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2nd Cancer Update

4-(1-Aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-N-substituted benzene sulfonamides: Synthesis, antimicrobial, anticancer evaluation and QSAR studies

Mahesh Kumar [a], Balasubramanian Narasimhan [a,*], Pradeep Kumar [a], Kalavathy Ramasamy [b], Vasudevan Mani [c], Rakesh Kumar Mishra [c], Abu Bakar Abdul Majeed [c]

[a] *Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak 124001, India*
[b] *Collaborative Drug Discovery Research Group, Faculty of Pharmacy, Campus Puncak Alam, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia*
[c] *Brain Research Laboratory, Faculty of Pharmacy, Campus Puncak Alam, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia*

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5-Chloroisatin;
Synthesis;
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Abstract A series of 4-(1-aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-substituted benzenesulfonamide derivatives (**1–20**) was synthesized and evaluated for its *in vitro* antimicrobial and anticancer activities. Antimicrobial results indicated that compounds *N*-(4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino) phenylsulfonyl)-4-isopropoxy benzamide (**9**) and *N*-(4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino) phenylsulfonyl)-4-isopropoxybenzamide (**19**) were found to be the most effective ones. The anticancer results indicated that almost all the synthesized compounds were more active than the standard drug carboplatin but less active than the standard drug 5-fluorouracil (5-FU) against both the cell lines (HCT116 and RAW 264.7). 4-(1-Benzoyl-5-

* Corresponding author. Tel.: +91 1262 393222; fax: +91 1262 274133.
E-mail address: naru2000us@yahoo.com (B. Narasimhan).
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chloro-2-oxoindolin-3-ylideneamino)-*N*-(pyrimidin-2-yl) benzenesulfonamide (**3**) was found to be most potent and exhibited selectivity toward HCT 116. QSAR studies indicated that antimicrobial activity of isatin derivatives against different microbial strains was governed by lipophilic parameter, log *P* and topological parameters valance zero and third order molecular connectivity indices ($^{0}\chi^{v}$ and $^{3}\chi^{v}$).

1. Introduction

The prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. Prudent use of antibiotics and development of novel antimicrobial agents seem to be the common strategies and action plans taken to combat this challenge. Consequently, the search for new antimicrobial agents will always remain an important task for medicinal chemists (Metwally et al., 2006).

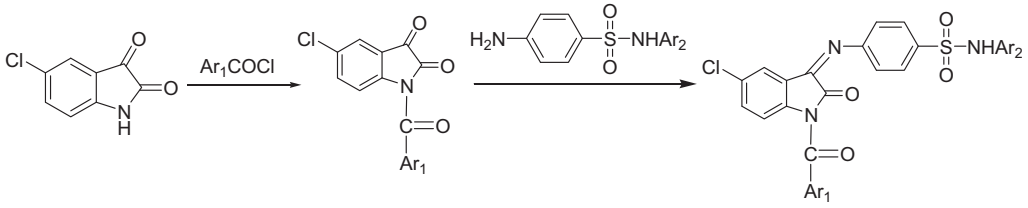
Cancer has become the second cause of mortality in the world and the development of potent and specific anticancer agents is urgently required due to problems associated with the existing drugs which include toxicity as well as resistance (Kamal et al., 2010). This undoubtedly underscores the need of developing novel anticancer agents.

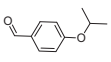
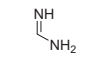
Isatin is a natural product found in a number of plants, including those of the genus Isatis. It is an endogenous compound isolated in 1988 that has been reported to possess a wide range of central nervous system activities. Several compounds containing an isatin moiety have also been documented to possess antimicrobial (Karthikeyan et al., 2010), anticonvulsant (Siddiqui et al., 2011), anti-inflammatory (Nirmal et al., 2010), anticancer (Liang et al., 2008) and anti-HIV (Banerjee et al., 2010) activities.

Aminopeptidase N (APN/CD13) is a zinc-dependent metalloprotease ectoenzyme widely expressed on hematopoietic cells of myeloid origin and non-hematopoietic cells and tissues, such as brain cells, fibroblasts, and epithelial cells of the kidney, liver, and intestine. Over-expression of APN is often associated with many diseases, such as cancer, viral infection and inflammation. In the process of tumorigenesis, it plays a crucial role in tumor invasion, metastasis and angiogenesis. Since the first marketed anti-APN drug Bestatin that was launched in 1976, many APN inhibitors (APNIs) which include probestin, lapstatin and AHPA-Val have been reported. Indole-2,3-dione (isatin) derivatives such as APNIs have also been described. Preliminary results demonstrated that most of the isatin derivatives exhibited better inhibition than bestatin (Jin et al., 2013).

Recently, it has been reported that 5,7-dibromoisatin is significantly more potent *in vitro* as a cytotoxin than the parent molecule (isatin) against U937 (human monocyte-like histiocytic lymphoma) cells (Matesic et al., 2008). The substitution of bromo and chloro groups at the C-5 and C-7 positions of isatin exhibited potent anticancer activity. These isatin analogs were also potent against a wide range of other human cancer cell lines, including MDA-MB-231 metastatic breast adenocarcinoma cells. Several isatin-*b*-thiosemicarbazones have displayed activity against a parental HeLa-derived cervical cancer cell line (KB-3-1) expressing the efflux pump multidrug resistance (MDR). The anticancer activity of a number of isatin derivatives conjugated to a cell targeting moiety *via* a spacer group has also been described (Penthala et al., 2010).

On the other hand, sulfonamides have a variety of biological activity which includes antibacterial, insulin releasing, carbonic anhydrase inhibition, anti-inflammation, and antitumor properties (Rohini et al., 2011). These agents block important metabolic steps of the microorganisms e.g. sulfonamides. Moreover, due to their metabolic inhibitory action, sulfonamide-based heterocycles (Selvam et al., 2006) represent an attractive target of contemporary organic synthesis. These earlier findings encouraged us to explore the synthesis of sulfonamides using isatin moiety and to examine their antibacterial, antifungal and anticancer properties. There are accumulating lines of evidence that hybridization of two or more different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often renders synergistic effects (Solomon et al., 2009).



Comp.	Ar_2	Comp.	Ar_2	Comp.	Ar_2	Comp.	Ar_2	Comp.	Ar_2	Comp.	Ar_1
1, 11		3, 13		5, 15		7, 17		9, 19		1-10	
2, 12		4, 14		6, 16		8, 18		10, 20		10-20	

Scheme 1 Synthetic scheme of the synthesis of 4-(1-aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-substituted benzenesulfonamide derivatives (1–20).

Table 1 Physicochemical characteristics and anticancer activity of the synthesized isatin derivatives.

Comp.	M. formula	M. Wt.	R_f value*	IC_{50} in μM	
				HCT116	Raw 264.7
1	$C_{25}H_{17}ClN_4O_5S$	520.94	0.62	109.42	57.59
2	$C_{23}H_{16}ClN_3O_5S$	481.91	0.78	124.50	87.15
3	$C_{25}H_{16}ClN_5O_4S$	517.94	0.84	17.38	54.06
4	$C_{26}H_{18}ClN_5O_4S$	531.97	0.76	24.44	45.12
5	$C_{24}H_{15}ClN_4O_4S_2$	522.98	0.68	99.43	15.30
6	$C_{27}H_{20}ClN_5O_6S$	578.00	0.74	112.46	25.95
7	$C_{26}H_{18}ClN_5O_5S$	547.97	0.78	80.30	9.12
8	$C_{26}H_{17}ClN_4O_4S$	516.96	0.80	96.72	9.67
9	$C_{31}H_{24}ClN_3O_6S$	602.06	0.80	101.32	13.29
10	$C_{22}H_{16}ClN_5O_4S$	481.91	0.72	124.50	16.60
11	$C_{25}H_{16}Cl_2N_4O_5S$	555.39	0.78	90.03	10.80
12	$C_{23}H_{15}Cl_2N_3O_5S$	516.35	0.66	38.73	11.62
13	$C_{25}H_{15}Cl_2N_5O_4S$	552.39	0.82	72.41	18.10
14	$C_{26}H_{17}Cl_2N_5O_4S$	566.42	0.70	61.79	8.83
15	$C_{24}H_{14}Cl_2N_4O_4S_2$	557.43	0.66	39.47	8.97
16	$C_{27}H_{19}Cl_2N_5O_6S$	612.44	0.70	42.45	8.16
17	$C_{26}H_{17}Cl_2N_5O_5S$	582.41	0.80	85.85	8.59
18	$C_{26}H_{16}Cl_2N_4O_5S$	551.40	0.64	97.93	10.88
19	$C_{31}H_{23}Cl_2N_3O_6S$	636.50	0.62	83.27	11.00
20	$C_{22}H_{15}Cl_2N_5O_4S$	516.36	0.78	133.63	13.56
	Carboplatin			> 100	> 100
	5-Flourouracil			4.6	4.6

* TLC mobile phase: chloroform: methanol (9:1).

QSAR models are highly effective in describing the structural basis of biological activity. The success of QSAR approach can be explained by the insight offered into the structural determination of chemical properties and the possibility to estimate the properties of new chemical compounds without the need to synthesize and test them (Sawant et al., 2013).

Prompted by the above facts and in continuation of our research efforts in the field of synthesis, antimicrobial, anticancer evaluation and QSAR studies (Sharma et al., 2012; Kumar et al., 2013; Sigraha et al., 2012), we hereby report synthesis, antimicrobial, anticancer evaluation and QSAR studies of 4-(1-aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-substituted benzene sulfonamides.

Table 2 Antimicrobial activity (μM/ml) of synthesized isatin derivatives.

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
1[a]	1.62	1.62	1.62	2.22	1.92
2	1.89	1.59	1.59	1.59	1.89
3[a]	1.92	1.92	1.62	2.22	1.92
4	1.93	1.93	1.63	2.23	1.93
5	1.62	1.92	1.92	2.22	1.92
6	1.67	1.67	1.97	2.27	1.97
7[a]	1.94	1.94	1.94	2.24	1.94
8	1.62	1.92	1.92	1.62	1.92
9	1.98	1.98	1.98	1.68	1.98
10	1.59	1.59	1.59	1.59	1.89
11[a]	1.65	1.65	1.95	1.65	1.95
12	1.92	1.62	1.92	2.22	1.92
13	1.65	1.95	1.95	1.65	1.95
14	1.96	1.96	1.96	1.96	1.96
15	1.95	1.95	1.95	1.95	1.95
16[a]	1.69	1.69	1.99	1.99	1.99
17[a]	1.67	1.97	1.97	1.97	1.97
18	1.95	1.95	1.95	2.25	1.95
19	2.01	2.01	2.01	2.31	2.01
20	1.92	1.62	1.92	2.22	1.92
SD	0.16	0.16	0.15	0.27	0.03
Std.	2.61*	2.61*	2.61*	2.64**	2.64**

SD – standard deviation.
* Norfloxacin.
** Fluconazole.
[a] Outliers.

2. Results and discussion

2.1. Chemistry

The synthesis of 4-(1-aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-substituted benzene sulfonamide derivatives (**1–20**) was accomplished (Scheme 1). The physicochemical characteristics of the synthesized compounds are presented in Table 1.

The synthesized compounds were characterized by IR and ^{1}H NMR spectroscopy and the results are in accordance with the assigned molecular structures. IR stretching band ranging from 1688 to 1681 cm^{-1} (C = O str., Ar–C = O) confirmed the acylation of isatin. IR stretching band at 1656–1650 cm^{-1} (C = N str.) confirmed the formation of a Schiff base. Further, peak of NH in plane bending at 1516–1491 cm^{-1}, O = S = O str. at 1186–1142 cm^{-1} confirmed the presence of sulfonamide moiety in the synthesized compounds.

In the ^{1}H NMR spectra the signals of the respective protons of the synthesized compounds were confirmed based on their chemical shifts, multiplicities and coupling constants. These spectra showed a singlet at 4.01–4.76 ppm, which corresponds to the SO_2NH protons and multipletes at 6.55–8.74 ppm, showed aromatic protons.

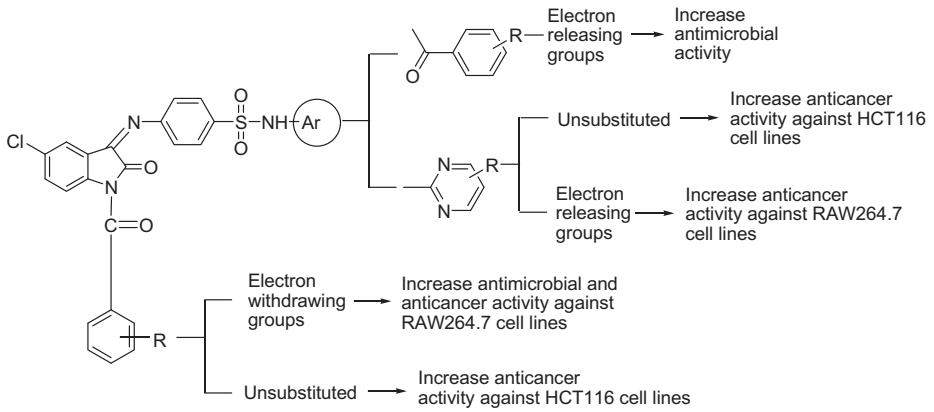


Figure 1 Structure–activity relationship for antimicrobial and anticancer activity of synthesized isatin derivatives.

Table 3 Values of selected parameters used in QSAR studies of synthesized compounds.

Comp.	log *P*	MR	$^{0}\chi^{v}$	$^{3}\chi^{v}$	k_1	*R*	Te	LUMO	HOMO	μ
1[a]	4.32	133.63	20.37	1.57	27.56	17.23	−6426.55	−1.48	−9.38	8.75
2	3.17	121.62	18.85	1.49	26.07	15.67	−5952.82	−1.54	−9.43	8.38
3[a]	4.11	133.31	20.07	1.44	27.56	17.33	−6300.99	−1.54	−9.42	9.29
4	4.30	138.24	20.99	1.57	28.53	17.73	−6456.81	−1.54	−9.40	9.14
5	4.70	133.32	20.33	1.54	26.60	16.83	−6146.78	−1.51	−9.24	8.72
6	4.54	146.89	22.73	1.55	31.43	19.20	−7252.72	−1.62	−9.45	8.86
7[a]	4.48	141.52	21.40	1.51	29.49	18.26	−6776.44	−1.39	−9.32	2.92
8	4.49	134.88	20.20	1.46	27.56	17.33	−6236.23	−1.51	−9.38	9.34
9	5.59	157.42	24.15	1.83	33.37	20.03	−7407.09	−1.52	−9.36	7.68
10	3.24	122.47	18.52	1.46	26.07	15.67	−5917.55	−1.29	−9.39	3.21
11[a]	4.84	138.44	21.49	1.73	28.53	17.64	−6786.57	−1.55	−9.44	9.31
12	3.69	126.42	19.97	1.65	27.05	16.08	−6312.81	−1.56	−9.47	8.92
13	4.63	138.11	21.19	1.60	28.53	17.74	−6660.98	−1.57	−9.46	10.30
14	4.82	143.04	22.11	1.73	29.49	18.14	−6816.80	−1.55	−9.45	10.38
15	5.22	138.13	21.45	1.70	27.56	17.24	−6506.78	−1.55	−9.24	8.31
16[a]	5.06	151.70	23.85	1.71	32.40	19.61	−7612.69	−1.35	−9.50	5.62
17[a]	5.00	146.33	22.52	1.67	30.46	18.67	−7136.47	−1.54	−9.24	9.04
18	5.01	139.69	21.32	1.62	28.53	17.74	−6596.22	−1.52	−9.38	9.52
19	6.10	162.23	25.26	1.99	34.34	20.44	−7767.09	−1.52	−9.39	8.05
20	3.76	127.28	19.64	1.62	27.05	16.08	−6277.56	−1.30	−9.39	2.82

2.2. Antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined by tube dilution method (Cappucino and Sherman, 1999) and the results are given in Table 2. Compound **19** demonstrated potent antibacterial activity against *Staphylococcus aureus* ($pMIC_{sa}$ = 2.01 μM). In the case of *Bacillus subtilis* compounds **9** and **19** emerged as most effective antibacterial agents with $pMIC_{bs}$ values of 1.98 and 2.01 μM, respectively. Compounds **9** and **19** ($pMIC_{ec}$ values 1.98 and 2.01 μM, respectively) also emerged as the most active candidates among the synthesized compounds against Gram-negative bacterium *Escherichia coli*. In the case of antifungal activity against *Candida albicans*, compound **19** emerged as the most active candidate among the synthesized compounds ($pMIC_{ca}$ values 2.31 μM) and against *Aspergillus niger* compounds, **9** and **19** ($pMIC_{an}$ values 1.98 and 2.01 μM, respectively) emerged as most active antifungal agents.

The antimicrobial results of the standard drugs, norfloxacin and fluconazole were almost similar against all the tested microorganisms. In the present study however, the standard drugs were more potent than the synthesized compounds.

In general, the results of MBC/MFC studies revealed that the synthesized compounds were bacteriostatic and fungistatic in action as their MFC and MBC values were (ranging from >0.08 to >0.10 μM/ml) 3-fold higher than their MIC values (a drug is considered to be bacteriosatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC value (Emami et al., 2004).

2.3. Anticancer activity

The anticancer activity (IC_{50}) of the synthesized isatin derivatives was determined against mouse leukemic monocyte macrophage (RAW 264.7) and colon cancer (HCT116) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983). The anticancer activity results are presented in Table 1. In general all the synthesized compounds were more active than the standard drug carboplastin (IC_{50} = >100 μM) but less active than the standard drug 5-FU (IC_{50} = 4.6 μM) against both the cell lines (HCT116 and RAW 264.7). Compounds, 4-(1-Benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-*N*-(pyrimidin-2-yl)benzene sulfonamide (**3**) and 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoin-

Table 4 Correlation matrix for the antifungal activity of synthesized compounds against *A. niger*.

	log *P*	MR	$^0\chi^v$	k_1	*R*	Te	LUMO	HOMO	μ	$pMIC_{an}$
log *P*	1.000	0.918	0.903	0.788	0.870	−0.814	−0.435	0.314	0.408	0.905
MR		1.000	0.989	0.962	0.991	−0.959	−0.439	0.033	0.346	0.963
$^0\chi^v$			1.000	0.966	0.976	−0.979	−0.470	−0.002	0.348	0.980
k_1				1.000	0.970	−0.984	−0.366	−0.153	0.224	0.928
R					1.000	−0.962	−0.474	−0.049	0.371	0.946
Te						1.000	0.426	0.153	−0.271	−0.970
LUMO							1.000	0.149	−0.925	−0.467
HOMO								1.000	−0.149	−0.036
μ									1.000	0.356
$pMIC_{an}$										1.000

dolin-3-ylideneamino)-*N*-(4,6-dimethoxypyrimidin-2-yl)benzene sulfonamide (**16**) were found to be most potent with IC_{50} values of 17.38 μM against HCT116 and 8.16 μM against RAW 264.7, respectively.

2.4. Structure–activity relationship

a. Results of the antimicrobial and anticancer screening indicated that phenyl nucleus attached to benzenesulfonamide moiety increased the antimicrobial potential, whereas its replacement with pyrimidine nucleus improved the anticancer potential of the synthesized isatin derivatives.

Table 5 Correlation of antibacterial, antifungal and antimicrobial activity of synthesized compounds with calculated molecular descriptors.

Descriptor	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$
Cos E	0.183	0.642	0.345	−0.062	0.325
log *P*	0.359	0.859	0.721	0.283	0.905
MR	0.365	0.701	0.617	0.266	0.963
$^0\chi$	0.383	0.552	0.559	0.253	0.946
$^0\chi^v$	0.433	0.647	0.643	0.316	0.980
$^1\chi$	0.300	0.653	0.582	0.238	0.946
$^1\chi^v$	0.388	0.760	0.659	0.290	0.950
$^2\chi$	0.399	0.623	0.565	0.248	0.952
$^2\chi^v$	0.469	0.760	0.667	0.311	0.931
$^3\chi$	0.623	0.197	0.354	0.255	0.742
$^3\chi^v$	0.706	0.505	0.564	0.326	0.832
κ_1	0.386	0.499	0.534	0.240	0.928
κ_2	0.298	0.538	0.522	0.195	0.911
κ_3	0.348	0.454	0.439	0.154	0.862
$\kappa\alpha_1$	0.388	0.519	0.581	0.296	0.959
$\kappa\alpha_2$	0.293	0.574	0.587	0.269	0.954
$\kappa\alpha_3$	0.352	0.500	0.516	0.237	0.924
R	0.300	0.653	0.582	0.238	0.946
J	−0.043	−0.836	−0.501	−0.135	−0.777
W	0.342	0.586	0.534	0.212	0.929
Te	−0.401	−0.506	−0.625	−0.317	−0.970
Ee	−0.341	−0.538	−0.585	−0.288	−0.955
Ne	0.335	0.541	0.580	0.285	0.953
SA	0.379	0.572	0.545	0.242	0.934
IP	0.050	−0.337	−0.106	−0.073	0.036
LUMO	−0.164	−0.438	−0.359	−0.161	−0.467
HOMO	−0.050	0.337	0.106	0.073	−0.036
μ	0.084	0.571	0.323	0.071	0.356

b. The high antimicrobial activity of compound **19** indicated that the presence of electron withdrawing group (2-chloro) on benzoyl portion and electron donating group (4-isopropoxy) on benzamide portion increased the antimicrobial activity of the synthesized isatin derivatives. The role of electron releasing groups in enhancing the antimicrobial activity of isatin derivatives is further supported by the study of Pandeya et al. (1999).

c. The anticancer activity results indicated that the presence of unsubstituted pyrimidine ring attached to benzenesulfonamide moiety (**3**) increased the anticancer potential of the synthesized compounds against HCT116 cancer cell lines.

d. The anticancer activity results also indicated that the presence of electron releasing methoxy group on the pyrimidine ring attached to benzene sulfonamide moiety (**16**) improved the anticancer activity of synthesized compounds against RAW 264.7 cancer cell lines which indicated that this substitution is beneficial for the binding with the receptor ANP. The role of electron releasing groups in improving anticancer activity is supported by the studies of Mologni et al. (2010).

e. From the above mentioned antimicrobial and anticancer activity results, it can be concluded that different structural requirements are necessary for a compound to be active against different microbial and cancer targets. This is in accordance with the findings of Sortino et al. (2007) and Yogeeswari et al. (2005).

The above findings are summarized in Fig. 1.

2.5. QSAR studies

In order to identify the substituent effect on the antimicrobial activity, quantitative structure–activity relationship (QSAR) studies were undertaken using the linear free energy relationship model (LFER) described by Hansch and Fujita (1964)). Biological activity data determined as MIC values were first transformed into pMIC values (i.e. −log MIC) and used as a dependent variable in the QSAR study. The different molecular descriptors (independent variables) like log of octanol–water partition coefficient (log *P*), molar refractivity (MR), Kier's molecular connectivity ($^0\chi$, $^0\chi^v$, $^1\chi$, $^1\chi^v$, $^2\chi$, $^2\chi^v$) and shape (κ_1, κ_2, κ_3, $\kappa_{1,}$ $\kappa_{2,}$ κ_3) topological indices, Randic topological index (*R*), Balaban topological index (*J*), Wiener topological index (*W*), Total energy (Te), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital

(LUMO), dipole moment (μ), nuclear repulsion energy (Nu.E) and electronic energy (Ele.E) were calculated for isatin derivatives and values of selected descriptors are presented in Table 3 (Hansch et al., 1973; Kier and Hall, 1976; Randic, 1975, 1993; Balaban, 1982; Wiener, 1947). Units of the energies and dipole were electron volts (eV), and atomic units (a.u.), respectively (Dai et al., 1999).

In the present study, a data set of 20 isatin derivatives was subjected to linear free energy regression analysis for model generation. During the regression analysis studies it was observed that the response values of compounds **1**, **3**, **7**, **11**, **16** and **17** were outside the limits of response values of other synthesized isatin derivatives. Thus compounds **1**, **3**, **7**, **11**, **16** and **17** were designated as outliers and were not involved in the data set for QSAR model generation. In multivariate statistics, it is common to define three types of outliers (Furusjo et al., 2006).

1. X/Y relation outliers are substances for which the relationship between the descriptors (X variables) and the dependent variables (Y variables) is not the same as in the (rest of the) training data.
2. X outliers. Briefly, a substance is an X outlier if the molecular descriptors for this substance do not lie in the same range as the (rest of the) training data.
3. Y outliers are only defined for training or test samples. They are substances for which the reference value of response is invalid.

As there was no difference in the activity (Table 2) as well as the molecular descriptor range (Table 3) of these outliers when compared to other isatin derivatives, these outliers belong to the category of Y outliers (Substances for which the reference value of response is invalid).

Preliminary analysis was carried out in terms of correlation analysis. A correlation matrix constructed for antifungal activity against *A. niger* is presented in Table 4. In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between topological parameter, Randic index (R) and steric parameter, molar refractivity (MR, $r = 0.991$) and low interrelationship was observed between electronic parameter, HOMO and topological parameter, valance zero order molecular connectivity index ($^0\chi^v$, r = -0.002). The correlations of different molecular descriptors with antibacterial and antifungal activity against different microorganisms are presented in Table 5.

The correlation matrix (Table 4) indicated the predominance of topological parameter, valance zero order molecular connectivity index ($^0\chi^v$) in describing the antifungal activity of the synthesized compounds against *A. niger*. Thus, QSAR model for antifungal activity against *A. niger* was developed using valance zero order molecular connectivity index ($^0\chi^v$).

$$\begin{aligned} &\mathrm{pMIC_{an}} = 0.0174\,{}^0\chi^v + 1.571 \\ &n = 14 \quad r = 0.980 \quad q^2 = 0.945 \quad s = 0.0069 \quad F = 296.78 \end{aligned} \tag{1}$$

Here and thereafter, n – number of data points, r – correlation coefficient, q^2 – cross validated r^2 obtained by leave one out method, s – standard error of the estimate and F – Fischer statistics.

Topological indices are numerical quantifiers of molecular topology and are sensitive to bonding pattern, symmetry, content of heteroatom as well as degree of complexity of atomic neighborhoods (Lather and Madan, 2005). The valence zero order molecular connectivity topological index ($^0\chi^v$) represents the molecules with branched structure. In this case, the positive correlation was observed between $^0\chi^v$ and antifungal activity against *A. niger*. Therefore, the antifungal activity of synthesized compounds against *A. niger* will increase with increase in their $^0\chi^v$ values. This is clearly evident from Table 3 that compound **19** having highest $^0\chi^v$ value of 25.26 is having highest antifungal activity against *A. niger* ($\mathrm{pMIC_{an}} = 2.01$, Table 2).

The QSAR model expressed by Eq. (1) was cross validated by its high q^2 value ($q^2 = 0.945$) obtained with leave one out (LOO) method. The value of q^2 greater than 0.5 is the basic requirement for qualifying a QSAR model to be a valid one (Golbraikh and Tropsha, 2002). The comparison of observed and predicted antifungal activities is presented in Table 6. It can be seen from the results that the observed and predicted antifungal activities against *A. niger* lie close to each other as evidenced by their low residual values Table 6. The plots of observed, predicted and residual $\mathrm{pMIC_{an}}$ values were also developed to check the statistical validity of QSAR models. The plot of predicted $\mathrm{pMIC_{an}}$ against observed $\mathrm{pMIC_{an}}$ (Fig. 2) also favors the model expressed by Eq. (1). Further, the plot of observed $\mathrm{pMIC_{an}}$ vs residual $\mathrm{pMIC_{an}}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero (Kumar et al., 2007).

Lipophilic parameter, log P was the most dominating parameter in describing the antibacterial activity of the synthesized compounds against *B. subtilis* and *E. coli*.

$$\begin{aligned} &\mathrm{pMIC_{bs}} = 0.172 \log \mathrm{p} + 1.058 \\ &n = 14 \quad r = 0.859 \quad q2 = 0.657 \quad s = 0.089 \quad F = 33.78 \end{aligned} \tag{2}$$

In order to improve the value of correlation coefficient, we coupled log P with dipole moment (μ), which resulted in best QSAR model for antibacterial activity of synthesized compounds against *B. subtilis* (Eq. (3)).

$$\begin{aligned} &pMIC_{\mathrm{bs}} = 0.150 \log \mathrm{P} + 0.195\mu + 0.997 \\ &n = 14 \quad r = 0.892 \quad q^2 = 0.711 \quad s = 0.083 \quad F = 21.51 \end{aligned} \tag{3}$$

As in case of antibacterial activity against *B. subtilis*, the antibacterial activity of the synthesized compounds against *E. coli* (Eq. (4)) was also positively correlated with log P. Hence, antibacterial activity of synthesized compounds against both bacterial strains will increase with increase in their log P values.

$$\begin{aligned} &\mathrm{pMIC_{ec}} = 0.128 \log \mathrm{P} + 1.297 \\ &n = 14 \quad r = 0.721 \quad q2 = 0.331 \quad s = 0.108 \quad F = 12.98 \end{aligned} \tag{4}$$

Antibacterial activity of synthesized compounds against *S. aureus* was best described by topological parameter, valance third order molecular connectivity index ($^3\chi^v$) which was positively correlated with antibacterial activity (Eq. (5)).

Progress in the use of quantitative structure–activity relationship (QSAR) methods has shown the importance of the hydrophobic or lipophilic nature of biologically active molecules. The lipophilicity modifies the penetration of bioactive molecules through the apolar cell membranes. This property is usually characterized by the partition coefficient (log P), which is essentially determined from distribution studies of the compound between an immiscible polar and non-polar solvent pair.

Table 6 Comparison of observed and predicted antimicrobial activity obtained by mt-QSAR models.

Comp.	$pMIC_{sa}$			$pMIC_{bs}$			$pMIC_{ec}$			$pMIC_{an}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.62	1.76	−0.14	1.62	1.82	−0.20	1.62	1.85	−0.23	1.92	1.93	−0.01
2	1.89	1.80	0.09	1.59	1.64	−0.05	1.59	1.70	−0.11	1.89	1.90	−0.01
3	1.92	1.63	0.29	1.92	1.80	0.12	1.62	1.82	−0.20	1.92	1.92	0.00
4	1.93	1.75	0.18	1.93	1.82	0.11	1.63	1.85	−0.22	1.93	1.94	−0.01
5	1.62	1.73	−0.11	1.92	1.87	0.05	1.92	1.90	0.02	1.92	1.92	0.00
6	1.67	1.72	−0.05	1.67	1.85	−0.18	1.97	1.88	0.09	1.97	1.97	0.00
7	1.94	1.66	0.28	1.94	1.73	0.21	1.94	1.87	0.07	1.94	1.94	0.00
8	1.62	1.65	−0.03	1.92	1.85	0.07	1.92	1.87	0.05	1.92	1.92	0.00
9	1.98	1.95	0.03	1.98	1.98	0.00	1.98	2.01	−0.03	1.98	1.99	−0.01
10	1.59	1.73	−0.14	1.59	1.55	0.04	1.59	1.71	−0.12	1.89B	1.89	0.00
11	1.65	1.92	−0.27	1.65	1.90	−0.25	1.95	1.92	0.03	1.95	1.94	0.01
12	1.92	1.96	−0.04	1.62	1.72	−0.10	1.92	1.77	0.15	1.92	1.92	0.00
13	1.65	1.79	−0.14	1.95	1.89	0.06	1.95	1.89	0.06	1.95	1.94	0.01
14	1.96	1.91	0.05	1.96	1.92	0.04	1.96	1.91	0.05	1.96	1.96	0.00
15	1.95	1.89	0.06	1.95	1.94	0.01	1.95	1.97	−0.02	1.95	1.94	0.01
16	1.69	1.85	−0.16	1.69	1.87	−0.18	1.99	1.94	0.05	1.99	1.99	0.00
17	1.67	1.84	−0.17	1.97	1.92	0.05	1.97	1.94	0.03	1.97	1.96	0.01
18	1.95	1.80	0.15	1.95	1.93	0.02	1.95	1.94	0.01	1.95	1.94	0.01
19	2.01	2.11	−0.10	2.01	2.07	−0.06	2.01	2.08	−0.07	2.01	2.01	0.00
20	1.92	1.89	0.03	1.62	1.62	0.00	1.92	1.78	0.14	1.92	1.91	0.01

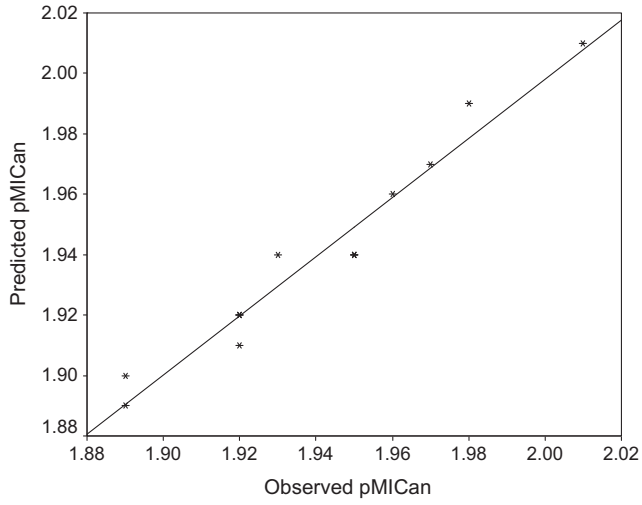


Figure 2 Plot of observed $pMIC_{an}$ against predicted $pMIC_{an}$ obtained by Eq. (1).

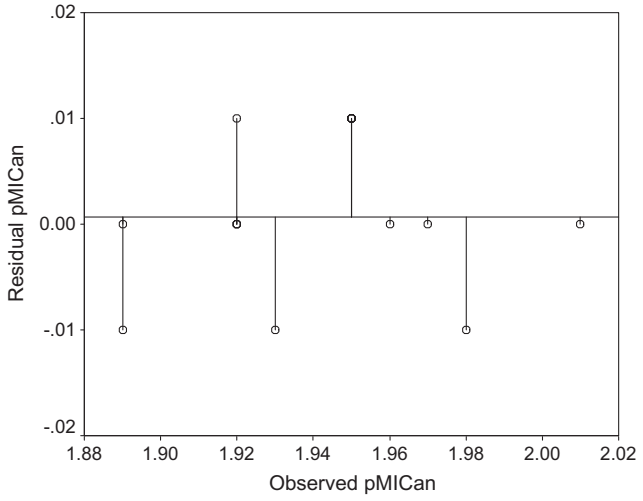


Figure 3 Plot of observed $pMIC_{an}$ against residual $pMIC_{an}$ obtained by Eq. (1).

This quantitative descriptor of lipophilicity (log *P*) is one of the key determinants of pharmacokinetic properties. Knowing the exact values for this parameter, it is possible to predict the inhibitory activity of the drugs (Podunavac-Kuzmanovic et al., 2008).

log *P* is the logarithm of the ratio of the concentrations of the un-ionized solute in two solvents, which is calculated according to the following equation, where *o* is octanol and *w* is un-ionized water.

$$\log P_{o/w} = \log([\text{solute}_o]/[\text{solute}_w])$$

The hydrophobic effect is the major driving force for the binding of drugs to their receptor targets in pharmacodynamics, and is based on the log *P* contribution of each atom. Each atom in a molecule contributes to the log *P* by the amount of its atomic parameter multiplied by the degree of exposure to the surrounding solvent (Park et al., 2008)

$$\begin{aligned} &pMIC_{sa} = 0.768\,{}^3\chi^v + 0.582 \\ &n = 14 \quad r = 0.760 \quad q^2 = 0.272 \quad s = 0.118 \quad F = 11.91 \end{aligned} \tag{5}$$

In order to improve the value of correlation coefficient, we coupled valance third order molecular connectivity index ($^3\chi^v$) with Balaban index (*J*) which resulted in best QSAR model for antibacterial activity of synthesized compounds against *S. aureus* (Eq. (6)).

$$\begin{aligned} &pMIC_{sa} = 0.934\,{}^3\chi^v + 0576J - 0.356 \\ &n = 14 \quad r = 0.769 \quad q^2 = 0.342 \quad s = 0.111 \quad F = 7.95 \end{aligned} \tag{6}$$

As in case of Eq. (1), the high q^2 value ($q^2 = 0.711$) supported the validity of developed QSAR model for antibacterial activity against *B. subtilis* (Eq. (3)). In case of antibacterial activity against *E. coli* (Eq. (4)) and *S. aureus* (Eq. (6)) the value q^2 is less than 0.5 (0.331 and 0.342, respectively), which shows that

the developed models are invalid ones. But according to the recommendations of Kim et al. (2007), the regression models are acceptable if the value of standard deviation (SD, Table 2) is not much larger than 0.3. As the value of standard deviation is less than 0.3 (Table 2), the developed QSAR models for antibacterial activity against *E. coli* (Eq. (4)) and *S. aureus* (Eq. (6)) are valid ones. Further, the low residual values (Table 6) supported the validity of QSAR models i.e. Eqs. (4) and (6).

It is important to note that no significant correlation was observed between the antifungal activity of synthesized compounds against *C. albicans* and their physicochemical parameters. Further, high residual activity values observed in case of outliers (compounds **1**, **3**, **7**, **11**, **16** and **17**) justified their removal before the development of QSAR models.

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of antibacterial and antifungal activities of the synthesized compounds is within one order of magnitude. It is important to note that the predictability of the QSAR models developed in the present study is high evidenced by their low residual values. This is in accordance with results suggested by the Bajaj et al. (2005), who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range. Further, recent literature reveals that the QSAR models have been applied to describe the relationship between narrow range of biological activity and physicochemical properties of the molecules (Narasimhan et al., 2007; Sharma et al., 2006; Hatya et al., 2006; Kumar et al., 2006). When biological activity data lie in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies (Kumar et al., 2007; Narasimhan et al., 2007). The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies its use in QSAR studies.

In summary, the antimicrobial activity of synthesized isatin derivatives against different microbial strains was governed by lipophilic parameter, log *P* and topological parameters valance zero and third order molecular connectivity indices ($^0\chi^v$ and $^3\chi^v$).

3. Conclusion

A series of 4-(1-aryl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-substituted benzenesulfonamide derivatives (**1–20**) was synthesized and evaluated for its *in vitro* antimicrobial and anticancer activities. Antimicrobial study indicated that compounds **9** and **19** were found to be the most effective antimicrobial agents. Anticancer results indicate that all the synthesized compounds were more potent than the standard drug carboplastin but less active than the standard drug 5-fluorouracil (5-FU) against both the cells (HCT116 and RAW 264.7). The compound **3** was found to be the most potent one against HCT116 and compound **16** against RAW 264.7.

4. Experimental

4.1. Chemistry

Starting materials were obtained from commercial sources and were used without further purification. Solvents were dried by standard procedures. Reaction progress was observed by thin layer chromatography. Melting points were determined in open capillary tubes on a Sonar melting point apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were determined by Bruker 500 MHz NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). NMR data are given as multiplicity (*s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet) and number of protons. IR spectra were recorded on a Varian Resolutions Pro spectrophotometer in a KBr disk.

4.2. General procedure for the synthesis of 4-(1-aryl-5-chloro-2-oxo-1, 2-dihydro-indol-3-ylideneamino)-N-substituted benzenesulfonamide (**1–20**)

Thionyl chloride 32.8 g (0.3 mol) was added to different aromatic acids (0.25 mol) in a round bottomed flask. After addition, the mixture was refluxed for 2 h. The excess of thionyl chloride was removed by distillation. To the solution of acid chloride (1 mol) 0.1 mol of 5-chloroisatin was added and the mixture was refluxed for 1 h and 30 min. Then the reaction mixture was cooled and the resultant precipitate (*N*-acyl 5-chloroisatin) was collected, washed with hexane and recrystallized from ethyl acetate. A solution of 0.05 mol of different sulfonamides in warm ethanol was added to the corresponding solution of *N*-acyl 5-chloroisatin (0.05 mol) in the presence of small amount of glacial acetic acid. The mixture was refluxed for 4–6 h. Then the reaction mixture was allowed to cool at room temperature and the precipitate obtained was filtered, dried and recrystallized from ethanol.

4.2.1. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(5-methylisoxazol-3-yl) benzenesulfonamide (**1**)

Mp (°C) 246–248; Yield – 85%; IR (KBr pellets)cm^{-1} 1494 (NH in plane bending, sec. amine), 1681 (C═O str., Ar–C═O), 1651 (C═N str.), 1179 (O═S═O str.), 730 (C–Cl str., Ar–Cl), 1247 (–C–O–N str., isoxazole), 894 (CH out of plane bending, isoxazole), 894–699 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.14–7.96 (12, m, ArH), 4.17 (1H, s, SO_2NH), 2.30 (3H, s, $ArCH_3$); ^{13}CNMR (DMSOd_6, δ ppm): 166.18, 161.62, 136.46, 133.62, 129.89, 129.30, 129.12, 128.06, 125.82.

4.2.2. N-(4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)phenylsulfonyl)acetamide (**2**)

Mp (°C) 88–90; Yield – 80%; IR (KBr pellets) cm^{-1} 1494 (NH in plane bending, sec. amine), 1682 (C═O str., Ar–C═O), 1651 (C═N str.), 1180 (O═S═O str.), 1494 (CH_3 bending vibration, $COCH_3$), 730 (C–Cl str., Ar–Cl), 894–699 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.58–7.97 (12H, m, ArH), 4.01 (1H, s, SO_2NH), 2.38 (3H, s, $COCH_3$).

4.2.3. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide (**3**)

Mp (°C) 252–254; Yield – 72%; IR (KBr pellets) cm^{-1} 1493 (NH in plane bending, sec. amine), 1682 (C═O str., Ar–C═O), 1651 (C═N str.), 1180 (O═S═O str.), 1604 (C═N str., pyrimidine), 700 (C–Cl str., Ar–Cl), 730 (CH out of plane bending, 4-sustituted pyrimidine), 894–657 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.58–8.71 (15H, m, ArH), 4.18 (1H, s, SO_2NH); ^{13}CNMR (DMSOd_6, δ ppm): 166.18, 161.63, 136.47, 133.64, 132.88, 129.91, 129.32, 129.13, 128.07, 128.83.

*4.2.4. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide (**4**)*

Mp (°C) 268–270; Yield – 76%; IR (KBr pellets) cm^{-1} 1493 (NH in plane bending, sec. amine), 1681 (C═O str., Ar–C═O), 1651 (C═N str.), 1180 (O═S═O str.), 1575 (C═N str., pyrimidine), 700 (C–Cl str., Ar–Cl), 752–795 (CH out of plane bending, 4-sustituted pyrimidine), 897–697 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.58–8.71 (14H, m, ArH), 4.17 (1H, s, SO_2NH), 2.33 (3H, s, $ArCH_3$).

*4.2.5. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(thiazol-2-yl) benzene sulfonamide (**5**)*

Mp (°C) 213–215; Yield – 82%; IR (KBr pellets) cm^{-1} 1493 (NH in plane bending, sec. amine), 1683 (C═O str., Ar–C═O), 1652 (C═N str.), 1150 (O═S═O str.), 1576 (C = N str., thiazole), 731 (C–Cl str., Ar–Cl), 750 (C–S–C str., thiazole), 897 (CH out of plane bending, thiazole), 897–699 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.55–7.95 (14, m, ArH), 4.15 (1H, s, SO_2NH); ^{13}CNMR (DMSOd_6, δ ppm): 166.34, 166.18, 161.65, 161.19, 141.49, 137.86, 136.64, 136.53, 133.67, 133.60, 132.87, 132.61, 130.57, 129.94, 129.21, 129.12, 128.91, 128.75, 128.06, 127.17, 125.80, 125.37, 124.89, 120.34, 108.64.

*4.2.6. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(4, 6-dimethoxypyrimidin-2-yl)benzenesulfonamide (**6**)*

Mp (°C) 124–126; Yield – 85%; IR (KBr pellets) cm^{-1} 1494 (NH in plane bending, sec. amine), 1684 (C═O str., Ar–C═O), 1652 (C═N str.), 1180 (O═S═O str.), 1286 (C–O–C str.), 1576 (C═N str., pyrimidine), 731 (C–Cl str., Ar–Cl), 1602 (C = C str., pyrimidine), 894–659 (CH out of plane bending, indole).^{1}H NMR (DMSO) δ; 6.55–7.95 (13H, m, ArH), 4.17 (1H, s, SO_2NH), 3.65 (6H, s, $ArOCH_3$).

4.2.7. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(6-methoxypyridazin-3-yl) benzenesulfonamide (7)

Mp (°C) 208–210; Yield – 74%; IR (KBr pellets)cm^{-1} 1493 (NH in plane bending, sec. amine), 1681 (C═O str., Ar–C═O), 1652 (C═N str.), 1183 (O═S═O str.), 1287 (C–O–C str.), 1400 (N–N str., pyridazine), 730 (C–Cl str., Ar–Cl), 887–656 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.56–8.74 (14H, m, ArH), 4.19 (1H, s, SO_2NH), 3.33 (3H, s, $ArOCH_3$); ^{13}CNMR (DMSOd_6, δ ppm):166.17, 161.65, 149.23, 141.01, 136.53, 133.67, 132.87, 129.94, 129.12, 128.93, 128.06, 125.80, 125.33, 120.43, 101.62.

*4.2.8. 4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(pyridin-2-yl) benzene sulfonamide (**8**)*

Mp (°C) 220–222; Yield – 68%; IR (KBr pellets) cm^{-1} 1495 (NH in plane bending, sec. amine), 1683 (C = O str., Ar–C = O), 1651 (C = N str.), 1182 (O = S = O str.), 1577 (C = N str., pyridine), 1602 (C = C str., pyridine), 731 (C–Cl str., Ar–Cl), 895–617 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.55–8.11 (16H, m, ArH), 4.19 (1H, s, SO_2NH).

*4.2.9. N-(4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino) phenylsulfonyl)-4-iso propoxybenzamide (**9**)*

Mp (°C) 208–210; Yield – 74%; IR (KBr pellets) cm^{-1} 1515 (NH in plane bending, sec. amine), 1684 (C═O str., Ar–C═O), 1653 (C═N str.), 1182 (O═S═O str.), 1166 ($CH(CH_3)_2$ bending), 1259 (C–O–C str.), 732 (C–Cl str., Ar–Cl), 886–645 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.09–7.95 (16H, m, ArH), 4.72 (1H, s, SO_2NH), 4.19 (1H, s, CH), 1.29 (6H, d, $CH(CH_3)_2$); ^{13}CNMR (DMSOd_6, δ ppm): 166.19, 164.75, 161.72, 161.66, 154.04, 136.52, 133.67, 133.61, 132.88, 130.92, 130.46, 129.94, 129.25, 128.07, 125.82, 124.55, 123.98, 115.42, 112.68.

*4.2.10. 1-(4-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)phenylsulfonyl)guanidine (**10**)*

Mp (°C) 88–90; Yield – 80%; IR (KBr pellets) cm^{-1} 1516 (NH in plane bending, sec. amine), 1681 (C═O str., Ar–C═O), 1650 (C═N str.), 1181 (O═S═O str.), 699 (C–Cl str., Ar–Cl), 898–641 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.46–7.95 (12H, m, ArH), 4.20 (1H, s, SO_2NH), 3.31 (3H, s, $COCH_3$).

*4.2.11. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(5-methyl isoxazol-3-yl)benzenesulfonamide (**11**)*

Mp (°C) 203–205; Yield – 76%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1687 (C═O str., Ar–C═O), 1656 (C═N str.), 1142 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 895 (CH out of plane bending, isoxazole), 895–665 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.47–8.06 (12H, m, ArH), 4.17 (1H, s, SO_2NH), 2.38 (3H, s, $ArCH_3$).

*4.2.12. N-(4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)phenyl sulfonyl)acetamide (**12**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1686 (C═O str., Ar–C═O), 1655 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1402 (CH_3 bending vibration, $COCH_3$), 895–665 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.58–7.97 (14H, m, ArH), 4.18 (1H, s, SO_2NH), 2.37 (3H, s, $COCH_3$); ^{13}CNMR (DMSOd_6, δ ppm): 165.17, 161.57, 137.74, 136.15, 133.59, 132.33, 130.01, 129.86, 129.63, 129.37, 129.24, 126.01.

*4.2.13. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide (**13**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1687 (C═O str., Ar–C═O), 1656 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1593 (C═N str., pyrimidine), 777 (CH out of plane bending, 4-sustituted pyrimidine), 896–665 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.65–7.97 (14H, m, ArH), 4.18 (1H, s, SO_2NH).

*4.2.14. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(4-methyl pyrimidin-2-yl)benzenesulfonamide (**14**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1686 (C═O str., Ar–C═O), 1656 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1593 (C═N str., pyrimidine), 777 (CH out of

plane bending, 4-sustituted pyrimidine), 896–666 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.58–8.02 (13H, m, ArH), 4.19 (1H, s, SO_2NH), 2.38 (3H, s, $ArCH_3$); ^{13}CNMR (DMSOd_6, δ ppm): 165.17, 161.56, 137.74, 136.14, 133.58, 132.33, 130.01, 129.85, 129.65, 129.37, 129.24, 126.02.

*4.2.15. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(thiazol-2-yl) benzenesulfonamide (**15**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1687 (C═O str., Ar–C═O), 1655 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1593 (C═N str., thiazole), 739 (C–S–C str., thiazole), 896–666 (CH out of plane bending, indole, thiazole). ^{1}H NMR (DMSO) δ; 7.64–7.96 (13, m, ArH), 4.18 (1H, s, SO_2NH).

*4.2.16. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(4,6-dimethoxy pyrimidin-2-yl)benzenesulfonamide (**16**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1686 (C═O str., Ar–C═O), 1656 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1250 (C–OC str.), 1594 (C═N str., pyrimidine), 896–666 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.51–8.06 (12H, m, ArH), 4.19 (1H, s, SO_2NH), 3.30 (6H, s, $ArOCH_3$).

*4.2.17. 4-(5-chloro-1-(4-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(6-methoxy pyridazin-3-yl)benzenesulfonamide (**17**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1687 (C═O str., Ar–C═O), 1655 (C═N str.), 1183 (O═S═O str.), 1250 (C–O–C str.), 1402 (N–N str., pyridazine), 777 (C–Cl str., Ar–Cl), 896–666 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.51–8.03 (13H, m, ArH), 4.19 (1H, s, SO_2NH), 3.31 (3H, s, $ArOCH_3$).

*4.2.18. 4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)-N-(pyridin-2-yl) benzenesulfonamide (**18**)*

Mp (°C) 220–222; Yield – 76.87%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1687 (C═O str., Ar–C═O), 1655 (C═N str.), 1183 (O═S═O str.), 777 (C–Cl str., Ar–Cl), 1593 (C═N str., pyridine), 896–665 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 7.51–8.02 (15H, m, ArH), 4.18 (1H, s, SO_2NH); ^{13}CNMR (DMSOd_6, δ ppm): 165.18, 161.58, 137.75, 136.16, 133.60, 132.34, 130.02, 129.88, 129.61, 129.38, 129.24, 126.01, 112.87.

*4.2.19. N-(4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino)phenyl sulfonyl)-4-isopropoxybenzamide (**19**)*

Mp (°C) 208–210; Yield – 74%; IR (KBr pellets) cm^{-1} 1492 (NH in plane bending, sec. amine), 1688 (C═O str., Ar–C═O), 1656 (C═N str.), 1186 (O═S═O str.), 1160 ($CH(CH_3)_2$ bending), 1259 (C–O–C str.), 742 (C–Cl str., Ar–Cl), 889–668 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.08–8.01 (15H, m, ArH), 4.76 (1H, s, SO_2NH), 4.18 (1H, s, CH), 1.30 (6H, d, $CH(CH_3)_2$); ^{13}CNMR (DMSOd_6, δ ppm): 165.18, 164.75, 161.71, 161.59, 154.04, 137.77, 136.18, 133.61, 132.34, 130.92, 130.46, 130.02, 129.89, 129.59, 129.39, 129.25, 126.00, 124.55, 123.98, 115.41, 112.61.

*4.2.20. 1-(4-(5-chloro-1-(2-chlorobenzoyl)-2-oxoindolin-3-ylideneamino) phenyl sulfonyl)guanidine (**20**)*

Mp (°C) 88–90; Yield – 80%; IR (KBr pellets) cm^{-1} 1491 (NH in plane bending, sec. amine), 1688 (C═O str., Ar–C═O), 1656 (C═N str.), 1186 (O═S═O str.), 741 (C–Cl str., Ar–Cl), 896–666 (CH out of plane bending, indole). ^{1}H NMR (DMSO) δ; 6.71–8.62 (11H, m, ArH), 4.19 (1H, s, SO_2NH), 3.30 (3H, s, $COCH_3$); ^{13}CNMR (DMSOd_6, δ ppm): 165.17, 161.56, 137.74, 136.13, 133.58, 132.33, 130.02, 129.85, 129.67, 129.38, 129.24, 126.03.

4.3. Evaluation of antimicrobial activity

4.3.1. Determination of MIC

The antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus*, *B. subtilis*, the Gram-negative bacterium *E. coli* and several fungal strains: *C. albicans* and *A. niger* using the tube dilution method (Cappucino and Sherman, 1999). Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth – I.P. (fungi) (Pharmacopoeia of India, 2007). The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. niger*) and at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC).

4.3.2. Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by sub culturing 100 µL of culture from each tube (which remained clear in the MIC determination) on fresh medium. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction (Rodriguez-Arguelles et al., 2005).

4.4. Anticancer evaluation

The anticancer activity of the synthesized compounds was determined against murine leukemia (RAW 264.7) and colon cancer (HCT116) cell lines. Cancer cell lines were purchased from the American Type Culture Collection (ATCC), Manassas, VA, USA. All cell lines were cultured in RPMI 1640 (Sigma) supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin (PAA Laboratories). Cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. Anticancer activity of the synthesized compounds at various concentrations was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann (1983) but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose–response curve of which the concentration of test compounds required to kill 50% of the cell population (IC_{50}) was determined. Anticancer activity was expressed as the mean IC_{50} of three independent experiments.

4.5. QSAR studies

The structures of synthesized isatin derivatives were first pre-optimized with the Molecular Mechanics Force Field (MM^+) procedure included in Hyperchem 6.0 (1993) and the resulting geometries were further refined by means of the semi-empirical method PM3 (Parametric Method-3). We chose a gradient norm limit of 0.01 kcal/Å for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows (TSAR 3D Version 3.3, 2000). Further, the regression analysis was performed using the SPSS software package (SPSS for Windows, 1999).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2013.03.002.

References

Bajaj, S., Sambi, S.S., Madan, A.K., 2005. Prediction of anti-inflammatory activity of *N*-arylanthranilic acids: computational approach using refined Zagreb Indices. Croat. Chem. Acta 78 (2), 165–174.

Balaban, A.T., 1982. Highly discriminating distance based topological indices. Chem. Phys. Lett. 89, 399–404.

Banerjee, D., Yogeeswari, P., Bhat, P., Thomas, A., Srividya, M., Sriram, D., 2010. Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. Eur. J. Med. Chem. 45, 1–16.

Cappucino, J.G., Sherman, N., 1999. Microbiology-a Laboratory Manual. Addison Wesley, California, p. 263.

Dai, J., Sun, C., Han, S., Wang, L., 1999. QSAR for polychlorinated compounds (PCOCs). I. Prediction of partition properties for PCOCs using quantum chemical parameters. Bull. Environ. Contam. Toxicol. 62, 530–538.

Emami, S., Falahati, M., Banifafemi, A., Shafiee, A., 2004. Stereoselective synthesis and antifungal activity of trans-3-azolyl-2-methylchromanone oxime ethers. Bioorg. Med. Chem. Bioorg. Med. Chem. 12, 5881–5889.

Furusjo, E., Svenson, A., Rahmberg, M., Andersson, M., 2006. The importance of outlier detection and training set selection for reliable environmental QSAR predictions. Chemosphere 63, 99–108.

Golbraikh, A., Tropsha, A., 2002. Beware of q^2! J. Mol. Graphics Modell. 20, 269–276.

Hansch, C., Fujita, T., 1964. A method for the correlation of biological activity and chemical structure. J. Am. Chem. Soc. 86, 1616–1626.

Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D., Lien, E.J., 1973. "Aromatic" substituent constants for structure-activity correlations. J. Med. Chem. 16, 1207–1216.

Hatya, S.A., Aki-sener, E., Tekiner-Gulbas, B., Yildiz, I., Temiz-Arpaci, O., Yalcin, I., Altanlar, N., 2006. Synthesis, antimicrobial activity and QSARs of new benzoxazine-3-ones. Eur. J. Med. Chem. 41, 1398–1404.

Hyperchem 6.0, Hypercube, Inc., Florida, 1993.

Jin, K., Zhang, X., Ma, C., Xu, Y., Yuan, Y., Xu, W., 2013. Novel indoline-2,3-dione derivatives as inhibitors of aminopeptidase N (APN). Bioorgan. Med. Chem. In press.

Kamal, A., Srikanth, Y.V.V., Khan, M.N.A., Shaik, T.B., Ashraf, M., 2010. Synthesis of 3,3-diindolyl oxyindoles efficiently catalysed by $FeCl_3$ and their in vitro evaluation for anticancer activity. Bioorg. Med. Chem. Lett. 20, 5229–5231.

Karthikeyan, K., Sivakumar, P.M., Doble, M., Perumal, P.T., 2010. Synthesis, antibacterial activity evaluation and QSAR studies of novel dispiropyrrolidines. Eur. J. Med. Chem. 45, 3446–3452.

Kier, L.B., Hall, L.H., 1976. Molecular Connectivity in Chemistry and Drug Research. Academic Press, NewYork.

Kim, Y.M., Farrah, S., Baney, R.H., 2007. Structure-antimicrobial activity relationship for silanols, a new class of disinfectants, compared with alcohols and phenols. Int. J. Antimicrob. Agents 29, 217–222.

Kumar, A., Sharma, P., Gurram, V.K., Rane, N., 2006. Studies on synthesis and evaluation of quantitative structure–activity relationship of 10-methyl-6-oxo-5-arylazo-6,7-dihydro-5H-[1,3]azaphospholo[1,5-d][1,4]benzodiazepin-2-phospha-3-ethoxycarbonyl-1-phosphorus dichlorides. Bioorg. Med. Chem. Lett. 16, 2484–2491.

Kumar, A., Narasimhan, B., Kumar, D., 2007. Synthesis, antimicrobial, and QSAR studies of substituted benzamides. Bioorg. Med. Chem. 15, 4113–4124.

Kumar, H., Kumar, P., Narasimhan, B., Ramasamy, K., Mani, V., Mishra, R.K., Majeed, A.B.A., 2013. Synthesis, *in vitro* antimicrobial, antiproliferative, and QSAR studies of *N*-(substituted phenyl)-2/4-(1*H*-indol-3-ylazo)-benzamides. Med. Chem. Res. 22, 1957–1971.

Lather, V., Madan, A.K., 2005. Topological models for the prediction of anti-HIV activity of dihydro(alkylthio)(naphthylmethyl)oxopyrimidines. Bioorg. Med. Chem. 13, 1599–1604.

Liang, Z., Zhang, D., Ai, J., Chen, L., Wang, H., Kong, X., Zheng, M., Liu, H., Luo, C., Matesic, L., Locke, J.M., Bremner, J.B., Pyne, S.G., Skropeta, D., Ranson, M., Vine, K.L., 2008. *N*-Phenethyl and *N*-naphthylmethyl isatins and analogues as *in vitro* anticancer agents. Bioorg. Med. Chem. 16, 3118–3124.

Matesic, L., Locke, J.M., Bremner, J.B., Pyne, S.G., Skropeta, D., Ranson, M., Vine, K.L., 2008. N-Phenethyl and N-naphthylmethyl isatins and analogues as in vitro anticancer agents. Bioorgan. Med. Chem. 16, 3118–3124.

Metwally, K.A., Aziz, L.M.A., Lashine, E.S.M., Husseiny, M.I., Husseiny, R.H., 2006. Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: synthesis and preliminary evaluation as antimicrobial agents. Bioorg. Med. Chem. 14, 8675–8682.

Mologni, L., Rostagno, R., Brussolo, S., Knowles, P.P., Kjaer, S., Murray-Rust, J., Rosso, E., Zambon, A., Scapozza, L., McDonald, N.Q., Lucchini, V., Gambacorti-Passerini, C., 2010. Synthesis, structure–activity relationship and crystallographic studies of 3-substituted indolin-2-one RET inhibitors. Bioorg. Med. Chem. 18, 482–1496.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Narasimhan, B., Judge, V., Narang, R., Ohlan, S., Ohlan, R., 2007. Quantitative structure–activity relationship studies for prediction of antimicrobial activity of synthesized 2,4-hexadienoic acid derivatives. Bioorg. Med. Chem. Lett. 17, 5836–5845.

Nirmal, R., Prakash, C.R., Meenakshi, K., Shanmugapandiyan, P., 2010. Synthesis and pharmacological evaluation of novel schiff base analogues of 3-(4-amino) phenylimino) 5-fluoroindolin-2-one. J. Young Pharm. 2, 162–168.

Pandeya, S.N., Sriram, D., Nath, G., DeClercq, E., 1999. Synthesis and antimicrobial activity of schiff and mannich bases of isatin and its derivatives with pyrimidine. Il Farmaco 54, 624–628.

Park, D.S., Kim, J.M., Lee, Y.B., Ahn, C.H., 2008. QSID tool: a new three-dimensional QSAR environmental tool. J. Comput.-Aided Mol. Des. 22, 873–883.

Penthala, N.R., Yerramreddy, T.R., Madadi, N.R., Crookset, P.A., 2010. Synthesis and *in vitro* evaluation of *N*-alkyl-3-hydroxy-3-(2-imino-3-methyl-5-oxoimidazolidin-4-yl)indolin-2-one analogs as potential anticancer agents. Bioorg. Med. Chem. Lett. 20, 4468–4471.

Pharmacopoeia of India, 2007 vol. 1. Controller of Publications, Ministry of Health Department, Govt. Of India, New Delhi. p. 37.

Podunavac-Kuzmanovic, S.O., Barna, D.J., Cvetkovic, D.D., 2008. Quantitative structure-activity relationships to predict antibacterial effect of some benzimidazole derivatives. APTEFF 39, 1–212.

Randic, M., 1975. On characterization of molecular branching. J. Am. Chem. Soc. 97, 6609–6615.

Randic, M., 1993. Comparative regression analysis: regression based on a single descriptor. Croat. Chem. Acta 66, 289–312.

Rodriguez-Arguelles, M.C., Lopez-Silva, E.C., Sanmartin, J., Pelagatti, P., Zani, F., 2005. Copper complexes of imidazole-2-, pyrrole-2- and indol-3-carbaldehyde thiosemicarbazones: Inhibitory activity against fungi and bacteria. J. Inorg. Biochem. 99, 2231–2239.

Rohini, R., Reddy, P.M., Shanker, K., Kanthaiah, K., Ravinder, V., Hu, A., 2011. Synthesis of mono, bis 2-(2-arylideneaminophenyl) indole azomethines as potential antimicrobial agents. Arch. Pharm. Res. 34, 1077–1084.

Sawant, R.L., Bansode, C.A., Wadekar, J.B., 2013. *In vitro* anti-inflammatory potential and QSAR analysis of oxazolo/thiazolo pyrimidine derivatives. Med. Chem. Res. 22 (4), 1884–1892.

Selvam, P., Murugesh, N., Chandramohan, M., Keith, K.A., Kern, E.R., 2006. Inhibitory activity of 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-*N*-(4,6-dimethylpyrimidin-2-yl) benzene sulphonamide and its derivatives against ortho poxvirus replication *in vitro*. Antiviral Chem. Chemother. 17, 107–110.

Sharma, P., Kumar, A., Sharma, M., 2006. Synthesis and QSAR studies on 5-[2-(2-methylprop1-enyl)-1*H*-benzimidazol-1yl]-4,6-diphenyl- pyrimidin-2-(5*H*)-thione derivatives as antibacterial agents. Eur. J. Med. Chem. 41, 833–840.

Sharma, S.K., Kumar, P., Narasimhan, B., Ramasamy, K., Mani, V., Mishra, R.K., Majeed, A.B.A., 2012. Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1H-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters. Eur. J. Med. Chem. 48, 16–25.

Siddiqui, M., Alam, M.S., Stables, J.P., 2011. Synthesis and anticonvulsant properties of 1-(amino-*N*-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea derivatives. Eur. J. Med. Chem. 46, 2236–2242.

Sigraha, S., Narasimhan, B., Kumar, P., Ramasamy, K., Mani, V., Mishra, R.K., Majeed, A.B.A., 2012. Design, synthesis, antimicrobial, anticancer evaluation, and QSAR studies of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones. Med. Chem. Res. 48, 16–25.

Solomon, V.R., Hua, C., Lee, H., 2009. Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach. Bioorg. Med. Chem. 18, 1563–1572.

Sortino, M., Delgado, P., Jaurez, S., Quiroga, J., Abonia, R., Insuasey, B., Rodero, M.N., Garibotto, F.M., Enriz, R.D., Zaccino, S.A., 2007. Synthesis and antifungal activity of (Z)-5-arylidenerhodanines. Bioorg. Med. Chem. Lett. 15, 484–494.

SPSS for Windows, version 10.05, SPSS Inc., Bangalore, India, 1999.

TSAR 3D Version 3.3, Oxford Molecular Limited, 2000.

Wiener, H., 1947. Structural determination of paraffin boiling points. J. Am. Chem. Soc. 69, 17–20.

Yogeeswari, P., Sriram, D., Kavya, R., Tiwari, S., 2005. Synthesis and *in-vitro* anticancerity evaluation of gatifloxacin mannich bases. Biomed. Pharmacother. 59, 501–510.