Design, synthesis, and antimicrobial screening of novel pyridyl-2-amidrazone incorporated isatin mannich bases

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INTRODUCTION

Isatin is an endogenous compound isolated in 1988[1] and reported to possess a wide range of biological activities.[2,3] Owing to these properties, derivatives of isatin have been developed for therapeutic applications. A recent example is the compound SU11248 (Sutent), a 5-fluoro-3-substituted-2-oxoindole that has received FDA approval for the treatment of gastrointestinal stromal tumors[4] and advanced renal cell carcinoma.[5] Isatin is the biologically active chemical produced by an Alteromonas sp. strain inhabiting the surface of embryos of the caridean shrimp Palaemon macrodactylus, which protects them from the pathogenic fungus Lagenidium callinectes.[6] Schiff bases and Mannich bases of isatin were reported to possess antibacterial,[7-9] antifungal,[10-12] antiviral,[13-15] anti-HIV,[16-18] antiprotozoal,[19,20] analgesic,[21] and anthelminthic[22,23] activities. Rapid development of resistance to clinically important Gram-positive bacteria is a serious public health threat. Staphylococcus aureus can produce a number of diseases affecting human beings and animals. Therefore, the search for novel bactericidal compounds is the object of continuous investigation.[24-30]

Numerous papers have shown that the pyridyl-2-amidrazone nucleus possesses a potent antimicrobial activity.[31-34]

Based on these prior observations, we postulated that a compound containing both isatin and pyridyl-2-amidrazone pharmacophores could be very effective for antimicrobial activity. Unfavorable adsorption, distribution, metabolism, and excretion (ADME) properties can in many cases lead to the clinical trials failure of potentially successful drug candidates. Their evaluation, therefore, at an earlier stage is desired. Here, we also present the predicted ADME properties of our ligands through computation. All the compounds (2a–5) exhibited a better solubility, diffusion, Log P, molecular weight, etc., with no violations making the ligands pharmacodynamically active and better oral absorptive series. Based on the results of computational design, a series of novel pyridyl-2-amidrazone-incorporated isatin Mannich bases were synthesized and screened for their antimicrobial activities. IR, 1H-NMR, and Mass Spectroscopy data were consistent with the assigned structures. The results exhibited that all of the lead compounds showed good antimicrobial activities; noticeably, the compound 2a2 showed the best activity against Candida albicans (16 µg/ml) and compound 2a3 was found to be the most active derivative against Staphylococcus aureus and Escherichia coli at minimal inhibitory concentration values of 4 and 32 µg/ml, respectively.

Key words: Antimicrobial activity, isatin, Lipinski’s rule of 5, pyridyl-2-amidrazone

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ORIGINAL ARTICLE

Abstract

Isatin is an endogenous compound and reported to possess a wide range of biological activities. Numerous papers have shown that the pyridyl-2-amidrazone nucleus possesses a potent antimicrobial activity. Based on these prior observations, we postulated that a compound containing both isatin and pyridyl-2-amidrazone pharmacophores could be very effective for antimicrobial activity. Unfavorable adsorption, distribution, metabolism, and excretion (ADME) properties can in many cases lead to the clinical trials failure of potentially successful drug candidates. Their evaluation, therefore, at an earlier stage is desired. Here, we also present the predicted ADME properties of our ligands through computation. All the compounds (2a–5) exhibited a better solubility, diffusion, Log P, molecular weight, etc., with no violations making the ligands pharmacodynamically active and better oral absorptive series. Based on the results of computational design, a series of novel pyridyl-2-amidrazone-incorporated isatin Mannich bases were synthesized and screened for their antimicrobial activities. IR, 1H-NMR, and Mass Spectroscopy data were consistent with the assigned structures. The results exhibited that all of the lead compounds showed good antimicrobial activities; noticeably, the compound 2a2 showed the best activity against Candida albicans (16 µg/ml) and compound 2a3 was found to be the most active derivative against Staphylococcus aureus and Escherichia coli at minimal inhibitory concentration values of 4 and 32 µg/ml, respectively.

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amidrazone pharmacophores could be very effective for antimicrobial activity. The synthesis of isatin-pyridyl-2-amidrazone conjugates could be possible by a pharmacophore hybrid approach. Hybridization of two different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects.\[35-39\] Therefore, we synthesized hybrid compounds by linking the main structural unit of the isatin ring system with the pyridyl-2-amidrazone ring system by a Schiff base reaction [Figure 1]. Furthermore, a new series of the corresponding N-Mannich bases was synthesized by reacting them with formaldehyde and various secondary amines and then examined their antimicrobial activities.

Unfavorable adsorption, distribution, metabolism, and excretion (ADME) properties can in many cases lead to the clinical trials failure of potentially successful drug candidates. Analysis of the structures of orally administered drugs, and of drug candidates, as pioneered by Lipinski in 1995,\[40\] has so far been the primary guide to correlating physical properties with successful drug development.\[41,42\] This analysis has been very useful and has led to a set of rules relating to the importance of lipophilicity (octanol-water partition), molecular weight (MW), and the number of hydrogen bond donors and acceptors.

This analysis led to the “Rule of Five” mnemonic. The name of this mnemonic refers to the observation that all the critical threshold values are multiples of five. The “Rule of Five” states that poor absorption is more likely when a compound possesses:
1. more than five H-bond donors
2. more than ten H-bond acceptors
3. \( \log P \) greater than five (or \( \log P \) greater than 4.15)
4. molecular mass over 500 Da

Their evaluation, therefore, at an earlier stage is desired. Here, we also present the predicted ADME properties of our inhibitors calculated using the Molinspiration online server which estimates both physically significant descriptors and pharmaceutically relevant properties.

**MATERIALS AND METHODS**

All the melting points were taken with the help of an open capillary tube and were uncorrected. The structures of prepared compounds were identified on the basis of their IR, \(^1\)\( \text{H} \) NMR, and mass spectra. The IR spectra (KBr) were recorded using JASCO FTIR-420 Series of department of Pharmaceutical Analysis, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore. \(^1\)\( \text{H} \) NMR spectra were recorded using a Bruker advance 500 MHz NMR spectrometer of Sophisticated Analytical Instrument Facility department, IIT, Chennai. Mass spectra were recorded on The JEOL GCMATE II GC-MS, The Sophisticated Analytical Instruments Facility (SAIF), Department of Science and Technology (DST), IIT, Chennai. Reactions were monitored by thin-layer chromatography on a precoated silica gel G plates using Iodine vapors as visualizing agent. Pharmacological Evaluation was done in the Department of Pharmaceutical Chemistry, Sankanth Reddy College of Pharmacy, Nellore, India. IR, \(^1\)\( \text{H} \) NMR, Mass Spectroscopy, and elemental analysis were consistent with the assigned structures.

**Adsorption, Distribution, Metabolism, and Excretion Property Prediction**

**Lipinski screening test**

Results from the Molinspiration ADME predictions are presented in Table 1. To test the drug-likeness of the ligands, we applied Lipinski’s rule of 5, requiring candidates to have no more than 5 and 10 hydrogen bond donors and acceptors, respectively, MW less than 500 amu, and partition coefficients between octanol and water (Q\( \log P \)) less than 5. An orally active compound/drug should have no more than one violation of these rules. All the test compounds with the violation of ‘0’ passed the Lipinski screening test. Poor absorption or permeation is more likely when a ligand molecule violates Lipinski’s rule of 5.

**General methods of synthesis**

In the present study, isatin is treated with the pyridyl-2-amidrazonel[43] to form Schiff base (1). Then, this intermediate undergoes reaction in presence of formaldehyde and various secondary amines to form N-Mannich Bases of isatin (2a1–2a5) as depicted in the Figure 2. All the synthesized compounds are soluble in dimethylformamide.

**General procedure for the synthesis of N-[[2-oxo-1H-indol-3-ylidene] amino] pyridine-2-carboximidamide (1)**

Indoline-2,3-dione (Isatin) (1.0 mmol) was dissolved in methanol (5 ml) with gentle heating, then pyridyl-2-
amidrazone (2.4 mmol) was slowly added with stirring at room temperature. After stirring overnight, the resulting precipitate was filtered and crystallized or the reaction mixture was evaporated to dryness and the residue suitably purified.

**General procedure for the synthesis of N-[(1-(dimethylamino)methyl)-2-oxo-1H-indol-3-ylidene]amino|pyridine-2-carboximidohydrazide (2a)***

To a solution of dimethylamine (0.02 mol) in ethanol (50 ml), isatin and its derivatives (0.02 mol) and 37% formalin (1 ml) were added. The reaction mixture was heated under reflux for 24 hours. On cooling, the precipitate was collected, washed with cold ethanol, and recrystallized from a mixture of DMF and water to give 2a. In an analogous way, the remaining drug molecules 2a1, 2a2, 2a3, and 2a4 have been prepared.

**Antimicrobial screening**

We have designed and synthesized novel pyridyl-2-amidrazone incorporated isatin mannich bases, in order to investigate their antimicrobial activity. The compounds (2a1,4) were tested for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The minimal inhibitory concentration (MIC) of the synthesized compounds was determined against *S. aureus*, *E. coli*, and *C. albicans* using a standard broth dilution technique. All the MIC results are presented in Table 2.

**RESULTS AND DISCUSSION**

**Chemistry**

IR, 1H-NMR, Mass Spectroscopy, and elemental analysis were consistent with the assigned structures.

*N’-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-2-carboximidohydrazide [2a]*

Percent yield: 66.22% (solid). Mp. 203°C. FTIR (KBr): cm⁻¹ 3438 (N-H Stretch); 3058 (Ar C-H Stretch); 1686 (C = O Stretch); 1488 (C = N Stretch); 3390 (imines N-H Stretch); 1096 (N-N Stretch); 1H-NMR (DMSO, d ppm); 7.92–8.43 (s, 1H, NH-CO). 1.62 (s, 1H, -NH); 7.0-8.6 (m, 8H, Ar-H), 7.07 (s, 1H, NH), MS mlz 266 [M+1]. Anal Calcd for C14H11N5O: C, 63.39; H, 4.18; N, 26.40; O, 6.03. Found: C, 63.41; H, 4.20; N, 26.43; O, 6.04.

*N’-[1-(dimethylamino)methyl]-2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-2-carboximidohydrazide [2a.]*

Percent yield: 73% (solid). Mp. 218°C. FTIR (KBr): cm⁻¹ 3045 (Ar C-H Stretch); 1785 (C = O Stretch); 1613 (C = N Stretch); 3381 (imines N-H Stretch); 1465 (-CH2-), 1072 (N-N Stretch); 1H-NMR (DMSO, d ppm); 2.27 (s, 6H, -N(CH3)).
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\[ N^+\text{[1-(diethylamino)methyl]-2-oxo-1,2-dihydro-3H-indol-3-ylidenepyridine-2-carboximidohydrazide}[2a] \]

Percent yield: 69.72\% (solid). Mp. 209 \(^{\circ}\)C. FTIR (KBr): cm\(^{-1}\) 3041 (Ar C-H Stretch); 1772 (C = O Stretch); 1604 (C = N Stretch); 1374 (amines N-H Stretch); 1441 (-CH\(_2\)), 1059 (N-N Stretch); 1\(^{\text{H}}\)-NMR (DMSO, d ppm): 1.50-2.24 (q, 4H, -N (CH\(_2\))), 7.0-8.6 (m, 8H, Ar-H), 4.03 (s, 2H, -NCH\(_3\)) N, 26.07; O, 4.96. Found: C, 63.38; H, 5.63; N, 26.11; O, 4.97.

\[ N^+\text{[2-oxo-1-(piperidin-1-ylmethyl)-1,2-dihydro-3H-indol-3-ylidenepyridine-2-carboximidohydrazide}[2a] \]

Percent yield: 71.54\% (solid). Mp. 252\(^{\circ}\)C. FTIR (KBr): cm\(^{-1}\) 3049 (Ar C-H Stretch); 1745 (C = O Stretch); 1645 (C = N Stretch); 1336 (amines N-H Stretch); 1434 (-CH\(_3\)), 1053 (N-N Stretch); 1\(^{\text{H}}\)-NMR (DMSO, d ppm): 1.50-2.24 (s, 4H, CH\(_3\)), Piperidinyl), 7.0-8.6 (m, 8H, Ar-H), 4.43 (s, 2H, -NCH\(_3\)), N, 26.07; O, 4.96. Found: C, 66.28; H, 6.12; N, 23.19; O, 4.43.

Antimicrobial Screening

Antibacterial screening

The investigation of antibacterial screening [Table 2] revealed that all the newly synthesized compounds were able to inhibit the growth of the selected microorganisms in vitro showing MIC values between 4 and 256 \(\mu\)g/ml. Among the synthesized compounds, 2a was found to be the most active derivative against S. aureus and E. coli at MIC values of 4 and 32 \(\mu\)g/mL, respectively. But the compounds 2a showed a good activity with MICs of 32 \(\mu\)g/ml. Also, other compounds exhibited moderate activity against the test microorganisms.

Antifungal screening

The investigation of antifungal screening [Table 2] revealed that all the newly synthesized compounds were able to inhibit the growth of the selected microorganisms in vitro showing MIC values between 4 and 256 \(\mu\)g/mL. Among the synthesized compounds, 2a showed the best activity against C. albicans (16 \(\mu\)g/ml). But the compound 2a also showed a good activity with MICs of 32 mg/ml. Also, the other compounds exhibited moderate activity against the test microorganisms.

CONCLUSION

It has been very clear from the above findings that the heterocyclic compounds containing both isatin and pyridyl-2-amidrazone rings have pharmacological activities as predicted. The design, synthesis, and biological screening had shown that among the compounds synthesized, the compound 2a, and 2a, can be a lead for the antibacterial and antifungal design, respectively, and further derivatives can be prepared with modification of these particular moieties.

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