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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NOVEL PYRIDINE QUATERNARY ANALOGS

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ABSTRACT

Resistance to known antimicrobial drugs has continued to emerge as a global human threat which can be solved by the discovery of fresh candidates. In this regard, we hereby discussed the synthesis of novel analogs of 4-Pyridine carbohydrazide PCH (I) which was prepared by quaternizing this molecule with substituted phenacyl halides (II a-k). The obtained products were structurally characterized and then screened for their *in-vitro* antimicrobial potential against different bacteria and fungi. It was observed that some of the prepared derivatives exhibited moderate to good antibacterial activity where *p*-phenyl and *p*-chloro substitutions induced activity in derivatives III a and III c against tested species of *E. coli*. Compound III a and III i was more active against *Candida albicans* than that of the standard antifungal drug Griseofulvin.

KEYWORDS: Pyridine carbohydrazide, Phenacyl halides, Quaternization, Antimicrobial activity, Agar Well method, Structure Activity Relationship.

INTRODUCTION

Microorganisms have a global existence of more than 3.8 billion years, shared half of the living biomass and secured a significant position in the biosphere where they are crucial for maintaining the ecosystem.[1-3] In contrast, the pathogenic microbes remained a health threat where mankind made efforts to eradicate those using antimicrobial agents. Now a day, infections became the 3rd in United States and 2nd primary source of death globally.

It has been recognized that microorganisms possessed natural ability to develop resistance against the used treatments, rendering these with reduced affectivity. There is a high demand of newer anti-infective agents because of several reasons and questioned when if not today. One possible reason could be the mortality rates associated with the emergence of antimicrobial resistance.[4, 5] Second is the lack of introduction of fresh anti-infective agents for the last 40 years.[6, 7] Another reason is that the pharmaceutical companies curtailed their research for the development of new antibiotic treatments.[8, 9] On the other hand, globalization also raised the risk of spread of infectious diseases among different countries.[10]

Medicinal chemistry remained highly fruitful in development of new anti-infective as well as in redesigning of already available natural and synthetic antibiotics. With this view, we attempt to synthesize derivatives with potential activity against bacteria and fungi by taking PCH as parent molecule. This compound remained therapeutically famous as Isoniazid and used as first line antitubercular drug. [11] Consequently antimicrobial action of PCH was also evident from the past studies where numerous of its analogs possessed this effect.[12-17]

MATERIALS AND METHODS

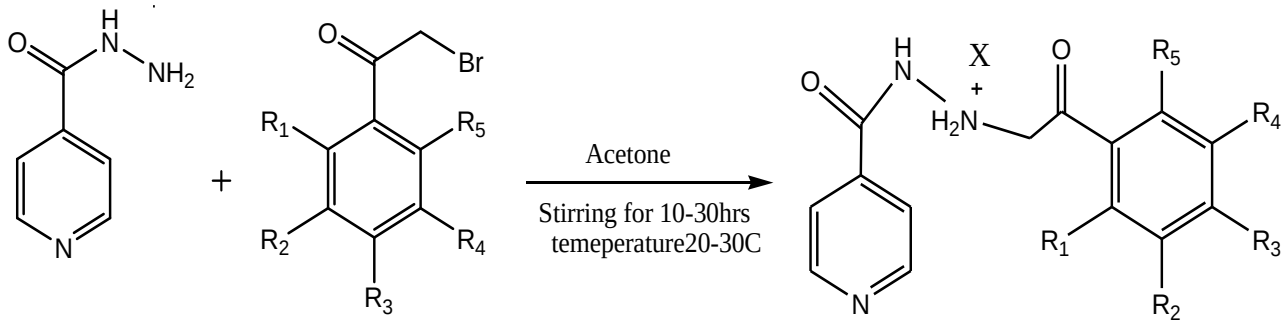
Analytical grade solvents (acetone & ethanol) were obtained from E. Merck and doubled distilled prior to use. Other chemicals were acquired from Sigma Aldrich Chemical Company. Reaction response was observed by means of TLC using pre-coated silica gel, GF-254. Gallen Kamp melting point apparatus was used to find out the melting points which were uncorrected. Spots were visualized under iodine vapors and ultraviolet light. Gravity filtration was done using Whatman Filter Paper-1.

Infrared (IR) spectra were determined on VECTOR$_{22}$FTIR Spectrophotometer using KBr discs. Varian Massen spectrometer MAT 331A was used to obtain mass spectra. Proton NMR(s) were measured via AVANCE 400-B/300 spectrophotometer using d_6-DMSO. Elemental analysis was performed on Perkin Elmer 2400 Series II, CHN/S Elemental Analyzer.

Synthetic procedure for quaternary derivatives III a- k

Equimolar solutions (3.6×10^{-6}mM) of I and II a-k were dissolved in acetone, mixed and stirred constantly for 10-30 hours (Scheme-1). Solid products III a-k were separated from the

reaction mixture via gravity filtration, washed with acetone and recrystallized with ethanol. After having round spots on TLC, products were kept in vacuum desicator over silica beads.



I II a-k III a-k

III a; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = C_6H_5
III b; X = Cl, R_1, R_4, R_5= H, R_2, R_3 = OH
III c; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Cl
III d; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = F
III e; X = Br, R_1, R_3, R_4, R_5 = H, R_2 = NO_2
III f; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = NO_2
III g; X = Cl, R_2, R_4, R_5 = H, R_1, R_3 = F
III h; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = Br
III i; X = Br, R_2, R_3, R_5= H, R_1,R_4 = OCH_3
III j; X = Br, R_1, R_2, R_4, R_5 = H, R_3 = OCH_3
III k; X = Br, R_1, R_2, R_3, R_4, R_5 = H

Scheme-1: Synthetic procedure for quaternary derivatives III a-k

[2-oxo-2-(4-phenylphenyl)ethyl](pyridin-4-yl formamido) azanium bromide III a

$C_{20}H_{18}BrN_3O_2$, Off-white solid, Yield (%): 46, M.P. (°C): 257-259. **UV λ_{max} (nm) (EtOH):** 223 and 292. **IR (KBr) cm^{-1}:** 3120.3 (NH str.), 1696.5 (C=O str.), 1668.6 (CO str., acyl), 1642.8 (C=C str. aromatic), 3050.3 (C-H str. aromatic), 2996.8 (C-H str., aliphatic). **EI-MS m/z (-HBr):** 313$[M]^+$. **^{1}H-NMR (d_6- DMSO, 300MHz) δ:** 9.06-9.16 (dd, 2H-pyridine; *J*=23.7), 8.35-8.56 (dd, 2H-pyridine; *J*=56.4), 8.13-8.16 (d, 2H-phenyl; *J*=8.1), 7.97-8.00 (d, 2H-phenyl; *J*=8.1), 7.80-7.83 (d, 2H-substituted phenyl; *J*=7.2), 7.44-7.56 (m, 3H-substituted phenyl), 6.53-6.57 (d, 2H-quaternary N; *J*=10.8), 11.23-11.37 (d, 1H-CONH; *J*=41.4), 3.32 (s, 2H-CH_2). **CHN analysis: Calculated** C 58.2; H 4.4; N 10.1, **Found** C 61.7; H 3.1; N 9.5

[2-(3,4-dihydroxyphenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium chloride III b

$C_{14}H_{14}ClN_3O_4$, Grey solid, Yield (%): 49, M.P. (°C): 215-217. **UV λ_{max} (nm) (EtOH):** 223 and 284. **IR (KBr) cm^{-1}:** 3174.2 (NH str.), 1703.0 (C=O str.), 1668.6 (CO str., acyl), 1643.3 (C=C str., aromatic), 3097.23 (C-H str., aromatic), 2990.1(C-H str., aliphatic), 3560.5 (OH stretching). **EI- MS m/z (-HCl):** 271 $[M\text{-}OH]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.04-9.11

(d, 2H-pyridine; *J*=22.8), 8.30-8.56 (dd, 2H-pyridine; *J*=71.1), 7.44-7.47 (d, 2H-phenyl; *J*=9.3), 6.97-7.00 (d, 1H-phenyl; *J*=8.1), 6.37-6.42 (d, 2H-quaternary N; *J*=9), 9.69 (s, 1H-ArOH), 10.40 (s, 1H-ArOH), 11.35-11.40 (d, 1H-CONH; *J*=13.8), 3.35 (s, 2H-CH_2). **CHN analysis: Calculated** C 45.6; H 3.8; N 11.4, **Found** C 51.5; H 4.2; N 10.5

[2-(4-chlorophenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium bromide III c

$C_{14}H_{13}BrClN_3O_2$, White solid, Yield (%), 72, M.P. (°C): 213-214 **UV λ_{max} (nm) (EtOH):** 224 and 262. **IR (KBr) cm^{-1}:** 3174.2 (NH str.), 1703.0 (C=O str.), 1681.3 (CO str., acyl), 1643.3 (C=C str., aromatic), 3097.2 (C-H str., aromatic), 2900.6 (C-H str., aliphatic). **EI-MS m/z (-HBr):** 292 $[M+2]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.02-9.12 (dd, 2H-pyridine; *J*=24.6); 8.34-8.55 (dd, 2H-pyridine; *J*=56.1), 8.06-8.09 (d, 2H-phenyl; *J*=8.4), 7.74-7.77 (d, 2H-phenyl; *J*=8.1), 6.47-6.51 (d, 2H-quaternary N; *J*=12), 11.22-11.36 (d, 1H-CONH; *J*=42.6), 3.32 (s, 2H-CH_2). **CHN analysis: Calculated** C 45.3; H 3.5; N 11.3 **Found** C 46.3; H 2.9; N 9.5

[2-(4-fluorophenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium bromide III d

$C_{14}H_{13}BrFN_3O_2$, White solid, Yield (%): 76, M.P. (°C): 230-232**UV λ_{max} (nm) (EtOH):** 222 and 255. **IR (KBr) cm^{-1}:** 3188.5 (NH str.), 1703.3 (C=O str.), 1670.5 (CO str., acyl), 1639.9(C=C str. aromatic), 3074.3 (C-H str. aromatic), 2999.2 (C-H str., aliphatic). **EI-MS m/z (-HBr):** 271.9 $[M-1]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.02-9.13 (dd, 2H-pyridine; *J*=24.3); 8.34-8.55 (dd, 2H-pyridine; *J*=56.4), 8.13- 8.17 (t, 2H-phenyl; *J*=17.4), 7.49-7.54 (t, 2H-phenyl; *J*=12), 6.47-6.51 (d, 2H-quaternary N; *J*=12), 11.22-11.36 (d, 1H-CONH; *J*=42.6), 3.31 (s, 2H-CH_2). **CHN analysis: Calculated** C 47.4; H 3.7; N 11.8 **Found** C 51.3; H 2.2; N 10.6

[2-(3-nitrophenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium bromide III e

$C_{14}H_{13}BrN_4O_4$, Dull white solid, Yield (%): 89, M.P. (°C): 221-223, **UV λ_{max} (nm) (EtOH):** 220 and 255. **IR (KBr) cm^{-1}:** 3122.7 (NH str.), 1705.2 (C=O str.), 1683.2 (CO str., acyl), 1641.8 (C=C str. aromatic), 3026.2 (C-H str. aromatic), 2950.0 (C-H str., aliphatic), 1526.63, 1352.7 (Ar-NO_2 str.). **EI-MS m/z (-HBr):** 281.9 $[M–H_2O]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.06-9.17 (dd, 2H-pyridine; *J*=19.5), 8.37-8.50 (dd, 2H-pyridine; *J*=38.7), 8.75 (s, 1H-phenyl), 8.56-8.62 (t, 2H-phenyl; *J*=18), 7.95-8.00 (t, 1H-phenyl; *J*=15.9), 6.628-6.672 (d, 2H-quaternary N; *J*=13.2), 11.25-11.37 (d, 1H-CONH; *J*=36.6), 3.33 (s, 2H-CH_2). **CHN analysis: Calculated** C 44.1; H 3.4; N 14.7 **Found** C 49.2; H 5.1; N 13.4

[2-(4-nitrophenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium bromide III f

$C_{14}H_{13}BrN_4O_4$, Off-white solid, Yield (%): 90, M.P. (°C): 214-218, **UV λ_{max} (nm) (EtOH):** 225and 263. **IR (KBr) cm^{-1}:** 3104.1 (NH str.), 1708.7 (C=O str.), 1688.4 (CO str., acyl), 1643.4 (C=C str. aromatic), 3079.6 (C-H str. aromatic), 2879.4 (C-H str., aliphatic), 1523.9, 1333.0 (Ar-NO_2 str.). **EI-MS m/z (-HBr):** 255.7$[M\text{-}NO_2]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.04-9.14 (dd, 2H-pyridine; *J*=24.6), 8.28-8.38 (dd, 2H-pyridine; *J*=30.9), 8.46-8.57 (dd, 4H-phenyl; *J*=32.7), 6.55-6.59 (d, 2H-quaternary N; *J*=13.2), 11.24-11.38 (d, 1H-CONH; *J*=41.4), 3.33 (s, 2H-CH_2). **CHN analysis: Calculated** C 44.1; H 3.4; N 14.7 **Found** C 46.1; H 5.3; N 12.4

[2-(2,4-difluorophenyl)-2-oxoethyl](pyridin-4-yl formamido) azanium chloride III g

$C_{14}H_{12}ClF_2N_3O_2$, White solid, Yield (%): 28, M.P. 228-231, **UV λ_{max} (nm) (EtOH):** 225 and 249. **IR (KBr) cm^{-1}:** 3118.8 (NH str.), 1687.4 (C=O str.), 1666.7 (CO str., acyl), 1643.4 (C=C str. aromatic), 2920.4 (C-H str. aromatic), 2829.2 (C-H str., aliphatic). **EI-MS m/z (-HCl):** 273.0 $[M\text{-}H_2O]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.04-9.14 (dd, 2H-pyridine; *J*=29.1), 8.52-8.59 (dd, 2H-pyridine; *J*=21.6), 7.35-8.14 (m, 3H-phenyl), 6.34-6.37(d, 2H-quaternary N; *J*=8.4), 11.38-11.45 (d, 1H-CONH; *J*=20.7), 3.33 (s, 2H-CH_2). **CHN analysis: Calculated** C = 51.3; H = 3.6; N = 12.8 **Found** C = 56.1; H = 4.8; N = 11.5

[2-(4-bromophenyl)-2-oxoethyl]pyridin-4-yl formamido) azanium bromide III h

$C_{14}H_{13}Br_2N_3O_2$, White solid, Yield (%), 45, M.P. (°C), 217-218, **UV λ_{max} (nm) (EtOH):** 220 and 245. **IR (KBr) cm^{-1}:** 3107.2 (NH str.), 1693.8 (C=O str.), 1658.8 (CO str., acyl), 1642.8 (C=C str. aromatic), 3039.8 (C-H str. aromatic), 2890.0 (C-H str., aliphatic). **EI-MS m/z (-HBr):** 319.2 $[M\text{-}CH_3]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.08-9.06 (d, 2H-pyridine; *J*= 6.6), 8.47-8.49 (d, 2H-pyridine; *J*=6.6), 7.88-8.00 (q, 4H-phenyl), 6.46 (s, 2H-quaternary N), 10.68 (s, 1H-CONH), 3.32 (s, 2H-CH_2). **CHN analysis: Calculated** C 40.5; H 3.1; N 10.1 **Found** C 41.8; H 2.4; N 9.8

[2-(2,5-dimethoxyphenyl)-2-oxoethyl] (pyridin-4-yl formamido) azanium bromide III i

$C_{16}H_{18}BrN_3O_4$, Yellow solid, Yield (%): 48, M.P. (°C): 161-162, **UV λ_{max} (nm) (EtOH):** 219 and 260. **IR (KBr) cm^{-1}:** 3176.3 (NH str.), 1693.9 (C=O str.), 1660.2 (CO str., acyl), 1643.3 (C=C str. aromatic), 3041.4 (C-H str. aromatic), 2892.9 (C-H str., aliphatic), 2834.7 (Ar-OCH_3 str.). **EI-MS m/z (-HBr):** 297.0 $[M\text{-}H_2O]^+$. **^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.08-9.13 (t, 2H-pyridine; *J*=14.4), 8.44-8.52 (dd, 2H-pyridine; *J*=22.2), 7.33-7.35 (d, 3H-phenyl; *J*=5.7), 6.24-6.26 (d, 2H-quaternary N; *J*=6.6), 3.75 (s, 6H-ArOCH_3), 11.22-11.35 (d, 1H-

CONH; *J*=39.3), 3.33 (s, 2H-CH_2). **CHN analysis: Calculated** C 48.5; H 4.5; N 10.6 **Found** C 52.3; H 6.4; N 9.5

[2-(4-methoxyphenyl)-2-oxoethyl]pyridin-4-yl formamido) azanium bromide III j

$C_{15}H_{16}BrN_3O_3$, Yellow solid, Yield (%): 60, M.P. (°C): 226-228, **UV λ_{max} (nm) (EtOH):** 228 and 287. **IR (KBr) cm^{-1}:** 3150.0 (NH str.), 1695.1 (C=O str.), 1673.2 (CO str., acyl), 1643.5 (C=C str. aromatic), 3020.1 (C-H str. aromatic), 2939.0 (C-H str., aliphatic), 2839.1 (Ar-OCH_3str.). **EI-MS m/z (-HBr):** 285 $[M]^+$.**^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.03-9.14 (dd, 2H-pyridine; *J*=24.9), 8.33-8.54 (dd, 2H-pyridine; *J*=57.3), 8.03-8.06 (d, 2H-phenyl; *J*=8.7), 7.17-7.20 (d, 2H-phennyl; *J*=8.7), 6.44-6.48 (d, 2H-quaternary N; *J*=12), 3.89 (s, 3H-ArOCH_3), 11.23-11.36 (d, 1H-CONH; *J*=41.4), 3.33 (s, 2H-CH_2). **CHN analysis: Calculated** C 49.2; H 4.4; N 11.4 **Found** C 54.7; H 6.2; N 10.5

(2-oxo-2-phenylethyl) (pyridin-4-yl formamido) azanium bromide III k

$C_{14}H_{14}BrN_3O_2$, White solid, Yield (%): 89, M.P. (°C): 235-236, **UV λ_{max} (nm) (EtOH):** 226 and 254. **IR (KBr) cm^{-1}:** 3174.1(NH str.), 1695.6 (C=O str.), 1677.5 (CO str., acyl), 1641.4 (C=C str. aromatic), 3057.8 (C-H str. aromatic), 2983.1 (C-H str., aliphatic). **EI-MS m/z:** 237.0 $[M-H_2O]^+$.**^{1}HNMR (d_6- DMSO, 300MHz) δ:** 9.03-9.13 (dd, 2H-pyridine; *J*=2), 8.34-8.55 (dd, 2H-pyridine; *J*=55.8), 8.05-8.08 (d, 2H-phenyl; *J*=7.8), 7.64-7.82 (m, 3H-phenyl), 6.48-6.52 (d, 2H-quaternary N; *J*=11.4), 11.22-11.37 (d, 1H-CONH; *J*=44.4), 3.32 (s, 2H-CH_2). **CHN analysis: Calculated** C 50.0; H 4.2; N 12.5 **Found** C 55.8; H 6.3; N 11.2

***In-vitro* Antibacterial Activity**

Agar well method was used to screen compounds III a-k against selected gram-positive and gram-negative bacteria which were identified using conventional purity techniques and maintained on nutrient agar at 4°C till use. Bacterial cultures were maintained on Autoclaved Muller Hinton broth. Later, wells were dug onto Muller Hinton Agar in which 10µL of the cultures and the test compounds were introduced.[18] After 24 - 48 hours of incubation at a temperature of 28 ± 2°C, each derivative was observed for diameter of zone of inhibition using vernier caliper. Gentamicin was employed as standard antibiotic.[19]

***In-vitro* Antifungal Activity**

3 yeast, 2 dermatophytes and 2 saprophytic fungi were selected to test the antifungal action of derivatives III a-k using agar-well method. Sabourd Dextrose Agar (SDA) (Oxoid, Basingstoke-UK) was used to keep the fungal isolates at 4°C until needed. Autoclaved

distilled water was used to prepare fungal spore suspensions which transferred aseptically into each SDA plates and then loaded with the samples.[20] After incubating the plates at 28 ± 2°C for 24 -48 hours, diameter of zone of inhibition was gauged via vernier caliper. Griseofulvin antifungal agent was used as a positive control.

RESULTS AND DISCUSSION

Chemistry

The IR spectra of derivatives III a-k exhibited NH-amide peak at around 3104-3188cm^{-1}. Carbonyl (or nicotinoyl) group appeared as stretching band at 1687 - 1708cm^{-1} while the spectra revealed CO acyl at 1658 - 1688cm^{-1}. Peak around 1624 - 1643cm^{-1} confirmed C=C aromatic stretching while C-H aromatic stretching showed the presence at 2920 - 3097cm^{-1}. Band at 2829 - 2999cm^{-1} suggested C-H aliphatic structures in the IR spectra of all these compounds. Aromatic nitro group in compounds III e and III f were confirmed by vibrational frequencies at 1523 - 1538cm^{-1} and 1333 - 1354 cm^{-1}. Presence of methoxy group confirmed at 2834cm^{-1} and 2839 cm^{-1} in III i and III j respectively. Vibrational band at 3560cm^{-1} presented aromatic hydroxyl group in III b.

^{1}H-NMR spectra revealed the resonance of four pyridine hydrogen atoms δ 8.3 - 9.1 while hydrogen atoms of substituted aromatic ring resonated at δ 6.7-8.7. ^{1}H-NMR spectra of III a exhibited aromatic hydrogens at δ 7.4 – 7.8 while aliphatic CH_2 hydrogens in all the derivatives appeared at δ 3.1 – 3.3. Singlet peaks at δ 9.6 and δ 10.4 indicated the presence of two hydroxyl groups in III b. Methoxy hydrogens resonated as singlet at δ 3.7 and δ 3.8 in III i and III j respectively. All products confirmed NH amide (CONH) at δ 10-11 while the quaternary hydrogens expressed at δ 6.2 - 6.6 in their ^{1}H-NMR spectra.

Antimicrobial Activity

Results for antibacterial and antifungal activity were presented in Table-1 and Table-2.

Table-1: Antibacterial activity of derivatives III a–k

Compounds / Bacteria	Zone of inhibition (mm)												
	Gentamicin	**I**	**III a**	**III b**	**III c**	**III d**	**III e**	**III f**	**III g**	**III h**	**III i**	**III j**	**III K**
Gram-positive Bacteria													
Bacillus cereus	22	08	18	-	15	08	-	-	09	-	13	06	11
Bacillus subtilis	23	10	14	-	17	08	-	-	06	-	18	08	09
Bacillus thruingiensis	15	10	16	-	12	07	-	-	10	-	16	08	12
Staphylococcus epidermidis	13	08	09	-	12	-	07	08	-	-	14	-	13
Streptococcus faecalis	13	-	-	-	-	-	-	-	-	08	10	-	-
Streptococcus pyogenes	20	13	18	-	14	12	-	-	-	-	15	06	13
Streptococcus saprophyticus	19	-	08	-	15	12	-	-	-	-	11	-	-
Gram-negative bacteria													
Enterobacter aerogenes	19	-	09	-	09	-	-	-	-	-	-	-	-
Escherichia coli	25	-	11	-	14	-	-	-	-	-	-	-	-
Escherichia coli ATCC 8739	20	07	18	-	15	-	-	-	07	-	08	-	-
E. coli MDR	21	-	12	-	10	-	-	-	-	-	-	-	-
Helicobacter pylori	-	-	06	-	05	-	-	-	-	-	-	-	-
Salmonella typhi	16	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio cholera	-	-	04	-	-	-	-	-	-	-	-	-	07

(-) indicates no activity,

Less than or equal to 10 = Mild,

11 – 15 = Moderate

Above 15 = Good

Table-2: Antifungal activity of derivatives III a- k

Compounds / Fungi	Zone of inhibitions (mm)												
	Griseofulvin	I	III a	III b	III c	III d	III e	III f	III g	III h	III i	III j	III k
Yeast													
Candida albicans	04	13	15	-	13	09	-	-	-	-	11-	-	17
Candida albicans ATCC 0383	06	08	08	-	-	-	-	-	-	-	18	-	-
Candida tropicalis	05	12	11	-	-	-	08	11	-	-	10	-	-
Dermatophytes													
Microsporum canis	-	-	07	-	06	-	-	-	-	-	-	-	-
Microsporum gypseum	-	-	05	-	-	-	-	-	-	-	-	-	-
Saprophytes													
Aspergillus flavus	04	13	-	-	08	-	-	-	-	-	-	-	-
Aspergillus niger	04	-	-	-	-	-	-	-	-	-	-	-	-

(-) indicates no activity

Less than or equal to 10 = Mild,

11 – 15 = Moderate,

Above 15 = Good

It was found that compound I was active against bacillus species, *S. epidermidis and S. pyogene*, but the action was only mild to moderate. On the other hand, some derivatives reflected promising antibacterial effects. III a, III c and III i displayed moderate to good antibacterial properties against the same species. The same compounds were also active against *S. saprophyticus*. In case of gram-negative bacteria, parent molecule expressed mild action against E. coli ATCC 8739 whereas products III a and III c demonstrated moderate to good antibacterial action against this strain.

According to Table-2, it can be seen that III i was active against *Candida albicans ATCC 0383* more than that of PCH and standard drug Griseofulvin.

Structural composition exemplified that the presence of electronegative atom caused to enhance antibacterial potential in compound III c. It can be said that two electron donating methoxy groups in derivative III i might result in improve antibacterial activity when compared to its parent. Similarly, the presence of bulky (lipophilic) group supported antibacterial action in compound III a.

CONCLUSION

It can be concluded that some synthetic analogues obtained from this study possessed improved antimicrobial activity than the parent drug. The encouraging results will help medicinal chemists in drug designing. Further research is needed to convert the active candidates into medicinal agents.

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