Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

Cytotoxicity Studies on Novel N-Mannich Bases of Isatin Bearing Heterocyclic Scaffolds Clubbed with Primaquine

Deepika Purohit[1], Sahil Kumar[2*], Rohit Dutt[3], Shailja Singh[4]
[1]School of Medical and Allied Science, GD Goenka University, Sohna-Gurgaon Road, Sohna -122103 Haryana, India
[2*]Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research, Delhi Pharmaceutical Sciences and Research University, New Delhi, 110017, India
[3]GMN College, Ambala Cantt, Haryana
[4*]Special Center for Molecular Medicine, Jawaharlal Nehru University, New Mehrauli Road, New Delhi, India
***Corresponding Author**
Dr Sahil Kumar

(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS
Mannich base, HepG2, cytotoxicity, isatin, primaquine

Abstract:
In this study, a series of novel N-Mannich bases isatin bearing heterocyclic scaffolds clubbed with primaquine were tested against HepG2 cancer cell lines. The conventional MTT assay was used to assess the in vitro cytotoxic activities against hepatocellular carcinoma cell lines. Various compounds showed potential cytotoxic effects on HepG2 cell lines in vitro. Additionally, compounds DSR 11 and DSR 40 exhibited the strongest cytotoxic effect on HepG2 cells in comparison to the conventional medication primaquine.

Introduction

As the only licensed agent for the radical treatment of Plasmodium vivax and P. ovale malaria as well as a single dose gametocytocidal in falciparum malaria, primaquine is one of the most commonly used antimalarial drugs among the various reported agents. It possesses an 8-aminoquinoline chromophore and has gained the trust of clinicians and policymakers since the 1950s [1]. Because of their potential to combat malaria, heterocyclic scaffolds made of nitrogen, sulfur, and oxygen have been increasingly popular recently. Fused pyran, aminoquinoline, quinazoline, indole, quinoxaline, pyrazoline, pyrimidines, triazole, benzimidazole, imidazole, isoxazolinepyrazole, isoquinoline, isatin, isoxazole, etc. are the most commonly found heterocyclic scaffolds in this category [2; 3]. Among these are isatin (1H-Indole-2,3-dione) and its N-bridged compounds, such as N-Mannich bases (a beta-amino-ketone produced by the reaction of an amine, formaldehyde, and a carbon acid), which are a promising heterocyclic scaffold with numerous promising biological activities, including anti-HIV, antimalarial, antiviral, antifungal, antitumor, antiangiogenic, anti-Parkinson's disease, and anticonvulsants, in addition to having good human tolerance and the capacity to bind with the target's hydrophobic sites [3; 4].

Mannich base is a beta-amino-ketone produced when an amine, formaldehyde, and carbon acid react together. The mannich base is the result of a nucleophilic addition reaction between a non-enolizable aldehyde and any primary or secondary amine to form a resonance stabilized imine. The Mannich base is obtained by adding a carbanion from a CH acidic molecule to the imine. Isatin forms the N-Mannich base by condensation of equimolecular properties of the appropriate isatin, secondary amine, and formaldehyde due to the presence of an acidic amino group. In this reaction, Hydrochloric acid is used as a catalyst. Aminoalkylation reactions, also referred to as Mannich reactions, involve combining an amine with an aldehyde component to produce the appropriate Mannich base. Secondary aliphatic amines that have distinct pKa values are the most often encountered amine reagents in the Mannich process, while primary amines and even ammonium salt can be used as reagents [**5**]. Alkylamines' pKa values increased in the following order: secondary > primary > tertiary. The hydrogen bonding and increased availability of lone pairs, as well as the stability of the protonated forms, are

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

the two factors that influence the pKa value. Several investigations have documented the synthesis of N-Mannich bases using various amines. The most often utilized secondary amines are morpholine, diethylamine, and dimethylamine; these compounds have shown potential antifungal, anticancer, and antimalarial properties [6].

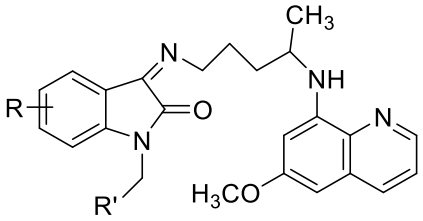
In the present work, cytotoxicity of a series of N-Mannich base derivatives of primaquine bearing isatin moiety as heterocyclic by Mannich reaction was carried out for finding out the best compound.

Experimental Work

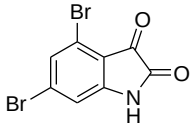
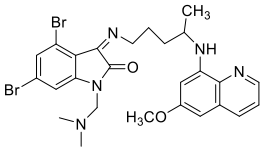
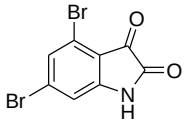
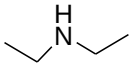
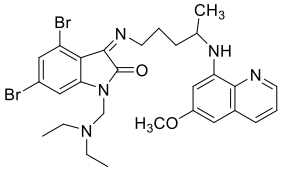
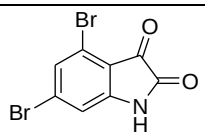
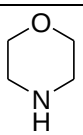
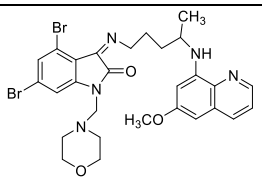
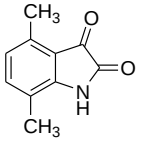
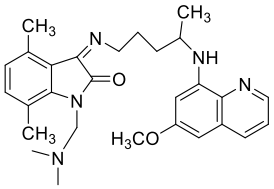
Virtual Combinatorial Library Preparation and in-silico studies

A collection of 75 compounds, which are N-Mannich base derivatives of primaquine with an isatin moiety and are heterocyclic, was created (refer to Table 1) and their anti-malarial potential was assessed through molecular docking studies against four target proteins: Cystein Protease Falcipain-2, Dipeptidyl Aminopeptidase-1, Dipeptidyl Aminopeptidase-3, and Glycogen Synthase Kinase-3β receptors. After applying Lipinski rules to assess the molecules' binding affinities and drug-like qualities, 30 hits were selected for further analysis, including pharmacokinetic profile analysis. Subsequently, the efficacy of these 28 hits against cerebral malaria was assessed by docking them against two target proteins, namely Plasmodium falciparum erythrocyte membrane protein-1 and intracellular adhesion molecule-1. The outcomes were documented. **(Reported previously [7])**.

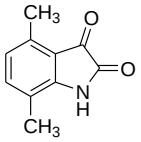
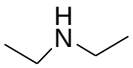
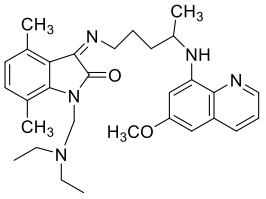
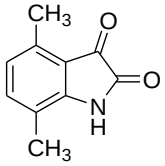
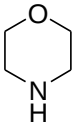
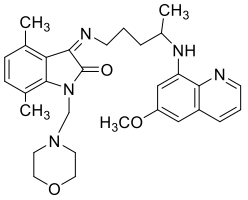
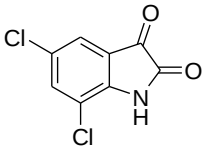
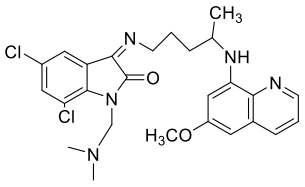
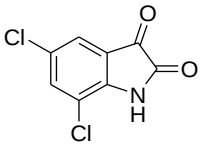
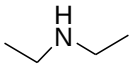
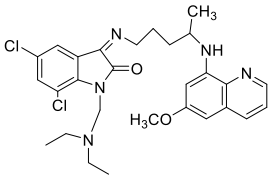
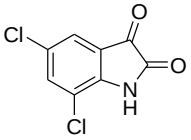
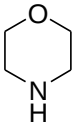
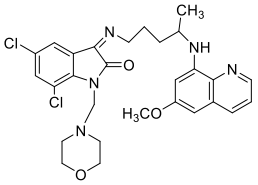
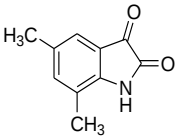
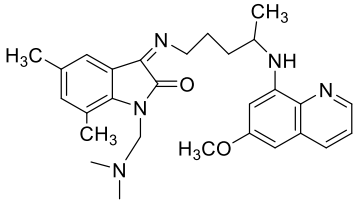
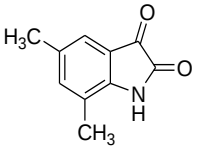
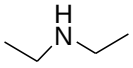
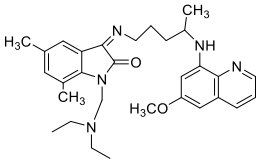
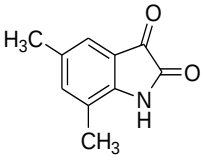
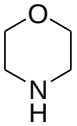
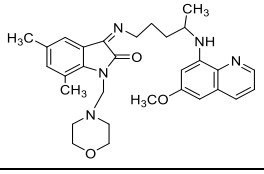
Table-1: List of designed virtual libraries of N-mannich base derivatives.



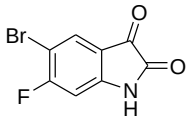
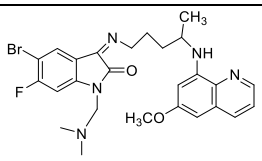
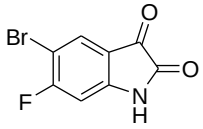
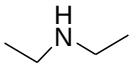
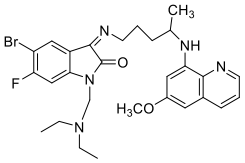
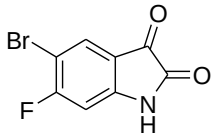
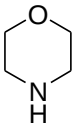
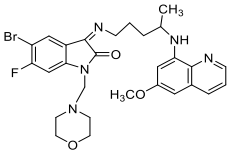
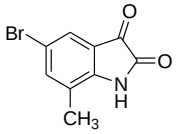
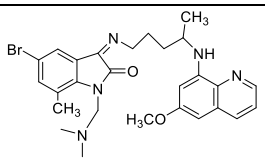
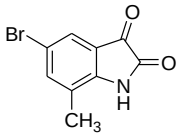
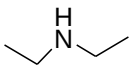
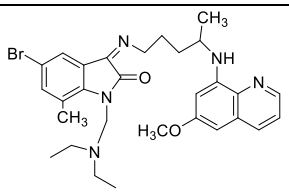
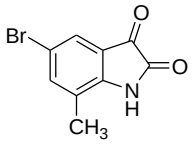
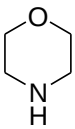
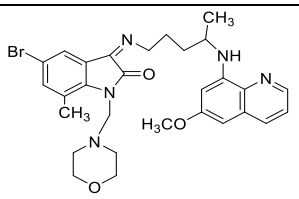
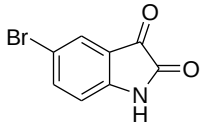
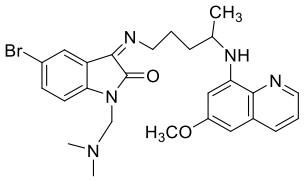
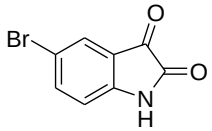
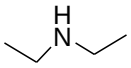
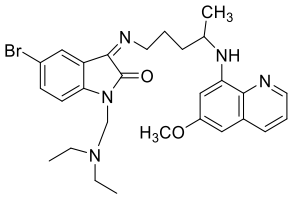
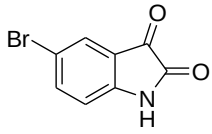
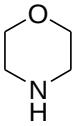
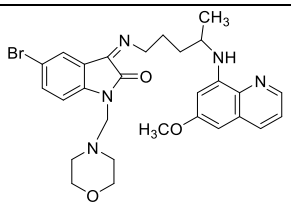
General Structure of N-Mannich base derivatives of Primaquine and Isatin

S. No.	R	Structure of Isatin derivative	R'	Final structure of N-mannich base
1.	4,6-Br			
2.	4,6-Br			
3.	4,6-Br			
4.	4,7-CH_3			

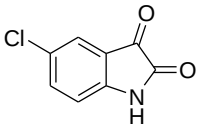
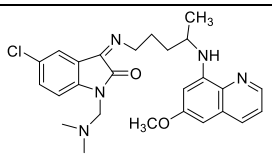
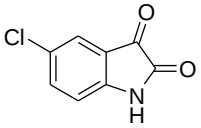
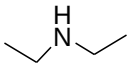
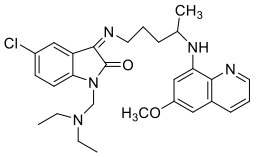
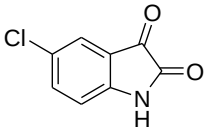
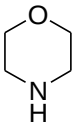
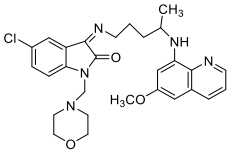
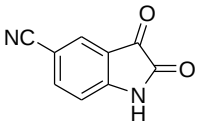
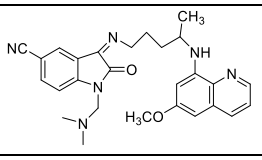
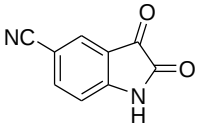
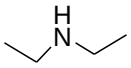
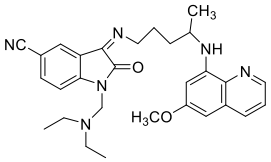
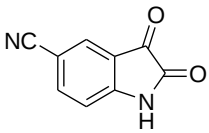
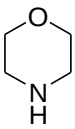
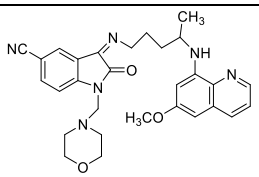
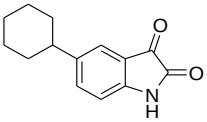
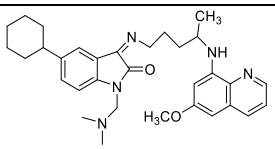
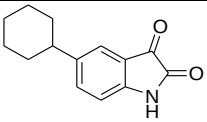
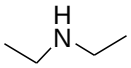
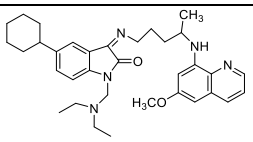
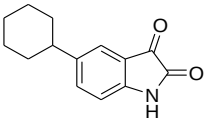
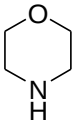
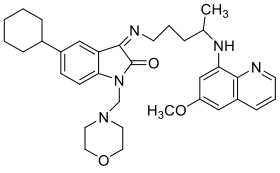
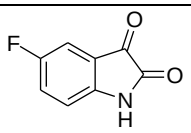
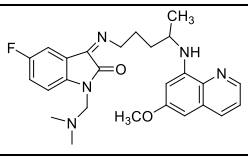
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

5.	4,7-CH_3			
6.	4,7-CH_3			
7.	5,7-Cl			
8.	5,7-Cl			
9.	5,7-Cl			
10.	5,7-CH_3			
11.	5,7-CH_3			
12.	5,7-CH_3			

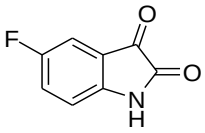
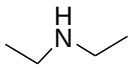
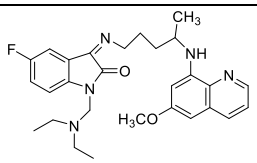
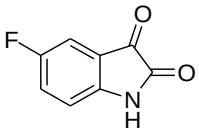
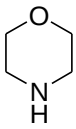
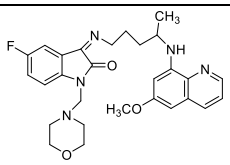
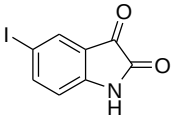
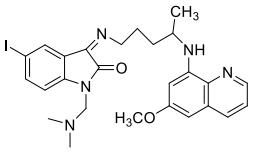
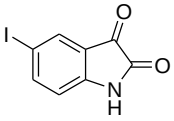
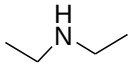
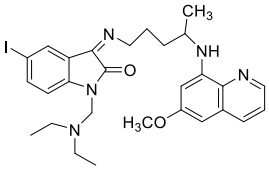
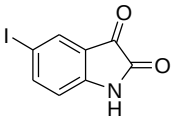
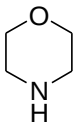
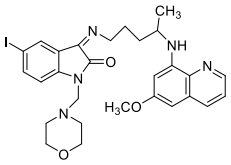
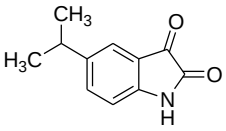
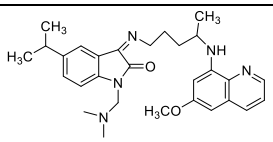
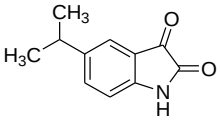
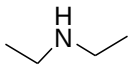
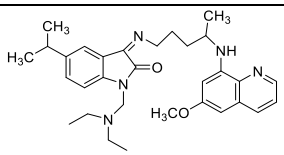
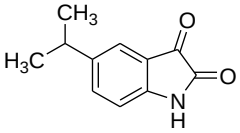
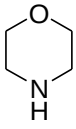
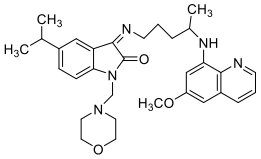
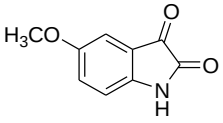
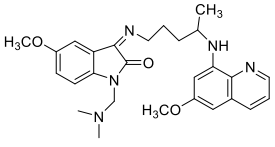
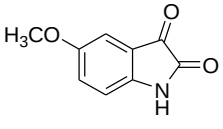
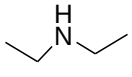
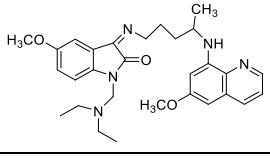
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

13.	5-Br-6-F			
14.	5-Br-6-F			
15.	5-Br-6-F			
16.	5-Br-7-CH_3			
17.	5-Br-7-CH_3			
18.	5-Br-7-CH_3			
19.	5-Br			
20.	5-Br			
21.	5-Br			

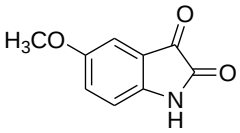
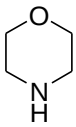
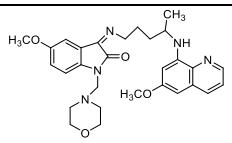
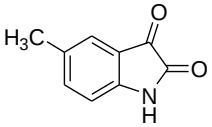
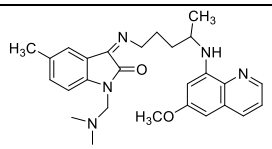
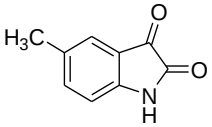
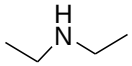
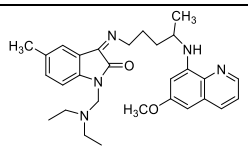
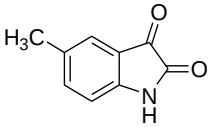
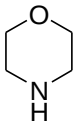
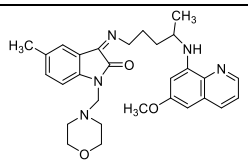
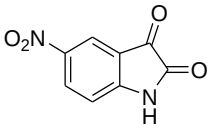
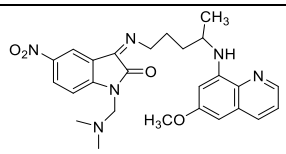
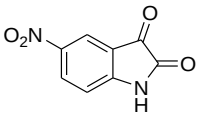
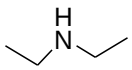
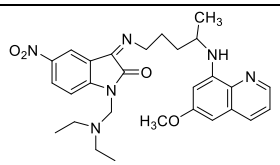
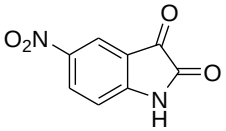
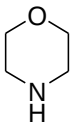
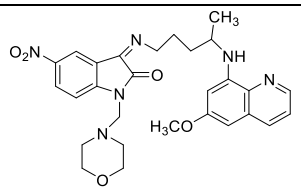
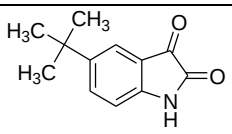
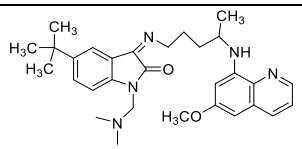
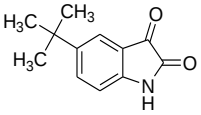
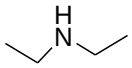
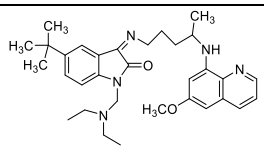
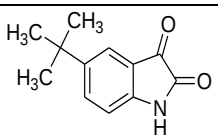
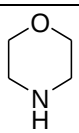
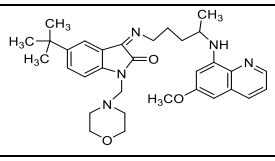
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

22.	5-Cl			
23.	5-Cl			
24.	5-Cl			
25.	5-CN			
26.	5-CN			
27.	5-CN			
28.	5-Cyclohexyl			
29.	5-Cyclohexyl			
30.	5-Cyclohexyl			
31.	5-F			

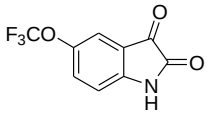
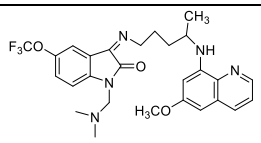
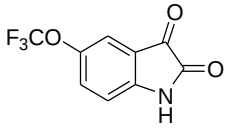
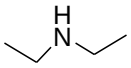
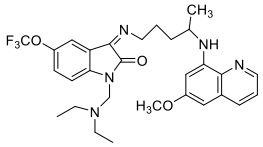
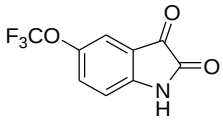
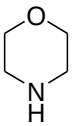
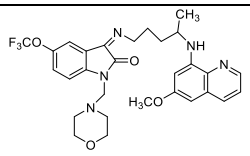
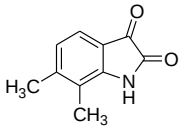
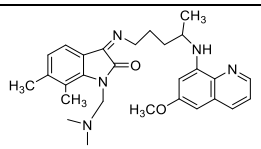
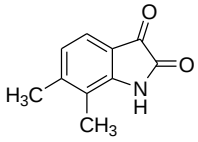
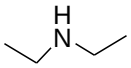
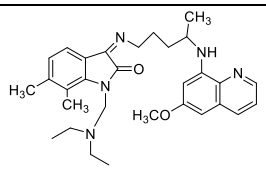
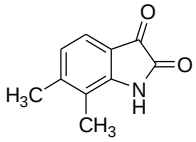
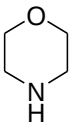
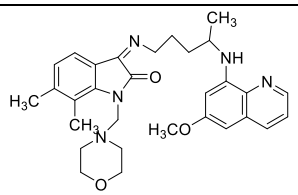
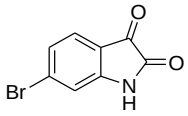
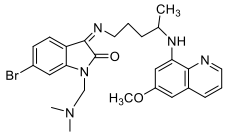
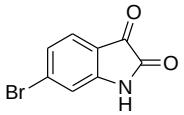
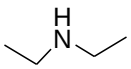
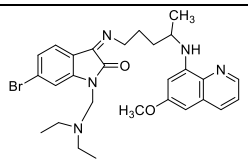
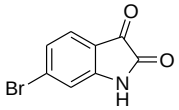
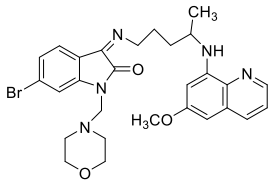
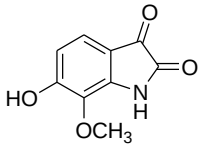
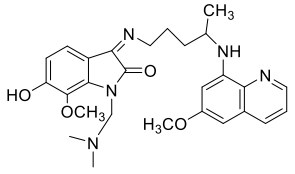
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

32.	5-F			
33.	5-F			
34.	5-I			
35.	5-I			
36.	5-I			
37.	5-Isopropyl			
38.	5-Isopropyl			
39.	5-Isopropyl			
40.	5-OCH_3			
41.	5-OCH_3			

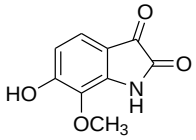
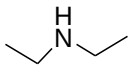
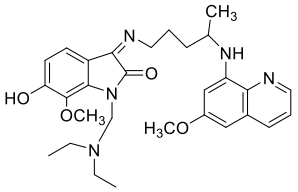
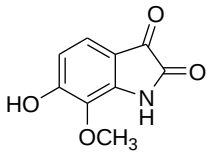
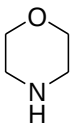
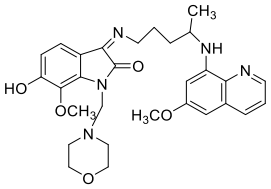
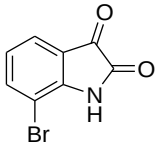
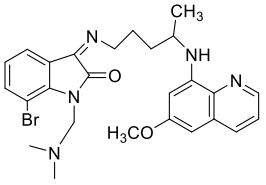
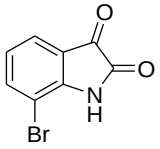
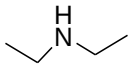
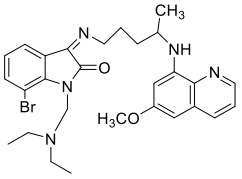
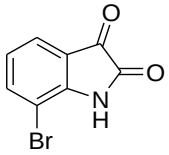
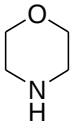
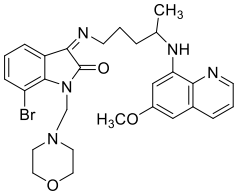
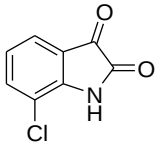
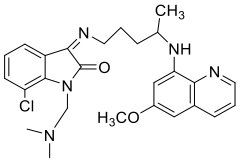
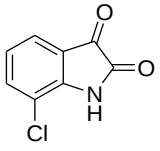
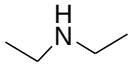
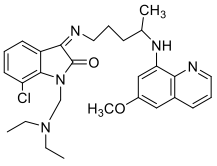
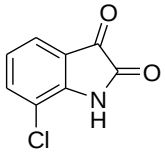
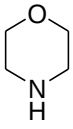
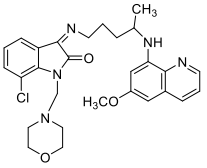
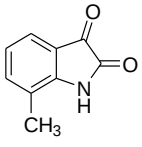
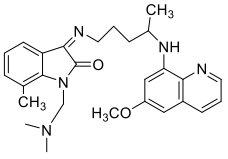
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

42.	5-OCH_3			
43.	5-CH_3			
44.	5-CH_3			
45.	5-CH_3			
46.	5-NO_2			
47.	5-NO_2			
48.	5-NO_2			
49.	5-Tert-butyl			
50.	5-Tert-butyl			
51.	5-Tert-butyl			

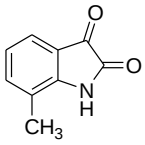
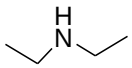
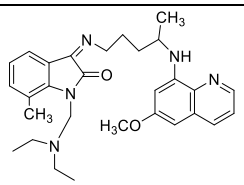
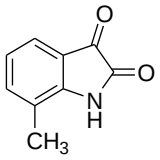
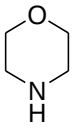
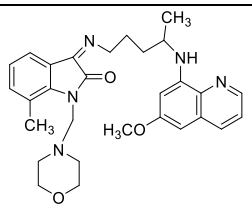
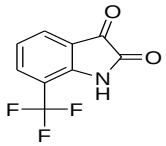
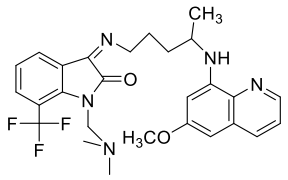
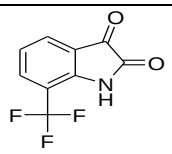
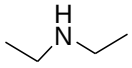
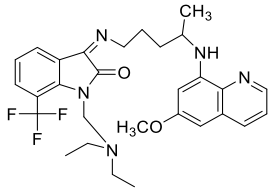
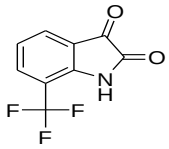
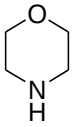
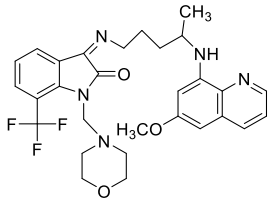
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

52.	5-OCF_3			
53.	5-OCF_3			
54.	5-OCF_3			
55.	6,7-CH_3			
56.	6,7-CH_3			
57.	6,7-CH_3			
58.	6-Br			
59.	6-Br			
60.	6-Br			
61.	6-Hydroxy-7-methoxy			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

62.	6-Hydroxy-7-methoxy			
63.	6-Hydroxy-7-methoxy			
64.	7-Br			
65.	7-Br			
66.	7-Br			
67.	7-Cl			
68.	7-Cl			
69.	7-Cl			
70.	7-CH_3			

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

71.	7-CH_3			
72.	7-CH_3			
73.	7-CF_3			
74.	7-CF_3			
75.	7-CF_3			

Materials

Analytical-grade chemicals were purchased from commercial suppliers and were utilized without additional purification. Media for MTT assay (RPMI 1640), Fetal Calf Serum (FCS), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (**MTT**) were obtained from Thermo Fisher Scientific, Albumax II and gentamicin sulphate [Invitrogen, USA], HEPES, $NaHCO_3$, hypoxanthine [Sigma-Aldrich, (USA)] and Human liver hepatocellular carcinoma cells (Hep-G2) cells were used as available in Dr. Shailja's Lab, Special Centre for Molecular Medicine, JNU, New Delhi.

Methods Cellular Cytotoxicity

Intracellular toxicity of synthesized N-mannich base derivatives of primaquine and isatin was assessed using MTT assay. For carrying out this colorimetric assay, Hep-G2 cells (2×10) were seeded in a 96-well plate. Cells were treated with synthesized compounds and standard reference drug primaquine at different concentration (1 µM, 10 µM, 100 µM, 1 mM and 5 mM) and incubated (37°C and 5% CO_2) for 24 hours. Cells were washed and 10 µL of MTT (5 mg/mL) was added in each well and again incubated for 3 hours. After 3 hours of incubation, 100 µL of DMSO was added to each well and shaken for 15 minutes. The absorbance was then measured by a Varioskan Flash multi-well plate reader (Thermo Fisher Scientific) at 570 nm and percentage cytotoxicity was calculated as follows [**Pre-clinical study of iron oxide nanoparticles fortified artesunate for efficient targeting of malarial parasite**]:

$$\%\ cytotoxicity = 1 - \frac{Average\ OD\ of\ treated\ cells}{Average\ OD\ of\ control\ cells}\ X\ 100$$

Results and Discussion

Virtual Combinatorial Library preparation and in-silico studies

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

It is anticipated that the Isatin moiety present in the developed N-Mannich bases of Primaquine will target brain-based parasites and aid in the successful treatment of CM. Figure 1 illustrates the suggested pharmacophore for creating unique N-Mannich Base derivatives of primaquine
[7].

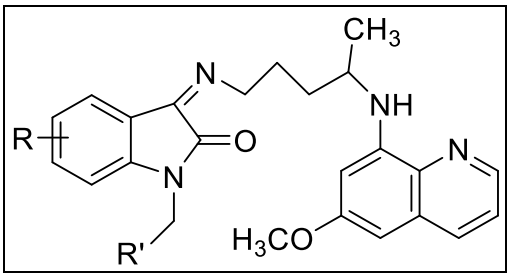


Figure 1: General structure of N-Mannich bases of Primaquine and isatin **(**where R = substituted Isatin derivatives, and R' = Dimethylamine, Diethylamine, Morpholine)

As indicated in Table 1, different derivatives were developed based on variations in isatin derivatives and secondary amines, such as dimethylamine, diethylamine, and morpholine, in accordance with the suggested pharmacophore of N-mannich base derivatives of primaquine.

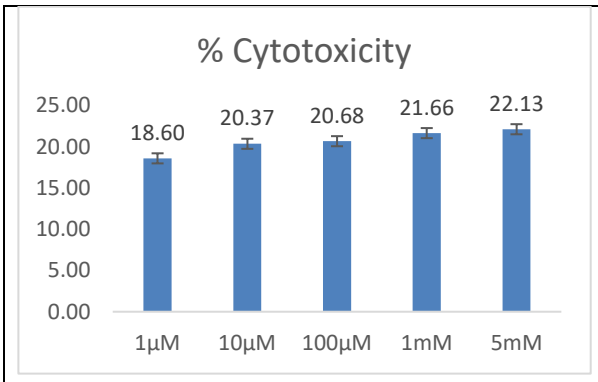
Using Lipinski criteria, all of the designed compounds were docked against different receptors and examined for binding affinity and drug-like qualities. The pharmacokinetic profile of the top 30 hits was then examined. After it was discovered that two of these hits were more hazardous than primaquine, they were left out of additional analysis. Subsequently, the efficacy of these 28 hits against cerebral malaria was examined by docking them against two target proteins, namely (a) Plasmodium falciparum erythrocyte membrane protein-1 and (b) intracellular adhesion molecule-1, and the results were recorded. Eight investigated molecules were identified as lead molecules through analysis of the docking results, and these molecules were chosen for further investigations.

Biological Evaluation

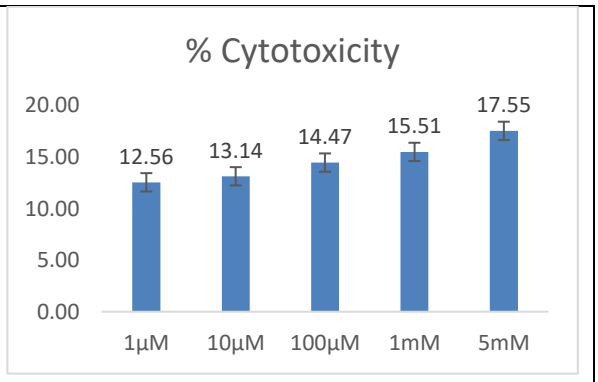
CELLULAR CYTOTOXICITY

Cytotoxicity of the selected derivatives and reference drug primaquine against human cells was determined at a different concentration (1µM, 10µM, 100µM, 1mM and 5mM) upon incubation with Hep-G2. Post 24 h incubation no cellular death was observed (Figure 2). The compounds were found to be non-toxic to human cells.

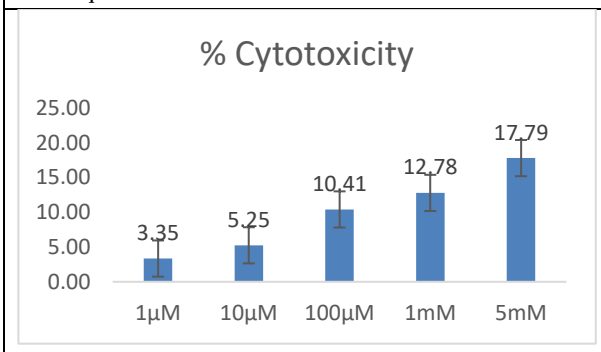
Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727



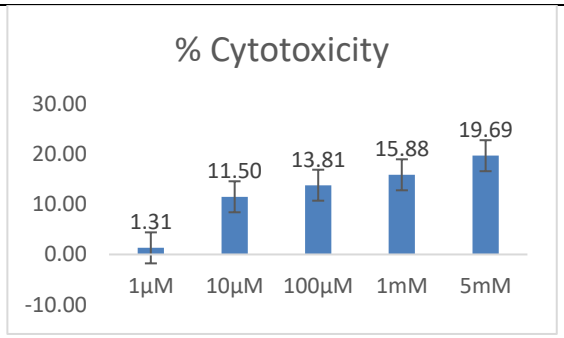
Primaquine



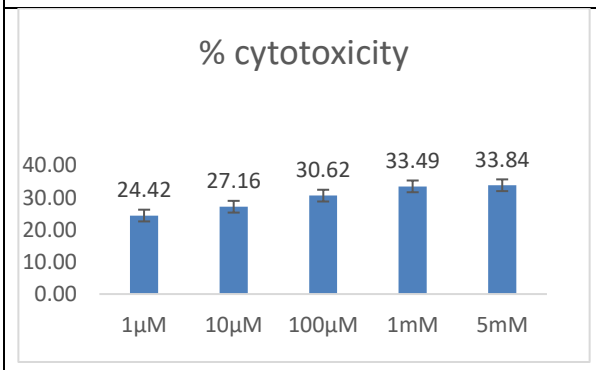
DSR 4



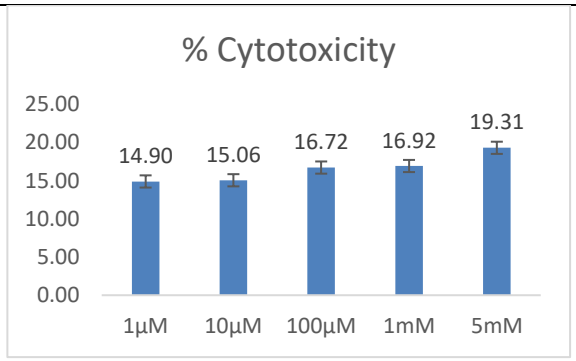
DSR 10



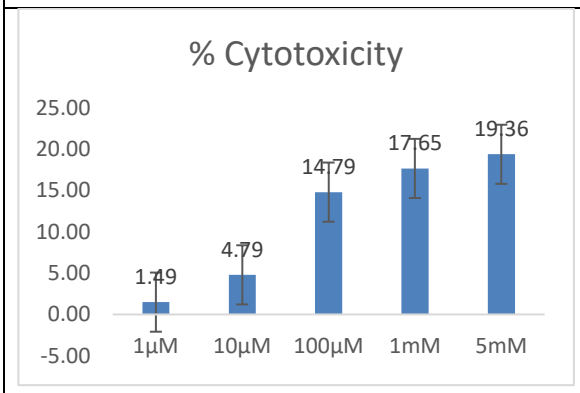
DSR 11



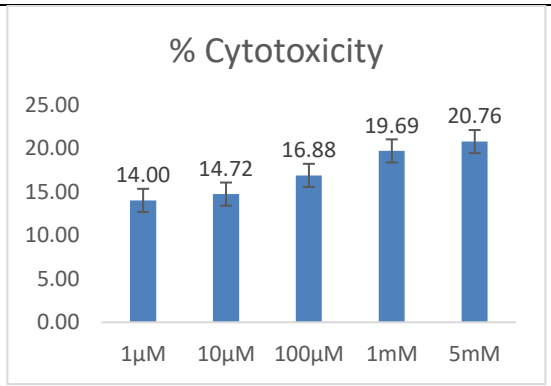
DSR 38



DSR 40



DSR 49



DSR 56

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1837-1849 | ISSN:2251-6727

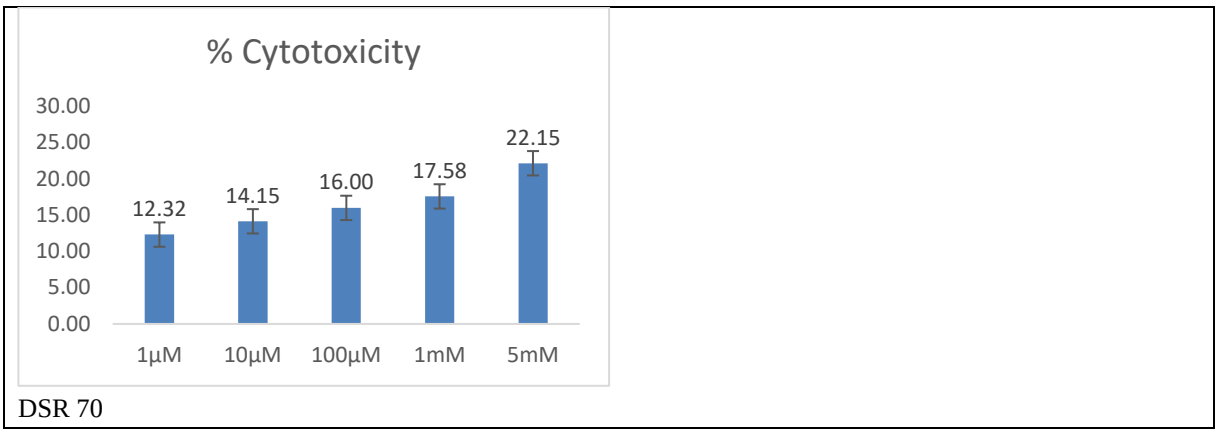


Figure 2: Cytotoxicity of the Novel N-Mannich Bases of Isatin Bearing Heterocyclic Scaffolds Clubbed with Primaquine

Conclusion

During the present research study, eight novel N-Mannich bases of Isatin Bearing Heterocyclic Scaffolds Clubbed with Primaquine were tested on the cytotoxic effects of hepatocellular carcinoma cell lines in vitro. The obtained data demonstrated the potential lethal effects of several produced compounds on HepG2 cell lines in vitro. The compounds DSR 11 and DSR 40 exhibited the strongest cytotoxic effects on HepG2 cells, indicating a strong and encouraging therapeutic potential for these novel compounds.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

Authors acknowledge facility of Special Center for Molecular Medicines (SCMM) and Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), for all the spectral analysis and biological activities carried out.

References

1. Policy brief on single-dose primaquine as a gametocytocide in Plasmodium falciparum malaria. https://www.who.int/malaria/publications/atoz/who_htm_gmp_2015.1.pdf?ua=1 (Accessed 12 December 2022)
2. Kalaria P.N., Karad S.C., Raval D.K. 2018 A review on diverse heterocyclic compounds as the privileged scaffolds in antimalarial drug discovery. Euro J Med Chem. 158, 917-936.
3. Raj R., Biot C., Carrère-Kremer S., et al. 2014. 7-Chloroquinoline-isatin Conjugates: Antimalarial, antitubercular, and cytotoxic evaluation. Chem Bio Drug Des. 83(5), 622-629.
4. Bekircan O., Bektas H. 2008. Synthesis of Schiff and Mannich bases of isatin derivatives with 4-Amino-4,5-dihydro-1H-1,2,4-triazole-5-ones. Mol. 13(9), 2126-2135.
5. Roman G. 2015. Mannich bases in medicinal chemistry and drug design. Eur J Med Chem. 89, 743-816.
6. Rajan V. K., Muraleedharan K., 2017. The pKa values of amine based solvents for CO2 capture and its temperature dependence-An analysis by density functional theory. Int J Greenhouse Gas Control. 58, 62-70.
7. Purohit D., Dutt R., Kumar P., Kumar S., Kumar A. 2023. Design and molecular docking studies of N-Mannich base derivatives of primaquine bearing isatin on the targets involved in the pathophysiology of cerebral malaria. CNS Neurol Disord Drug Targets. 22 (6), 932-943.