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Synthesis, antimicrobial and antitubercular screening of new azetidinone derivatives

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ABSTRACT

Some N-[(2-oxo-3-chloro-4-substituted phenyl-azetidineimino)-propyl] derivatives bearing indole moieties have been synthesized and evaluated in vitro for their antitubercular activity against Mycobacterium tuberculosis H37Rv and antimicrobial activity against a panel of bacteria and fungi. All these compounds were fully characterized by spectroscopic methods and elemental analysis. Some of the tested compounds showed moderate to excellent antitubercular and antimicrobial activities.

Keywords: Antimicrobial, Antitubercular, Azetidinones, Indole, Toxicity.

INTRODUCTION

Diseases caused by microorganisms still attract significant attention of medicinal chemists and biologists because of growing antimicrobial and antimycobacterial resistance. β-Lactam antibiotics are the most important antimicrobial agents for human health and it began with the discovery of penicillin by Alexander Fleming in 1928. The b-lactam class of antibiotics is well known for their neurotoxicity. The azetidinone nucleus is a pharmacophoric scaffold and represents a class of heterocycles with a wide range of biological applications. Many of them are widely used as anti-inflammatory [1,2], antitubercular [3], antiproliferative [4], DNA cleavage [5], cholesterol absorption inhibitors [6], Antiplasmodial [7], antidepressant [8] and antimicrobial [9-11]. The study of indole derivatives is of considerable current interest as a result of their important biological properties. Compounds containing indole ring have been reported to possess various types of biological activity such as anti-inflammatory and analgesic [12-14], antitumorl [15], COX-2 inhibitors [16], anti-hyperlipidemic [17], antioxidant [18] and antimicrobial[19-20] etc.

The biological significance of this class of compounds impelled us to synthesis of new azetidinone derivatives of indole. It has been noticed that introduction of azetidinone ring to the indole core tends to exert profound influence in conferring novel biological activities in these molecules. In view of these facts, in connection with our studies we turn our attention to synthesis some new azetidinone derivatives of indole as biological agents.

EXPERIMENTAL SECTION

All the melting points were determined by open capillary method. All reagents were obtained from Sigma-Aldrich chemicals Pvt. Ltd. Solvents were commercially obtained as laboratory grade. All chemicals were used after further purification (recrystallization or distillation). The progress of reactions and the purity of the compounds were controlled by thin layer chromatography (TLC). TLC was carried out on silica gel G coated glass plates. The purification of the compounds was crried out by column chromatography using 100-200 mesh Silica gel. ^{1}H-NMR spectra were recorded on a Bruker DRX 300 instrument at 300 MHz in $CDCl_3$ on δ scale in ppm using TMS as a reference. ^{13}C-NMR spectra were recorded on a Varian AMX 400 spectrophotometer at 50 MHz using $CDCl_3$. The

FTIR spectra were recorded on a Perkin-elmer IR spectrophotometer using KBr disc of the sample in cm^{-1}. Mass spectra of the synthesized compounds have been recorded on a JEOL SX 102/DA-6000 spectrometer.

General Procedure of the synthesis

Synthesis of N-(chloropropyl)-indole, compound 1.

Indole (0.21 mol) was dissolved in methanol (100 ml) and 1-bromo-3-chloropropane (0.21mol) was added. The mixture was refluxed on a water bath for about 6 hrs, filtered and the solvent was removed to dryness *in vacuo*. The product was purified over the column of silica gel, eluted with chloroform: methanol mixture (7:3 v/v) as eluent and recrystallized by chloroform to furnish compound **1**.

Yield (%):72; M.P.(^{0}C): 62-64; IR (KBr, cm^{-1}): 3082 (C-H str. in Ar-H), 2856, 2882 (C-H str. in CH_2), 1512, 1490 (C=C str.), 1416 (C-H bend. in CH_2), 1318 (N-CH_2 str.) and 732 (C-Cl str.); ^{1}HNMR ($CDCl_3$, ppm): 7.41-6.82 (m, 4H, Ar-H), 6.52 (d, 1H, H_2 J=4.0), 6.26 (d, 1H, H_3 J=4.0), 2.98 (t, 2H, J=7.20, $\underline{CH_2}CH_2CH_2$), 2.42 (t, 2H, J=7.20, $CH_2CH_2\underline{CH_2}$) and 1.61-1.58 (m, 2H, $CH_2\underline{CH_2}CH_2$); ^{13}CNMR ($CDCl_3$, ppm): 148.13, 144.28, 129.74, 127.36, 125.67, 119.14, 114.87, 110.01, 42.46, 36.32 and 32.13; Mass: 194 $(M)^+$. Elemental analysis: Calc. for $C_{11}H_{12}NCl$: C, 68.21; H, 6.24; N, 7.23. Found: C, 68.17; H, 6.18; N, 7.19.

Synthesis of N-(hydrazinopropyl)-indole, compound 2.

An equimolar mixture of compound **1** (0.072mol) and hydrazine hydrate (0.072mol) in methanol (35 ml) was stirred for about 2 hrs and refluxed on a water bath for about 7 hrs. The solvent was removed under *in vacuo* to obtain a solid product. The product was purified over the column of silica gel, eluted with acetone: methanol mixture (6:4 v/v) as eluent and recrystallized from ethanol to furnish compound **2.**

Yield (%): 65; M.P.(^{0}C): 74-75; IR (KBr, cm^{-1}): 3362 (NH str. in NH_2), 3319 (NH str.); ^{1}HNMR ($CDCl_3$, ppm): 8.43 (s, 1H, NH), 4.26 (s, 1H, NH_2); ^{13}CNMR ($CDCl_3$, ppm): 150.18, 145.34, 132.65, 128.32, 125.44, 120.26, 114.22, 109.76, 42.28, 36.99 and 32.32; Mass: 189$(M)^+$; Elemental analysis: Calc. for $C_{11}H_{15}N_3$: C, 69.81; H, 7.98; N, 22.20. Found: C, 69.77; H, 7.94; N, 22.17.

Synthesis of N-[(benzylidene hydrazino)-propyl]-indole, compound 3a.

A mixture of compound **2** (0.01 mol) and benzaldehyde (0.01 mol) in methanol (20ml) in the presence of a catalytic amount of glacial acetic acid was refluxed for about 5 hrs on a water bath. After cooling and filtration the solvent was removed under *in vacuo* to obtain a solid product. The product was purified over the column of silica gel, eluted with chloroform: methanol mixture (8:2 v/v) and recrystallized from chloroform to give compound **3a**.

Compounds **3 (b-h)** have also been synthesized by treating the **compound-2** with selected aldehydes using similar method as above.

Characterization of N-[(benzylidene hydrazino)-propyl]-indole, 3a:

Yield (%):68; M.P.(^{0}C): 90-91; IR (KBr, cm^{-1}): 3356 (NH str.), 1588 (N=CH str. azomethine); ^{1}HNMR ($CDCl_3$, ppm): 8.74 (s, 1H, N=CH, azomet.), 8.14 (s, 1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 148.32, 144.56, 142.16, 137.24, 133.13, 130.26, 128.48, 126.82, 125.34, 121.16, 118.47, 115.78, 109.11, 42.17, 36.75 and 32.20; Mass: 277$(M)^+$; Elemental analysis: Calc. for $C_{18}H_{19}N_3$: C, 77.94; H, 6.90; N, 15.14. Found: C, 77.90; H, 6.87; N, 15.11.

Characterization of N-[(2-chlorobenzylidene hydrazino)-propyl]-indole, 3b:

Yield (%): 62; M.P.(^{0}C): 95-96; IR (KBr, cm^{-1}): 3368 (NH str.), 1593 (N=CH str. azomethine) and 712 (Ar-Cl str.); ^{1}HNMR ($CDCl_3$, ppm): 8.83 (s, 1H, N=CH, azomethine), 8.02 (s, 1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 151.34, 143.34, 141.56, 138.28, 135.25, 131.12, 130.36, 128.48, 127.22, 125.92, 123.04, 119.14, 117.22, 115.98, 108.68, 43.11, 37.89 and 34.33; Mass: 312$(M)^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_3Cl$: C, 69.33; H, 5.81; N, 13.47. Found: C, 69.27; H, 5.78; N, 13.43.

Characterization of N-[(4-chlorobenzylidene hydrazino)-propyl]-indole, 3c:

Yield (%): 64; M.P.(^{0}C): 104-105; IR (KBr, cm^{-1}): 3364 (NH str.), 1588 (N=CH str. azomethine) and 703 (Ar-Cl str.); ^{1}HNMR ($CDCl_3$, ppm): 8.74 (s, 1H, N=CH, azomethine), 8.06 (s,1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 149.56, 144.34, 142.66, 139.38, 136.22, 133.14, 126.44, 124.92, 122.14, 120.85, 118.44, 114.12, 108.24, 43.42, 37.85 and 34.40; Mass: 312$(M)^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_3Cl$: C, 69.33; H, 5.81; N, 13.47. Found: C, 69.25; H, 5.73; N, 13.42.

Characterization of N-[(2-Bromobenzylidene hydrazino)-propyl]-indole, 3d:

Yield (%): 65; M.P.(^{0}C): 91-92; IR (KBr, cm^{-1}): 3361 (NH str.), 1595 (N=CH str. azomethine) and 672 (Ar-Br str.); ^{1}HNMR ($CDCl_3$, ppm): 8.75 (s, 1H, N=CH, azomethine), 8.09 (s, 1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 150.46,

144.58, 142.58, 139.12, 135.22, 132.38, 129.88, 127.92, 126.12, 125.08, 122.88, 118.42, 114.98, 113.45, 108.22, 43.11, 37.89 and 34.33; Mass: 356(M)$^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_3Br$: C, 60.68; H, 5.09; N, 11.79. Found: C, 60.62; H, 5.05; N, 11.73.

Characterization of N-[(3-Bromobenzylidene hydrazino)-propyl]-indole, 3e:
Yield (%): 68; M.P.(^{0}C): 99-100; IR (KBr, cm^{-1}): 3358 (NH str.), 1592 (N=CH str. azomethine) and 656 (Ar-Br str.); ^{1}HNMR ($CDCl_3$, ppm): 8.72 (s, 1H, N=CH, azomethine), 8.08 (s, 1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 150.34, 146.14, 143.87, 138.12, 136.22, 133.76, 129.17, 127.55, 125.22, 122.18, 119.16, 115.78, 106.88, 43.34, 37.83 and 34.84; Mass: 356(M)$^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_3Br$: C, 60.68; H, 5.09; N, 11.79. Found: C, 60.62; H, 5.05; N, 11.73.

Characterization of N-[(3-Nitrobenzylidene hydrazino)-propyl]-indole, 3f:
Yield (%): 63; M.P.(^{0}C): 107-108; IR (KBr, cm^{-1}): 3374 (NH str.), 1585 (N=CH str. azomethine) and 1358 (Ar-NO_2 str.); ^{1}HNMR ($CDCl_3$, ppm): 8.89 (s, 1H, N=CH, azomethine), 8.05 (s,1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 152.48, 145.11, 143.16, 139.74, 135.46, 133.44, 132.12, 130.46, 128.28, 125.34, 122.64, 120.78, 118.46, 115.88, 109.22, 43.86, 38.01 and 34.89; Mass: 322(M)$^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_4O_2$: C, 67.06; H, 5.62; N, 17.39. Found: C, 67.00; H, 5.56; N, 17.35.

Characterization of N-[(4-Nitrobenzylidene hydrazino)-propyl]-indole, 3g:
Yield (%): 65; M.P.(^{0}C): 111-112; IR (KBr, cm^{-1}): 3372 (NH str.), 1584 (N=CH str. azomethine) and 1361 (Ar-NO_2 str.); ^{1}HNMR ($CDCl_3$, ppm): 8.78 (s, 1H, N=CH, azometine), 8.08 (s,1H, N-H); ^{13}CNMR ($CDCl_3$, ppm): 151.20, 146.38, 143.42, 141.12, 137.54, 135.46, 130.48, 127.78, 125.82, 122.85, 119.44, 116.94, 109.78, 44.14, 37.78 and 34.12; Mass: 322(M)$^+$; Elemental analysis: Calc. for $C_{18}H_{18}N_4O_2$: C, 67.06; H, 5.62; N, 17.39. Found: C, 67.02; H, 5.58; N, 17.34.

Characterization of N-[(2-Methoxybenzylidene hydrazino)-propyl]-indole, 3h:
Yield (%): 65; M.P.(^{0}C): 98-99; IR (KBr, cm^{-1}): 3358 (NH str.), 2961 (OCH_3 str.), 1582 (N=CH str. azomethine); ^{1}HNMR ($CDCl_3$, ppm): 8.72 (s, 1H, N=CH, azomethine), 7.92 (s,1H, N-H) and 3.84 (s, 3H, O-CH_3); ^{13}CNMR ($CDCl_3$, ppm): 149.22, 144.46, 141.82, 139.24, 135.64, 133.33, 131.66, 129.18, 126.88, 124.46, 121.55, 119.46, 118.12, 114.72, 107.84, 56.82, 43.62, 37.11 and 34.28; Mass: 307(M)$^+$; Elemental analysis: Calc. for $C_{19}H_{21}N_3O$: C, 74.23; H, 6.88; N, 13.66. Found: C, 74.18; H, 6.82; N, 13.63.

Synthesis of N-[(2-oxo-3-chloro-4-phenyl-azetidineimino)-propyl]-indole, Compound 4a.
A mixture of compound **3a** (0.01 mol) and $(C_2H_5)_3N$ (0.01 mol) in methanol (50 ml), $ClCH_2COCl$ (0.01 mol) was added drop wise in it. The reaction mixture was first stirred for about 2 hours followed by refluxing on a steam bath for about 4 hours. The reaction mixture was cooled and excess of solvent was removed under reduced pressure. The product was purified over the column of silica gel, eluted with chloroform: methanol mixture (9:1 v/v) and recrystallized from chloroform to furnish compound **4a**.

Compounds **4 (b-h)** have also been synthesized by treating the compounds **3 (b-h)** using similar method as above.

Characterization of N-[(2-oxo-3-chloro-4-phenyl-azetidineimino)-propyl]-indole, 4a.
Yield (%): 69; M.P.(^{0}C) 105-106; IR (KBr, cm^{-1}): 3361 (NH str.), 2948 (CH-Cl str.), 1742 (C=O str.); ^{1}HNMR ($CDCl_3$, ppm): 8.32 (s, 1H, N-H), 5.53 (d, 1H, J=4.50, N-CH-Cl) and 5.21 (d, 1H, J = 4.50, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 165.05, 148.64, 143.84, 136.48, 134.87, 131.88, 128.22, 126.44, 124.76, 121.52, 119.38, 113.58, 109.18, 64.21, 58.46, 43.84, 38.78 and 33.38; Mass: 354(M)$^+$; Elemental analysis: Calc. for $C_{20}H_{20}N_3OCl$: C, 67.88; H, 5.69; N, 11.80. Found: C, 67.83; H, 5.65; N, 11.77.

Characterization of N-[(2-oxo-3-chloro-4-(2-chlorophenyl)-azetidine imino)-propyl]-indole, 4b.
Yield (%): 71; M.P.(^{0}C) 108-109; IR (KBr, cm^{-1}): 3367 (NH str.), 2942 (CH-Cl str.), 1748 (C=O str.) and 735 (Ar-Cl); ^{1}HNMR ($CDCl_3$, ppm): 8.35 (s, 1H, N-H), 5.52 (d, 1H, J=4.50, N-CH-Cl) and 5.24 (d, 1H, J = 4.50, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 166.21, 150.46, 145.86, 139.44, 136.62, 134.74, 132.12, 131.28, 127.48, 126.12, 123.94, 121.38, 118.32, 114.86, 108.72, 65.56, 58.38, 45.38, 38.74 and 33.24; Mass: 388(M)$^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_3OCl_2$: C, 61.86; H, 4.93; N, 10.82. Found: C, 61.83; H, 4.89; N, 10.77.

Characterization of N-[(2-oxo-3-chloro-4-(4-chlorophenyl)-azetidine imino)-propyl]-indole, 4c.
Yield (%): 71; M.P.(^{0}C) 102-103; IR (KBr, cm^{-1}): 3361 (NH str.), 2945 (CH-Cl str.), 1746 (C=O str.) and 731 (Ar-Cl str.); ^{1}HNMR ($CDCl_3$, ppm): 8.35 (s, 1H, N-H), 5.58 (d, 1H, J=4.22, N-CH-Cl) and 5.21 (d, 1H, J = 4.22, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 168.22, 149.66, 145.76, 140.87, 138.82, 135.28, 131.46, 128.18, 125.94, 122.36,

118.68, 111.14, 108.94, 65.72, 58.12 , 44.92, 38.52 and 33.44; Mass: 388$(M)^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_3OCl_2$: C, 61.86; H, 4.93; N, 10.82. Found: C, 61.82; H, 4.88; N, 10.79.

Characterization of N-[(2-oxo-3-chloro-4-(2-bromophenyl)-azetidine imino)-propyl]-indole, 4d.
Yield (%): 68; M.P.(^{0}C) 101-102; IR (KBr, cm^{-1}): 3372 (NH str.), 2946 (CH-Cl str.), 1744 (C=O str.) and 653 (Ar-Br str.); ^{1}HNMR ($CDCl_3$, ppm): 8.38 (s, 1H, N-H), 5.51 (d, 1H, J=4.30, N-CH-Cl) and 5.22 (d, 1H, J=4.30, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 165.64, 150.18, 143.38, 136.78, 135.12, 133.84, 131.46, 129.92, 128.24, 126.84, 122.64, 121.02, 117.38, 112.56, 108.72, 64.89, 56.92, 45.72, 39.88 and 32.36; Mass: 433$(M)^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_3OClBr$: C, 55.51; H, 4.42; N, 9.71. Found: C, 55.47; H, 4.38; N, 9.66.

Characterization of N-[(2-oxo-3-chloro-4-(3-bromophenyl)-azetidine imino)-propyl]-indole, 4e.
Yield (%): 70; M.P.(^{0}C) 111-112; IR (KBr, cm^{-1}): 3365 (NH str.), 2942 (CH-Cl str.), 1742 (C=O str.) and 662 (Ar-Br str.); ^{1}HNMR ($CDCl_3$, ppm): 8.33 (s, 1H, N-H), 5.54 (d, 1H, J= 4.28, N-CH-Cl) and 5.27 (d, 1H, J= 4.28, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 166.24, 149.88, 143.82, 138.62, 136.42, 134.76, 131.18, 129.28, 126.84, 121.34, 119.38, 113.36, 108.74, 65.48, 57.18, 44.66, 38.74 and 33.12; Mass: 433$(M)^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_3OClBr$: C, 55.51; H, 4.42; N, 9.71. Found: C, 55.47; H, 4.36; N, 9.65.

Characterization of N-[(2-oxo-3-chloro-4-(3-nitrophenyl)-azetidine imino)-propyl]-indole, 4f.
Yield (%): 74; M.P.(^{0}C) 116-117; IR (KBr, cm^{-1}): 3361 (NH str.), 2949 (CH-Cl str.), 1747 (C=O str.) and 1321 (Ar-NO_2 str.); ^{1}HNMR ($CDCl_3$, ppm): 8.36 (s, 1H, N-H), 5.59 (d, 1H, J= 4.24, N-CH-Cl) and 5.26 (d, 1H, J= 4.24, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 169.52, 150.46, 146.38, 141.42, 138.34, 135.26, 131.48, 130.12, 128.56, 126.48, 124.88, 122.64, 119.12, 112.78, 107.56, 66.96, 58.30, 45.98, 37.72 and 33.16; Mass: 399$(M)^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_4O_3Cl$: C, 60.22; H, 4.80; N, 14.04. Found: C, 60.18; H, 4.76; N, 14.01.

Characterization of N-[(2-oxo-3-chloro-4-(4-nitrophenyl)-azetidine imino)-propyl]-indole, 4g.
Yield (%): 72); M.P.(^{0}C) 121-122; IR (KBr, cm^{-1}): 3365 (NH str.), 2941 (CH-Cl str.), 1749 (C=O str.) and 1324 (Ar-NO_2 str.); ^{1}HNMR ($CDCl_3$, ppm): 8.38 (s, 1H, N-H), 5.57 (d, 1H, J= 4.25, N-CH-Cl) and 5.22 (d, 1H, J= 4.25, CH-Ar); ^{13}CNMR ($CDCl_3$, ppm): 169.58, 151.14, 145.26, 141.46, 139.78, 133.56, 131.48, 126.22, 125.02, 123.54, 118.18, 112.98, 107.58, 66.38, 57.16 , 45.67, 37.82 and 33.24; Mass: 399$(M)^+$; Elemental analysis: Calc. for $C_{20}H_{19}N_4O_3Cl$: C, 60.22; H, 4.80; N, 14.04. Found: C, 60.17; H, 4.78; N, 14.00.

Characterization of N-[(2-oxo-3-chloro-4-(2-methoxyphenyl)-azetidine imino)-propyl]-indole, 4h.
Yield (%): 68; M.P.(^{0}C) 112-113; IR (KBr, cm^{-1}): 3366 (NH str.), 2965 (OCH_3 str.), 2946 (CH-Cl str.) and 1743 (C=O str.); ^{1}HNMR ($CDCl_3$, ppm): 8.35 (s, 1H, N-H), 5.59 (d, 1H, J=4.30, N-CH-Cl), 5.21 (d, 1H, J = 4.30, CH-Ar) and 3.74 (s, 3H, -OCH_3); ^{13}CNMR ($CDCl_3$, ppm): 164.88, 148.62, 141.58, 135.72, 134.08, 132.46, 130.96, 129.12, 127.23, 125.42, 122.88, 121.46, 119.82, 112.56, 106.28, 64.82, 56.42, 54.12, 46.24, 38.94 and 33.48; Mass: 384$(M)^+$; Elemental analysis: Calc. for $C_{21}H_{22}N_3O_2Cl$: C, 65.70; H, 5.77; N, 10.94. Found: C, 65.65; H, 5.73; N, 10.91.

RESULTS AND DISCUSSION

The target compounds were synthesized into two steps. In the first step **(scheme-1)** N-(chloropropyl)-indole **(1)** was synthesized by reaction of indole with 1-bromo-3-chloro propane. Then compound **(1)** was reacted with hydrazine hydrate to afford the compound **(2) (scheme-1)**. The new compounds **3(a-h)** were obtained through reaction of compound **(2)** with various aromatic aldehydes in presence of glacial acetic acid **(scheme-1)**.

In the last step target compounds **4(a-h)** were synthesized by a cylisation reaction of compounds **3(a-h)** with chloroacetyl chloride in presence of triethyl amine **(scheme-2)**. The spectroscopic data supported the formation of the products in end step.

All the synthesized compounds of series **4(a-h)** were screened for their antibacterial and antifungal activity against some selected microorganisms, antitubercular activity against *Mycobacterium tuberculosis* H37Rv and toxicity.

Pharmacology
Evaluation of antitubercular screening
All the synthesized compounds of series **4(a–h)** were evaluated for their antitubercular activity. Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H37Rv was performed by agar microdilution method where twofold dilutions of each test compound were added into 7H10 agars supplemented with OADC and organism. A culture of *M. tuberculosis* H37Rv growing on L-J medium was harvested in 0.85%

saline with 0.05% Tween-80. A suspension of compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentration of compound keeping the volume constant, that is, 0.1 ml. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 ^{0}C for 24 h followed by streaking of M. tuberculosis H37Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37 ^{0}C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. Streptomycin and ethambutol were used as standard drug. The MIC levels of some active compounds **4(a–h)** against these organisms are given in table-**I.**

Evaluation of antimicrobial screening

The synthesized compounds of series **4(a-h)** were investigated for their antimicrobial activity. For antibacterial screening *Bacillus subtilis, Pseudomonas aeruginosa* and *Streptococcus pneumoniae* were used. For antifungal activity *Candida albicans, Aspergillus fumigatus* and *Aspergillus niger* was taken. The minimum inhibitory was carried out by broth micro dilution method as described by Rattan. The MICs of the investigated compounds for antibacterial and antifungal activities are given in the table **II** and **III** respectively. Antibacterial and antifungal screenings were performed by dilution method using nutrient agar media. The tubes were incubated at 37ºC for 48 hrs. DMSO was used as solvent. Norflaxacin was used as standard drug for antibacterial screening and miconazole was used as standard drug for antifungal screening.

Cytotoxicity analysis:

All the synthesized products were analysed for cytotoxicity using neutral red uptake by using vero-c-1008 cell line at various concentrations, none of them were found toxic. Hence the activities of the above synthesized compounds were not due to cytotoxicity of the compounds.

Table-I- Antitubercular activity results of series 4(a-h)

S.No.	Compounds	MIC (µg/ml) H37Rv
1	**4a**	**25.0**
2	**4b**	**3.125**
3	**4c**	**1.56**
4	**4d**	**3.125**
5	**4e**	**6.125**
6	**4f**	**1.56**
7	**4g**	**1.56**
8	**4h**	**12.5**
9	Streptomycin	**3.0**
10	Ethambutol	**1.0**

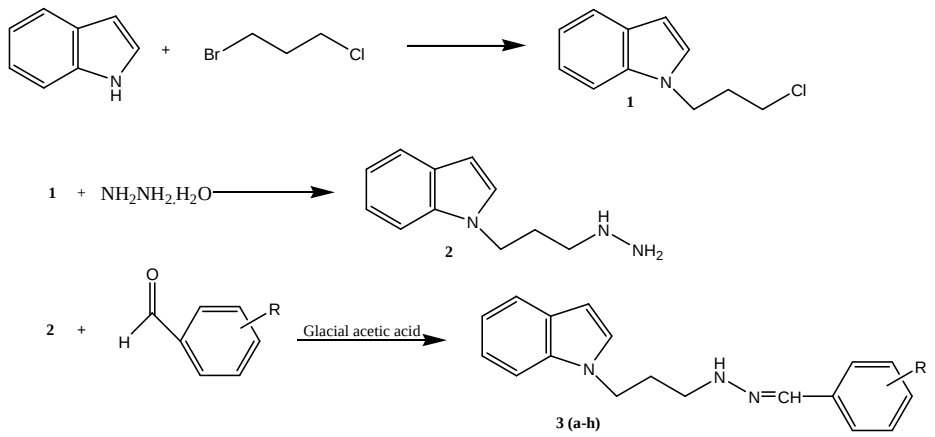
Table-II- Antibacterial activity data of series 4(a-h), MIC (µg/ml).

S.No	Compounds	*B. Subtilis*	*P. aeruginosa*	*S. pneumoniae*
1	**4a**	**50**	**50**	**12.5**
2	**4b**	**6.25**	**12.5**	**6.25**
3	**4c**	**12.5**	**6.25**	**6.25**
4	**4d**	**25**	**25**	**12.5**
5	**4e**	**25**	**12.5**	**12.5**
6	**4f**	**6.25**	**12.5**	**6.25**
7	**4g**	**25**	**12.5**	**12.5**
8	**4h**	**25**	**25**	**12.5**
9	Ciproflaxacin	**6.25**	**6.25**	**3.12**

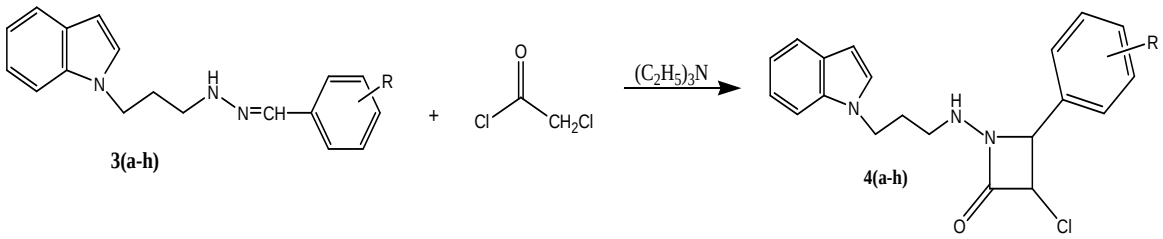
Table-III- Antifungal activity data of series 4(a-h) , MIC (µg/ml).

S.No.	Compounds	*C. Albicans*	*A. fumigatus*	*A. niger*
1	**4a**	**50**	**12.5**	**50**
2	**4b**	**12.5**	**12.5**	**12.5**
3	**4c**	**25**	**12.5**	**6.25**
4	**4d**	**6.25**	**6.25**	**12.5**
5	**4e**	**12.5**	**6.25**	**6.25**
6	**4f**	**12.5**	**25**	**6.25**
7	**4g**	**6.25**	**12.5**	**6.25**
8	**4h**	**12.5**	**12.5**	**6.25**
9	Fluconazole	**6.25**	**3.12**	**3.12**

Scheme-1



Scheme-2



Com.	R	Com.	R
3a, 4a	-H	3e, 4e	3-Br
3b, 4b	2-Cl	3f, 4f	3-NO_2
3c, 4c	4-Cl	3g, 4g	4-NO_2
3d, 4d	2-Br	3h, 4h	2-OCH_3

CONCLUSION

We described herein design, synthesis and characterization of N-[(2-oxo-3-chloro-4-substituted phenyl-azetidineimino)-propyl]-indoles **4(a-h)**. The structure of the synthesyzed compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, MASS and analytical methods.

Newly synthesyzed compounds of the series **4(a-h)** were screened for their antibacterial, antifungal activities against selected bacteria and fungi. Some compounds having chloro and nitro groups exhibited acceptable antibacterial activity and compounds having bromo and methoxy, groups showed good antifungal activity. Compounds of the series **4(a-h)** were also tested for their antitubercular analysis. Some compounds showed moderate to good antitubercular activity as comparision to standard drug.

Finally, we expect that these results will contribute to the development of newer antitubercular and antimicrobial compounds with less toxicity.

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