Synthesis, Characterization, Antimicrobial Investigation and *in-silico* Screening of Di-imines Derived from 4,4'-Diaminoazobenzene

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ABSTRACT

Eight new di-imines were synthesized in good yield simply by refluxing substituted benzaldehyde/pyridyl carboxaldehydes and diamine using methanol. Diamine was synthesized from acetanilide. The chemical structure of di-imines were confirmed by FT-IR, NMR, MS and elemental microanalysis. The compounds were assayed for anti-bacterial and anti-fungal activities by the disc diffusion method. Also, the *in-silico* ADMET study, logP prediction, biological activity score, molecular properties and Drug likeness score of these compounds were determined using Pre-ADMET, molinspiration and Molsoft online softwares respectively. ADMET study revealed that from absorption parameters human intestinal absorption value for all the synthesized di-imine was good. Similarly, the distribution property in plasma protein binding predicted that these di-imines have greater binding ability. The physical properties were determined using MolSoft. These physical properties were used to evaluate the oral bioavailability of compounds using "Lipinski's rule of Five" and Molecular polar surface area for drug transportation through membranes penetration.

Keywords: 4,4'-diaminoazobenzene; Schiff base; Antibacterial activity; Antifungal activity; *in-silico*

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INTRODUCTION

Azo-groups are relatively robust and chemically stable and have prompted extensive study of azobenzene-based structures such as dyes and colorants. Furthermore, the light-induced interconversion allows systems incorporating azo-group to be used as photo switches, effecting rapid and reversible control over a variety of chemical, mechanical, electronic, and optical properties [1, 2]. Schiff bases possess physiological and pharmacological activities. Thev constitute an interesting class of chelating agents capable of coordination with metal ions for the development of complex that serves as models for biological system [3-5]. Salicylaldehyde derivatives, with one or more halo-atoms in the aromatic ring, showed variety of biological activities like antibacterial and antifungal activities [6]. A considerable work has been done for the synthesis of Schiff bases [7-12] however, these procedures have some limitations such as low yield, long duration, and cause high environmental pollution regarding to solvent. Keeping in view structureactivity relationship (SAR) of Schiff bases [13-16] and in continuation of our investigations in this area [17-19], we hereby report the synthesis of new Schiff bases from various substituted benzaldehydes/pyridylcarboxaldehydes and 4,4'-diaminoazobenzene by simply refluxing in methanol. The title compounds were also evaluated for their antimicrobial activity.

The *in-silico* study was done using Pre-ADMET, Molinspiration and MolSoft online softwares. All these properties were determined to predict the drug like properties of the synthesized Schiff bases. Moreover, the Lipinski rule of five and Molecular polar surface were also estimated using data obtained from molinspiration and MolSoft.

MATERIAL AND METHODS Material

Acetanillide, HNO₃, H₂SO₄, Ammonium formate, Zn dust, CHCl₃, NaCl ,Acetic acid, boric acid, sodium perborate tetrahydrate, 4-chlorobenzaldehyde, 3.4dichlorobenzaldehyde, 2,4-dihydroxybenzaldehyde, 2hydroxybenzaldehyde, 4-methoxybenzaldehyde, 3-ethoxypyridine-3-carboxaldehyde, 4-hydroxybenzaldehyde, pyridine-4-carboxaldehyde, were obtained commercially from Lancaster Research Chemicals. Solvents used were of analytical grade. TLC was run on the silica get coated aluminium sheets (silica gel 60 F254, Merck) and visualized under UV. 1H-NMR spectra were recorded on a Bruker DPX 400 instrument. Chemical shifts were reported in ppm reference to the residual solvent signal. Mass spectra were recorded on a JEOL SX-102 instrument and IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer. Melting points were recorded on a Stuart Scientific- SMP3 apparatus and are uncorrected.

Methods

Preparation of *p*-nitroacetanilide

1.5 g (11 mmol) of acetanilide was taken in an Erlemnyer flask of 125 ml. In this flask 2.5 ml of conc. H_2SO_4 was added drop wise with constant stirring. Once the solution was formed, then the flask was put in ice-bath to decrease the temperature up to 0 °C. To check temperature a thermometer was placed in the flask. In another flask about 0.9 ml conc. HNO₃ and 2.5 ml of conc. H_2SO_4 was mixed. This solution of acids was added drop wise in the cold solution of acetanilide. The flask was stirred after each addition. The temperature was maintained below 5 °C. Approximately after 20 minutes, the reaction mixture was taken out from ice bath. Then, 12.5ml of ice water was taken in a beaker and the mixture was poured into ice cold water. Yellow

precipitates were formed immediately. These precipitates were then allowed to stand for about 5 min and meanwhile it was swirled. Using buckner funnel the precipitates were collected on a filter paper and were washed three times with water. Once the precipitates were dried, they were recrystallized with ethanol [20]. The melting point was found to be 210-213 $^{\circ}$ C which was almost near to reported M.P 215 $^{\circ}$ C. Yield was 60%.

Preparation of *p*-aminoacetanilide

In a 100 ml Round bottom flask 0.23 g (1.7 mmol) of *p*nitroacetanilide were taken, in that flask 0.1 g (1.5 mmol) activated Zn dust, 5 ml methanol and about 0.12 g of Ammonium formate were added. The mixture was stirred till the completion of reaction. After 5 h stirring the reaction was stopped and it was filtered. The residues were collected and dissolved in a CHCl₃. This solution was poured in separating funnel and washed with brine solution of NaCl (3 X 10 mL) was added in it [21]. The M.P. of product was 160-162°C and the reported M.P. is 164-167°C. Yield was 75%.

Preparation of 4,4'-diaminoazobenzene

p-aminoacetanilide (29.0 g, 0.19 mol), sodium perborate tetrahydrate (4 0g, 0.26 mol), boric acid (10 g, 0.16 mol) and glacial acetic acid (500 ml) were placed in a 1-L three-necked round-bottomed flask equipped with an efficient stirrer, a reflux condenser, and a thermometer. The mixture was heated to 50-60 °C with continuous stirring for 6 hours. Initially the solid had dissolved, but after heating for approximately 40 minutes, the product began to separate. The mixture was collected on a Büchner funnel. It was washed with water until the washings were neutral to pH paper and then dried in an oven at 110° . The yield of 4,4'-bis(acetamido)azobenzene is 16.5g (57.7%); m.p. 288–293° (dec.). It was used as such for the hydrolysis step.

Equal volumes of MeOH and 6*N* HCl (150 ml each), and the total yield of 4,4'-bis(acetamido)azobenzene were placed in 500-ml round-bottomed flask. The mixture was refluxed for 1.5 hours. Upon cooling the reaction mixture, the violet solid was collected on a Büchner funnel. The damp product was suspended in water (500 ml) in a 1-L beaker equipped with a stirrer, and the mixture was slowly neutralized by the addition of 2.5*N* sodium hydroxide. In the course of neutralization, the salt was dissolved, and the free base was separated. The 4,4'-diaminoazobenzene was collected on a Büchner funnel, washed with water, and dried under reduced pressure. The product thus obtained was yellow crystalline with 56% yield, m.p: 238–241° (dec.); IR (KBr, cm⁻¹): 3350, 3110, 1585, 1400; ¹H-NMR (DMSO-*d*₆; 400 MHz): δ (ppm): 7.82 (d, 4H), 6.74 (d, 4H), 5.72 (s, 4H).

General Procedure for Preparation of Schiff bases 1a-h

4,4'-diaminoazobenzene (1.00 mmol) in dry MeOH (15 ml) was stirred at 70 °C and after cooling to 10 °C, benzaldehydes (2.00 mmol) dissolved in MeOH (15 ml) were added dropwise to it in a period of 20 minutes. After addition, contents were refluxed for a period of 40 minutes. When the reaction was over, contents were cooled, the precipitates obtained were washed with MeOH and dried.

4,4'-((Z)-diazene-1,2-diyl)-bis(N-(4-

chlorobenzylidene)aniline) 1a

Yield 91%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 7.48 (d, *J* 6.8 *Hz*, 4H), 7.62 (d, *J* 6.9 *Hz*, 4H), 7.97 (d, *J* 6.8 *Hz*, 4H), 7.99 (d, *J* 6.9 *Hz*, 4H), 8.75 (s, 2H); MS: *Calcd*. 456.1, Found: 456.1; FT-IR (KBr, cm⁻¹): 1625,

1570, 1460, 1370. Anal. *Calcd*. For C₂₆H₁₈Cl₂N₄: C 68.28%, H 3.97%, N 12.25%. Found: C 68.20%, H 3.99%, N 12.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(N-(3,4dichlorobenzylidene)aniline) 1b

Yield 93%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.66 (d, *J* 9.4 Hz, 4H), 7.43 (d, *J* 8.8 Hz, 2H), 7.66 (d, *J* 8.8 Hz, 2H), 7.81 (d, *J* 9.2 Hz, 4H), 8.17 (s, 2H), 8.73 (s, 2H); MS: *Calcd*. 526.2, Found: 526.2; FT-IR (KBr)cm⁻¹: 1619, 1583, 1543, 1469, 1383. Anal. *Calcd*. For C₂₆H₁₆Cl₄N₄: C 59.34%, H 3.06%, N 10.65%. Found: C 59.20%, H 3.00%, N 10.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(N-(2,4-

dichydroxybenzylidene)aniline) 1c

Yield 90%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.31 (d, *J* 2.0 *Hz*, 2H), 6.42 (dd, *J* 2.1, 8.4 *Hz*, 2H), 7.48 (d, *J* 8.6 *Hz*, 4H), 7.55 (d, *J* 8.6 *Hz*, 4H), 7.96 (d, *J* 8.6 *Hz*, 2H), 8.91 (s, 2H); MS: *Calcd*. 452.1, Found 452.1; FT-IR (KBr)cm⁻¹: 3410, 1615, 1550, 1470, 1260, 1170. Anal. *Calcd*. For C₂₆H₂₀N₄O₄: C 69.02%, H 4.46%, N 12.38%. Found: C 69.20%, H 4.30%, N 12.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(N-(2-

hydroxybenzylidene) aniline) 1d

Yield 91%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.67 (d, *J* 8.7 Hz, 4H), 6.93 (t, *J* 8.3 Hz, 2H), 6.98 (t, *J* 8.4 Hz, 4H), 7.43 (d, *J* 8.6 Hz, 2H), 7.55 (d, *J* 8.6 Hz, 4H), 8.02 (s, 2H), 12.98 (s, 2H); MS: *Calcd*. 420.2, Found: 420.2; FT-IR (KBr)cm⁻¹: 3420, 1617, 1563, 1483, 1278, 1184. Anal. *Calcd*. For C₂₆H₂₀N₄O₂: C 74.27%, H 4.79%, N 13.33%. Found: C 74.20%, H 4.50%, N 13.20%.

4,4'-((*Z*)-diazene-1,2-diyl)-bis(*N*-(4-methoxybenzylidene)aniline) 1e

Yield 95%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 3.30 (s, 6H), 6.66 (d, *J* 7.0 Hz, 4H), 7.09 (t, *J* 6.6 Hz, 4H), 7.35 (d, *J* 6.9 Hz, 4H), 7.42 (d, *J* 6.8 Hz, 4H), 8.62 (s, 2H); MS: *Calcd*. 448.2, Found: 448.2; FT-IR (KBr)cm⁻¹: 1610, 1550, 1460, 1235, 1115. Anal. *Calcd*. For C₂₈H₂₄N₄O₂: C 74.98%, H 5.39%, N 12.49%. Found: C 74.50%, H 5.12%, N 12.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(*N*-(3-ethoxy-4-hydroxybenzylidene) aniline 1f

Yield 92%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 2.49 (t, *J* 2.5 *Hz*, 6H), 4.10 (q, *J* 2.6 *Hz*, 4H), 6.61 (d, *J* 6.9 *Hz*, 2H), 6.67 (d, *J* 6.9 *Hz*, 2H), 7.37 (t, *J* 6.9 *Hz*, 2H), 7.52 (d, *J* 8.4 *Hz*, 4H), 7.92 (d, *J* 8.4 *Hz*, 4H), 8.52 (s, 2H); FT-IR (KBr)cm⁻¹: 3415, 1620, 1550, 1450, 1235, 1125. MS: *Calcd.* 508.2, Found: 508.2; Anal. *Calcd.* For C₃₀H₂₈N₄O₄: C 70.85%, H 5.55%, N 11.02%. Found: C 70.50%, H 5.30%, N 11.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(*N*-(pyridine-3-ylmethylene) aniline) 1g

Yield 91%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.67 (d, *J* 8.4 Hz, 2H), 7.51 (t, *J* 8.4 Hz, 2H), 7.58 (t, *J* 7.4 Hz, 4H), 7.99 (d, *J* 8.4 Hz, 4H), 8.35 (d, *J* 7.6 Hz, 2H), 8.73 (d, *J* 3.3 Hz, 2H), 8.82 (s, 2H); FT-IR (KBr)cm⁻¹: 1620, 1560, 1417, 1364, 1145. MS: *Calcd*. 390.2, Found: 390.2; Anal. *Calcd*. For C₂₄H₁₈N₆: C 73.83%, H 4.65%, N 21.52%. Found: C 74.00%, H 4.42%, N 21.20%.

4,4'-((Z)-diazene-1,2-diyl)-bis(N-(pyridine-4-ylmethylene) aniline) 1h

Yield 94%; decomposition point: >300 °C; ¹H-NMR (400 MHz, DMSO-d₆, δ ppm): 6.67 (d, *J* 8.6 *Hz*, 4H), 7.47 (d, *J* 8.5 Hz, 4H), 7.66 (d, *J* 8.7 *Hz*, 4H), 8.50 (d, *J* 8.6 *Hz*, 4H), 8.76 (s,

Scheme 1 Synthesis of *p*-aminoacetanilied

2H); FT-IR (KBr)cm⁻¹: 1632, 1596, 1403, 1131. MS: *Calcd*. 390.2, Found: 390.2; Anal. *Calcd*. For C₂₄H₁₈N₆: C 73.83%, H 4.65%, N 21.52%. Found: C 73.90%, H 4.52%, N 21.40%.



Antimicrobial Activity

The newly synthesized compounds were screened for their antibacterial activity against locally isolated gram (ve) bacterial strain i.e Escherichia coli (AMJ-2006a), Shigella sonnei (AMJ-2006b) and gram (+ve) bacterial strain i.e Staphylococcus *aureus* (AM/-2005a), Pseudomonas aeruginosa (AMJ-2005b), Bacillus subtilus (AMJ-2005c) and Salmonella typi (AMJ-2005d), while antifungal activity was tested against Saccharomyces cerevisiae (AMJ-2007) by the disc diffusion method [22-23]. Overnight incubated cultures of these microbes were introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs were prepared from Whatman No.1 filter paper and sterilized by dry heat at 140 °C for an hour. The sterile discs previously soaked in a 100 µg/ml of the test compound dissolved in DMSO were placed on the inoculated nutrient agar medium. The plates were inverted and incubated for one day at 37 °C. DMSO was used as standard. Growth inhibition zones were measured and compared with the controls. The inhibition zone values are summarized in Table 1.

In-silico Study

Pre-ADMET online available server was used to calculate ADME (Absorption, Distribution, Metabolism, Excretion) and Toxicity properties of newly synthesized Schiff base compounds. Pre-ADMET uses certain pharmacokinetics properties, which are important in the development of drugs [24]. The synthesized compounds were evaluated for Human Intestinal absorption (HIA), Caco-2 cell permeability, MDCK permeability, skin permeability, Plasma protein binding (PPB), Blood Brain barrier (BBB) penetration. For toxicity evaluation Ames test and Carcinogenicity (in rat and mouse) were used. Mostly the Schiff bases have proved very effective against biological activity. In silico ADMET study helps in determining the most favorable compound by evaluating it with pharmacokinetic properties in the search of drug study [25].

Molinspiration online available source was used to calculate the logP (Octanol/water partition coefficient) values. It is based on group contribution for predicting logP values [26]. This value was used for determination of molecular hydrophobicity. Molinspiration calculates these values by fitting calculated logP with experimental twelve thousand logP values, which are almost all drug like molecules. Biological activity score of the Schiff based compounds was calculated using this online available source. For this purpose, G-protein coupled receptor, Kinase inhibitor, Nuclear receptor, protease inhibitor and enzyme inhibitor all types were predicted.

MolSoft was used to determine the molecular properties such as hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), molecular weight, logP, water solubility (logS), Molecular polar surface is (PSA) and volume of synthesized Schiff bases. Determination of Molecular Polar Surface (PSA) value is important for drug transport capacity of compound. These values predict the compound's property as a drug transport. PSA is the sum of surfaces of polar atoms, which are Oxygen, Nitrogen, and their hydrogens present in the compound. Greater value of more than 140 square angstrom suggests poor permeability. Whereas, less than 60 square angstrom value of PSA is considered good for penetration through HIA, Caco-2 permeability and BBB penetration [27]. Also, the drug likeness score was calculated using molSoft. Its training set mode consist of 5K marketed drugs from World Drug Index gives positive value), and 10K non-drug molecule gives negative value). This method of prediction is very robust and efficient. The obtained drug-likeness score determined the drug-likeness of compounds.

Lipinski rule of five is important and widely used in pharmaceutical industries as a first step to determine the good compound for drugs. This rule predicts the bioavailability of the compounds by determining its physiochemical properties. According to Lipinski's rule, good membrane permeability contains log $P \le 5$, molecular weight ≤ 500 , number of hydrogen bond donor ≤ 5 , and number of hydrogen bond acceptor ≤ 10 [28].

RESULTS AND DISCUSSION

Chemistry

In the present study, the 4,4'-diaminoazobenzene was prepared from *p*-aminoacetanilide. This *p*aminoacetanilide was synthesized from acetanilide. First the nitration of acetanilide was done for the synthesis of *p*nitroacetanilide then, the reduction of *p*-nitroacetanilide was attempted to prepare *p*-aminoacetanilide (**Scheme I**). After that, the various substituted benzaldehydes were separately treated with 4,4'-diaminoazobenzene in methanol to prepare the corresponding Schiff bases (**Scheme II**). All the compounds gave satisfactory chemical analysis.

The overall yield of the diamine (1) from paminoacetanilide is 52 %. The Schiff bases (1a-h) were prepared by refluxing 4,4'-diaminoazobenzene with substituted benzaldehyde/pyridyl carboxaldehydes in MeOH, Reaction was monitored by IR and confirmed by the lack of NH₂ stretching bonds in the 3300-3420 cm⁻¹ and the presence of strong stretching bonds in the 1635-1605 cm⁻¹ IR region in 1a, 1b, 1e, 1g and 1h. While, in 1c, 1d and **1f**, strong absorption was observed in O-H stretching region i-e, 3410-3420 cm⁻¹ All the title compounds due to conjugation on either side of CH=N-, exhibited absorption in the 1635-1605 cm⁻¹region. Absorption at 1125-1383 cm-1 indicates the presence of azo group in compounds 1a**h**. In the ¹H-NMR spectra of all the titled compounds, a singlet appeared in the range of δ 8.02-8.91 ppm due to CH=N. The ¹H-NMR spectra of **1a-h** contains multiplet signals due to aromatic protons in the region δ 6.11-8.73 ppm. Six protons singlet appears at δ 3.30 ppm in **1e** while six protons triplet at δ 2.49 ppm and four protons quartet indicate the presence of two OC_2H_5 group in **1f**.

Antimicrobial Activity

Different Schiff bases usually interact with bacteria and fungi and are dependent on their structures. Such structure-activity analysis might be helpful in the design of better inhibitors. The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, survival in the medium of application, and state of the target organism [29]. In this study, we focused our attention on the structure-activity relationship.

All the compounds tested (Table-1) may not be suitable for controlling gram (-) bacterial contaminations/infections as no inhibitory effect could be detected for the *Escherichia coli* as well as for *Shigella sonnei*. Same results appeared for the eukaryotic model *Saccharomyces cerevisiae* and for gram (+ve) bacteria *Pseudomonas aeruginosa*, *Bacillus subtilus* and *Salmonella typhi*. However, the synthesized compounds exerted in general elaborative antibacterial effects only on gram (+) bacterium *Staphylococcus aureus*. Compound **1a** contains chloro group at *p*-position while **1b** bears two chloro groups at *m*- and *p*- position showing complete inhibition of bacterial zone against gram (+) strain. Compound **2c** possesses two OH groups at *o*- and *p*-positions fails to exert any inhibitory effect but interestingly compound **1d** having OH group at *o*-position shows complete inhibition. Similarly >42 mm and 21 mm growth inhibition zones were calculated in compounds **1e** and **1f** respectively containing OMe- group at *p*-position in **1e** and OH group at *p*-position and OEt- group at *m*position in **1f**. Compounds **1g** and **1h** derived from pyridine carboxyaldehyde show significant activity against gram (+) strain. Thus, from this structure-activity relationship, it can be envisaged that compounds having chloro group at *m*- and *p*-positions are biologically active against gram (+) strain. Whereas, compound possessing OH group at *o*-position only shows biologically activity and by inserting OH group at *p*-position minimizes its activity. Compound **1e** containing OMe group at *p*-position is more potent as compared to compound **1f** containing hydroxyl group at *p*position and OEt- group at *m*-position. Compounds **1g-h** are also found highly effective against gram (+) strain and probably due to pyridyl group. Study on structure-activity relationship of these Schiff

Study on structure-activity relationship of these Schiff bases has indicated that halo group and heteroatom enhance activity.

Compound	Escharichia coli	Staphylococcus aureus	Saccharomyces cerevisiae
1a	NE a	СІь	NE
1b	NE	CI	NE
1c	NE	NE	NE
1d	NE	CI	NE
1e	NE	>42mm ^c	NE
1f	NE	21mm	NE
1g	NE	CI	NE
1h	NE	CI	NE

Table 1 Antimicrobial Activity of di-imines.

a = No antimicrobial effect was noticed

b = Complete inhibition; the zones of growth inhibition were indistinguishable and found to occupy the entire plate. (Six discs were loaded on each inoculated Petri plate with intra discs distance of 20 mm).

c = For the two discs complete growth inhibition was noticed, however, on one side of a disc presence of bacterial growth at the margin enabled to estimate the growth inhibition zone.

In-silico study

It is predicted from the ADME study presented in Table 2 that Human intestinal absorption (HIA) property of all the synthesized Schiff bases was found to have good absorption. All have absorption values greater than 90%. Caco-2 cell permeability of these Schiff bases compounds predicted to possess medium permeability. And MDCK values for all the derivatives were low as the predicted values were less than 25. The skin permeability of all the compounds was not in the range of -0.8 to -1.0. The distribution property such as PPB was good because all derivatives gave values greater than 90%, which predicted greater binding ability. Moreover, the BBB property suggested that compounds 1a, 1b, 1d, and 1f were CNS active whereas compounds 1c, 1e, 1g, and 1h were CNS inactive. All the values are mentioned in the Table 2.

The toxicity predicted by Pre-ADMET as mentioned in Table 2 suggested that all the set of synthesized derivatives have mutagenic property. Carcinogenicity result in rat was positive for all Schiff bases, while in mouse only 1a, 1c, 1d and 1f gave positive result other compounds 1b, 1e, 1g and 1h were predicted as negative result for carcinogenicity.

LogP values were determined using Molinspiration and MolSoft online servers and is reported in Table 3 and 4

respectively. For bioactivity score all compounds were moderately active for GPCR ligand and ion channel ligand modulator. For kinase inhibitor compounds 1g and 1h were active whereas 1a, 1b, 1c, 1d, 1e and 1f were all moderately active. And for nuclear receptor ligand compounds 1a, 1b, 1c, 1d, 1e and 1f were all moderately active but compounds 1g and 1h were inactive. Also, for protease inhibitor and enzyme inhibitor all synthesized compounds were moderately active.

Some physical properties along with drug likeness model score were calculated using MolSoft. HBA for all the compounds were found in the permissible range. Also, the number of HBD and MW were all in the acceptable range except for 1a, 1b and 1f. Drug likeness score predicted that all the synthesized Schiff base compounds were non-drug like. All values have been reported in Table 4. For Lipinski's rule of five, the log P values were determined from molinspiration software and its other parameters were determined from MolSoft. It was predicted that only compounds 1g and 1h is obeying Lipinski's rule of 5. Molecular PSA values of compounds 1a, 1b, 1e, 1g, 1h were found less than 60 square angstrom and of compounds 1c, 1d, and 1f were more than this.

Compounds	Absorption Parameters				Distribution		Toxicity		
	^a Caco2 cell permeability	bHIA	¢MDCK	^d Skin_Permeability	•BBB	fPPB	Ames test	Carcinogenicity	
								Mouse	Rat
1a	51.95	98.2	0.23	-1.45	3.83	100.0	Mutagen	Positive	Positive
1b	53.59	98.3	0.24	-1.44	12.11	100.0	Mutagen	Negative	Positive
1c	6.18	90.8	0.06	-2.6	0.68	100.0	Mutagen	Positive	Positive
1d	11.43	95.7	0.22	-1.78	2	100.0	Mutagen	Positive	Positive
1e	43.28	97.6	0.11	-1.69	0.1	100.0	Mutagen	Negative	Positive
1f	15.09	95.7	1.87	-1.77	1.06	99.25	Mutagen	Positive	Positive
1g	21.63	97.6	0.16	-1.96	0.12	100.0	Mutagen	Negative	Positive
1h	19.75	97.6	0.16	-1.96	0.05	100.0	Mutagen	Negative	Positive

Table 2. ADMET prediction of all the synthesized Schiff bases (from 1a-1h).

aCaco-2 cell permeability less than 4 low permeability, between 4-70 medium, greater than 70 higher permeability. bHIA 70-100% well absorbed, 20-70% moderate, 0-20% poorely absorbed. cMDCK less than 25 low, 25-500 moderate, more than 500 higher cell permeability. dSkin permeability should be in tha range of -0.8 to -1.0 for 95% drugs. ePPB% greater than 90 strongly bound, less than 90 weakly bound. fBBB more than 1 CNS active compound, less than 1 CNS inactive compound. (http://preadmet.bmdrc.org/)

Table 3. logP value and bioactivity score of 1a to 1h derivatives using Molinspiration.

Compounds	^a Log P	GPCR ligand	Ion channel ligand modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	^b Enzyme inhibitor
1a	8.44	-0.29	-0.34	-0.07	-0.51	-0.31	-0.16
1b	9.02	-0.26	-0.31	-0.08	-0.48	-0.29	-0.15
1c	6.07	-0.24	-0.36	-0.03	-0.34	-0.23	-0.1
1d	7.08	-0.27	-0.38	-0.05	-0.42	-0.24	-0.12
1e	7.31	-0.31	-0.37	-0.09	-0.47	-0.3	-0.17
1f	6.62	-0.29	-0.43	-0.1	-0.39	-0.31	-0.16
1g	4.72	-0.25	-0.31	0.01	-0.58	-0.28	-0.07
1h	4.62	-0.27	-0.3	0.05	-0.55	-0.27	-0.09

more than 0.00 activity score means active, between -0.50 to 0.00 moderately active, and if less than -0.50 then inactive. blogP value must be greater than 2.00. (http://www.molinspiration.com/cgi-bin/properties)

 Table 4. Calculation of physical properties and drug likeness score using MolSoft for 1a-1h compounds.

Compounds	Mol. Formula	MW	No of HBA	No of HBD	MolLogP	MollogS	MolPSA	MolVol	No of stereo center	Drug likeness
1a	$C_{26}H_{18}C_{12}N_4$	456.06	4	0	8.95	-11.76	38.18	408.7	0	-1.29
1b	$C_{26}H_{16}C_{l4}N_4$	524.01	4	0	10.14	-13.02	38.18	437.5	0	-1.04
1c	$C_{26}H_{20}N_4O_4$	452.15	8	4	6.24	-8.76	106.5	416.6	0	-0.94
1d	$C_{26}H_{20}N_4O_2$	420.16	6	2	6.76	-8.94	71.28	395.4	0	-1.35
1e	$C_{28}H_{24}N_4O_2$	448.19	6	0	7.7	-10.37	53.27	438	0	-1.61
1f	$C_{30}H_{28}N_4O_4$	508.21	8	2	7.9	-10.26	85.7	498	0	-0.8
1g	C24H18N6	390.16	6	0	5.42	-7.58	57.22	365.3	0	-0.7
1h	C24H18N6	390.16	6	0	5.42	-8.55	57.04	364.9	0	-0.93

(physical Properties and drug likeness score was calculated using online source at: (http://molsoft.com/mprop/)

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