Research Paper

Synthesis Computational Studies and Biological Evaluation of Novel Isatin Derived Schiff Bases as Potential Antimicrobial Antianxiety and Muscle Relaxant Activity

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Abstract

Schiff bases are wide group of compounds characterized with the presence of a double bond attached along with carbon and nitrogen atoms, the utility which is generated in the different ways to combined a variety of alkyl or aryl substituents. We describe here computational investigations, synthesis, and biological assessments of numerous new Schiff Bases as antianxiety, skeletal muscle relaxants, and antibacterial medicines in light of the risks associated with drug resistance. Here we synthesized six novel isatin derived schiff bases they are having different biological applications.The target compounds (A1-6) were produced from Isatin (1) and 1,4 diaminobenzene (2) with glacial acetic acid. Melting point, TLC , Infrared spectroscopy, and NMR spectroscopy were used to characterize 3-(-4-aminophenylimino)indoline-2-one (3), which was chloroacetylated with chloroacetyl chloride and then treated with substituted phenylpiperazines. The target substances were examined for their ability to reduce anxiety, relax Swiss albino mice's skeletal muscles, and fight against bacteria including S .aureus, E. Coli, and C. albicans. The outcomes were contrasted with those of the reference medications, clotrimazole and diazepam. The intended molecules included substantial biologically active compounds.

Key Words: Schiff Base; Phenylpiperazine; Antianxiety; Skeletal Muscles Relaxants Activity; Antimicrobial Activity.

1. **Introdction**:

"Schiff bases are the compounds containing an azomethine group (HC = N). These are condensation products of ketones and aldehydes (aldehydes and ketones) with primary amines, first reported by Hugo schiff in 1864". Schiff bases generally in acid or "base catalysis" or heating. These bases have crystalline solids that are generally weak so in chief form insoluble salts with Strong acids (Xavier et al; 2014). In medicine, the family of chemicals known as Schiff bases is significant. Schiff bases are almost used organic compounds. They are used as an pigments and dyes, catalysts, intermediates in organic synthesis, and polymer stabilizers. Schiff bases have been shown to have a different activities, including antibacterial, antifungal, and antiviral antianxiety & skeletal muscle relaxent. Imine and azomethine containing groups are found in many natural, contained compounds. In class of compounds,the imine group is important for their activity (C.M. da Silva et al., 2011). Schiff bases, nitrogen heterocycles, 4 thiazolidinediones, benzoxazines, etc. It was used as a compound for the preparation of different commercial and

biological products such as ring closing, cycloaddition, and substitution. For the creation of new environmentally friendly technologies, Schiff base derivatives in diverse syntheses can readily create novel heterocyclic/aryl Schiff bases (Anu et al., 2011).The outcome its potential use in transition metal ion complexes, particularly those with various molecular topologies and atomic groups, has become an emerging study field.(Debdulal Maity et al. 2019) Due to their excellent stability when combined with various oxidation states, Schiff bases are frequently utilized as ligands. The indicated bases are some used an organic compounds. They are used as an pigments and dyes, catalyst intermediates and polymer stabilizers in organic compounds synthesis (Dhar et al., 1982). Synthesis of pharmaceuticals, antibiotics, anti-inflammatory drugs. According to the literature study, Schiff base, a substance created by metal complexing with certain bacteria, has been regarded as a good antibiotic.Increased antibiotic resistance in infectious illnesses is closely correlated with an increase in infectious disease-related fatalities. .The development of new antibiotics with new and more effective methods is an immediate medical need. Like hydroxyproline, This have been regarded as effective anti-inflammatory compounds. “Schiff bases” obtained from isatin has also been reported to have antibacterial properties (de Souza et al., 2007).He had been repoted the synthesis and in vitro antibacterial activities of 11 morpholine-originated Schiff bases (Panneer Selvam et al., 2005). Inhibitory effects of bases containing the 2,4-dichloro-5-fluorophenyl of bacteria development. Such Schiff bases completely stop the growth of Staphylococcus aureus and Escherichia coli. Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae (Karthikeyan et al., 2006). Unnecessary or pathological stress is a well known condition that causes a lot of stress in individuals, families and communities. Anxiety disorders come in certain types, but they may all share certain nerve pathways. Although some psychotherapies have been shown to be effective (Beck et al, 1988), drugs therapy still the most effective remedy, basically in dengerous cases. Anxiety is an undesirable but normal and active function that gives us warning signs of threats. It includes physical and mental symptoms that prepare us to face or avoid threats (Marks, 1987).

1. Experimental

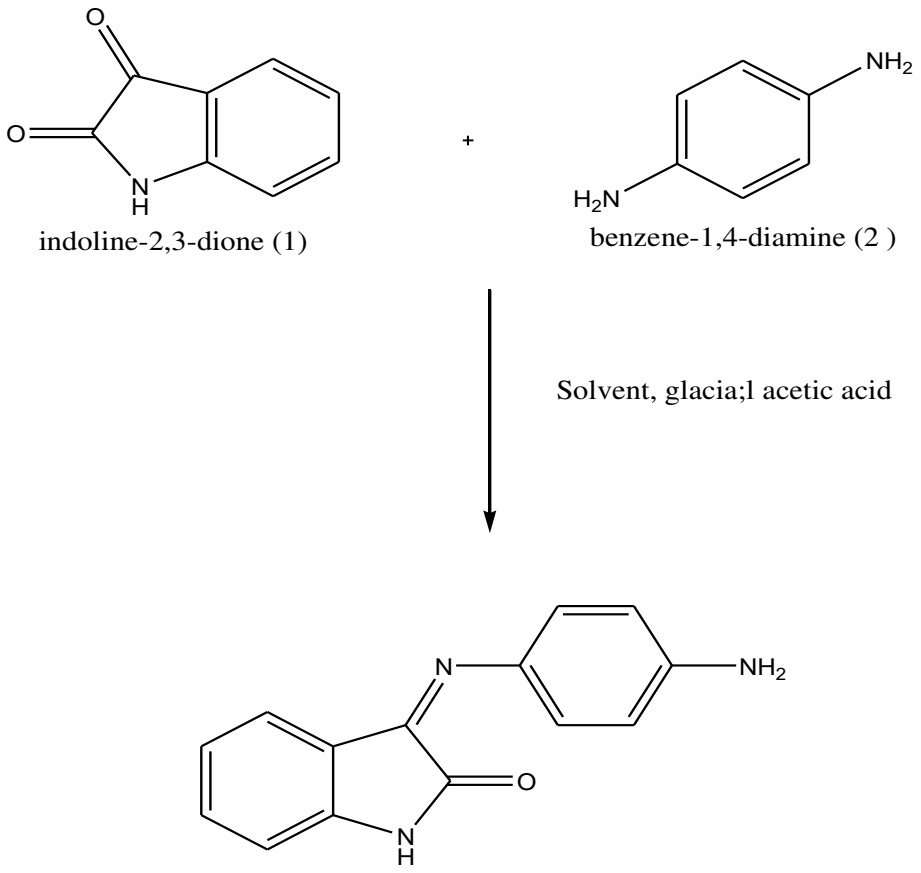
1.1 Material and Methods

Indolin-2,3-dione, benzene-1,4-diamine, chloroacetyl chloride, potassium carbonate and substituted piperazine were arranged from CDH. Chemicals used for this work was laboratory grade. Glassware’s washed and dried prior use. By using the open capillary approach, melting points of synthetic substances have been identified. On Shimadzu instrument was using as KBr discs, synthetic compound infrared spectra have been captured.1^{H} NMR spectrum were observed on a “Bruker Advance neo” 500 MHz Spectrophotometer in Dimethylsulphoxide with TMS as a standard. All the NMR & IR were got from Central University Punjab .All frequency of NMR were reported in ppm (δ) . TLC was used to track reaction's development while silica gel was used as adsorbent in Thin layer chromatography plates.The synthesized compounds have recrystallized, dried and stored in a “vacuum desiccator”. Using free online tool Chem Draw 12, the physical characteristics (Physicochemical characteristics) of the target compounds were determined, and chemical properties were added to the antibacterial and stress properties of the targets and attributed to the microbial resistance.

1.2 Synthetic procedure for obtained Schiff base

Isatin and 1,4-diaminobenzidine were dissolved in 100 ml of ethanol in clean dried RBF, and a catalytic quantity of glacial acetic acid was mixed . The reaction solution was refluxed for 8 hours. If solvent is still present in the reaction mixture after keep aside for the night, extract it under decreased pressure. A collection of the obtained residue (3) was dried.

Step I: Synthesis of 3 -(4-aminophenylimino)indolin-2-one



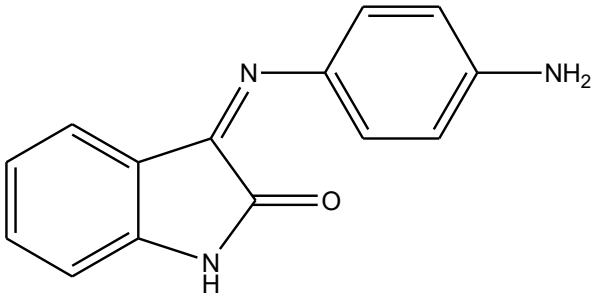
(*Z*)-3-(4-aminophenylimino)indolin-2-one

Step II : "N (4 - (2-oxoindolin-3-ylideneamino) phenyl)-2-chloroacetamide"-

Procedure-

0.008 moles (1.10gm) of anhydrous K2CO3 were added to 0.004 moles (0.94gm) of 3-(-4-aminophenyl imino) indolin-2-one (3) that had been dissolved in 30 ml of anhydrous dimethylformamide. After end of reaction, dimethylformamide mixture was transferred &kept in to cold water. 0.008 moles (0.63 ml) of chloroacetylchloride were added drop by drop over the course of 20 minutes while being stirred continuously for 8 hours. In order to get the crude residue, the reaction mixture was filtered and dried.

Synthesis of "N-(4-(2-oxoindolin-3-ylideneamino)phenyl)-2-chloroacetamide"

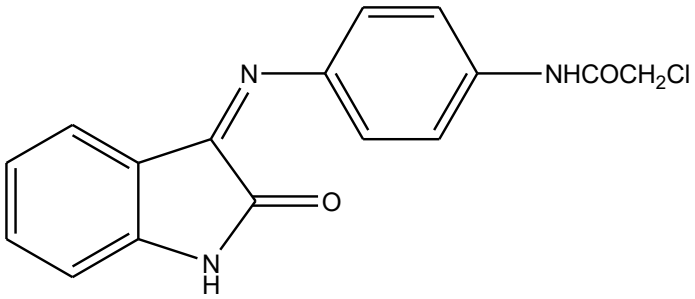


(*Z*)-3-(4-aminophenylimino)indolin-2-one (3)

\+ $ClCOCH_2Cl$

Chloroacetylchloride (4)

Solvent, Base



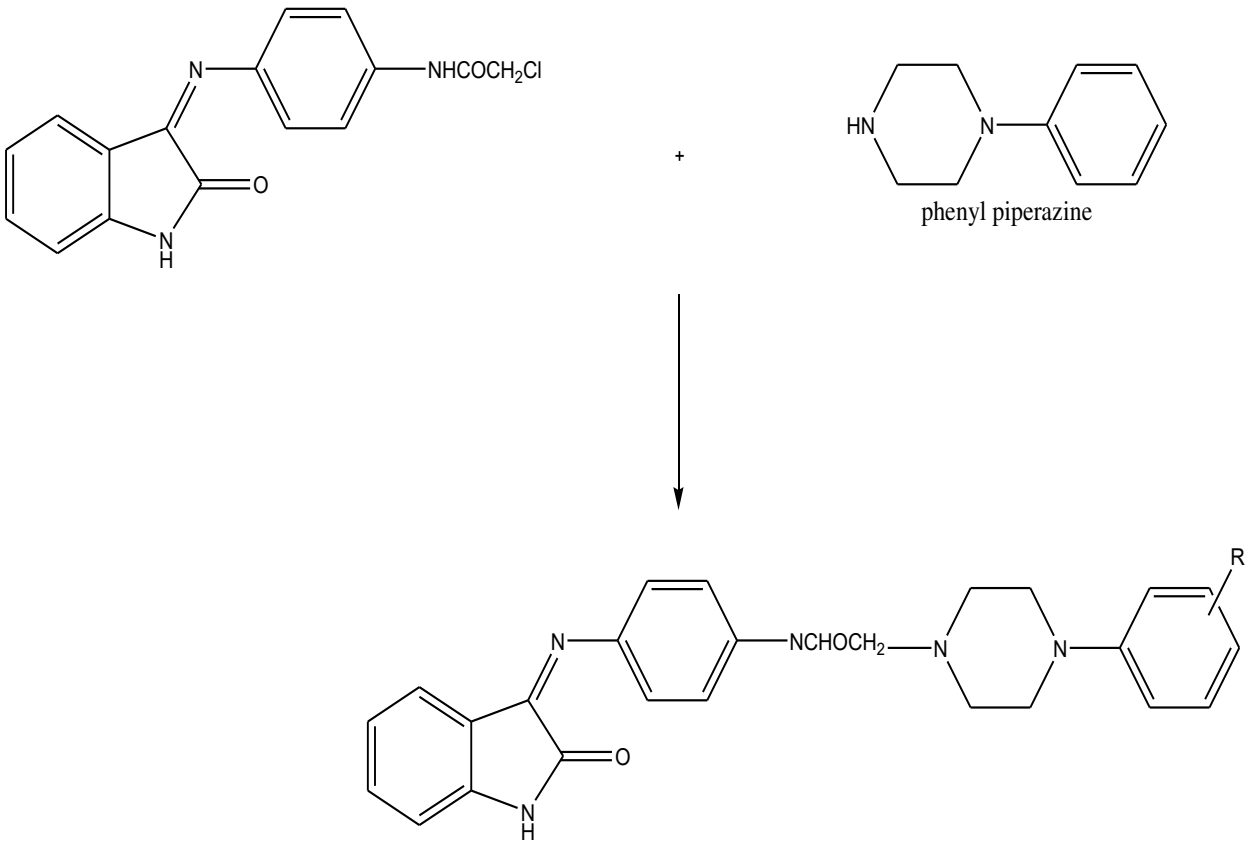
N-(4-(2-oxoindolin-3-ylideneamino)phenyl)-2-chloroacetamide (5)

Step III: Synthesis of Phenylpiperzines hybrids (A1-A6)

General Procedure –

N-(4-(2-oxoindolin-3-ylideneamino)phenyl)-chloroacetamide (5) mixed in 30 ml of anhydrous dimethylformamide (without any moisture) in a RBF that had been well cleaned and dried. K_2CO_3 and phenylpiperazines to 0.0025 moles; they were also added. For ten hours, the reaction mixture was warmed. After being placed into ice-cold water, the reaction solution was left overnight. For purpose of obtaining crude product (A1-6), the reaction mixture was filtered and dried.

Synthesis of Phenylpiperazines hybrids



Target compounds (A1-6)

2.3.1 Chemistry

(Z)-2-(4-(phenyl)piperazin-1-yl)-N-(4-((2-oxoindolin-3-ylidene)amino)phenyl acetamide (A1)

Yeild 74% m.p 213-216 °C ^{1}H NMR (300 MHz, DMSO-D6): δ (ppm) 4.87 (1H ,-SH), 5.30(2H,-OCH_2) 6.91-8.17 (14 H, Ar-H) IR (KBr, cm−1): 3289 (CH Str. Aromatic), 3094 (CH Str. Aliphatic), 2833(C=O str), 1514 (C=C str. in aromatic), 1469 (C-O str. in C-O-C)

Z)-2-(4-(methylphenyl)piperazin-1-yl)-N-(4-((2-oxoindolin-3ylidene)amino)phenyl) acetamide (A-2)

Yeild 85 % m.p 226-229 °C ^{1}H NMR (300 MHz, DMSO-D6): δ (ppm) 5.30 (2H, -OCH_2),6.63-8.24 (14 H, Ar-H) IR (KBr,cm−1 ,3173CH (Str. Aromatic), 3091(CH Str. Aliphatic),1713 (C=O str),1664 (C=C str. in aromatic),1588 (C-O str. in C-O-C)

(Z)-2-(4-(4-nitrophenyl) piperazin- 1-yl)-N-(4-((2-oxoindolin-3 ylidene)amino) phenyl) acetamide (A-3)

Yeild 89 % m.p 197-201 °C ^{1}H NMR (300 MHz, DMSO-D6): δ (ppm) ,5.28 (2H, OCH_2), 65.4-8.05 (10 H- Ar-H) IR (KBr, cm−1 3845 (CH Str. Aromatic) , 1696(CH Str. Aliphatic),1597(C=O str), 1565 (C=C str. in aromatic), 1555 (C-O str. in C-O-C)

(Z)-2-(4-(2,3-dimethyl)piperazin-1-yl)-N-(4-((2-oxoindolin-3- ylidene)amino) phenyl) acetamide (A-4)
Yeild 76 % m.p 207-210 °C ^{1}H, NMR (300 MHz, DMSO - D6): δ (ppm) 5.28 (2H, OCH_2), 6.62-8.54 (10H,Ar-H) IR (KBr, cm−1 3076 (CH Str. Aromatic),1715 (CH Str. Aliphatic),1700 (C=O str) 1696(C=C str. in aromatic) , 1664 (C -O str. in C -O- C)

Z)-2-(4-(chlorophenyl)piperazin- 1-yl)-N-(4-((2-oxoindolin-3- ylidene)amino) phenyl) acetamide (A-5)
Yeild 81 % m.p. 235-239 °C ^{1}H NMR (300 MHz, DMSO-D6): δ (ppm) 3.51 (1H,CH), 3.80 (1H CH), 5.30 (2H-OCH_2), 6.79-8.24 (10H Ar-H) IR (KBr, cm−1 1700 (CH Str Aromatic), 1696 (CH- Str Aliphatic), 1658 (C=O Str) ,1649 (C=C Str in Aromatic), 1565 (1565 (C=O Str in C-O-C)

(Z)-2- (4-(3,4-dichlorophenyl) piperazin-1-yl) -N-(4-((2-oxoindolinylidene) amino)phenyl) acetamide (A-6)
Yeild 73 % m.p 185-188 °C ^{1}H NMR (300 MHz, DMSO-D6): δ (ppm) 5.30 (2H - OCH_2), 6.63-8.24 (14 H Ar-H) IR (KBr, cm−1 3064 (CH Str in aromatic), 2900 (CH Str in Aliphatic), 1705 (C=O Str), 1580 (C=C Str in Aromatic), 1093 (C=O Str in C-O-C)

2.3.2 Computational studies

Computational studies was performed by using the free online software Chemdraw 12 and Swiss ADME software, the physiochemical properties of target compounds were determined with the help of these software and results were compared with standard drugs such as diazepam, clotrimazole. In the computational studies we have checked the drugs with different parameters such as their log P values, Connolly molecular surface area (CMSA), Cannolly Solvent Excluded Volume , Molecular Weight, Molar Refractivity Index and Ovality. In these studies was suggest that all parameters of our synthesized compounds near similar to the standard drugs diazepam as an antanxiety drugs and clotrimazole as antimicrobial drugs.

2.3.3 Similarity calculation

A similarity study plays an important role in the research studies. These calculation suggest that novel synthesized compounds how similar to standard drugs. A set of the physiochemical parameters that were computed using software were used to determine the taerget synthesized compounds physiochemical simarity to standard drugs. Formula used for to caluculation of similarity of certain compounds such as standard drugs the formula given below

$$di^2 = \sum_{j=1}^{n} \frac{\left(\frac{1 - X_{i,j}}{X_{i,std}}\right)^2}{n}$$

Where, X_i Standard is a values of the similar molecular spacification to the reference medication as an diazepam and X_j value for same parameters for compound j.Then, it was determined how similar thsese compound to reference drug using formula

Percetage Similarity = (1- r) X 100
Where R= d2, a measured tendency

Synthesized new compoundwere shown strong comparability to reference drugs. All the physiochemical parameters were calculated by the software.

2.4 Biological evaluation:

The most popular and fundamental techniques for calculating the antimicrobial activity disk diffusion and agar diluation methods. The other methods are also using if depth analysis of antimicrobial agents such methods as time kill test, flow cytoflurometric methods suggest the type of effect of inhibition (bacteristatic and bacteriocidal), this method is concentration time dependent. The another method was cup diffusion were used to access the antibacterial and antifungal properties (Brillion,2016). The target compounds were determined in dimethylformamide (DMF) solutions at concentration 1mg/ml (Selly HW,1975; Al- Gendy AO 2012). Using the Agar Diffusion method the to checked the anti-microbial activities against Staphylococcus aureus, Candida albicans etc. active compounds were those with inhibitory zones at the least of 18 mm. Typical antibacterial agents were ampicillin and clotrimazole. The test organism were seeded into sterile plate after being added to nutrient agar (100 ml of media at 24 hours broth culture).On the agar cup 8 mm diameter cut,(0.1 ml) test solution was transfer to every cup. After the process was over after thatincubated for 24 hours at 37^0c. Width (mm) was used to measure the activity shown in table 3,4.

2.5 Antianxiety Evaluation

Swiss albino mice were used for these studies, Rota rod techniques used to measure the muscle relaxant activity, the antixiolytic activity (Sinoriya et al.,2100) was measured by popular elevated plus maize method. All tests were carried out with the Animal Ethics Committee's prior approval and in accordance with the CPSEA-approved procedure. An industry standard (positive control) was diazepam. A one-way ANOVA with a fixed significance level of 0.05 was used to statistically compare the test group's findings to those of the control group.

2.5.1 "Elevated plus maize method"

Swiss albino mice that have weight about 21–25 g were selected from stock colony held in the major animal facilities available with water food. The place where the animals were kept had air conditioning. The room was maintained at a cozy 25°–20°C using natural light. Each medication was used at a concentration 10 mg / kg in freshly produced suspensions in 1% tween 80. Each solution was newly prepared and administeredintraperitoneally (I.P.) In test days the volume equivalent to 0.5/ml of mouse body weight.The experimental animal received either the test compounds (10 mg/kg) or the benzodiazepine (2 mg/kg, n = 6) 60 minutes prior to their evaluation in the labyrinth. The control group was adminstered saline with 1% tween 80. The Plus Maze apparatus, according to Moser et. al ; (1989) and Pellow et al. (1985), and Rabbani et al. (2004), contains of two open (16 x 5 cm^2) and two closed arms (16 x 5 x 12 cm^3) facing one another with an open roof. The elevation of the maze is 25 cm all around. Test group of each mice the test group was determined in this apparatus for five minutes at a time.On the platform in the center, each mouse was placed such that it faced the open arm. Over the course of a five-minute period, time, the how many entries into open and closed arms, and the duration of time spent with open arms were recorded..Percentage of (number of entries into (open arms + closed arms x 100 for every mice).

2.6 Rotarod Method

The "Rotarod method" was used to assess relaxation potential of the target compounds. In a Wooden rod that was rotating at horizontally on a speed of 25 round per minute , mice were placed. The study used mice that could balance on top for at least one minute over the course of three sessions. The chosen animals were arranged into twelve groups (N = 6). Each test sample was created as a stock solution by suspension in 1% tween 80, together with the standard. 1% twen 80, and 2 mg/kg, i.p diazepam were administered to the control and standard groups. Test subject Each test group got a 10 mg/kg injection. After a 30-minute break, each group of animals was again added to the rod.Animals were regarded to have passed the test if they not able to pass the Rotarod test more than once within the course of one minute. skeletal muscle relaxant activity results are listed in table.

2.7 Result & Discussions

A survey of the literature demonstrates that Schiff bases are utilized in the creation and design of several significant medicinal medicines. These results imply that Schiff bases include several biological properties, such as antibacterial, antifungal, anti-anxiety, anti-inflammatory, antidepressant, antipsychotic, and antipyretic action. The interaction of isatin (1) with 1,4 diaminobenzene (2) with glacial acetic acid produced the target compounds (A1-6). To produce 3-(-4-aminophenyl imino)indoline-2-one (3), which was then replaced phenylpiperazines and chloroacetylated using chloracetylchloride.Compounds A1-6 had yields ranging from 60% to 90%.The designs of these On the basis of 1H-NMR and IR spectrum analyses, substances were identified. The sharp melting point and TLC of all compounds proved their purity and homogeneity. The production of the synthetic compounds and, consequently, the accuracy of the predicted structures created for synthetic compounds were positively validated by all of the aforementioned outcomes. The target compounds have demonstrated strong physio-chemical characteristics in computational tests, as well as good parallels to conventional medications The target substances were examined for their ability to reduce anxiety, relax skeletal muscles, and fight against B. subtilis, E. coli, and C. albicans. Table 1 displays the results of anti-anxiety, skeletal muscle relaxants, and antibacterial activities.Its remarkable stability is indicated by research on the reaction between isatin and the Schiff base generated from benzene 1,4 diamine. This motivates the use of physicochemical methods of analysis in the synthesis and thorough examination of the type of bonding between the Schiff bases and their significant biological significance. From the discussion above, it is evident that the complex's fragmentation pattern and spectrum investigations support and provide examples for the hypothesized geometry determined by elemental analysis, IR, 1H NMR.

2.8 Aknowlwgwments

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2.9 Conflicts of interests

According to the authors, there are no conflicts of interest related to the publication of this work.

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Table 1-Physical properties of synthesized Phenylpiperzines (6)

S. NO	R	Compound Code.	M.P (^{0}C)	Rf(n-Hexane:Ethylacetate, 1:1)
1	H	A1	213-216 °C	0.66
2	1-(4 - methyl)	A2	226-229°C	0.77
3	4-Nitro	A3	197-200°C	0.58
4	2,3 - dimethyl	A4	207-210°C	0.51
5	1(-4 -chloro)	A5	235-239°C	0.85
6	3,4 dichloro	A6	185-188°C	0.60

Tabal 2 Physicochemical properties of target compounds (A1-6)

Cpd. Code	MWa	MRb	tPSAc	CAAd	CMAe	CSEVf	Ovg	Log P
A1	440	128.48	61.83	717.47	397.21	344.53	1.67	3.01
A2	454	133.52	61.83	748.80	416.38	361.58	1.69	3.50
A3	485	134.12	62.44	750.67	418.24	363.41	1.69	1.74
A4	468	138.56	61.42	754.60	426.69	374.68	1.62	3.99
A5	474	133.29	133.64	741.81	412.29	359.00	3.57	3.57
A6	508	138.09	69.11	762.00	425.84	373.22	1.69	4.13
Ampicillin	349.40	89.37	112.73	526.59	282.00	285.27	1.34	0.2
Diazepam	288.71	84.81	36.67	466.26	243.16	203.28	1.51	2.67

MWa : Molecular Weight.
MRb : Molar Refractivity.
tPSAc: Topological Polar Surface Area.
CAAd: Connolly Accessible Area.
CMAe: Connolly Molecular Area.
CSEVf:Connolly Solvent Excluded Volume.

Table3 : Similarity of target compounds (A1-6) with respect to the standard drugs:

Compound Code.	Similarity to Ampicillin	Similarity to Clotrimazole	Similarity to Diazepam
A1	57.60	66.60	56.50
A2	60.80	46.70	61.30
A3	56.10	60.80	57.90
A4	44.20	52.80	60.30
A5	37.20	58.30	59.35
A6	67.60	69.15	63.17

Table 4 The antimicrobial activity of Target compounds (A1-6)

Compound code	Zone of Inhibition B. Subtilis (mm)	Zone of Inhibition E. Coli (mm)	Zone of Inhibition C.albicans (mm)
A1	18	17	17
A2	17	17	21
A3	16	15	15
A4	14	12	16
A5	20	21	16
A6	16	14	15
Clotrimazole	-	-	26
Ampicillin	23	26	-

Table5 : Anti anxiety activity against target compounds (A1-6) on method using elevated plus maze

Compond code.	Spent time in open arm	No. of entries	% No.of entries
A1	63.68 ± 1.72	6.14±0.34	49.03
A2	52.67±1.61	6.69±0.51	45.16
A3	55.26±3.47	7.08±0.73	43.39
A4	83.76±1.98	8.71±0.29	54.72
A5	46.23±1.67	5.82±0.69	36.31
A6	50.28±1.78	5.29±0.78	40.47
Daizepam	89.84±2.38	10.09±0.79	64.49
vehicle	43.12±3.29	2.97±0.91	23.21

Table 6 Skeletal muscle relaxant activity of novel synthesized compounds (A1-6) by Rotarod method

Compond code.	**Dose**	**Rotarod test**
A1	10 mg/kg	60.14±1.27
A2	10 mg/kg	60.29±2.19
A3	10 mg/kg	62.29± 2.12
A4	10 mg/kg	45.34± 1.17
A5	10 mg/kg	66.79± 2.46
A6	10 mg/kg	67.56±1.31
Diazepam	2 mg/kg	36. 59± 1.39
vehicle	1% tween 80	86.07± 1.69