate results in the 4-(1H)-benzotriazoyl methyl amino benzoyl hydrazide **(IV)**. This compound on condensation with various aromatic aldehyde **(Va-j)** to afford the corresponding 4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-arylidenebenzohydrazide **(Va-j)**. All the compounds **(VIa-j)** were characterized by spectral studies. The compounds showed significant antimicrobial activity against various bacteria and fungi.

Keywords: Synthesis, heterocyclic substituted benzoyl hydrazide derivatives, schiff's bases, derivatives and antimicrobial activity.

INTRODUCTION

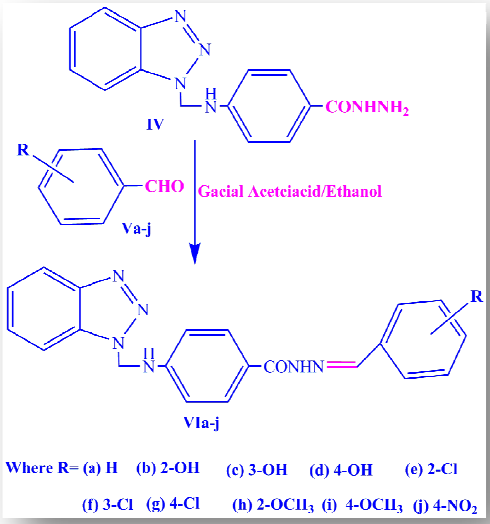
Schiff bases resultant from an amino and carbonyl compound are an significant class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied extensively[i]. Schiff bases derived from various heterocycles have been reported to acquire cytotoxic, anticonvulsant, antiproliferative, antimicrobial, anticancer and antifungal activities[ii-vii]. Mannich bases have expanded significance due to their application in pharmaceutical chemistry. They have been come acrossed with antibacterial, anticancer, analgesic and anti-inflammatory, anticonvulsant, antimalarial, antiviral and CNS depressant activities [viii-xiv]. Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical and biological activities[xv]. Hence the present paper comprises the synthesis and characterization of novel schiff's bases derivatives shown in Scheme 1. Futher work in these directions are in progress.

MATERIALS AND METHODS

Measurements

All chemicals used were of laboratory grade. Ethyl-p-amino benzoate and Benzotriazole were prepared by reported method[xvi]. Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded in KBr pellets on a Nicolet 760D spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046 instrument.

REACTION SCHEME



PREPARATION OF 4 - (1H) - BENZOTRIAZOLYL METHYL AMINO BENZOATE III

A mixture of 1H-Benzotriazole **I** (0.02mole), formaldehyde (0.02mole) and ethyl-4-amino benzoate **II** (0.02mole) in ethanol (50ml) was heated under reflux for 4hrs. Subsequently, ethanol was distilled off and the pasty mass obtained, which was triturated with petroleum ether (40-60°C). The solid 4-(1H)-benzotriazolyl methyl amino benzoate **III**, which was isolated and dried. Yield 68% , m.p.146-147°C. IR [v,cm^{-1},KBr]: 3086-3034(C-H aromatic), 2965(CH_2), 2910-2890,1456(C-H), 1725(C=O of ester),1255-1197(C-N). ^{1}HNMR [400 MHz, ð, ppm, DMSO- d_6] :8.21-6.56 (m, 8H, Ar-H), 5.7 (s, 2H, CH_2), 4.32(q,2H,-O-CH_2), 3.2(s,1H,NH), 1.32 (t,3H,-CH_3).^{13}C NMR [100 MHz, ð, *ppm*, DMSO]: 170.4(CO),149.1-114.3(Ar-C), 75.7 (CH_2), 62.1(CH_2),13.9(CH_3). LC-MS: *m/z* 305(M^+).Anal. Calcd for $C_{16}H_{16}N_4O_2$ (296)C, 64.85; H, 5.44; N, 18.91.Found; C, 64.8; H, 5.4; N, 18.9.

PREPARATION OF 4-(1H) - BENZOTRIAZOLYL METHYL AMINO BENZOYL HYDRAZIDE IV

4-(1H)-benzotriazolyl methyl amino benzoate **III** (0.05mole) was refluxed with hydrazine hydrate (0.05mole) in absolute ethanol for 8 to10 hours. It was cooled and kept overnight. The solid so obtained was filtered and recrystallized from ethanol. Yield 63%, m.p.77-78°C. IR [v,cm^{-1},KBr]: 3450(NH_2),3086-3034(C-H aromatic),2965(CH_2), 1725 (C=O of ester), 1630 (-CO-NH-NH_2), l255-1197 (C-N). ^{1}H NMR [400MHz, ð ,ppm, DMSO- d_6] : 9.66(s,1H,CONH), 8.21-6.56 (m, 8H,Ar-H), 5.7(s,2H,CH_2), 3.95(s,2H,NH_2), 3.2(s,1H, NH). ^{13}C NMR [100 MHz, ð, *ppm*, DMSO]: 170.4 (CO), 149.1-114.3 (Ar-C),75.7 (CH_2). LC-MS: *m/z* 291 (M^+). Anal. Calcd for $C_{14}H_{14}N_6O$ (282): C, 59.56; H, 5.00; N, 29.77.Found : C, 59.5; H, 4.9; N, 29.7.

PREPARATION OF 4-((1H-BENZO[D][1,2,3]TRIAZOL-1-YL)METHYLAMINO)-N'-ARYLIDENE BENZOHYDRAZIDE (VIa-j)

A compound **IV** (0.01 mole) and a various aromatic aldehyde **Va-j** (0.01 mole) was refluxed in absolute ethanol (30 mL) in presence of a catalytic amount of glacial acetic acid for 3 to 5 hours. The reaction mixture was cooled and the precipitate was filtered and recrystallized from methanol to give compounds **VIa-j**.

VIa: 4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-benzylidenebenzohydrazide (VIa).

Yield 72%, m.p.184–186 °C, IR[ν, cm^{-1}, KBr]: 3445(NH),3086-3034(C-H aromatic), 2965(CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N). ^1H NMR[400MHz,ð,ppm,DMSO-d_6]:9.66 (s, 1H, CONH), 8.21-6.56 (m, 13H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH),8.56(s,1H, HC=N-). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),149.1-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{18}N_6O$ (370.18): C, 68.09; H, 4.90; N, 22.69. Found: C68.07; H, 4.89; N, 22.67.

VIb:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(2-hydroxybenzylidene)benzo hydrazide (VIb).

Yield 68%, m.p.171–172 °C, IR[ν,cm^{-1}, KBr]: 3445-3435(NH,OH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N). ^1H NMR[400MHz, ð,ppm, DMSO-d_6]: 9.66 (s, 1H, CONH), 8.21-6.56 (m, 12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH),8.56(s,1H, HC=N-),5.47(s,1H,OH). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),156.8-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{18}N_6O_2$ (386.16): C, 65.27; H, 4.70; N, 21.75. Found: C, 65.25; H, 4.68; N, 21.74.

VIc:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(3-hydroxybenzylidene)benzo hydrazide (VIc).

Yield 65%, m.p.168–170 °C, IR[ν,cm^{-1}, KBr]: 3447-3435(NH,OH),3086-3034(C-H aromatic), 2965 (CH_2),1670 (amide C=O),1630 (NH),1596-1548(C=N). ^1H NMR[400MHz, ð,ppm,DMSO-d_6]: 9.66(s,1H,CONH),8.21-6.56(m,12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH),8.56(s,1H, HC=N-),5.38(s,1H,OH). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),159.1-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{18}N_6O_2$ (386.16): C, 65.27; H, 4.70; N, 21.75. Found: C, 65.26; H, 4.67; N, 21.73.

VId:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(4-hydroxybenzylidene)benzo hydrazide (VId).

Yield 66%, m.p.169–171 °C, IR[ν,cm^{-1}, KBr]: 3447-3435(NH,OH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N). ^1H NMR[400MHz, ð,ppm,DMSO-d_6]: 9.66 (s, 1H, CONH), 8.21-6.56 (m, 12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH),8.56(s,1H, HC=N-),5.36(s,1H,OH). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),160.4-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{18}N_6O_2$ (386.16): C, 65.27; H, 4.70; N, 21.75. Found: C, 65.27; H, 4.68; N, 21.74.

VIe:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(2-chlorobenzylidene)benzo hydrazide (VIe).

Yield 69%, m.p.174–176 °C, IR[ν,cm^{-1}, KBr]: 34425(NH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),846(C-Cl). ^1H NMR[400MHz, ð,ppm, DMSO-d_6]: 9.66 (s, 1H, CONH), 8.17-6.56 (m, 12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH), 8.56 (s,1H, HC=N-). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),151.6-111.3 (Ar-C), 134.3 (ArC-Cl),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{17}N_6OCl$ (404.5): C, 62.30; H, 4.23; N, 20.76; Cl, 8.76. Found: C, 62.28; H, 4.23; N, 20.75; Cl, 8.75.

VIf:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(3-chlorobenzylidene)benzo hydrazide (VIf).

Yield 70%, m.p.170–172 °C, IR[ν,cm^{-1}, KBr]: 3443(OH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),848(C-Cl). ^{1}H NMR[400MHz, ð,ppm, DMSO-d_6]: 9.66 (s, 1H,CONH),8.17-6.56(m,12H,Ar-H),5.7(s,2H,CH_2),3.2(s,1H, NH), 8.56 (s,1H, HC=N-). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),151.6-111.3 (Ar-C), 134.5 (ArC-Cl),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{17}N_6OCl$ (404.5): C, 62.30; H, 4.23; N, 20.76; Cl, 8.76. Found: C, 62.29; H, 4.22; N, 20.75; Cl, 8.74.

VIfg:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(4-chlorobenzylidene)benzo hydrazide (VIg).

Yield 71%, m.p.169–170 °C, IR[ν,cm^{-1}, KBr]: 3443(OH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),848(C-Cl). ^{1}H NMR[400MHz, ð,ppm, DMSO-d_6]: 9.66(s,1H,CONH),8.17-6.56(m,12H,Ar-H),5.7(s,2H,CH_2),3.2(s,1H, NH), 8.56 (s, 1H, HC=N-). ^{13}C NMR[100 MHz, ð, ppm,DMSO]:170.4(CO),151.6-111.3(Ar-C),134.6 (ArC-Cl),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{17}N_6OCl$ (404.5): C, 62.30; H, 4.23; N, 20.76; Cl, 8.76. Found: C, 62.28; H, 4.23; N, 20.74; Cl, 8.74.

VIh:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(2-methoxybenzylidene)benzo hydrazide (VIh).

Yield 66%, m.p.165–167 °C, IR[ν,cm^{-1}, KBr]: 3435(NH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),1298-1090(OCH_3). ^{1}H NMR[400MHz, ð,ppm,DMSO-d_6]: 9.66 (s, 1H, CONH), 8.21-6.56 (m, 12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH), 8.56(s,1H,HC=N-),3.96(s,3H,OCH_3). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),157.4-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N),56.2(OCH_3). Anal. Calcd. for $C_{22}H_{20}N_6O_2$ (400): C, 65.99; H, 5.03; N, 20.99. Found: C, 65.97; H, 5.03; N, 20.97.

VIi:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(3-methoxybenzylidene)benzo hydrazide (VIi).

Yield 67%, m.p.168–169 °C, IR[ν,cm^{-1}, KBr]: 3435(NH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),1296-1094(OCH_3). ^{1}H NMR[400MHz, ð,ppm,DMSO-d_6]: 9.66 (s, 1H, CONH), 8.21-6.56 (m, 12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH), 8.56(s,1H,HC=N-),3.94(s,3H,OCH_3). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),157.4-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N),56.4(OCH_3). Anal. Calcd. for $C_{22}H_{20}N_6O_2$ (400): C, 65.99; H, 5.03; N, 20.99. Found: C, 65.99; H, 5.01; N, 20.98.

VIj:4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-(4-nitrobenzylidene) benzohydrazide (VIj).

Yield 69%, m.p.169–170°C, IR[ν,cm^{-1}, KBr]: 3435(NH),3086-3034(C-H aromatic), 2965 (CH_2), 1670 (amide C=O),1630 (NH),1596-1548(C=N),1540,1380(NO_2). ^{1}H NMR[400MHz, ð,ppm, DMSO-d_6]: 9.66 (s, 1H, CONH), 8.35-6.56 (m,12H,Ar-H),5.7(s,2H,CH_2), 3.2(s,1H, NH), 8.56 (s, 1H,HC=N-). ^{13}C NMR[100 MHz, ð, ppm,DMSO]: 170.4 (CO),152.4-114.3 (Ar-C),75.7 (CH_2),143.2 (C=N). Anal. Calcd. for $C_{21}H_{17}N_7O_3$ (415): C, 60.72; H, 4.12; N, 23.60. Found: C, 60.70; H, 4.11; N, 23.59.

RESULTS AND DISCUSSION

The compound **IV** (hydrazide) has been synthesized successfully as the Mannich reaction reported previously[xvii,xviii] and shown in Scheme 1. Its IR spectrum showed absorption bands

in the 3450cm^{-1} (hydrazide NH-NH$_2$), 3086-3034 cm^{-1} (aromatic C-H), 1725 cm^{-1}(-C=O carbonyl stretching) and 1630 cm^{-1} (-CO-NH-NH$_2$ groups), respectively. The ^{1}H-NMR spectrum exhibited a singlet due to the –CO-NH-NH$_2$ at ð 3.95 ppm. Methylene protons (-CH$_2$-) resonated as singlets at 5.7 ppm. The ^{13}CNMR spectrum exhibited 170.4 and 75.7 ppm for CO, CH$_2$ respectevely.

The series of Schiff's bases **VIa-j** was prepared by refluxing solutions of different suitable aromatic aldehydes and hydrazide **IV** in absolute ethanol for 3 to 5 hours, in a presence of catalytic amount of glacial acetic acid.The structures of the products **VIa-j** were inferred from their analytical and spectral data.The IR spectra of compounds **VIa-j** showed characteristic bands at 3,445–3,278 cm^{-1} (NH), 1670 (amide C=O),1630 (NH) and 1596-1548(C=N). The ^{1}HNMR spectra did not only show the absence of NH$_2$ protons at 3.95, but also the presence of N=CH proton at 8.56 ppm.^{13}C NMR exhibitd 75.7 ppm for CH$_2$ and 143.2 ppm for C=N.

All the compounds were confirmed on the basis of the elemental analysis and spectroscopic investigation. The examination of these data reveals that the IR band and ^{1}H NMR signals are appropriate to the correspond structure of compound.The final structure of all compounds was confirmed by ^{13}C NMR and LC- MS data, i.e. The compounds **VIIa** shows the molecular ion peak *m/z* 398 give the molecular weight of **VIIa** i.e. 370.18. All these facts confirm the structures (**VIIa-j)**.

ANTIMICROBIAL ACTIVITY

Antibacterial activities of all the compounds were studied against Gram–positive Bacteria (*Bacilus subtilis* and *Staphyllococcus aureus*) and Gram-negative Bacteria (*E.coil, Salmonella typhi* and *Klebsiella promioe*) at a concentration of 50µg/ml by Agar cup plate method[xix-xx]. Methanol system was used as control in this method. Under similar condition using sulphonamide as a standard for comparison carried out control experiment. The area of inhibition of zone measured in mm. Compound **VIIg** and **VIIi** found more active against the above microbes. Other compounds were found more active against the above microbes. The antibacterial activities of all the compounds are shown in Table-1.

Table 1: Antibacterial activity of the compounds **(VIa-j)**

Compounds	**Activity Index**				
	Gram +ve		**Gram –ve**		
	Bacillus Subtilis	***Staphylococcus Aureus***	***Kllebsiella promioe***	***Salmonella typhl***	***E.coil***
VIa	0.6	0.8	0.78	0.79	0.82
VIb	0.54	0.86	0.84	0.82	0.83
VIc	0.76	0.83	0.81	0.81	0.78
VId	0.74	0.85	0.8	0.84	0.82
VIe	0.8	0.81	0.84	0.85	0.8
VIf	0.81	0.83	0.83	0.82	0.79
VIg	0.84	0.88	0.86	0.89	0.87
VIh	0.75	0.8	0.8	0.84	0.83
VIi	0.83	0.86	0.85	0.9	0.84
VIj	0.8	0.78	0.81	0.85	0.83
Sulphonamide	1	1	1	1	1

(Activity Index) ^{std} ***= Zone of Inhibition of the sample/ Zone of Inhibition of the standard***

Conclusion:

The present study reports the synthesis of novel Schiff's bases 4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-arylidenebenzohydrazide **(VIa-j)** from the corresponding precursors various aromatic aldehyde **(Va-j)** and 4-(1H)- benzotriazolyl methyl amino benzoyl hydrazide **(IV)**. The antimicrobial activity of 4-((1H-benzo[d][1,2,3]triazol-1-yl)methylamino)-N'-arylidenebenzo hydrazide **(VIa-j)** was carried out against some strain bacteria. The results show that the synthesized compounds were toxic against the bacteria. The investigation of antibacterial screening reveals that the compounds **VIg** and **VIi** have exhibited good antibacterial activity comparable to the standard drugs.

References

i. Kumar S.; Niranjan M.S.; Chaluvaraju K.C.; Jamakhandi C.M. and Dayanand K.,*J Curr Pharm Res.,*2010,**01**,39.
ii. Tarafder, M.T.; Kasbollah, A.; Saravan, N.; Crouse, K.A.; Ali, A.M.; Tin, O.K. , *J.Biochem. Mol. Biol. Biophs.,*2002,**6**,85.
iii. Küçükgüzel, I.; Küçükgüzel, S.G.; Rollas, S.; Ötük-Sanis, G.; Özdemir, O.; Bayrak, I.; Altug, T. and Stables, J.P. , *Farmaco.* **2004**, *59*, 893.
iv. Vicini, P.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Kabras, C.A. and Colla, P.L. *Bioorg.Med. Chem.* 2003, **11**, 4785.
v. Kahveci, B.; Bekircan, O. and Karaoglu, S.A. , *Indian J. Chem*. 2005, **44B**, 2614.
vi. Bekircan, O.; Kahveci, B. and Kucuk, M.,*Tur. J. Chem.* 2006, **30,** 29.
vii. Singh, W.M. and Dash, B.C., *Pesticides,* 1988,**22**,33.
viii. Holla, B.S.; Shivananda, M.K.;Shenoy, M.S. and Antony, G.,*Farmaco.*1998,**53**,531.
ix. Holla, B.S.; Veerendra, B.; Shivananda, M.K.and Poojary, B.,*Eur. J. Med.Chem.,*2003,**38**,759.
x. Gokce, E.; Bakir, G.; Sahin, M.F.; Kupeli, E. and Yesilada, E., *Arzneim. Forsch.* 2005, **55,318**.
xi. Dimmock, J.R.; Jonnalagadda, S.S.; Phillips, O.A.; Erciyas, E.; Shyam, K. and Semple, H.A.,*J. Pharm. Sci*.,1992,**81**,436.
xii. Lopes, F.; Capela, R.; Goncaves, J.O.; Horton, P.N.; Hursthouse, M.B.; Iley, J.; Casimiro, C.M.;Bom, J. and Moreire, R.,*Tetrahedron Lett.,*2004,**45**,7663.
xiii. Sriram, D.; Bal, T.R. and Yogeesswari, P.,*Med. Chem. Res*.,2005,**14**,11.
xiv. Knabe, J.; Buch, H.P. and Schmitt, W., *Arch. Pharm. Chem. Life Sci.,* 1983, **316**, 1051.
xv. Gemi, M.J.; Biles,C.; Keiser, B.J.; Poppe,S.M.; Swaney, S.M.;Tarapley, W.G.;Romeso, D.L. and Yage, Y.,*J.Med.Chem.*,2000,**43(5)**,1034.
xvi. Vogel, A. I.,*A Texts Book of Practical Organic Chemistry,5th edn*.,1989,701,1162,883.
xvii. Amir, M.; Siddiqui, A. A. and Rizwan, S., *Oriental J Chem*., 2003, **19(3),** 629.
xviii. Patel, K. V. and Singh, A., *E-Journal of Chem*., 2009, **6(1),** 281.
xix. Baily, W. R. and Scott, E. G., *Diagnostic Microbiology, The C.V. Moshy Co. St. Lovis*, 1966,257.
xx. Banty,A. L.,*The Antimicrobial Susceptibility test; The Principal and practice edited by Illus lea and Febiger (Philadelphia. Pa USA),*1976,180.

Received on November 12, 2014.