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Synthesis, Characterization and Antioxidant Activity Some of Novel Isatin-Based N-Mannich Bases

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Abstract:
Free radical or reactive oxygen species (ROS) damage healthy cells in many ways and may cause various diseases like cancer, diabetes, cardiac disorders etc. Antioxidants play a significant role in improving immunity and preventing tissue damage by free radicals. A new series of isatin based N-mannich bases derived and synthesized the MM1-9 derivative for their antioxidant activity. The DPPH method was used to screen the antioxidant activity of these compounds using ascorbic acid. The results revealed that compound MM4 (3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-chloro-indolin-2-one) showed the highest IC_{50} values amongst the tested compounds. This may be probably due to electron withdrawing and the morpholine substituent on isatin.

Keywords: Isatin, Mannich bases, Antioxidant, DPPH Assay.

Introduction:
Free radical or reactive oxygen species (ROS)-induced cell spoilage has been predicted to play a key role in the course of disease and aging. Higher concentrations of free radicals become toxic, then results in oxidative stress, which damages the cells and causes the many diseases [1]. Body cells keep themselves healthy by maintaining the level of reactive oxygen species (ROS) by a normal cellular metabolism. When cells fail to normalize the high level of ROS, due to harmful species or low antioxidant level, they enter into a state of oxidative stress and are spoiled. An increased level of ROS results in damage to cell organelles, nucleic acids, membrane associated lipids, proteins, purine and pyrimidine bases of DNA, which leads to mutation. High levels of ROS can cause oxidative stress on a cell, which can result in a number of diseases include cancer, neurodegenerative disorders, atherosclerosis, diabetes, cataracts and aging [2-4]. These radicals can form on their own or as a result of certain biological functions including phagocytosis, controlling cell development, producing substances, and cell-to-cell communication. Antioxidants that have the ability to scavenge free radicals are therefore crucial to the treatment and prevention of various disorders [5]. Therefore, the discovery of antioxidants for use in preventative medicine has been the subject of extensive discussion. When provided through nutrition, antioxidants are thought to be boosting health because of their vast variety of biological and pharmacological effects. Antioxidants play a role in activities including boosting immunity, control tissue damage and stop growth or development brought on by free radicals [6,7]. Antioxidants are often utilized as catalysts in antibiotics, including those that are anti-inflammatory, anti-fungal, antibacterial, and antiviral, in the prevention and treatment of Parkinson's and Alzheimer's disease [8,9], as well as in sectors that deal with anticorrosion. Due to their affordability and effectiveness, synthetic antioxidants are more widely used nowadays than natural antioxidants [10].

Heterocycles are an accepted category in organic chemistry with several uses in both biology and business. Their heterocyclic nucleus plays a key role in medicinal chemistry by acting as the base for therapeutic drugs. The therapeutic value of several synthetic indole derivatives, including isatin, has increased in medicinal chemistry. The pyrrole nucleus with keto groups at positions 2 and 3 and the benzene ring are joined in the isatin ring system. In the 1841, first isatin was prepared by oxidizing indigo with nitric and chromic acids [11,12]. It is present in many plants, like Couroupita guianensis, Isatis tinctoria and Calanthe discolour; in humans as a metabolic product of adrenaline. It possesses numerous biological activities, including antimicrobial, antioxidant, anticancer, analgesic and anti-inflammatory, anticonvulsant, MAO inhibitor, anti-TB, anti-HIV and alpha-glucosidase inhibitor [13]. The majority of isatin derivatives have also been shown to have potential antioxidant activity, and the lactam ring of isatin is thought to have free radical scavenging properties because of its N-H and C=O moieties [14,15].

The final products of the Mannich reaction are compounds bearing beta-amino ketones. A molecule having active

hydrogen(s), an amine (primary or secondary), and formaldehyde (any aldehyde) condense to create the Mannich reaction, which is a nucleophilic addition process. Mannich bases also serve as significant pharmacophores or bioactive leads that are employed to synthesize a variety of possible significant drugs molecules using aminoalkyl chains [16,17]. Mannich bases are well recognized for their effective activities like anti-inflammatory, anticancer, antifilarial, antibacterial, anticonvulsant, anti-HIV, antitubercular, analgesic, antifungal, antimalarial, antipsychotic, antiviral activities, anthelmintic and other therapeutic properties [18-21].

So, we aimed to synthesize some novel N-Mannich bases of indole-2-one, that may have potential biological activities.

Material and Method

All chemicals were procured of AR grade and used without further purification. M.P. were obtained by the open capillary tube method and are uncorrected. The completion of the reaction was confirmed by TLC using chloroform: methanol (9:1) as solvent. JASCO FTIR 460+ was used to record the IR spectra using the KBr technique, while MHzJEOL JNM ECS 400 was used for ^{1}H NMR spectra using DMSO-d_6 solvent.

General Procedure

Preparation of N^2-Benzylidenepyridine-2,6-Diamine(M):

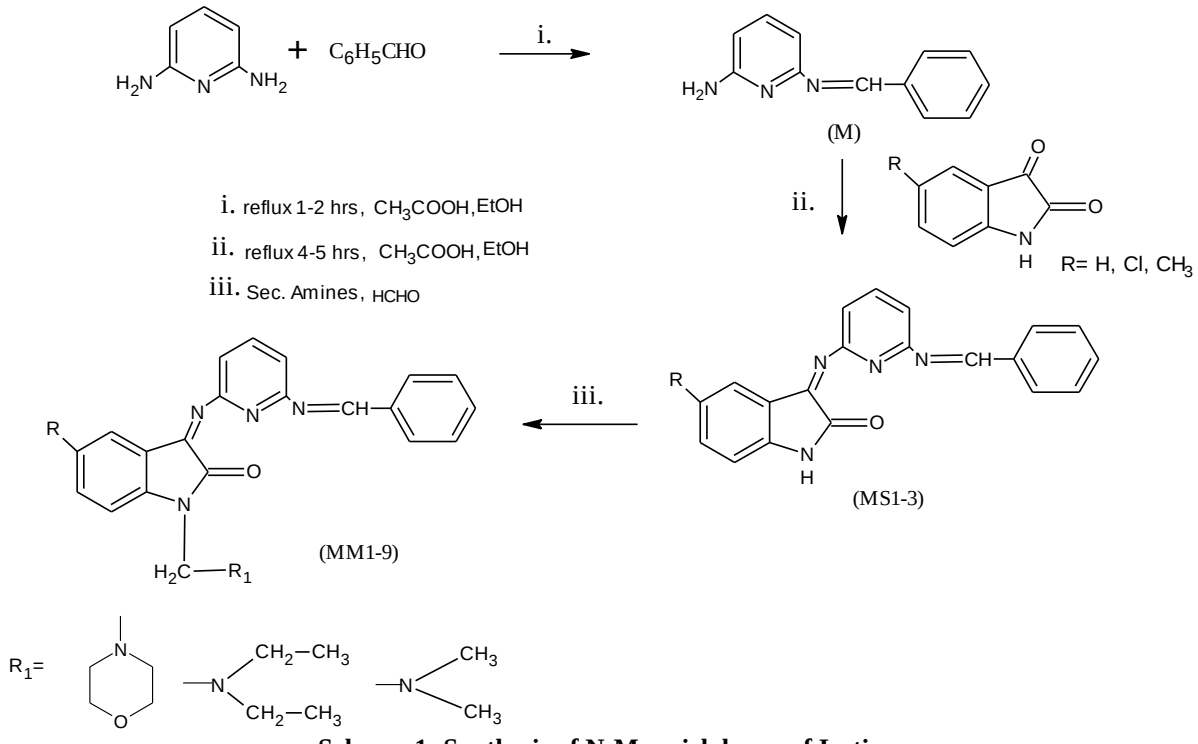
N^2-Benzylidenepyridine-2,6-Diamine was synthesized by the reaction of 2,6-diaminopyridine (2mmol) and benzaldehyde (2mmol) in ethanol using a few mL of glacial acetic acid. After the completion of the reaction, the resultant solid was filtered and re-crystallized with methanol to produce a pure product.

Preparation of Schiff bases (MS1-3):

By refluxing an equimolar quantity (2mmol) of 5-substituted isatin and N^2-benzylidenepyridine-2,6-diamine in ethanol containing 1 mL of glacial acetic acid, Schiff bases of isatin were prepared. The Resulting compounds were filtered and re-crystallized from suitable solvent.

Preparation of Mannich bases(MM1-9):

Preparing a slurry of Schiff bases (2mmol), 5 ml of THF and a few ml of formaldehyde solution, to which 2º amines (2mmol) were added with cooling and shaking. After standing at r.t. for one hr, it was warmed for 15 min on water bath. The final compound was obtained by cooling, followed by recrystallization from suitable solvent.



Scheme-1: Synthesis of N-Mannich bases of Isatin

Antioxidant Activity: (DPPH method):
A fast and simple method for evaluating the antioxidant activity of the antioxidants is the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test. The antioxidant activities were assessed using a measurement of IC_{50}, the concentration that is effective in which 50% of the free radicals were scavenged. Antioxidant activity was higher when the IC_{50} value was lower [22].

The Mannich base of 2,3-dioxoindole (MM1-9) was shown to exhibit DPPH radical scavenging activity by measuring the changes in DPPH absorbance at 517 nm using a spectrophotometer. Different concentrations of compounds (20-100 g/mL) were prepared in methanol and a 3 ml solution of was prepared with methanol, containing 1 ml of compound solution and 1 ml of DPPH solution in methanol (4 mg in 100 ml). After vigorous shaking of the mixture, it was left to remain at room temperature in the dark for 30 minutes. Using DPPH and methanol as a blank, the mixture's absorbance was measured at 517 nm, and the percentage of radical scavenging action was calculated using ascorbic acid as a reference for activity comparison.

% Radical Scavenging Activity: $[(Abs_0-Abs_1)/Abs_0] \times 100$
Where,
Abs_0 =Absorbance of control; Abs_1= Absorbance of sample

The IC_{50} value was calculated and compared with the standard drug.

Result and Discussion:
By reacting substituted isatin Schiff derivatives with aldehydes, and secondary amines, in THF, to create novel Mannich bases of isatin derivatives (scheme-1). The synthesized novel Mannich bases (MM1-9) were evaluated for antioxidant activities using the DPPH method. The synthesized compounds were characterized as MM1-9 via the physio-chemical data presented in Table 1 and satisfactory analytical and spectral data including IR and ^{1}H NMR data. A distinct and prominent band was seen in the IR spectra between 1730 and 1720 cm^{-1}, which verified the existence of the carbonyl C=O group. The CH=N (imine) group of the Schiff base was verified by an absorption band that was seen between 1590 and 1570 cm^{-1}. The absorption band at 2970-2950 cm^{-1}, confirmed the N-CH_2-N link of Mannich bases. Imine group of Schiff base and –CH_2- group of Mannich bases 1H NMR peak was shown at at δ 8.26-8.27 ppm and δ 4.60-4.62 ppm, respectively.

DPPH, a stable free radical with a purple color that transforms into a diamagnetic molecule by accepting an electron or hydrogen radical from other compounds, is frequently used to test antioxidant activity in vitro. This color change causes a reduction in the absorbance of 517 nm [23-25]. From Fig.-1 and Table 2 show that the compounds' percentage scavenging activity rises with their concentration in the measured range, and that the Mannich base of isatin having a morpholine group was found to be most active. The compound with the Cl group, MM4, exhibited an IC50 value 20.76µg/ml in comparison to ascorbic acid 10.07µg/ml, which was the highest amongst all the tested compounds. The better activity showed by compound MM6 having diethylamine in the structure followed by the dimethylamine group MM5. The compounds MM5 and MM1 demonstrate same the effect in scavenging the radicals, as do the compounds MM3 and MM9. The compounds MM1, MM6 and MM5 exhibited a mild to moderate effect, while the MM2 compound had least effect. Generally, electron-withdrawing substituents with morpholine groups showed significant scavenger activity as compared to aliphatic secondary amines`s Mannich bases of isatin.

Table 1: Physio-chemical Properties of the compounds

S. No.	Compound	M.P. (°C)	%Yield
1	MM1	269-271	80
2	MM2	258-260	59
3	MM3	248-251	73
4	MM4	284-286	79
5	MM5	270-272	58
6	MM6	279-281	63
7	MM7	261-263	75
8	MM8	270-273	66
9	MM9	274-276	61

Spectral Data: On the JASCO FTIR 460+, IR spectra were captured using the KBr. Tetramethylsilane (Me4Si) was used as an internal standard and DMSO-d6 as the solvent for the 400 MHz JEOL JNM ECS 400 used to record ^{1}H NMR spectra.

N-Mannich bases of isatin:

MM1:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-indolin-2-one: IR (KBr, cm^{-1}):

3058 (v, Ar-H), 2937 (v, N-CH_2-N), 1720 (v, C=O), 1595 (v, CH=N), 1112 (v, C-O-C); ^{1}H NMR (DMSO-d_6, 400MHz): δ 3.46-3.67 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 6.68-8.10 (8H, m, Ar).

MM2:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-indolin-2-one: IR (KBr, cm^{-1}): 3061 (v, Ar-H), 2984 (v, N-CH_2-N),1718 (v, C=O), 1596 (v, CH=N); ^{1}H NMR (DMSO-d_6, 400MHz): δ 0.89-0.99(6H, t, CH_3), 2.26-2.68 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 7.17-8.16(8H, m, Ar).

MM3:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-indolin-2-one: IR (KBr, cm^{-1}): 3058 (v, Ar-H), 2892 (v, N-CH_2-N), 1721 (v, C=O), 1595 (v, CH=N); ^{1}H NMR (DMSO-d_6, 400MHz): δ 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 6.70-7.80 (8H, m, Ar).

MM4:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-chloro-indolin-2-one: IR (KBr, cm^{-1}): 3060 (v, Ar-H), 2957 (v, N-CH_2-N), 1726 (v, C=O), 1587 (v, CH=N), 1113 (v, C-O-C), 749 (v, C-Cl); ^{1}H NMR (DMSO-d_6, 400MHz): 3.94-3.98 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 7.12-7.88 (8H, m Ar).

MM5:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-chloro-indolin-2-one: IR (KBr, cm^{-1}): 3058 (v, Ar-H), 2971 (v, N-CH_2-N), 1732 (v, C=O), 1590 (v, CH=N), 816 (v, C-Cl); ^{1}H NMR (DMSO-d_6, 400MHz): δ 0.96-0.99(6H, t, CH_3), 2.20-2.40 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 6.89-8.15(8H, m, Ar).

MM6:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]- 5-chloro-indolin-2-one: IR (KBr, cm^{-1}): 3061 (v, Ar-H), 2939 (v, N-CH_2-N), 1731 (v, C=O), 1578 (v, CH=N), 815 (v, C-Cl); ^{1}H NMR (DMSO-d_6, 400MHz): δ 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 6.60-7.88 (8H, m, Ar).

MM7:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-methyl-indolin-2-one: IR (KBr, cm^{-1}): 3058 (v, Ar-H), 2956 (v, N-CH_2-N), 2861 (v, C-H), 1723 (v, C=O), 1589 (v, CH=N), 1110 (v, C-O-C); ^{1}H NMR (DMSO-d_6, 400MHz): δ 1.51(3H, s, CH_3), 3.49-3.53 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 7.10-7.98 (8H, m, Ar).

MM8:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-methyl-indolin-2-one: IR (KBr, cm^{-1}): 3061 (v, Ar-H), 2973 (v, N-CH_2-N), 2909 (v, C-H), 1718 (v, C=O), 1589 (v, CH=N); ^{1}H NMR (DMSO-d_6, 400MHz): δ δ 0.94-0.99 (6H, t, CH_3), 1.19 (3H, s, CH_3), 2.40-2.60 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 6.89-7.88(8H, m, Ar).

MM9:3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]- 5-methyl-indolin-2-one: IR (KBr, cm^{-1}): 3061 (v, Ar-H), 2946 (v, N-CH_2-N), 2879 (v, C-H), 1720 (v, C=O), 1588 (v, CH=N); ^{1}H NMR (DMSO-d_6, 400MHz): δ 1.51(3H, s, CH_3), 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 7.25-8.02 (8H, m, Ar).

Table 2: In-vitro Antioxidant activity of synthesized Mannich bases of isatin (MM1-9)

S. No	Compound	IC_{50} (μg/ml)
1	MM1	35.03
2	MM2	50.45
3	MM3	44.36
4	MM4	20.76
5	MM5	34.48
6	MM6	30.11
7	MM7	39.72
8	MM8	49.89
9	MM9	44.26
10	Standard	10.07

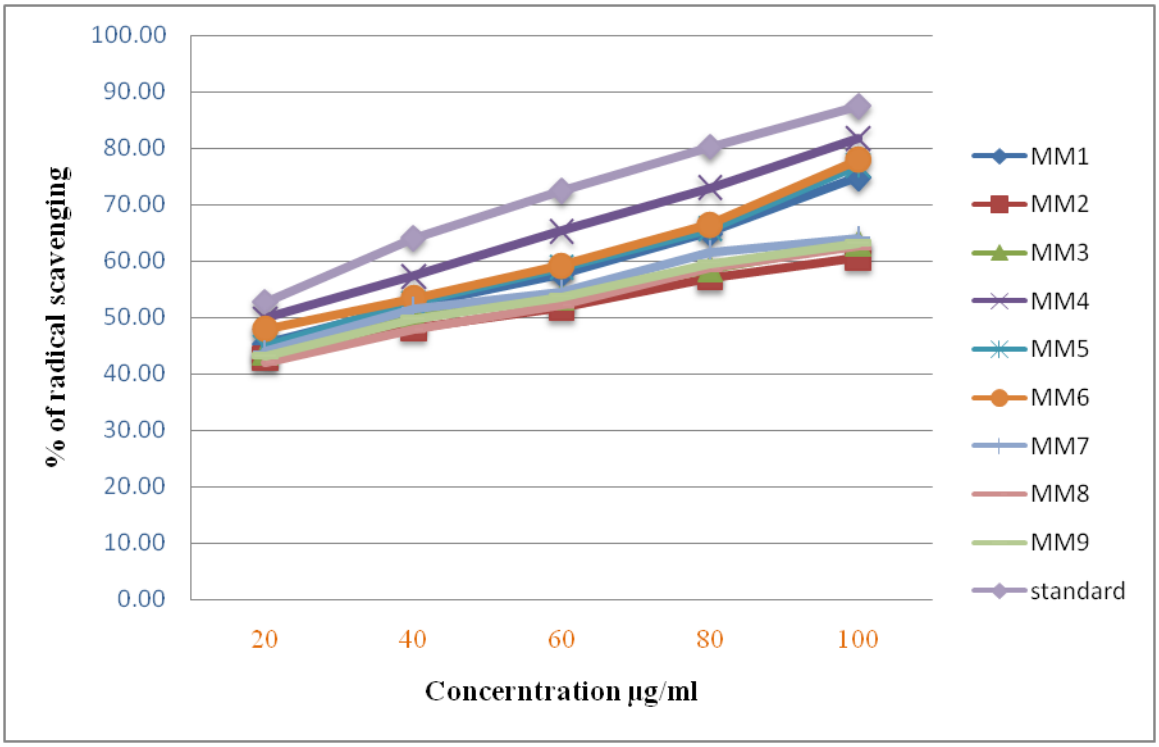


Fig-1: Effect of concentration on % of scavenging

Conclusion:

A novel series of N-mannich bases was synthesized and characterized with suitable analytical data. The DPPH method was used to assess the antioxidant activity of the resultant molecules. The significant antioxidant activity of the investigated compounds appears to be associated with the presence of electron-withdrawing groups and morpholine substituents in the isatin. Therefore, compound MM4-MM6 exhibited stronger radical scavenging activities than the others. The compound MM7-MM9 showed the least antioxidant activity. Future work will include more trials using various substituents.

References:

1. Prakash, C. R., Panneerselvam, T., & Raja, S. (2010). Synthesis and evaluation of antioxidant activities of some indole-2, 3-dione derivatives and analogs. Toxicological and Environ Chemistry, 92(8), 1397-1407.
2. Valko, M., Rhodes, C. J. B., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions, 160(1), 1-40.
3. Rakesh, K. P., Manukumar, H. M., & Gowda, D. C. (2015). Schiff's bases of quinazolinone derivatives: Synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants. Bioorganic & medicinal chemistry letters, 25(5), 1072-1077.
4. Shantharam CS, Swaroopa M, Darshini N, Mallesha N, Rakesh KP (2017). Synthesis and SAR Studies of Potent Antioxidant and Anti-Inflammatory Activities of Imidazole Derived Schiff Base Analogues. Biochem Anal Biochem, 6: 314. doi: 10.4172/2161-1009.1000314.
5. Suleman, M., Khan, A., Baqi, A., Kakar, M. S., & Ayub, M. (2019). 2. Antioxidants, its role in preventing free radicals and infectious diseases in human body. Pure and Applied Biology (PAB), 8(1), 380-388.
6. Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs & aging, 18, 685-716.
7. Sudha, K., Rao, A., Rao, S., & Rao, A. (2003). Free radical toxicity and antioxidants in Parkinson's disease. Neurology India, 51(1), 60.
8. Asplund, K. (2002). Antioxidant vitamins in the prevention of cardiovascular disease: a systematic review. Journal of internal medicine, 251(5), 372-392.
9. Prasad, K. N., Cole, W. C., & Kumar, B. (1999). Multiple antioxidants in the prevention and treatment of Parkinson's disease. Journal of the American College of Nutrition, 18(5), 413-423.
10. Ibrahim, M., Khan, A., Ikram, M., Rehman, S., Shah, M., Nabi, H., & Ahuchaogu, A. (2017). In vitro antioxidant properties of novel Schiff base complexes. Asian Journal of Chemical Sciences, 2(2), 1-12.
11. Da Silva, J. F., Garden, S. J., & Pinto, A. C. (2001). The chemistry of isatins: a review from 1975 to 1999. Journal of the Brazilian Chemical Society, 12, 273-324.
12. Bhrigu, B., Pathak, D., Siddiqui, N., Alam, M. S., & Ahsan, W. (2010). Search for biological active isatins: a short review. Int. J. Pharm. Sci. Drug Res, 2(4), 229-235.

13. Suvaiv, Singh, K., Kumar, P., Hasan, S. M., Kushwaha, S. P., Kumar, A., Zaidi, S. M. H. (2023). Antibacterial Potentiality of Isatin-Containing Hybrid Derivatives: A Review. Asian Journal of Chemistry, 35(4), 815–827. https://doi.org/10.14233/ajchem.2023.27632.
14. Pakravan, P., Kashanian, S., Khodaei, M. M., & Harding, F. J. (2013). Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity. Pharmacological Reports, 65(2), 313-335.
15. Visagaperumal, D., Ezekwem, J. E., Munji, H., & Chandy, V. (2018). Isatin schiff base-an overview. PharmaTutor, 6(5), 38-47.
16. V. J. Belinelo, G. T. Reis, G. M. Stefani, D. L. Ferreira-Alves, and D. Pil´o-Veloso (2002). Synthesis of 6α,7β-dihydroxyvouacapan- 17β-oic acid derivatives. Part IV: mannich base derivatives and its activities on the electrically stimulated guinea-pig ileum preparation, Journal of the Brazilian Chemical Society, (13)6, 830–837.
17. Joshi, S., Khosla, N., & Tiwari, P. (2004). In vitro study of some medicinally important Mannich bases derived from antitubercular agent. Bioorganic & medicinal chemistry, 12(3), 571-576.
18. Bala, S., Sharma, N., Kajal, A., Kamboj, S., & Saini, V. (2014). Mannich bases: an important pharmacophore in present scenario. International journal of medicinal chemistry, 2014.
19. Roman, G. (2015). Mannich bases in medicinal chemistry and drug design. European journal of medicinal chemistry, 89, 743-816.
20. Raoof, S. S., & Sadiq, A. S. (2022). Mannich Bases: Synthesis, Pharmacological Activity, and Applications: A Review. Iraqi Journal of Science, 5086-5105.
21. Raju, S. K., Vengadhajalaphathy, P., Sundaram, R., Periyasamy, S., Chinnaraj, T., & Sekar, P.(2023) Recent advances in biological applications of mannich bases—An overview. International Journal of Pharmaceutical Chemistry and Analysis, 10(1):15–27.
22. Lee, Y. L., Yen, M. T., & Mau, J. L. (2007). Antioxidant properties of various extracts from Hypsizigus marmoreus. Food chemistry, 104(1), 1-9.
23. Shpakovsky, D. B., Banti, C. N., Mukhatova, E. M., Gracheva, Y. A., Osipova, V. P., Berberova, N. T., ... & Hadjikakou, S. K. (2014). Synthesis, antiradical activity and in vitro cytotoxicity of novel organotin complexes based on 2, 6-di-tert-butyl-4-mercaptophenol. Dalton Transactions, 43(18), 6880-6890.
24. Jabbar, S., Shahzadi, I., Rehman, R., Iqbal, H., Qurat-Ul-Ain, Jamil, A., ... & Qanungo, K. (2012). Synthesis, characterization, semi-empirical study, and biological activities of organotin (IV) complexes with cyclohexylcarbamodithioic acid as biological active ligand. Journal of Coordination Chemistry, 65(4), 572-590.
25. Ramírez-Jiménez, A., Luna-García, R., Cortés-Lozada, A., Hernández, S., Ramírez-Apan, T., Nieto-Camacho, A., & Gómez, E. (2013). Dinuclear heptacoordinate dibutyltin (IV) complexes derived from Schiff bases and dicarboxylates: Synthesis, cytotoxicity, and antioxidant activity. Journal of Organometallic Chemistry, 738, 10-19.