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Short communication

Identification of antibacterial and antifungal pharmacophore sites for potent bacteria and fungi inhibition: Indolenyl sulfonamide derivatives☆

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ABSTRACT

Synthesis of seven new indolenyl sulfonamides, have been prepared by the condensation reaction of indole-3-carboxaldehyde with different sulfonamides such as, sulphanilamide, sulfaguanidine, sulfathiazole, sulfamethoxazole, sulfisoxazole, sulfadiazine and sulfamethazine. These synthesized compounds have been used as potential ligands for complexation with some selective divalent transition metal ions (cobalt, copper, nickel & zinc). Structure of the synthesized ligands has been deduced from their physical, analytical (elemental analyses) and spectral (IR, ^{1}H NMR and ^{13}C NMR & UV–vis) data. All the compounds have also been assayed for their *in vitro* antibacterial and antifungal activities examining six species of pathogenic bacteria (*Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis*) and six of fungi (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium soloni* and *Candida glabrata*). Antibacterial and antifungal results showed that all the compounds showed significant antibacterial activity whereas most of the compounds displayed good antifungal activity. Brine shrimp bioassay was also carried out for *in vitro* cytotoxic properties against *Artemia salina*.

1. Introduction

Sulfonamides represent an important class of medicinally important compounds which are extensively used as antibacterial agent. It interferes with PABA (*p*-aminobenzoic acid) in the biosynthesis of tetrahydrofolic acid, which is a basic growth factor essential for the metabolic process of bacteria. Many activities apart from carbonic anhydrase have been recently reviewed that include endotelin antagonism, anti-inflammatory, tubular transport inhibition, insulin release and saluretic activity [1]. It is well documented [2–5] that toxicological and pharmacological properties are enhanced when sulfonamides are administered in the form of their metal complexes [6–10]. Bulk of literature reveals that several compounds containing indole ring form compounds of pharmacological interest [11].

Based on the significant biological and pharmacological properties [12] & Ref therein the indole moiety possesses, a new class of such compounds is reported by combining the chemistry of sulfonamides [13] with indole-3-carbaldehyde and to explore their biological activities with the aim of obtaining more potent antibacterial and/or antifungal compounds.

These indolenol sulfonamides reported in this paper formulate a new class of antibacterial and antifungal agents that may become excellent candidates for globally alarming drug resistance issues in clinically used therapeutics.

There is considerable interest in these compounds as *combined* antibacterial and antifungal agents, exhibiting potency similar pharmacophore pockets to that observed for imipenem (**IMP**). Several groups have reported on the failed attempts to get a clear idea concerning the origin of each activity in this standard reference, casting doubt over the structural needs assignment. We present here the results of our virtual screening investigation into possible alternative structures for these compounds. A comparison between experiment and theoretical predictions of the antibacterial and antifungal activity has enabled us to identify alternative combined pharmacophore sites structures.

☆ This work is dedicated to Prof. Dr. Johann Gasteiger for his bioinformatic multiple services.

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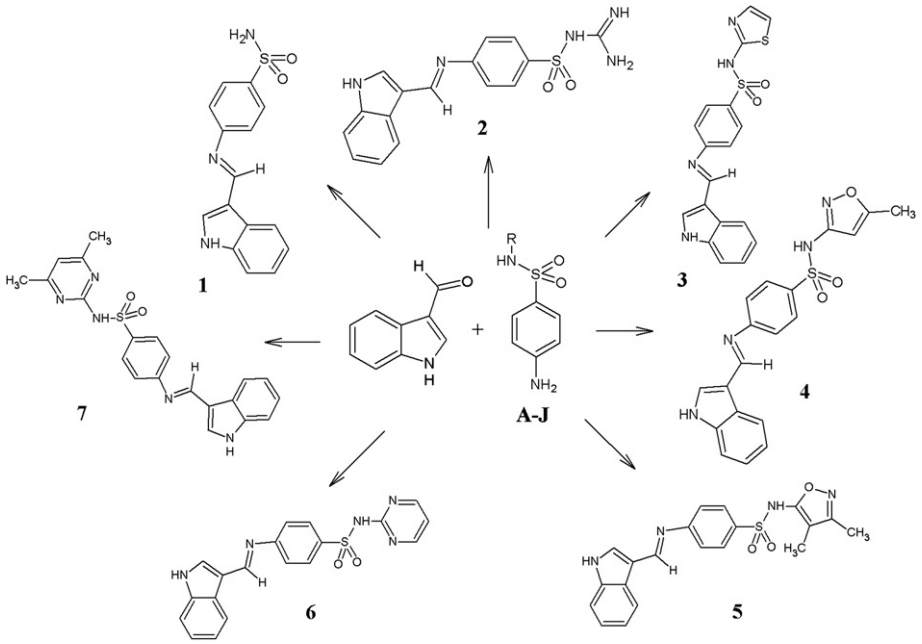


Scheme 1. Preparation of sulfonamides **(1)–(7)**.

The most predicted potent of these compounds **(2)**, **(3)** and **(5)** (high inhibition) were tested in bacterial cultures for their ability to present a potential antibacterial pharmacophore site. Compounds **(3)** and **(5)** were the most antibacterial agents. All these synthesized indolenyl sulfonamides **(1)–(7)** were then tested for their antifungal activity. They display too a potential antifungal activity against six fungi, *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium soloni* and *Candida glabrata*; may be because they have an antifungal pharmacophore site. Results from all aspects of this bioinformatic approaches will be discussed, as will our experience with screening candidates.

2. Chemistry

2.1. Synthesis of compounds *(1)–(7)*

All synthesized compounds were prepared by refluxing an equimolar ratio of indole-3-carbaldehyde and the respective sulfonamide such as sulphanilamide, sulfaguanidine, sulfathiazole, sulfamethoxazole, sulfisoxazole, sulfadiazine and sulfamethazine in ethanol (Scheme 1) which successfully lead to a new series of sulfonamides, containing indole moiety in good yield (80–86%). The reaction was rapid, and no observation supported any kind of side product. All the products were obtained as solids and their purities were checked by thin layer chromatography (eluent = n-hexane/chloroform/ethylacetate, 1/2/1 v/v). All the synthesized compounds were characterized by spectroscopic techniques (IR ^{1}H NMR & ^{13}C NMR) and their elemental analyses.

3. Pharmacology

3.1. Biological activity

3.1.1. Antibacterial activity (in vitro)

All compounds were tested against four Gram-negative (*E. coli*, S. *flexneri*, *P. aeruginosa*, *S. typhi*) and two Gram-positive (*S. aureus*, *B. subtilis*) bacterial strains according to the literature protocol [14,15]. The results were compared with those of the standard drugs imipenem (Fig. 1). All the synthesized compounds exhibited varying degree of inhibitory effect on the growth of different tested strains (Tables 1 and 2). A significant activity was observed by all the compounds against *E. coli*. Compound **(1)** & **(6)** showed moderate activity. Whereas, compounds **(2), (3), (4), (5)** & **(7)** showed significant activity against *S. flexenari*. Compound **(2) & (4)** showed moderate activity whereas compounds **(1), (3), (5), (6)** & **(7)** showed significant activity against *P. aeruginossa*. All the compounds showed significant activity against *S. typhi* except compound **(6),** where moderate activity was observed. Compound **(1)** against *S. aureus* copoumnd **(5)** & **(7)** against *B. subtilis* showed moderate activity, whereas, all other compounds showed significant activity against both the positive bacterial stains. Although most of the compounds showed moderate to significant activity against all Gram-negative and Gram-positive bacterial strains but compounds **(3)** was the most active one (Figs. 2 and 3).

3.1.2. Antifungal activity (in vitro)

The antifungal screening of all compounds was carried out against *T. longifusus, C. albicans, A. flavus, M. canis, F. soloni and*

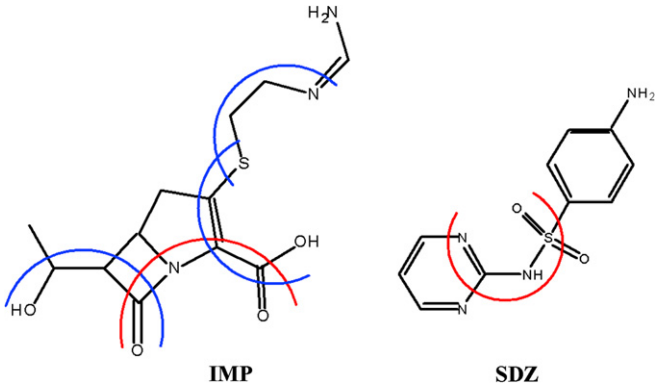


Fig. 1. Structure of standard reference (Imipenum) and indolenol SDZ analogue of sulfonamides **(1)–(7)**. The entity in blue color are a potential antibacterial pharmacophore site. That one in red color is a potential antifungal pharmacophore site.IMP: Imipenum; SDZ: 4-amino-N-(pyrimidin-2yl)-benzenesulfonamide.

Table 1
Antibacterial bioassay (concentration used 1 mg/mL in DMSO) and Antifungal bioassay (concentration used 200 μg/mL).

Compd	Gram (−)[a]				Gram (+)[a]		Fungi (%Inhibition)					
	E. coli	*S. flexenari*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*B. subtilis*	*T. longifucus*	*C. albicans*	*A. flavus*	*M. canis*	*F.soloni*	*C. glaberata*
1	21	18	19	20	18	20	00	40	55	00	43	25
2	23	22	18	23	22	23	20	35	30	00	35	55
3	24	23	20	23	23	24	35	40	30	25	80	60
4	22	20	17	19	19	23	00	25	45	10	45	40
5	23	19	19	20	20	17	20	45	55	00	65	55
6	19	18	20	17	19	19	00	35	25	20	70	20
7	24	19	20	22	23	18	25	60	55	00	75	28
SD	30	27	26	27	30	28	A	B	C	D	E	F

[a] [zone of inhibition (mm)]: <10: weak; >10:moderate; >16: Significant. SD = Standard Drug (Imipenum); MIC μg/mL; A = Miconazole (70 μg/mL: 1.6822×10^{-4} M), B = Miconazole (110.8 μg/mL; 2.6626×10^{-4} M), C = amphotericin B (20 μg/mL; 2.1642×10^{-5} M), D = Miconazole (98.4 μg/mL; 2.3647×10^{-4} M), E = Miconazole (73.25 μg/mL; 1.7603×10^{-4} M), F = Miconazole (110.8 μg/mL; 2.66266×10^{-4} M).

C. glabrata fungal strains according to the literature protocol [15]. All synthesized compounds showed good antifungal activity against different fungal strains. Although compound **(3)** showed good antifungal activity against all the fungal strains but significant activity against *F. solani*, compounds **(5), (6)**, and **(7)** also showed significant activity against *F. solani*. No activity was observed for compounds **(1), (4)**, and **(6)** against *T. longifusus* and **(1)**, **(2)**, **(5)** and **(7)** against *M. canis*. All other compounds showed weak to moderate activity against all other fungal stains. All individual synthesized compounds were compared with each other (Fig. 2) and compound **(3)** was found to be the most active one (Fig. 4). The inhibition results were compared with those for the standard drugs miconazole and amphotericin B (Table 1).

4. Results and discussion

4.1. Spectroscopic characterization of *(1)–(7)*

4.1.1. IR spectra

In the IR spectra of compounds **(1)–(7),** a sharp band at 1595 cm^{-1} is assigned [16] to the stretching of ν(C═N). Presence of this band is an evidence of the formation of condensation product. $\nu_{asymm}(SO_2)$ and $\nu_{symm}(SO_2)$ appeared at 1345 and 1110 cm^{-1} respectively [17,18]. ν(C–S), ν(C–N), ν(NH) of indole ring appeared at 833, 952, 3230 cm^{-1} respectively, in all compounds. The IR spectra of compounds **(1)** and **(2)** exhibited peaks at 3390 and 3395 cm^{-1} respectively, due to $-NH_2$ stretching. In addition, the spectra of **(2)–(7)** showed bands resulting from the guanidine (C═N), thiazole (C═N), isoxazolyl (C═N), isoxazolyl (C═N), pyrimidinyl (C═N), and pyrimidinyl (C═N), stretching at 1580, 1615, 1610, 1605, 1585 and 1580 cm^{-1} respectively. The IR spectra of **(2)–(7)**, exhibited the $-SO_2NH$ stretchings at 3255 cm^{-1}, the presence of all these frequencies was supportive [19,20] of the formation of compounds **(1)–(7)**.

4.1.2. ^1H NMR spectra

^1H NMR spectra of the compounds **(1)–(7)** displayed azomethine (CH═N) peak at δ 9.4 and H-4, H-7 protons of indole ring at 8.1 and 7.6 ppm as doublets. H-5 and H-6 protons appeared at 7.1 and 7.3 ppm as doublets of triplet and, H-2, H-1 protons at 8.3 and 12.1 ppm as singlet, respectively. A multiplet was observed at 7.7–7.8 ppm assigned for four protons of phenyl ring. The SO_2NH_2 or SO_2NH- protons in all cases appeared as a singlet in the region at 11.2–11.30 ppm ^1H NMR spectrum of compound **(2)** also displayed –C═NH and HN═C–NH_2 protons as a singlet at 8.1 and 7.6 ppm respectively. Also, compound **(3)** displayed thiazole, H-4 and H-5 protons as doublet at 3.3 and 3.1 ppm and in the case of compound **(4)**, the isoxazolyl, H-4 proton appeared as a singlet at 5.8 ppm. In compound **(6)**, the pyrimidinyl, H-4, H-5, H-6 protons appeared as multiplet at 7.0–7.4 ppm and in compound **(7),** the pyrimidinyl, H-5 proton appeared as a singlet at 6.7 ppm. The spectra of compounds **(4), (5)** and **(7)** also displayed the methyl proton peaks as singlet. Furthermore, the number of the proton calculated from the integration curves [21] and those obtained from the values of the expected CHN analysis agreed well with each others.

4.1.3. ^{13}C NMR spectra

^{13}C NMR spectra of compounds **(1)–(7)** displayed azomethine-C (CH═N) peak at 162.0 ppm which further supported the results obtained in IR and ^1H NMR. Compounds **(1)–(7)** displayed C_2, C_3, C_4, C_5, C_6, C_7, C_8 and C_9 carbons of indole moiety at 139.7, 115.4, 120.2, 121.3, 121.6, 114.4, 138.7 and 126.8 ppm respectively. Also, C_1-phenyl, C_2, C_6-phenyl, C_3, C_5-phenyl, C_4-phenyl appeared at 138.2, 128.6, 122.6 and 156.4 respectively in all the compounds. Carbamimidoyl carbon in compound **(2)** appeared at 167.0 ppm and in compound **(3)**, C_4, C_5, C_2 carbons of thiazole appeared at 108.0, 137.8, 117.7 respectively. In compound **(4)**, CH_3, C_5, C_4, C_3 of isoxazole carbons appeared at 12.8, 159.6, 95.0 and 150.0 ppm respectively. In compound **(5)**, CH_3-isoxazole, CH_3-isoxazole C_3-isoxazole, C_4-isoxazole, C_5-isoxazole carbons appeared at 15.1, 9.5, 159.9, 100.5 and 158.9 respectively. Pyrimidine carbons, C_4, C_6, C_5, C_2 appeared at 157.9, 110.2, 159.3 ppm respectively. In compound **(6)** and **(7)**, $2CH_3$- pyrimidine, C_4, C_6-pyrimidine, C_5-pyrimidine, C_2-pyrimidine carbons appeared at 25.1, 165.2, 103.0, 168.5 respectively. Furthermore, the number of carbons calculated from the integration curves [22]. And those obtained from the values of the expected CHN analyses agree well with each other.

4.2. Molecular properties calculations

For SDZ and certainly for its analogues **(1)–(7)**, depending on the pH and position of the dissociate amidogen hydrogen atom, nine possible SDZ conformations can be described for the neutral (N),

Table 2
Antibacterial minimum inhibitory concentration (MIC in M) and Brine shrimp bioassay data for compounds **(1)–(7)**.

Compd.	Gram (−)			Gram (+)	LD_{50} (M)
	E. coli	*S. flexenari*	*S. typhi*	*B. subtilis*	
1	–	–	–	–	>3.341
2	–	1.465×10^{-4}	2.929×10^{-4}	1.465×10^{-4}	>2.929
3	2.615×10^{-4}	1.307×10^{-4}	2.615×10^{-4}	6.537×10^{-5}	0.980
4	–	–	–	2.629×10^{-4}	>2.629
5	–	–	–	–	>2.535
6	–	–	–	–	>2.650
7	1.233×10^{-4}	–	2.466×10^{-4}	–	>2.466

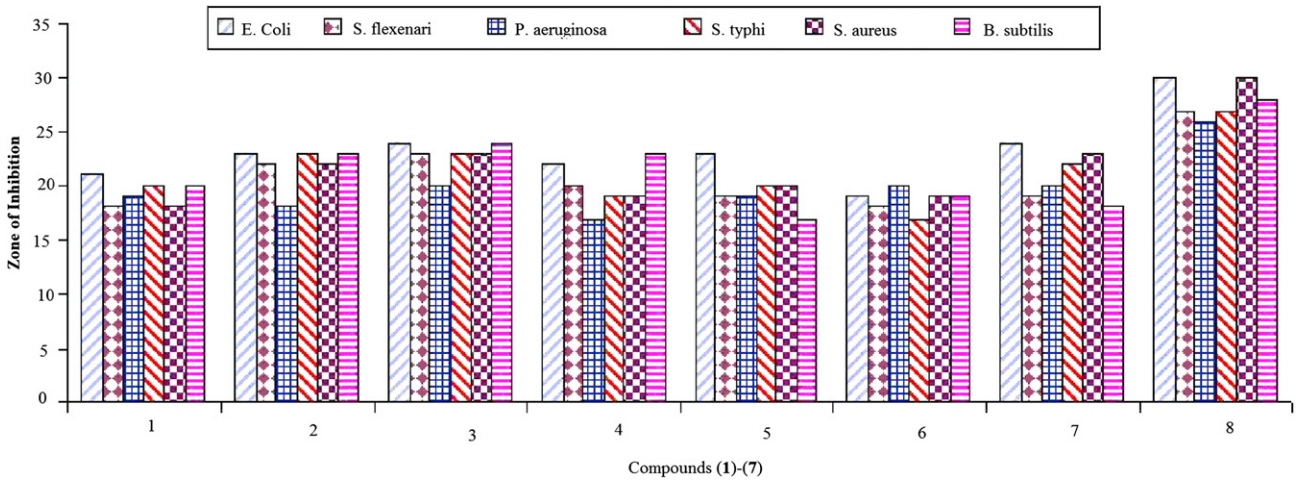


Fig. 2. Comparison of antibacterial activity of compounds **(1)–(7)**.

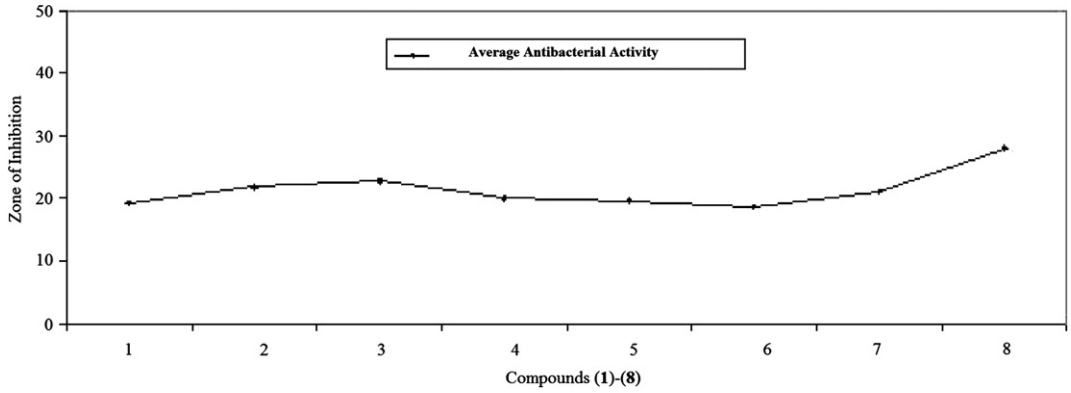


Fig. 3. Average antibacterial activity..

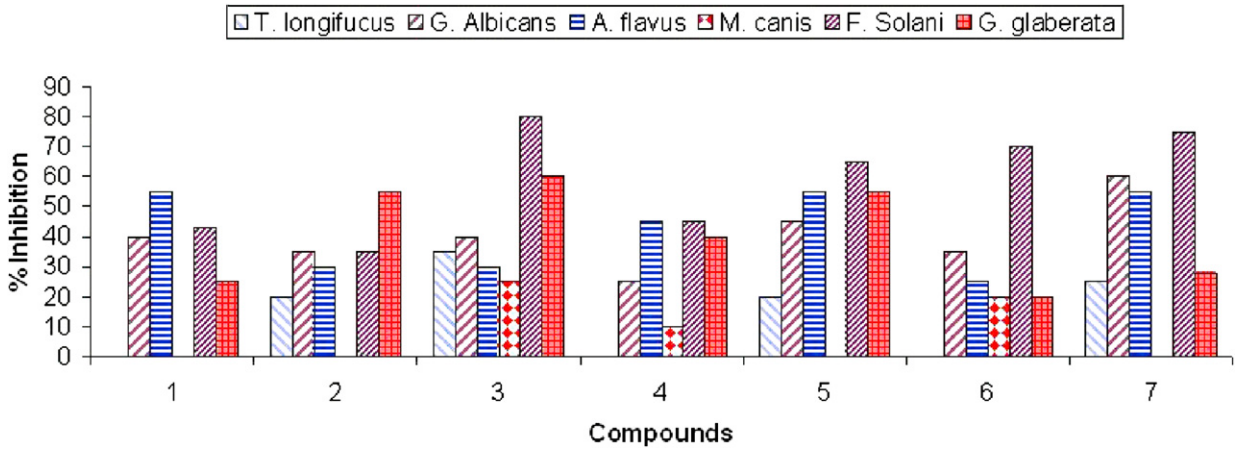


Fig. 4. Comparison of antifungal activity of compounds **(1)–(7)**.

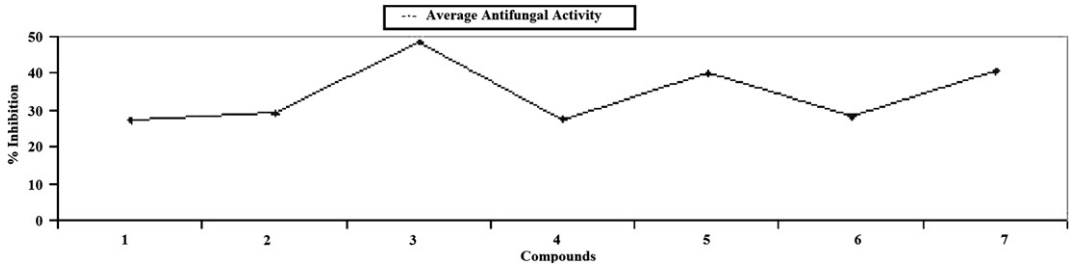


Fig. 5. Average antifungal activity.

deprotonated (D), and protonated (P) forms. These relevant structures are sketched in Fig. 5. In past, attention was mainly devoted to the N-1 structure. However, from a chemical point of view, all other structures are possible.

For the development of binding approaches for SDZ and its analogues **(1)–(7)** in the environment, the identification of the active sulfonamide structures present is important. Neither experimental nor theoretical data is available for the identification of water-solved SDZ species. Theoretically, NMR spectroscopy could be useful for identifying chemical structures. Theoretical ab initio studies could supplement these measurements. Additionally, calculations of energetics, atomic charges, minimum energy structures, geometry, and natural bond orbital (NBO) could indicate the electronic density distribution of each atom. Finally, by taking NBO results showing the presence of S–O single bonds in consideration, realistic Lewis structures can be determined. These systematic data, regarding the variation of molecular properties, are important for the chemical structure and could therefore provide first insights into the still poorly understood chemical bonding of SDZ complexes to soil [23 & ref therein].

In brief, the objective of this study is to investigate the potential pharmacophore sites of SDZ species using antibacterial and antifungal screenings dependence on pH and comparison with the calculated molecular properties. To verify these structures, further Petra/Osiris/Molinspiration (POM) analyses were carried out for example calculation of net atomic charges, bond polarity, atomic valence, electron delocalization and lipophicity.

Finally, to investigate the combined antibacterial/antifungal bioactivity of the SDZ species, tautomeric structure were performed.

Current thinking in the generation of specific drug leads embodies the concept of achieving high molecular diversity within the boundaries of reasonable drug-like properties [24]. Natural and semi-natural products, examples penicillin and, imipenem have high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead and standard references (SR) structures for drug discovery, and which serve to differentiate them from libraries of synthetic and combinatorial compounds.

Various investigators have used computational methods to understand differences between natural products and other sources

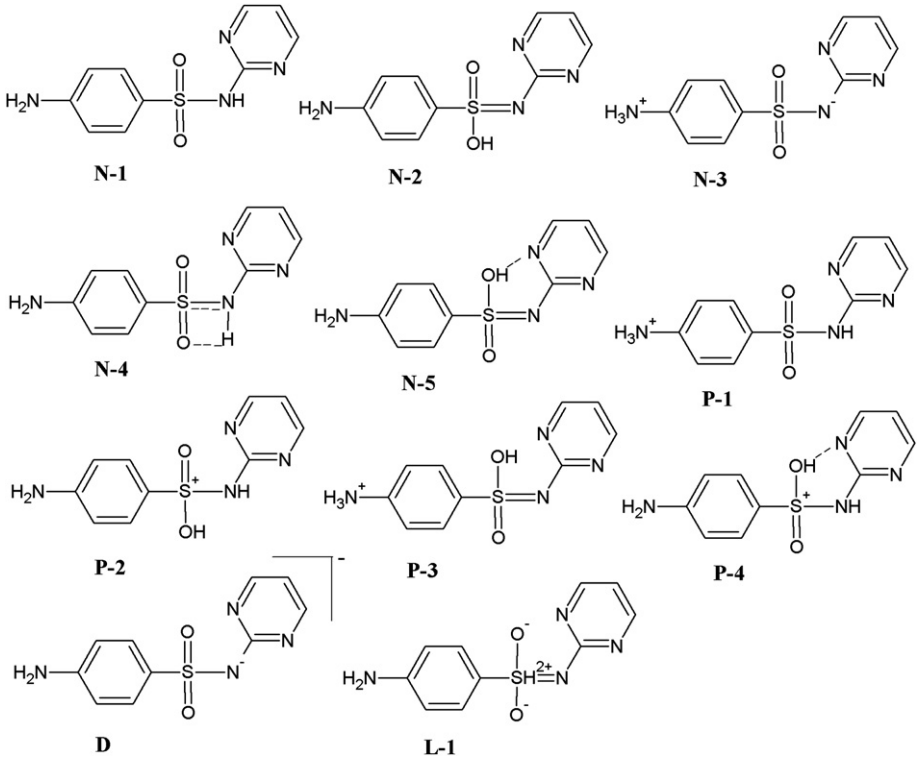


Fig. 6. Protonated (P), neutral (N), deprotonated (D) and Lewis (L) structures of sulfadiazine.

of drug leads [25]. Modern drug discovery is based in large part on high throughput screening of small molecules against macromolecular disease targets requiring that molecular screening libraries contain drug-like or lead-like compounds. We have analyzed known standard references (SR) for drug-like and lead-like properties. With this information in hand, we have established a strategy to design specific drug-like or lead-like indolenol sulfonamides **(1)–(7)**.

4.2.1. Petra calculations

PETRA is a program package comprising various empirical methods for the calculation of physicochemical properties in organic molecules. All methods are empirical in nature and have been developed over the last 20 years in the research group of Prof. J. Gasteiger. The following chemical effects can be quantified: heats of formation, bond dissociation energies, sigma charge distribution, π-charge distribution, inductive effect, resonance effect and delocalization energies and polarizability effect [26].

The series **(1)–(7)** of indolenol sulfonamides have been subjected to delocalised-charge calculations using Petra method of the non-hydrogen common atoms (Fig. 6), obtained from the partial pi-charge of the heteroatoms, have been used to model the bioactivity against *bacteria and fungi*. We give here, as example, the compound 3.

It is found that the negative charges of the oxygen of SO_2 group and sulfur contribute positively in favour of an antibacterial activity, more, and this is in good agreement with the mode of antbacterial action of the compounds bearing ($X^{\delta-}\cdots Y^{\delta+}$) pharmacophore site. It was hypothesized that difference in charges between two heteroatoms of the same dipolar pharmacophore site ($X^{\delta-}\cdots Y^{\delta+}$) may facilitate the inhibition of bacteria, more than viruses. It is further found that the activity increases with increase in negative charge of one heteroatom of the common pharmacophore fragment of the potential tautomers (III and VI). The presence of tautomerism phenomena in sulfonamide analogues was presented previously since 1999, in few papers by some rare authors [27] (Figs. 7 and 8).

On the basis of this analogue system described above, in compound **3**, sets of isomeric and tautomeric thiazole derivatives I–IV could be generated in-situ in the presence of bacteria of fungi.

This synergistic and streamlined working procedure led to highly active isomeric/tautomeric Gram(+/−) and fungi receptor ligands. However, a little difference in their respective binding affinities was consistently found for all isomeric pairs I–IV. The analysis of conformational differences due to heteroatom interactions in tautomers I–IV revealed a favorable (S═O···S) interaction in tautomers I and III, whereas thiazoles II and IV showed a repulsive (S═O···N) interaction.

So the antifungal activity is related with possible secondary electronic interaction with the positively charged side chains of the

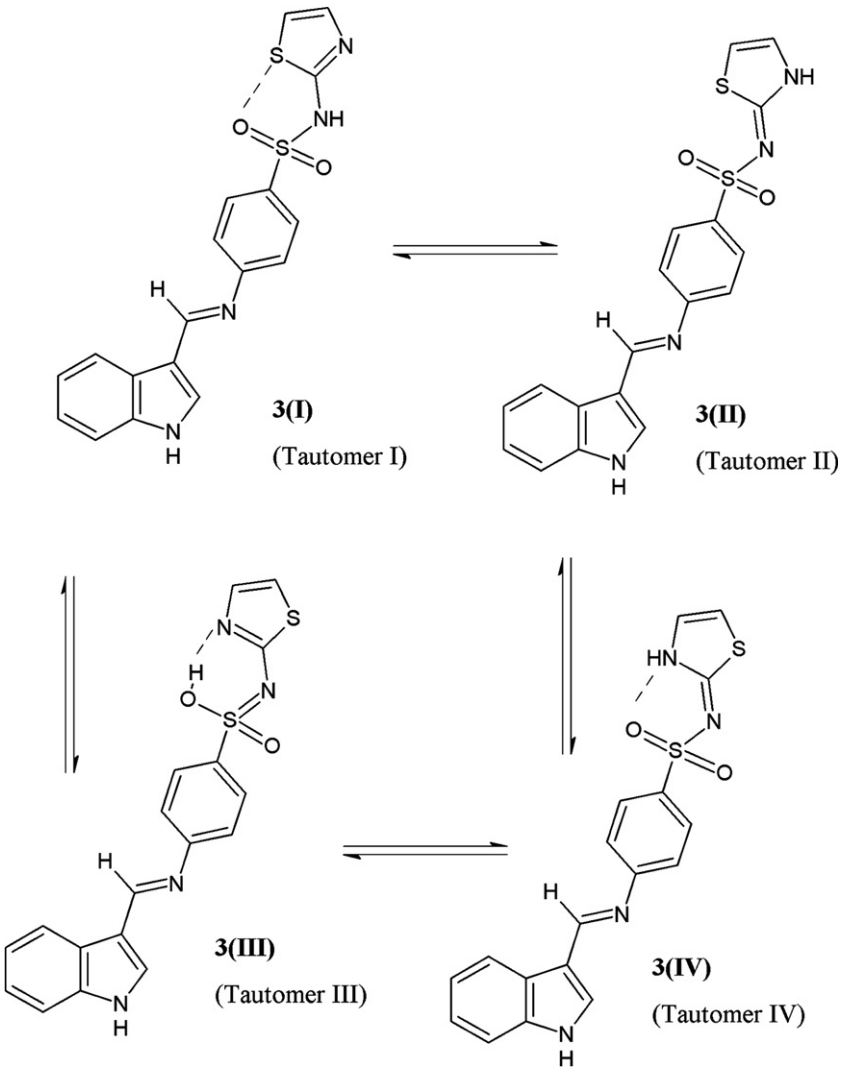
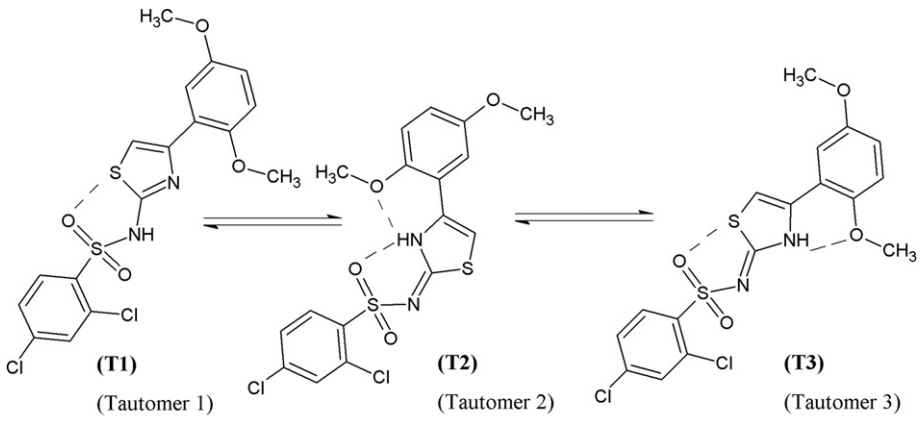


Fig. 7. Possible potential tautomeric forms of compound **3**.



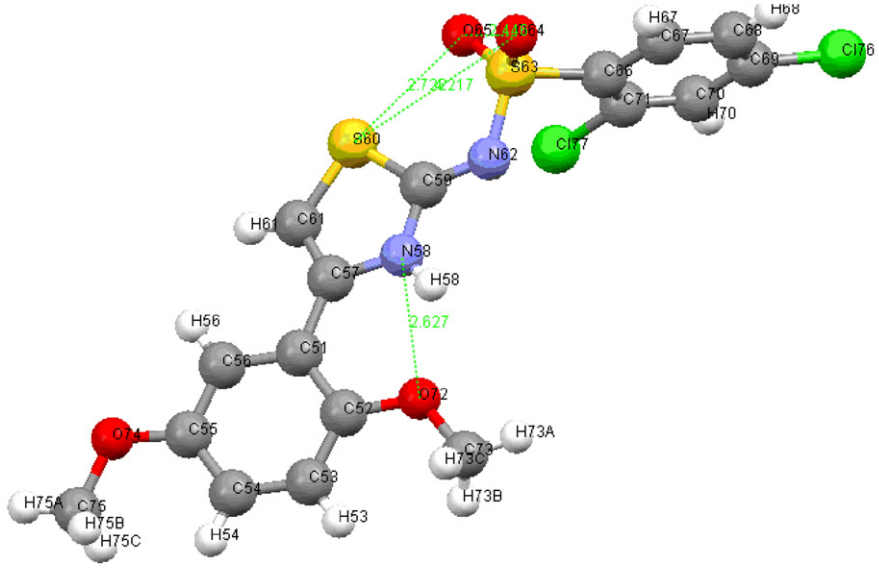


Fig. 8. The structural parameters do not indicate a tautomeric equilibrium but a single imino form. The main differences between the two crystalline forms lie in the intramolecular hydrogen bonding and the relative orientation of the methoxy groups. Attractive intermolecular interactions occur and are responsible for the crystalline cohesion.

virus target(s). Attempt was made to evaluate steric and indicator parameters which emerged as important contributors from previous pharmacologic analysis. The present results support the previous observations that heterocyclic ring in adjacent position of NH could generate four tautomeric forms, in less and two distinct four-member pharmacophore sites are conducive to the activity to both antibacterial ($O^-\cdots X^+$) and antifungal activity ($O^-\cdots X^-$).

4.2.2. *Osiris calculations [28]*

Structure based design is now fairly routine but many potential drugs fail to reach the clinic because of ADME-Tox liabilities. One very important class of enzymes, responsible for many ADMET problems, is the cytochromes P450. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions. Of the most important program, Osiris is already available online.

With our recent publication of the drug design combination of various pharmacophore sites by using spiro-heterocyclic structure, it is now possible to predict activity and/or inhibition with increasing success in two targets (bacteria and HIV). This is done using a combined electronic/structure docking procedure and an example will be given here (Table 4). The remarkably well behaved

Table 3
Selected Petra calculations of compounds.

Tautomer	Partial π-charge of heteroatoms (in e−)							
	N_1	N_2	N_3	N_4	S_1O2	S_2	O_1	O_2
3 (I)	0.339	−0.065	0.225	−0.088	−0.002	0.049	−0.187	−0.187
3 (II)	0.337	−0.066	−0.155	0.329	−0.009	0.106	−0.233	−0.233
3 (III)	0.3431	−0.0656	−0.099	−0.139	−0.024	0.047	−0.201	0.158
3 (IV)	0.3374	−0.0655	−0.155	0.329	−0.009	0.106	−0.233	−0.233
Imipenem	0.187	−0.145	0.128	–	0.087	0.082	−0.147	−0.217

Table 4
Osiris calculations of compounds.

Tautomer	Toxicity Risks				Osiris calculations				
	Mutagenic	Tumorigenic	Irritant	Reproductive effective	MW	CLP	S[a]	DL[a]	D-S[a]
3 (I)					382	2.87	−4.75	1.96	0.63
3 (II)					382	2.87	−4.89	0.9	0.56
3 (III)					382	2.13	−3.97	−0.94	0.49
3 (IV)					382	2.87	−4.89	0.9	0.56
Imipenem					299	−1.36	−1.93	5.34	0.93

[a] CLP: cLogP, S: Solubility, DL: Druglikness, DS: Drug-Scor.

Table 5
Molinspiration calculations of compounds.

Tautomer	Molinspiration calculations					Drug-likeness			
	cLogP	TPSA	OH···NH	N viol.	Vol.	GPCRL	ICM	KI	NRL
3 (I)	3.44	87.22	2	0	309	−0.71	1.07	−1.05	−1.36
3 (II)	4.37	90.45	2	0	309	−0.79	−0.84	−1.25	−1.30
3 (III)	1.93	90.71	2	0	309	−0.73	−0.86	−0.94	−1.16
3 (IV)	4.37	90.45	2	0	309	−0.79	−0.84	−1.25	−1.30
Imipenem	−0.86	116.22	4	0	255	−0.38	−0.38	−0.57	−0.41

mutagenicity of divers synthetic molecules classified in data base of CELERON Company of Swiss can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA.

4.2.3. Molinspiration calculations [29]

CLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors (Table 5).

The method is very robust and is able to process practically all organic, and most organometallic molecules. Molecular Polar Surface Area TPSA is calculated based on the methodology published by Ertl et al. as a sum of fragment contributions. O– and N– centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration. Prediction results of compounds **1–7** molecular properties (TPSA, GPCR ligand and ICM) are valued (Table 5).

5. Conclusions

The results of present investigation support the suggested structures of indolenyl sulfonamides. It has been suggested that some functional groups such as azomethine or hetero-aromatics present in these compounds displayed [30–34] role of biological activity that may be responsible for the increase of hydrophobic character and liposolubility of the molecules. This in turn, enhances activity of the compounds and biological absorbance, so as, all the synthesized sulfonamides have good antibacterial and antifungal properties.

A number of important points emerge concerning the electronic and steric factors which have direct impact on bioactivity properties. The positive results we have recorded, while encouraging for purposes of new drug design, confirm that very likely most of these compounds could be used as potential antibacterial activity after minor modifications. Based on their structural properties, these compounds may be useful as chelating agents with potential activity. These results prompt several pertinent observations: (i) This type of sulonamides can furnish an interesting model for studying the interaction of antibiotics with viral target because the possible charge modification of substituents and O/N of pharmacophore group; (ii) The future flexible pharmacophore site (s) geometric conformation enables us to prepare molecules for multi-therapeutic materials with high selectivity.

6. Experimental protocols

6.1. Materials and methods

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. Thin layer chromatography was performed using aluminium sheets (Merck) coated with silica gel 60 F_{254}. Elemental analysis was carried out with a LECO-CHNS-9320 model. ^{1}H NMR and ^{13}C NMR spectra of compounds were recorded with a Bruker Spectrospin Avence DPX-400 using TMS as internal standard and DMSO as solvent. IR spectra of compounds were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer. The melting points of compounds were determined with a Gallenkamp melting point apparatus. *In vitro* antibacterial, antifungal and cytotoxic properties were studied at HEJ research Institute of Chemistry, International Center for Chemical Sciences, university of Karachi, Pakistan.

6.2. Minimum inhibitory concentration (MIC) and cytotoxic activity (in vitro)

The preliminary antibacterial screening showed that compound **(2)**, **(3)**, **(4)** and **(7)** were the most active one. Above 80% these compounds were therefore selected for minimum inhibitory concentration (MIC) studies (Table 3). The MIC of all the four active compounds varied from 6.537×10^{-5} to 1.33×10^{-4} M compound **(3)** again proved to be the most active. It inhibited the growth of *B. subtilis* at 6.537×10^{-5} M.

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [35] From the data recorded in Table 5, it is evident that only compound **(3)** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive in this assay. Compound **(3)** showed activity ($LD_{50} = 0.980$ M) in the present series of compounds.

6.3. General procedure for the synthesis of compounds **(1)–(7)**

To an ethanol (20 mL) solution of the respective sulfanilamide (0.005 mol), an indole-3-carbaldehyde (0.005 mol) solution in ethanol (10 mL) was added with stirring. Later on the solution was refluxed for 2 h. The precipitates formed during refluxing, were cooled at room temperature and collected by suction filtration. Washing thoroughly with ethanol (2×8 ml), afforded TLC pure products in good yield. The same procedure was used for the preparation of all ligands.

6.3.1. 4-[(1H-indole-3-ylmethylene)amino] benzenesulfonamides **(1)**

Yellow powder. Mp = 220–221 °C. Yield: 1.29g (86%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 3399 (NH_2), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.62 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 7.6–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 2H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{15}H_{13}N_3O_2S$] [299.3]. Elemental analyses Found (Calcl.): C: 60.2 (60.1); H: 4.4 (4.3); N:14.1 (14.0).

6.3.2. N-Carbamimidoyl-4-[(1H-indol-3-ylmethylene)-amino]benzenesulfonamide **(2)**

Yellow powder. Mp = 255–260 °C. Yield: 1.43g (84%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 3399 (NH_2), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1580 (guanidine, C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 7.6–7.8 (m, 4H, N-Ph), 7.6 (s, 2H, NH_2), 8.1 (s, 1H, NH), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 167.0 (C-carbamimidoyl), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{16}H_{15}N_5O_2S$] [299.4]. Elemental analyses Found (Calcl.): C: 56.3 (56.2); H: 4.4 (4.5); N: 20.5 (20.4).

6.3.3. N-(thiazole-2-yl)-4-[(1H-indol-3-ylmethylene)-amino]benzenesulfonamide **(3)**

Yellow powder. Yiels : 1.62 g (85%). Mp = 268–273 °C. IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1615 (thiazol, C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 3.3 (d, H-4, thiazol), 3.1 (d, H-4, thiazol), 7.7–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 108.0 (C_4-thiazol), 137.8 (C_5-thiazol), 171.7 (C_2-thiazol), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{18}H_{14}N_4O_2S_2$] [382.5]. Elemental analyses Found (Calcl.): C: 56.5 (56.7); H: 3.7 (3.6); N: 14.7 (14.6).

6.3.4. N-(5-methylisoxazol-3-yl)-4-[(1H-indol-3-yl methylene)amino]benzenesulfonamide **(4)**

Yellow powder. Mp = 262–264 °C. Yield: 1.64 g (86%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1610 (isoxazolyl, C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 2.3 (s, 3H, CH_3), 5.8 (S, 1H, isoxazol), 7.7–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 12.8 (CH_3-isoxazol), 159.6 (C_5-isoxazol), 95.0 (C_4-isoxazol), 150.0 (C_3-isoxazol), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{19}H_{16}N_4O_3S$] [380.4]. Elemental analyses Found (Calcl.): C: 60.0 (59.9); H:4.2 (4.3); N: 14.7 (14.6).

6.3.5. N-(3,4-dimethylisoxazol-5-yl)-4-[(1H-indol-3-yl methylene)amino]benzenesulfonamide **(5)**

Yellow powder. Mp = 299–304 °C. Yield: 1.64 g (83%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1605 (isoxazolyl, C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 2.4 (m, 6H, CH_3), 7.7–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 15.1 (CH_3-isoxazol), 9.5 (CH_3-isoxazol), 159.6 (C_3-isoxazol), 100.5 (C_4-isoxazol), 158.9 (C_5-isoxazol), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{20}H_{18}N_4O_3S$] [394.5]. Elemental analyses Found (Calcl.): C: 60.9 (60.8); H: 4.6 (4.6); N: 14.2 (14.2).

6.3.6. N-(pyrimidin-2-yl)-4-[(1H-indol-3-yl methylene)amino]benzenesulfonamide **(6)**

Yellow powder. Mp = 277–283 °C. Yield: 1.51g (80%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 1595 (HC=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1585 (pyrimidine, C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 7.0–7.4 (m, 3H, pyrimidine), 7.7–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C=N, azomethine), 157.9 (C_4, C_6-pyrimidine), 110.2 (C_5-pyrimidine), 159.3 (C_2-pyrimidine), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{19}H_{15}N_5O_2S$] [377.4]. Elemental analyses Found (Calcl.): C: 60.5 (60.4); H: 4.0 (3.9); N: 18.6 (18.5).

6.3.7. N-(4,6-dimethylpyrimidin-2-yl)-4-[(1H-indol-3-yl methylene)amino]benzene-sulfonamide **(7)**

Yellow powder. Mp = 283–288 °C. Yield: 1.70 g (84%). IR (KBr, cm^{-1}): 3230 (NH), 3255 (NH), 1595 (HC=N), 1345, 1110 (S=O), 952

(S–N), 833 (C–S), 1580 (pyrimidine, C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.1 (d, H-4, indole), 7.1 (dt, H-5, indole), 7.3 (dt, H-6, indole), 7.6 (d, H-7, indole), 8.3 (s, H-2, indole), 12.1 (s, NH, indole), 2.3 (s, 6H, CH_3), 6.7 (s, 1H, pyrimidine), 7.7–7.8 (m, 4H, N-Ph), 9.4 (s, 1H, azomethine), 11.3 (s, 1H, SO_2NH_2); ^{13}C NMR (δ, ppm): 139.7 (C_2-indole), 115.4 (C_3-indole), 120.2 (C_4-indole), 121.3 (C_5-indole), 121.6 (C_6-indole), 114.4 (C_7-indole), 138.7 (C_8-indole), 126.8 (C_9-indole), 162.0 (C═N, azomethine), 25.1($2CH_3$-pyrimidine), 165.2 (C_4, C_6-pyrimidine), 103.0 (C_5-pyrimidine), 168.5 (C_2-pyrimidine), 138.2 (C_1-phenyl), 128.6 (C_2, C_6-phenyl), 122.6 (C_3, C_5-phenyl), 156.4 (C_4-phenyl). SM [$C_{21}H_{19}N_5O_2S$] [405.5]. Elemental analyses Found (Calcl.): C: 62.2 (62.3); H: 4.7 (4.7); N: 17.3 (17.3).

7. Biological properties

7.1. Antibacterial activity (in vitro)

All the synthesized compounds **(1)–(7)** were screened *in vitro* for their antibacterial activity against four Gram-negative (*E. coli, S. flexneri, P. aeruginosa, S. typhi*) and two Gram-positive (*S.aureus, B. subtilis*) bacterial strains by the agar-well diffusion method [36,37]. The wells (6 mm in diameter) were dug in the media with the help of a strile metallic borer with center at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of sterile cotton swab. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenem, served as negative and positive controls respectively. The plates were incubated immediately at 37 °C for 24 h activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the bacterial screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

7.2. Antifungal activity (in vitro)

All compounds were studied against six fungal cultures for antifungal activities. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) mL^{-1} fungal spore suspensions and transferred to Petri plates. Discs soaked in 20 mL (200 μg/mL in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for seven days. The results were recorded [14] as % of inhibition and compared with standard drugs miconazole and amphotericin B.

7.3. Minimum inhibitory concentration (MIC)

Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The MICs were determined using the disc diffusion technique [15] by preparing discs containing 10, 25, 50, and 100 μg/mL of the compounds and applying the protocol [38].

7.4. Cytotoxicity (in vitro)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plates dish (22 × 32 cm), filled with artificial sea water, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the light side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50 and 5 ug/mL were transferred to 9 vials (three for each dilution were used for each test sample and LD_{50} is the mean of three values) and one vial is kept as control having 2 mL of DMF only. The solvent is allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and ten shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by Finny computer program to determine the LD_{50} values [35,39].

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