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Advances in Bioresearch

ORIGINAL ARTICLE

Synthesis and exploration of novel Schiff bases of isatin derivatives as potentials towards antibacterial, antifungal, and antioxidative properties

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ABSTRACT
Isatin is a versatile precursor to numerous biologically active molecules. Many Schiff bases are known to be medically important and are used in designing medicinal compounds. In recent times, isatin and its derivatives have attracted considerable interest in organic, heterocyclic as well as medicinal chemistry because of their potent biological activities. In the present study, novel isatin-Schiff base analogs were designed and synthesized. Based on the existing reports on the bioactive isatin derivatives, a collection of Schiff bases was synthesized by condensation of isatin with bio active primary amines, whose chemical structures were verified by IR, ^1H-NMR, C^{13}-NMR and mass. The present work provides an efficient and feasible reaction route for the synthesis of Schiff base derivatives. The antimicrobial activity of compounds was investigated by the disk diffusion technique. Among these synthesised compounds 1a and 3a behaves the most favorable antibacterial and antifungal activity. Furthermore, the novel synthesis compound 1a shows highest antioxidant properties.
Keywords: *Isatin, Schiff base, Antibacterial activity, Antifungal activity, Antioxidant*

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INTRODUCTION

Isatin (1H-indole-2,3-dione) has been observed to be a common structural precursor in a most of paints, polymer, dyes, pharmacological, and agrochemicalsactive compounds by virtue of its unique size and structural behavior [1]. Isatin and its derivatives has ubiquitous in drugs shown interesting structural and mechanistic diversity. The literature found that, isatin has evolved into versatile building blocks in organic-heterocyclic synthesis. In this regards, the demand of pharmaceutical and antimicrobial active isatin derivatives, it seemed to be reasonable to synthesize novel Schiff bases and N-alkylated isatin for their biological properties[2]. According to the literature, the α-keto amide substructure found in isatins is amenable to a large number of different chemical transformations [3] [4]. The electron-deficient center at C_3 carbon in isatin enables them to be excellent carbonyl electrophiles equivalent to ketone and aldehydes. During the past several decades, an enormous and various of synthesis reactions have been formulated and reported with isatin as major building block. Isatin molecule has diverse kinds of biological activity; exploration of this moiety in the antiviral and anticonvulsant areas will be especially fruitful [5]–[9]. Schiff base of isatin derivatives was reported to exhibit a broad range of biological properties of isatin including protection against certain kinds of infections [10]. Similarly, Schiff bases and N-alkylated isatin are known to possess a extensive range of pharmacological properties likewise antibacterial, anticonvulsant, anti-HIV,antifungal, and antiviral activity [11]–[14]. Thus, isatin hybrids are

considered potential agents for clinical application in the treatment of various bacterial infections. The analysis of biological and catalytic activities of Schiff bases derived from isatin and imesatin derivatives is a topic of ongoing research in our laboratory.

However, one of the most important approaches to synthesizing more active and newer chemically bound molecules is molecular hybridization. This includes a combination of two or more pharmacophoric units or any other active heterocyclic molecules into a single unit to enhance their activities [15][16]. Hence, we opted for this approach for the building of novel isatin hybrid derivatives. The antioxidant properties of novel molecules play a major role in the mechanism to defend the body by controlling the production and removal of reactive oxygen species (ROS). The controlled mechanism includes the removal of toxins of excess reactive oxygen species, and if not done then the high concentrations of free radicals can damage proteins, carbohydrates, lipids, and normal cell structures and also destroys the nitrogen bases of nucleic acids which lead to certain transformations [17]. It also causes aging, cancer, and neurodegenerative muddles such as Parkinson's and Alzheimer's diseases [18], [19]. Because of the aforementioned facts, we hereby report the synthesis of a new Schiff base derived from isatin based on in-silico studies. Furthermore, we have assessed these compounds for antibacterial activity, antifungal activity, and antioxidant properties. Additionally, the binding of the most active molecules to bacterial and fungal targets was explored using molecular docking studies

MATERIAL AND METHODS

Design and physicochemical descriptors properties of isatin Schiff base derivatives.

Initially, twenty-four isatin Schiff base derivatives were designed by using ChemDraw and Molinspiration software. However, based on ***in silico*** structure-based evaluation, twelve effective compounds were selected for further studies.

Chemistry

All chemicals and reagents were purchased from Sigma Aldrich Chemical Company, and E. Merck India Ltd, India. All the reactions were performed in RBF with a heating batch, and all glassware was dried using dried and distilled solvents. The progress of the reaction was monitored by thin layer chromatography (TLC) using silica gel 60 F254 aluminum sheets, and visualized by ultraviolet light at 254 nm. Melting points were measured on an Electrothermal 9100 antom par apparatus, and IR spectra were measured on a Shimadzu IR-470 spectrophotometer. 1H-NMR and 13C-NMR spectra were determined on BRUKER–AV400 Avance instrument at 500 MHz in DMSO-D6 respectively. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiple). Mass spectra were recorded on a Finnigan-MAT 8430, Method DLC-ms50-1200mz-10min-0.120 ml flow-95 b.m mass spectrometer operating at an ionization potential of 70 eV.

General Procedure for Synthesis of Isatin Schiff bases

General Procedure for Synthesis of N-Alkylated Derivatives [1a, 2a and 2c]

For the synthesis of N-Alkylated Derivatives, equimolar quantities of isatin (0.004 mol) and available different heterocyclic with protected amines were dissolved in dimethyl formaldehyde (15 ml) and added dry K_2CO_3 . The resulting reaction mixture was heated at 65-70°C for 8-10hr.The progress of the reaction was checked by TLC. After the completion of the reaction, the reaction mixture was cooled at 25-30°C and poured into ice cold water. The precipitate was filtered, washed with water, and dried in an oven at 45-50°C. The product was then purified by using ethanol: water mixture to give title compounds

General Procedure for Synthesis of isatin to amino methanol [2b]

For the synthesis of amino methanol, equimolar quantities of isatin (0.004 mol) and were dissolved in ethanol (20 ml) and 2.0 ml 37% formaldehyde solution. The pH of reaction mass adjusted to 8-10 using 1N NaOH solution and resulting reaction mixture was heated at 50-55°C for 10-12 hr. The progress of the reaction was checked by TLC. After the completion of the reaction, the reaction mixture was cooled at 25-30°C and poured in to ice cold water and stir for 2-3 hrs. The precipitate was filtered, and washed with ethanol, and dried in an oven at 45-50°C. The product was then purified by using ethanol: water mixture to give title compounds.

Procedure for Synthesis of isatin to Schiff base [3a-3f]

For the synthesis of Schiff base, equimolar quantities of isatin (0.004 mol) and available different heterocyclic amines were dissolved in 20 ml acetic acid. The resulting reaction mixture was heated at 70-75°C for 6-8 hr. The progress of the reaction was checked by TLC. After the completion of the reaction, the reaction mixture was cooled at 25-30°C and poured into ice cold water and basified with 1 M NaOH (pH 8-9) resulting in the formation of a precipitate. The precipitate was filtered, washed with water, and dried in an oven at 50-55°C. The product was then purified by using ethanol: water mixture to give title compounds. .

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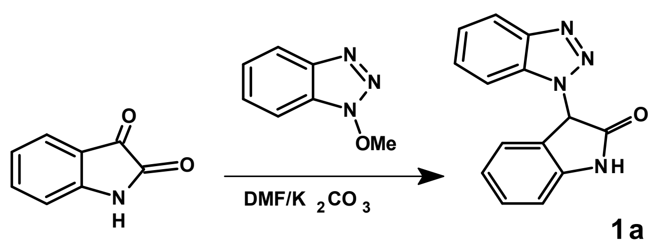
Scheme-1:



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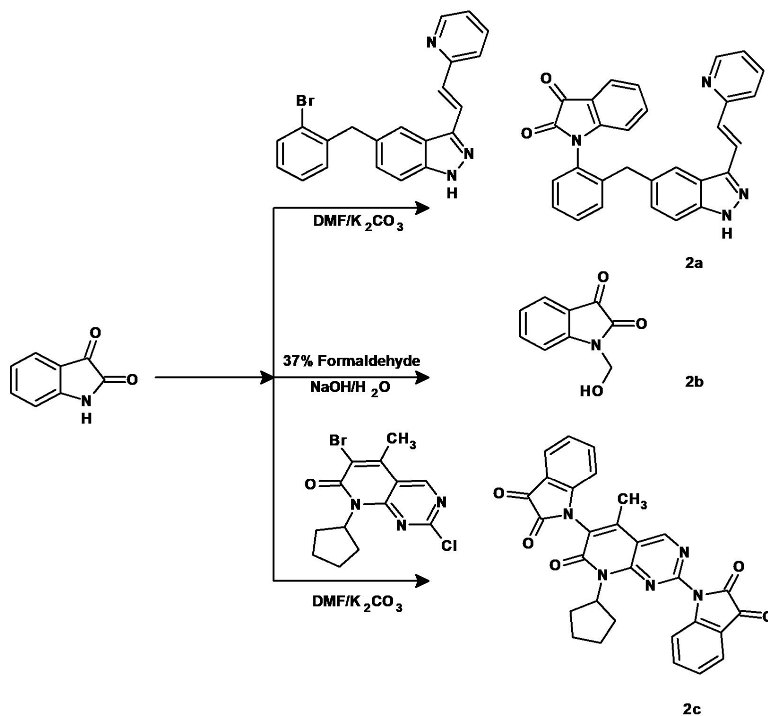

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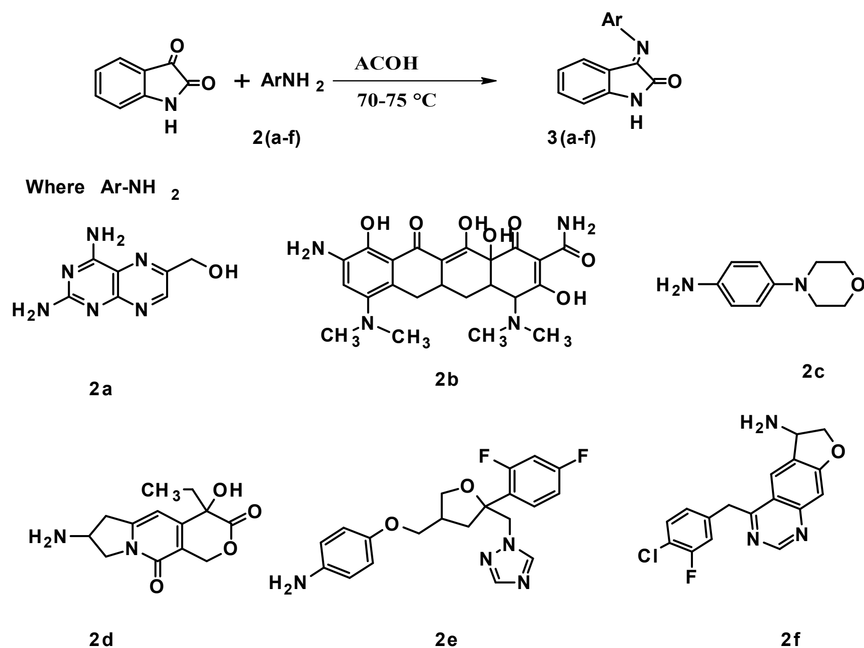

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Synthesis of 3-(1H-benzo[d][1,2,3]triazol-1-yl)indolin-2-one (1a)
Yield: 74.5 %; m.p. 182 °C; IR (KBr in cm^{-1}): 3456 ((NH)), 2922-3113, (Ar-CH), 1801(C=O), 1445-1617 (C=C); ^{1}H-NMR (400 MHz, DMSO-d6) δ ppm 6.31 (1H, s), 6.90 (1H, dd), 7.14-7.36(4H, dd), 7.25 (1H, dd), 7.28 (1H, dd), 7.76-8.04(3H, dd), 7.89 (1H, dd), 7.98 (1H, dd);^{13}C NMR ($CDCl_3$, 400 MHz): δ ppm 56.6 (1C, s), 110.0 (1C, s), 110.5 (1C, s), 119.8 (1C, s), 127.8 (1C, s),128.0 (1C, s), 128.1-128.3(3C, s), 128.4 (1C, s), 131.8 (1C, s), 145.8 (1C, s), 148.4 (1C, s), 168.7 (1C, s); MS (m/z): 250.25(M^+)

Synthesis of (Z)-1-(2-((3-(2-(pyridin-2-yl)vinyl)-1H-indazol-5-yl)methyl) phenyl) indoline-2,3-dione (2a)
Yield:71.2%;m.p. 177 °C; IR (KBr in cm^{-1}): 3438(N-H),3043-3090 (Ar-CH), 1740 (C=O), 1480-1659(C=C), 1516-1579 (C=N); ^{1}H-NMR(400 MHz, DMSO-d_6): δ ppm 0.71-0.83 (6H,q), 1.82 (2H, dd), 2.51 (1H, dt), 3.52 (1H, dd), 3.97 (1H, dd), 4.42-4.54 (2H,d), 4.63-4.72 (2H, s), 6.89-7.53 (10H, dd), 7.86-8.04 (2H, dd), ^{13}C NMR ($CDCl_3$, 400 MHz): δ 37.2 (1C, s), 114.8 (1C, s), 116.0 (1C, s), 117.3 (1C, s), 117.7 (1C, s), 118.4 (1C, s), 122.5 (1C, s), 122.7 (1C, s), 124.5 (1C, s), 125.9 (1C, s), 127.3 (1C, s), 127.4 (1C, s), 128.1-128.3 (2C, 128.2 (s), 128.2 (s)), 128.3-128.5 (2C, 128.4 (s), 128.4 (s)), 128.9 (1C, s), 129.9 (1C, s), 131.0 (1C, s), 135.5 (1C, s), 136.7 (1C, s), 140.8 (1C, s), 141.5 (1C, s), 142.5 (1C, s), 149.1 (1C, s), 151.7 (1C, s), 155.9 (1C, s), 158.2 (1C, s), 183.3 (1C, s);MS (m/z): 456.49 (M^+)

Synthesis of 1-(hydroxymethyl)-1H-indole-2,3-dione (2b)
Yield: 67.9 %;m.p.156 °C; IR (KBr in cm^{-1}): 3105-3554(O-H),2854-3097(Ar-CH), 1760-1801(C=O), 1437-1592(C=C); ^{1}H NMR: δ 5.45 (2H, s), 7.23-7.40 (2H,dd), 7.57-7.77 (2H, dd)
^{13}C NMR: δ 64.7 (1C, s), 114.8 (1C, s), 117.3 (1C, s), 124.5 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 142.5 (1C, s), 158.2 (1C, s), 183.3 (1C, s); MS (m/z): 177.15 (M^+)

Synthesis of 1,1-(8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-d] pyrimidine-2,6-diyl)bis(indoline-2,3-dione) (2c)
Yield: 62.35 %; m.p. 167 °C; IR (KBr in cm^{-1}):2910-3100 (Ar-CH), 1597-1798(C=O), 1521-1590(C=C), 1521-1554 (C=N; ^{1}H NMR: δ 1.63-2.04 (8H,dd), 2.48 (3H, s), 4.58 (1H, dd), 7.27-7.43 (2H,dd), 7.48-7.76 (6H, dd), 8.41 (1H, s); ^{13}C NMR: δ 17.9 (1C, s), 23.1 (2C, s), 32.8 (2C, s), 55.2 (1C, s), 114.8(2Cs), 117.3 (2Cs), 124.5s), 127.3 (1C, s), 128.0 (1C, s), 128.1-128.3 (2Cs), 128.3-128.5 (2Cs), 133.7(1C, s), 142.4-142.6 (2Cs), 149.2 (1C, s), 150.6 (1C, s), 157.3 (1C, s), 158.1-158.3 (2Cs), 165.2 (1C, s), 183.3-183.4 (2Cs); MS (m/z): 518.31 (M^+)

Synthesis of (Z)-3-((4-amino-6-(hydroxymethyl)pteridin-2-yl)imino)indolin-2-one (3a)
Yield: 65.9 %; m.p. 108 °C; IR (KBr in cm^{-1}):3452-3480 (N-H),2853-3116 (Ar-CH), 1773 (C=O), 1436-1606 (C=C), 1498-1736 (C=N); ^{1}H-NMR (400 MHz, DMSO-d6,): δ ppm 4.38 (2H, s), 7.23 (1H,dd), 7.37 (1H, dd), 7.67 (1H, dd), 8.73 (1H, s), 9.08 (1H, dd); ^{13}C NMR ($CDCl_3$, 400 MHz: δ ppm 64.2 (1C, s), 110.0 (1C, s), 122.5 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 131.9 (1C, s), 141.3 (1C, s), 143.7 (1C, s), 148.4 (1C, s), 151.5 (1C, s), 151.7 (1C, s), 152.5 (1C, s), 153.2 (1C, s), 162.7 (1C, s), 168.7 (1C, s);MS (m/z): 321.29(M^+)

Synthesis of (E)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-((2-oxoindolin-3-ylidene)amino)-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (3b)
Yield: 71.6 %; m.p. 119 °C; IR (KBr in cm^{-1}):3469 (N-H),2884-3116 (Ar-CH), 1608-1771 (C=O), 1440-1640 (C=C),1287 (C=N), 2942-3501(O-H); ^{1}H-NMR (400 MHz, DMSO-$d_{6,}$): δ ppm 1.54-1.73 (2H,dd), 1.65 (dd), 2.28 (6H, s), 2.70 (1H, dd), 2.79 (2H,dd), 2.87 (dd), 3.07-3.25 (7H,dd), 4.57(1H, d), 6.79 (1H, s), 7.28-7.46 (3H,dd), 7.38 (dd), 7.63 (1H, dd); ^{13}C NMR ($CDCl_3$, 400 MHz): δ ppm 28.3 (1C, s), 33.9 (1C, s), 35.3 (1C, s), 39.6 (1C, s), 40.3 (2C, s), 41.8 (2C, s), 70.6 (1C, s), 74.2 (1C, s), 97.2 (1C, s), 101.4 (1C, s), 107.1 (1C, s), 110.0 (1C, s), 115.4 (1C, s), 121.5 (1C, s), 122.5 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 131.9 (1C, s), 137.7 (1C, s), 148.4 (1C, s), 149.3 (1C, s), 151.7 (1C, s), 160.0 (1C, s), 167.1 (1C, s), 168.7 (1C, s), 173.3 (1C, s), 173.8 (1C, s), 193.6-193.8 (2Cs); MS (m/z): 601.60 (M^+)

Synthesis of *(Z)-3-((4-morpholinophenyl) imino) indolin-2-one* (3c)
Yield: 56.92 %; m.p. 126 °C; IR (KBr in cm^{-1}): 3452 (N-H),3082-3111 (Ar-CH), 1740 (C=O), 1366-1607(C=C),1652 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 3.03-3.20 (2H, dd), 3.38-3.54 (4H, dd), 3.45 (dd), 3.66-3.83 (2H, dd)), 7.09-7.38 (7H, dd), 7.79 (1H, dd); ^{13}C NMR ($CDCl_3$, 400 MHz): δ ppm 49.4 (2C, s), 66.5 (2C, s), 110.0 (1C, s), 114.4 (2C, s), 122.5 (1C, s), 123.1 (2C, s), 128.2 (1C, s), 128.4 (1C, s), 131.9 (1C, s), 148.0 (1C, s), 148.3 (1C, s), 148.4 (1C, s), 151.7 (1C, s), 168.7 (1C, s); MS (m/z): 307.34 (M^+)

Synthesis of *(Z)-4-ethyl-4-hydroxy-7-((2-oxoindolin-3-ylidene)amino)-1,6,7,8-tetrahydro-10H-pyrano [3,4-f]indolizine-3,10 (4H)-dione*(3d)
Yield: 70.5%; m.p. 208 °C; IR (KBr in cm^{-1}): 3451 (N-H),3087-3115 (Ar-CH), 1703-1757 (C=O), 1363-1630 (C=C), 1695 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 0.85-0.97 (3H,t), 1.91-2.03 (2H, q), 2.81-3.04 (2H, dd), 4.01 (1H, t), 4.23 (2H, dd), 5.20-5.44 (2H, d), 6.22 (1H, s), 7.20-7.35 (2H,dd), 7.67 (1H, dd), 7.88 (1H, dd); ^{13}CNMR ($CDCl_3$, 400 MHz): δ ppm 8.0 (1C, s), 29.8 (1C, s), 32.1 (1C, s), 54.0 (1C, s), 56.6 (1C, s), 64.5 (1C, s), 71.3 (1C, s), 106.9 (1C, s), 110.0 (1C, s), 122.0 (1C, s), 122.5 (1C, s), 128.2 (1C, s), 128.4 (1C, s),

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131.9 (1C, s), 148.4 (1C, s), 149.2 (1C, s), 151.7 (1C, s), 157.7 (1C, s), 163.1 (1C, s), 168.7 (1C, s), 171.9 (1C, 91.6 (1C, s), 104.7 (1C, s), 110.0 (1C, s), 114.5 (2C, s), 115.4 (1C, s), 122.1 (2C, s), 122.5 (1C, s), 127.8 (1C, s), 128.0 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 131.9 (1C, s), 143.5 (1C, s), 148.0 (1C, s), 148.4 (1C, s); MS (m/z): 393.39 (M^+)

Synthesisof (Z)-3-((4-((5-((1H-1,2,4-triazol-1-yl)methyl)-5-(2,4 difluorophenyl) tetrahydrofuran-3-yl) methoxy)phenyl)imino)indolin-2-one (3e)

Yield: 76.45 %; m.p. 178 °C; IR (KBr in cm^{-1}): 3453(N-H),3086-3112 (Ar-CH), 1750 (C=O), 1330-1607(C=C), 1667 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 2.08-2.35 (2H, dd), 2.82 (1H, dt), 3.60 (1H, dd), 3.79 (1H, dd), 4.45-4.55 (2H), 6.97-7.56 (10H), 7.92 (1H, dd), 8.14-8.28 (2H, d); ^{13}C NMR ($CDCl_3$, 400 MHz): δ ppm 29.2 (1C, s), 30.7 (1C, s), 69.8 (1C, s), 71.3 (1C, s), 151.5-151.8 (2Cs), 158.5 (1C, s), 160.8-161.0 (2Cs), 168.7 (1C, s); MS(m/z): 515.51 (M^+)

Synthesisof (E)-3-((4-(4-chloro-3-fluorobenzyl)furo[3,2-g]quinazolin-6-yl) imino) indolin-2-one (3f)

Yield: 65.82 %; m.p. 148 °C ; IR (KBr in cm^{-1}): 3091-3531(N-H), 2854-3083(Ar-CH), 1592-1801(C=C),1642 (C=N); ^{1}H NMR: δ 4.28 (2H, s), 7.12-7.49 (6H, dd), 7.61 (1H, dd), 7.76 (1H, dd), 8.56-8.75 (2H,t), 8.84 (1H, d); ^{13}C NMR: δ 41.7 (1C, s), 104.4 (1C, s), 110.0 (1C, s), 115.1 (1C, s), 118.2 (1C, s), 121.1 (1C, s), 122.5 (1C, s), 124.5 (1C, s), 126.3 (1C, s), 126.9 (1C, s), 128.2 (1C, s), 128.4 (1C, s), 130.9 (1C, s), 131.9 (1C, s), 133.7 (1C, s), 134.4 (1C, s), 144.4 (1C, s), 147.3 (1C, s), 148.4 (1C, s), 151.7 (1C, s), 152.9 (1C, s), 155.1 (1C, s), 156.3 (1C, s), 159.3 (1C, s), 168.7 (1C, s); MS (m/z):457.32(M^+)

Antimicrobial Screening:

Agar disc diffusion assay was employed for the assessment of antimicrobial activities or the inhibitory potential of the synthesized chemical moieties. In vitro, antibacterial and antifungal activities were determined by the agar well diffusion method. Exponentially grown cultures of bacteria and fungi were mixed with sterile 0.85% saline solution to make the final volume up to 105- 106 CFU/ml. Microbiological media used for bacteria is Nutrient agar (Hi-media) Composition (gL^{-1}): Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2). Microbiological media for fungi and yeast is Potato dextrose agar (all ingredients of Hi media) Composition (gL^{-1}): Potatoes infusion, 200; Dextrose, 20; Agar, 15; Final pH (at 25°C) 5.6±0.2. The inoculum of bacterial and fungal cultures was spread separately on the surface of their respective solidified media. Schiff bases (40 µg/ml) were dissolved in dimethyl sulfoxide (DMSO) and Whatman no.-1 filter paper discs (5mm in diameter) impregnated with the test compound (5 µl/disc) were placed on the plates. Chloramphenicol (10 µg/disc) was used for bacteria and Amphotericin-B was used for the fungi as a positive control. A paper disc impregnated with dimethyl sulfoxide (DMSO) was used as a negative control. Plates inoculated with thebacteria were incubated for 24hr at 30±2 ºC and the fungalcultures were incubated for 5-6 days at 27±2ºC. Theinhibition zone diameters were measured in millimeters. Antimicrobial activity was determined by measuring the diameter of the clear zones of inhibition and three replicates were done for each case. The antimicrobial activity of all synthesized compounds 1a-3f was screened against a panel of two Gram-positive bacteria (Methicillin resistance *Staphylococcus aureus* ATCC 25923) and (*Bacillus subtilis* NCIM 2063), twoGram-negative bacteria (*Escherichia coli* NCIM 2109) and *Proteus vulgaris* NCIM 2172) and four fungi (*Aspergillus niger* NCIM 545) (*Candida albicans* NCIM 3471) (*Aspergillus flavus* NCIM 555) and (*Malassezia furfur* MTCC 1374) using the agar well diffusion method [24]. Chloramphenicol and Amphotericin-B were used as antibacterial and antifungal standards, respectively.

Anti-oxidant activity using DPPH Radical Scavenging Method.

Blois[20] showed that α,α-diphenyl-β-picryl hydroxyl radical (DPPH) can be used for determining antioxidant activity. DPPH in ethanol shows a strong absorption band at 517 nm (independent of pH from 5.0 to 6.5), and the solution appears to be deep violet. As the DPPH radical is scavenged by the donated hydrogen from the antioxidant, the absorbance is diminished according to stoichiometry. Briefly, 0.5 ml of DPPH solution (0.2mM) was mixed with 0.1 ml of various concentrations of test compounds, and 1.5 mlof ethanol was added. The mixture was kept at room temperature for 30 min, and then the absorbance (OD) was read at 517 nm against blank. The % reduction of free radical concentration (OU) with different concentrations of test compounds was calculated and compared with standard, ascorbic acid. The results were expressed as IC 50 values (the concentration of test required to scavenge 50 % free radicals).

Preparation of ligands and receptors for docking

All computational studies were performed on Windows 10 platform. The ligand structures were sketched using Biovia's Discovery Studio Visualizer. The ligands were energy minimized by the steepest descent method with the UFF force field [19], using Avogadro v 1.2. [21]. The crystal structure of the enzymes dihydropteroate synthase in complex with sulfanilamide (PDB ID: 1AJ0) [22]. and Glucosamine-6-phosphate synthase in complex with glucosamine-6-phosphate (PDB ID: 2VF5) was downloaded from the

protein data bank. The protein structures were cleaned by deleting the water molecules, and the ligands and adding hydrogens using the 'Dock Prep' module of Chimera v 1.1. The protein structures were energy minimized by 100 steps of steepest descent method and 10 steps of conjugate gradient method with the Amber ff14SB force field in Chimera v 1.1.4 [23], [24]

Docking using AutoDockVina

Auto Dock Vina requires pdbqt files for docking. The pdbqt files for proteins and ligands were prepared using the Graphical User Interface program Auto Dock Tools (ADT) [25]. by assigned Auto Dock atom types, adding Kollman charges merging polar hydrogens to the proteins and ligands. Auto Grid was used for the preparation of the grid map using a grid box size of 64 × 64 × 64 xyz points with a grid spacing of 0.375 A°. The grid center was designated at dimensions (x, y, z): 41.317, 4.855, 6.199, and 29.843, 19.888, -3.143 for 1AJ0 and 2VF5 respectively. Auto Dock Vina [26] was employed for docking using protein and ligand information along with grid box properties in the configuration file. During the docking procedure, both the protein and ligands were considered rigid, the number of modes and exhaustiveness was set to 10 and 9 respectively. The pose with the lowest energy of binding i.e., highest binding affinity were extracted and aligned with receptor structure in Biovia's Discovery Studio Visualizer for further analysis.

RESULTS AND DISCUSSION

Antimicrobial Screening

The results of this study were recorded as the average diameter of the inhibition zone (IZ) in (Table-1: Antibacterial activity of the synthesized compounds) and (Table-2: Antifungal activity of the synthesized compounds). It was found that compounds 1a, 2a, 2b, 3a and 3f exhibit excellent improved antimicrobial activity against all Gram-positive & Gram-negative bacteria (3d and 3e show no activity), and the fungal strain *M. furfur* while moderate activity against *C. Albicans*. Moreover, compound 1a display higher activities against Gram-positive bacteria (*S. aureus and B. subtilis*) and Gram-negative P. Vulgaris than the remaining strains. The remaining derivatives demonstrate potencies from moderate to weak in comparison with the reference drugs. The antifungal potency against *C. Albicans* for all tested derivatives ranges from weak to no activity at all. Additionally, it was noticed that compounds 3b and 3c show no activity against all the screened strains.

Table-1: Antibacterial activity of the synthesized compounds.

Compound Bacteria	Gram Negative		Gram Positive	
	E. coli	*P. vulgaris*	*MRSA*	*B. subtilis*
1a	14.17	17.21	10.24	13.15
2a	11.34	15.64	9.81	10.84
2b	13.12	14.54	7.21	6.54
2c	9.16	12.24	7.95	9.33
3a	12.54	16.98	9.26	15.22
3b	7.85	7.45	6.52	8.25
3c	8.45	11.84	7.24	9.43
3d	-	-	-	-
3e	-	-	-	-
3f	14.34	13.54	11.45	13.76
Chloramphenicol	18.68	21.43	12.13	17.64

Diameter in 'mm' calculated by Vernier Caliper '-' means no zone of inhibition, NA Not applicable.

Table-2: Antifungal activity of the synthesized compounds

Compound Fungi	*A. niger*	*C. albicans*	*A. flavus*	*M. furfur*
1a	14.26	11.08	9.12	12.77
2a	15.24	8.65	6.54	7.45
2b	16.54	10.51	5.21	6.12
2c	18.42	7.02	6.29	9.73
3a	12.01	13.11	9.18	12.35
3b	-	-	-	-
3c	-	-	-	-
3d	8.33	6.45	5.14	8.26
3e	7.62	5.21	6.43	11.24
3f	14.21	10.28	4.38	13.27
Amphotericin-B	19.13	17.16	11.31	19.22

Diameter in 'mm' calculated by Vernier Caliper '-' means no zone of inhibition, NA Not applicable.

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Antioxidant activity using DPPH method

The use of DPPH provides an easy and rapid way to evaluate antioxidants. we used the stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) to investigate the radical scavenging features of the Schiff bases in terms of antioxidant reactivity (rate constants) and stoichiometry (number of radicals trapped per antioxidant molecule).

DPPH Scavenging effect (%) or Percent inhibition = A0-A1 /A0 x 100 %

Where A_0 was the Absorbance of the control reaction and A_1 was the Absorbance in presence of the test.

The antioxidant activity of all the synthesized compounds was performed using the DPPH method and the results are given in Table-3 (Antioxidant activity of the synthesized compounds). All the synthetic compounds produce concentration-dependent scavenging of free radicals. The IC values of all the synthetic test compounds were found between 2 and 60 μg/mL. Among all the test compounds Schiff bases 1a, 3a, 3b, 3d and 3e had more potent antioxidant activity against DPPH free radicals. It is proposed that DPPH may be scavenged by an antioxidant through a donation of hydrogen to form a stable DPPH-H molecule that does not absorb at 517nm. Thus, the results show that synthesized compounds possess antioxidant activity. In a biological system, an antioxidant is defined as "any substance that when present at low concentrations compared to that of an oxidizable substrate would delay or prevent oxidation of the substrate."

Table-3: Antioxidant activity of the synthesized compounds

Sample Conc.	code	Radical Scavenging activity[%]
0.1 mg	1a	36.50
	2a	-2.85
	2b	5.17
0.2 mg	2c	11.25
	3a	15.09
	3b	26.64
	3c	2.13
	3d	20.63
	3e	34.70
	3f	6.33

Docking Studies

The active compounds 1a and 3a among the series were docked in two antimicrobial targets namely dihydropteroate synthase (DHPS, 1AJ0) and glucosamine-6-phosphate (GlcN-6-P) synthase (2VF5) to predict their mechanism of action on bacteria and fungi respectively. DHPS is an essential component of the folate pathway for nucleic acid synthesis. Sulfonamides inhibit the DHPS resulting in bacterial death to starvation of nucleic [27].

Whereas, GlcN-6-P synthase is a key enzyme in the first step of the pathway for synthesis of UDP-GlcNAc, a building block for mannoproteins in fungi. Inactivation of GlcN-6-P synthase is reported to have lethal effects on the fungal cell [28]. The compounds 1a and 3a exhibit stereoisomerism, thus R and S stereoisomers of 1a while E and Z stereoisomers check the effect of stereoisomerism on binding affinity to the antimicrobial targets. An analysis of binding energy values (Table-4: Binding energy values in kcal/mol) indicates that all the stereoisomers bind effectively to both targets (Figure 1A & 1B). The R stereoisomer of 1a shows more affinity for the bacterial target DHPS (1AJ0) than its S stereoisomer. However, the R isomer displays the same binding affinity towards the fungal target GlcN-6-P synthase (2VF5) as that for DHPS. On the contrary, the S isomer exhibits more affinity than the R isomer for GlcN-6-P synthase. The S isomers also exhibit a higher affinity for the fungal target in comparison with the bacterial target. In the case of 3a, both the stereoisomers E and Z demonstrate higher affinity towards the bacterial target as compared to the fungal target. Furthermore, the Z stereoisomer of 3a has more affinity than its counterpart Z isomers in both antimicrobial targets.

Table-4: Binding energy values in kcal/mol

Sr. No.	Molecule	1AJ0	2VF5
1	1aR	-7.8	-7.8
2	1aS	-7.4	-8.1
3	3aE	-8.1	-7.5
4	3aZ	-8.8	-8.1

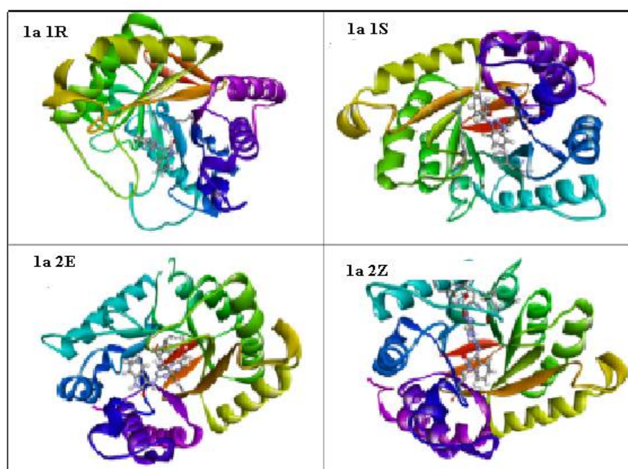

Figure 1A: Binding of stereoisomers of 1a&3a in bacterial target, DHPS (1AJ0)

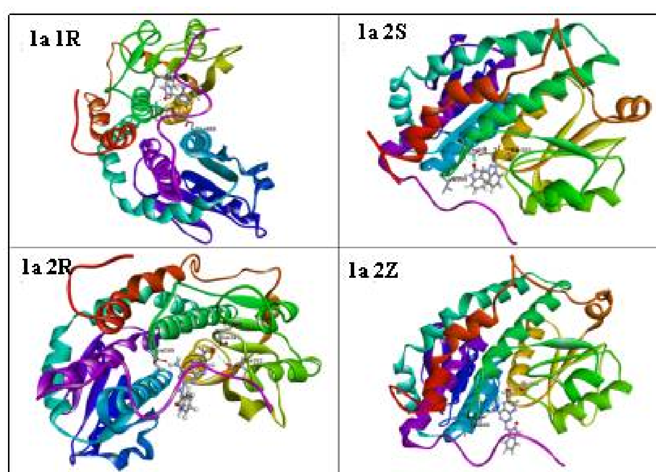

Figure 1B: Binding of stereoisomers of 1a&3a in fungal target, GlcN-6-P synthase (2VF5)

CONCLUSION

The isatin derivatives have contributed enormously act as antibacterial, anti-viral, anti-oxidant, anti-carcinogenic and many fungal diseases to treat various types infections, but antimicrobial resistance appeared gradually due to the long-term, broad, inappropriate use and even abuse of their modification in antibiotics. Isatin derivatives possess a variety of pharmacological properties and some of isatin-based derivatives have already used for clinical development in the control and eradication of variety of diseases. Thus, isatin derivatives are reasonable choice for the development of new anti-bacterial agents. The present study highlights the importance of the structural features responsible for the antibacterial, antifungal, and antioxidative properties.

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