STRATEGIC APPROACHES TO THE SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SUBSTITUTED PIPERAZINYL-IMIDAZOLE CONJUGATES

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ABSTRACT

A series of hybrid molecules bearing 5-nitroimidazole linked with substituted piperazine molecules have been synthesized. The cytotoxicity of all the synthesized compounds has been evaluated on HEK293 cells with the help of MTT assay. It was found that the treatment of these compounds shows a variable toxicity profile for HEK293 cells. The results of cytotoxicity showed that in the tested concentration range, compound NJ3, NJ5, NJ8 and NJ10 show considerable cytotoxicity towards HEK293 cells.

Keywords: Piperazine, Nitroimidazole, Cell Viability Assay, MTT Assay, Cytotoxicity

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INTRODUCTION

Nitroimidazole is a class of heterocyclic compounds where the nitro group is placed at position 5 of the imidazole ring. They are well known for antimycobacterial action through the bioreduction mechanism and have been found to show resistance against protozoal. They synthesize to metabolize which prevents intracellular bacterial synthesis¹. There has been a quest among the chemical fraternity to find a new alternative of this drug that has a minimum side effect and at the same time uses a similar nitroimidazole motif². Further, 5-nitroimidazole has proven bioactivity and toxicity concerning FABH enzyme. FABH has evolved as a major potential source for new antibacterial drug discovery. Since nitroimidazole compounds can get fully absorbed even through oral administration, it is much effective in a bacterial infection based on its action. Nitroimidazole has gained interest among researchers for its peculiar characteristic of penetrating deep with bacteria and getting completely submerged.³ Piperazine plays a dominant role in making tiny molecular therapeutic compounds. It is also one of the most common nitrogen-bearing heterocycles, which form the core of most approved pharmaceutical drug⁴. It has been reported that 51 out of 1175 drugs duly approved by FDA contain piperazines⁵. Piperazine in the current scenario is the key constituent of drug discovery with the highest number of biological actions reported as a heterocyclic compound.

Moreover, piperazine is the most important content of therapeutic molecule, which has a remarkable binding property where it can form motifs to select ligands in the different biological mechanisms. Further, piperazine scaffolds and their associated derivatives have formed renowned pharmacophores, which are present in numerous biologically potential compounds and are proven antifungal, antibacterial, anti-psychotic, anti-HIV, anti-malarial and antidepressant agents.⁶ We have designed a hybrid molecule bearing 5-nitroimidazole linked with substituted piperazine molecules. The diagrammatical model of the designed hybrid is shown in Fig.-1.

EXPERIMENTAL

Material and Methods

Prerequisite chemicals, reagents other necessary raw materials were procured from Merck and Aldrich chemical company, USA. The thin-layer chromatography (TLC) required fully coated aluminium sheets (Silica gel 60 F254, Merck Germany) viewed under UV light for specific marks. Veego instrument model no. REC-22038 A2 is specifically used to track melting points. Further, the Elementar Vario analyzer has

been used to record elemental observation, which gave results with $\pm 0.4\%$ variation of standard value. Bruker FT-IR spectrophotometer was deployed for the IR spectrum. Bruker Spectrospin DPX 300 MHz gave a reading of ^1H , while Bruker Spectrospin DPX 100 MHz showed ^{13}C NMR reading. In this NMR, CDCl_3 worked like a solvent while trimethylsilane worked as a local standard. In this method, the splitting trend has been designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The values of the chemical shift have been recorded in ppm while mass spectrum by ESI-MS (AB-Sciex 2000, Applied Biosystems). All the values observed have been provided in the experimental segment.

Standard Procedure for the Preparation of Different Substituted Chloro acetamides (J1-J11)

These compounds have been synthesized by the reported method in our previous research.⁷

Standard Procedure for the Synthesis of Piperazinyl-imidazole ethenone (NJ1-NJ11)

The method of synthesized compounds has been reported in our earlier research.⁷

1-[4-(2,3-dichlorophenyl)piperazin-1-yl]-2-(5-methyl-2-nitro-1H-imidazol-1-yl)ethanone(NJ-1)

Pale yellow color, solid (yield:96%); mp:132 °C; ^1H NMR (300 MHz, CDCl_3 , δ ppm) = 7.73 (s, 1H), 7.27-7.16 (m, J=5.5Hz, 2H), 6.96-6.94 (m, J=1Hz, 1H), 4.84 (s, 2H), 3.83-3.70 (m, J=6.5Hz, 4H), 3.13-2.95 (m, J=9Hz, 4H), 2.377 (s, 3H); ^{13}C NMR (75MHz, CDCl_3 , δ ppm) = 163.05, 150.08, 146.37, 145.88, 134.31, 127.82, 127.71, 125.64, 121.39, 118.88, 51.39, 50.88, 47.92, 45.26, 42.78, 13.01; IR (KBr) ν_{max} = 1657(C=O), 1539(N=O), 3142(C=C), 2907(C-C), 782, 668(C-Cl) cm^{-1} ; ESI-MS (m/z): 398.2[M+1]⁺; Anal. Calcd for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_3\text{Cl}_2$: C 48.25, H 4.30, N 17.59; Found: C 48.27, H 4.27, N 17.48%.

1-[4-(3-chlorophenyl)piperazin-1-yl]-2-(5-methyl-2-nitro-1H-imidazol-1-yl)ethanone(NJ-2)

Pale yellow color, solid (yield:95%); mp:123 °C; ^1H NMR (300 MHz, CDCl_3 , δ ppm) = 7.69 (s, 1H), 7.26-7.19 (m, J=3.5Hz, 2H), 6.90-6.79 (m, J=5.5Hz, 2H), 4.78 (s, 2H), 3.81-3.66 (m, J=15Hz, 4H), 3.43-3.09 (m, J=17Hz, 4H), 2.38 (s, 3H); ^{13}C NMR (75MHz, CDCl_3 , δ ppm) = 168.63, 156.51, 150.86, 139.54, 135.07, 127.11, 124.70, 120.91, 119.22, 53.49, 52.75, 49.22, 46.72, 45.42, 44.59, 17.74; IR (KBr) ν_{max} = 1647(C=O), 1539(N=O), 3163(C=C), 2923(C-C) 676(C-Cl) cm^{-1} ; ESI-MS (m/z): 364.2[M+1]⁺; Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{N}_5\text{O}_3\text{Cl}$: C 52.82, H 4.99, N 19.25; Found: C 52.78, H 4.96, N 19.45%.

1-[4-(2-methoxyphenyl)piperazin-1-yl]-2-(5-methyl-2-nitro-1H-imidazol-1-yl)ethanone(NJ-3)

Pale yellow color, solid (yield:93%); mp:127 °C; ^1H NMR (300 MHz, CDCl_3 , δ ppm) = 7.72 (s, 1H), 7.26-7.04 (m, J=11Hz, 4H), 6.97-6.89 (m, J=4Hz, 3H), 4.82 (s, 2H), 3.89-3.66 (m, J=11.5Hz, 3H), 3.15-2.95 (m, J=10Hz, 4H), 2.37 (s, 3H); ^{13}C NMR (75MHz, CDCl_3 , δ ppm) = 162.85, 152.28, 146.36, 145.86, 140.13, 123.97, 121.38, 121.13, 118.58, 111.47, 55.49, 50.75, 50.31, 47.88, 45.33, 42.82, 13.02; IR (KBr) ν_{max} = 1648(C=O), 1538(N=O), 3108(C=C), 2908(C-C), 2836(O-CH₃) cm^{-1} ; ESI-MS (m/z): 360.3[M+1]⁺; Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{O}_4$: C 56.82, H 5.89, N 19.49; Found: C 56.66, H 5.72, N 19.51%.

1-[4-(2-hydroxyphenyl)piperazin-1-yl]-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanone(NJ-4)

Brown color, solid (yield:56%); mp:121 °C; ^1H NMR (300 MHz, CDCl_3 , δ ppm) = 8.43 (s, 1H), 7.40-7.25 (m, J=7.5Hz, 4H), 5.28 (s, 1H), 4.70 (s, 2H), 3.85-3.63 (m, J=11Hz, 4H), 3.42-3.35 (m, J=3.5Hz, 4H), 2.29 (s, 3H); ^{13}C NMR (75MHz, CDCl_3 , δ ppm) = 162.75, 151.98, 146.41, 145.95, 140.12, 124.07, 121.19, 117.92, 55.50, 50.65, 50.33, 47.78, 44.93, 13.00; IR (KBr) ν_{max} = 1649(C=O), 1543(N=O), 3136(C=C), 2919(C-C) cm^{-1} ; ESI-MS (m/z): 346.3[M+1]⁺; Anal. Calcd for $\text{C}_{16}\text{H}_{19}\text{N}_5\text{O}_4$: C 55.64, H 5.55, N 20.28; Found: C 55.87, H 5.57, N 20.41%.

2-(2-methyl-5-nitro-1H-imidazol-1-yl)-1-[4-(4-nitrophenyl)piperazin-1-yl]ethanone(NJ-5)

Yellow color, solid (yield:95%); mp:165 °C; ^1H NMR (300 MHz, CDCl_3 , δ ppm) = 8.18-8.08 (m, J=5Hz, 3H), 7.06-7.03 (m, J=1.5Hz, 2H), 5.18 (s, 2H), 3.67-3.38 (m, J=14.5Hz, 8H), 2.26 (s, 3H); ^{13}C NMR (75MHz, CDCl_3 , δ ppm) = 165.03, 154.75, 146.68, 145.53, 137.55, 126.21, 123.81, 113.23, 48.30, 46.18, 43.81, 41.48, 40.81, 39.98, 12.99; IR (KBr) ν_{max} = 1660(C=O), 1537(N=O), 3076(C=C), 2916(C-C) cm^{-1} ; ESI-MS (m/z): 374.1[M]⁺; Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{N}_6\text{O}_5$: C 51.33, H 4.85, N 22.45; Found: C 51.43, H 4.77, N 22.33.

1-(4-acetylpiperazin-1-yl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanone(NJ-6)

Beige color, solid (yield:76%); mp:143 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 8.19 (s, 1H), 5.15 (s, 2H), 3.54-3.53 (m, J=0.5Hz, 5H), 3.45-3.37 (m, J=4Hz, 3H), 2.31-2.24 (s, J=3.5Hz, 3H), 2.05-2.01 (m, J=2Hz, 3H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 168.65, 163.44, 145.54, 121.57, 47.41, 45.21, 44.12, 43.86, 20.74, 12.36; IR (KBr) ν_{max}= 1640(C=O), 1538(N=O), 3115(C=C), 2927(C-C)cm⁻¹; ESI-MS (*m/z*): 296.3[M+1]⁺; Anal. Calcd for C₁₂H₁₇N₅O₄: C 48.81, H 5.80, N 23.72; Found: C 48.61, H 5.88, N 23.62%.

1-[4-(1,3-benzodioxol-5-yl)piperazin-1-yl]-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanone(NJ-7)

Brown color, solid (yield:79%); mp: 182 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 7.64 (s, 1H), 6.83-6.80 (m, J=1.5Hz, 1H), 6.67-6.63 (s, J=2Hz, 2H), 6.00 (s, 2H), 5.00 (s, 2H), 3.71-3.60 (m, J=5.5Hz, 4H), 2.74-2.59 (m, J=7.5Hz, 3H), 2.42-2.39 (s, J=1.5Hz, 3H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 164.12, 148.65, 148.22, 146.37, 144.90, 135.21, 127.96, 121.28, 119.93, 108.86, 107.74, 101.61, 50.81, 46.37, 13.32; IR (KBr) ν_{max}= 1606(C=O), 1540(N=O), 3155(C=C), 2916(C-C)cm⁻¹; ESI-MS (*m/z*): 373.3[M]⁺; Anal. Calcd for C₁₇H₁₉N₅O₅: C 54.69, H 5.13, N 18.76; Found: C 54.71, H 5.43, N 18.62 %.

2-(2-methyl-5-nitro-1H-imidazol-1-yl)-1-[4-(pyridin-3-yl)piperazin-1-yl]ethanone(NJ-8)

Pale yellow color, solid (yield:67%); mp: 149 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 8.21-8.19 (m, 1H), 7.75 (s, 1H), 7.56-7.50 (m, J=3Hz, 1H), 6.72-6.67 (m, J=2.5Hz, 2H), 4.87 (s, 2H), 3.77-3.55 (m, J=10Hz, 8H), 2.35 (s, 3H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 163.23, 158.70, 147.99, 146.23, 146.02, 137.83, 121.68, 114.31, 107.39, 47.99, 45.06, 44.86, 44.63, 42.03, 12.97; IR (KBr) ν_{max}=1651(C=O), 1537(N=O), 3113(C=C), 2913(C-C)cm⁻¹; ESI-MS (*m/z*): 331.2[M+1]⁺; Anal. Calcd for C₁₅H₁₈N₆O₃: C 54.54, H 5.49, N 25.44; Found: C 54.42, H 5.43, N 25.41%.

1-(4-benzhydrylpiperazin-1-yl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanone(NJ-9)

Beige color, solid (yield:88%); mp: 204 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 7.66-7.65 (s, J=0.5Hz, 1H), 7.43-7.40 (m, J=1.5Hz, 4H), 7.33-7.19 (m, J=7Hz, 6H), 4.69 (s, 1H), 4.30 (s, 2H), 3.65-3.49 (m, J=8Hz, 4H), 2.49-2.46 (m, J=1.5Hz, 4H), 2.35 (s, 3H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 170.29, 148.46, 146.47, 144.86, 127.29, 121.28, 119.93, 100.92, 58.33, 50.81, 13.32; IR (KBr) ν_{max}= 1654(C=O), 1532(N=O), 3105(C=C), 2922(C-C)cm⁻¹; ESI-MS (*m/z*): 420.3[M+1]⁺; Anal. Calcd for C₂₃H₂₅N₅O₃: C 65.85, H 6.01, N 16.70; Found: C 65.88, H 6.23, N 16.71%.

tert-butyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carboxylate(NJ-10)

Brown color, solid (yield:60%); mp: 192 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 8.04 (s, 1H), 5.08 (s, 2H), 3.54-3.00 (m, J=27Hz, 8H), 2.15 (s, 3H), 1.73 (s, 9H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 167.91, 163.25, 154.35, 145.97, 121.58, 80.72, 80.31, 79.79, 52.85, 47.95, 44.72, 43.11, 42.25, 41.66, 29.64, 28.32, 12.93; IR (KBr) ν_{max}= 1661(C=O), 1539(N=O), 3128(C=C), 2923(C-C)cm⁻¹; ESI-MS (*m/z*): 353.3[M]⁺; Anal. Calcd for C₁₅H₂₃N₅O₅: C 50.98, H 6.56, N 19.82; Found: C 50.67, H 6.43, N 19.82%.

1-(4-benzylpiperazin-1-yl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanone(NJ-11)

Brown color, solid (yield:67%); mp:143 °C; ¹HNMR (300 MHz, CDCl₃, δ ppm) = 7.65 (s, 1H), 7.39-7.27 (m, J=6Hz, 3H), 7.15-7.13 (m, J=1Hz, 2H), 5.10 (s, 2H), 4.12(s, 2H), 3.61-3.41 (m, J=10Hz, 4H), 3.32-3.12 (m, J=10Hz, 4H) 2.41 (s, 3H); ¹³C NMR (75MHz, CDCl₃, δ ppm) = 163.70, 146.49, 145.03, 142.18, 135.13, 133.78, 129.47, 129.00, 128.32, 127.22, 120.07, 109.47, 52.95, 50.96, 35.96, 13.29; IR (KBr) ν_{max}= 1659(C=O), 1536(N=O), 3125(C=C), 2912(C-C) cm⁻¹; ESI-MS (*m/z*): 344.1[M+1]⁺; Anal. Calcd for C₁₇H₂₁N₅O₃: C 59.46, H 6.16, N 20.40; Found: C 59.10, H 6.42, N 20.65%

RESULTS AND DISCUSSION

The different substituted 2-chloro-1-(piperazin-1-yl)ethanones (J1-J11) were synthesized by aromatic nucleophilic substitution of substituted piperazines on readily available chloroacetyl chloride in good yield. The synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-1-(4-phenylpiperazin-1-yl)ethanones (NJ1-NJ11) was obtained by reported methods⁷(Scheme-1). The acetamides emerged as final compounds (NJ1-

NJ11), which was due to the nucleophilic substitution of commercially available 2-methyl-5-nitroimidazol. Aprotic solvent, being DMF, undergoes S_N2 inversion by stereochemical evidence that changes in configuration. Potassium iodide (KI) as a catalyst, potassium carbonate (K_2CO_3) being a weak base, needs the reaction to be refluxed that is heating with the condenser. The reaction undergoes drastic conditions and yield is affected. All the compounds were soluble in MeOH, EtOH, DCM, DMF, DMSO and were recrystallized using appropriate solvent and were stable in the solid-state at room temperature.

Scheme-1: A Proposed Route for the Synthesis of Final Compounds (NJ1-NJ11)

Cytotoxicity Activity

Cytotoxicity of synthesized compounds (NJ1-NJ11) has been investigated along with the support of MTT assay as described earlier^{8,9}. In brief, nearly 9000-10000 cells/well were placed in a 96-well plate and allowed to grow for 12 hours. After 12 hours, cells were reacted with higher concentrations (0–200 μ M) of synthesized compounds for 48 hours at 37°C in a CO_2 incubator. After 48 hours, the medium of cells was aspirated and cells were rinsed two times with phosphate buffer saline (pH 7.4). Following the washing, MTT (20 μ l of MTT solution from 5mg/ml stock solution in PBS) and 100 μ l of DMEM were added to each well and plates were incubated additionally for 4-5 hours at 37°C in the CO_2 incubator. After 4-5 hours, incubation, the cell supernatant was aspirated without disturbing any purple color crystals of formazan yielded via reduction of MTT were then absorbed by adding 150 μ l DMSO. The absorbance was then measured at 570nm on a multiplate ELSIA reader (BioRad). The Percentage of cell usefulness has been determined by comparing the treated cells with non-treated ones (control cells) and plotted as a function of the concentration of the compound. As the compounds are DMSO soluble, so to calculate actual cell death, respective concentrations of DMSO has been taken for the treatment of cell and subtracted from the compound treated values.

Cell Viability Assay (MTT)

The cytotoxicity of these synthesized compounds has been evaluated on HEK293 cells with the help of MTT assay. The cytotoxicity of each compound was studied in a 0-200 μ M concentration range, for 48 hours. It has been observed that the clinical action of these compounds shows a variable toxicity profile for HEK293 cells. The results of cytotoxicity showed that in the tested concentration range, compound NJ3, NJ5, NJ8 and NJ10 shows considerable cytotoxicity towards HEK293 cells, the respective IC_{50} values of these compounds are shown in Table-1.

Fig.-1: Diagrammatic Model of Compounds

Whereas other compounds never altered the relevance of HEK293 cells remarkably, as nearly 70% of cells are active at 200 μ M concentration of each compound. It has been observed that four of the

synthesized compounds are toxic (NJ3, NJ5, NJ8 and NJ10), some are less toxic towards normal human cells (HEK) in the tested concentration range.

Table-1: Cytotoxicity Profile of Synthesized Compounds on HEK293 Cells

S.No.	Name of Compound	IC ₅₀ (μM)	Structure
1.	NJ1	> 200
2.	NJ2	> 200
3.	NJ3	175±4.22
4.	NJ4	> 200
5.	NJ5	102±5.12
6.	NJ6	> 200
7.	NJ7	> 200
8.	NJ8	147±3.45
9.	NJ9	> 200
10.	NJ10	184±4.23
11.	NJ11	> 200

CONCLUSION

The present study indicated that the complex consisting of 2-methyl-5-nitroimidazole and piperazine substituted derivatives showed results of cytotoxicity that in the tested concentration range compound NJ3, NJ5, NJ8 and NJ10 show the considerable cytotoxicity towards HEK293 cells. The final compounds (NJ1-NJ11) have been found to possess encouraging structure, which can be put under intensive research to yield biologically active compounds.

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