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Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters

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ABSTRACT

A series of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters (**1**–**16**) were synthesized and evaluated *in vitro* for their antimicrobial and anticancer potential. 6-Methyl-4-{1-[2-(4-nitro-phenylamino)-acetyl]-1*H*-indol-3-yl}-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**15**, $pMIC_{ec} = 2.50$ μM/mL) was found to be almost equipotent to the standard drug, norfloxacin ($pMIC_{ec} = 2.61$ μM/mL) against *Escherichia coli* and emerged as most potent antimicrobial agent ($pMIC_{am} = 1.84$ μM/mL). 4-{1-[2-(2-Chloro-4-nitro-phenylamino)-acetyl]-1*H*-indol-3-yl}-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**4**, $IC_{50} = 5$ μg/mL) was more potent than the standard drug 5-fluorouracil ($IC_{50} = 6$ μg/mL) against HCT-116 a colon cancer cell line, and emerged as the most potent anticancer agent. The QSAR studies demonstrated the importance of topological parameter, Balaban index (*J*) followed by lipophillic parameter, log *P* in describing the antimicrobial activity of the synthesized compounds.

1. Introduction

The microbial resistance to the currently available antimicrobial agents is the major problem in the treatment of microbial infections. These resistant strains curtail the life span of the drug [1]. Cancer on the other hand is one of the leading causes of death worldwide. It accounted for 7.9 million deaths (around 13% of all deaths) in 2007 and with an estimated 12 million deaths in 2030. Over the last 50 years, many drugs have been developed with the aim to impair this pandemic illness. However, a high percentage of them failed due to lack of selectivity and adverse events. Considering this, the development of novel chemotherapeutic agents, which selectively act on the target without side effects, has become a primary objective for medicinal chemists [2].

Recent literature revealed that indole derivatives have been reported to have wide spectrum of biological activities *viz.* antimicrobial [3], analgesic [4], anticancer [5], antihypertensive [6], anti-asthmatic [7], anti-Alzheimer [8], antimalaria [9], antidiabetic [10] and anti-HIV activities [11]. Quantitative structure–activity relationships (QSAR) are predictive tools for preliminary evaluation of the activity of chemical compounds using computer-aided models, which increase the probability of success, reduced time and cost involvement in drug discovery process [12]. A recent study by Heda et al. discussed the synthesis and antimicrobial activity of 5-substituted indole derivatives [13].

Tetrahydropyrimidenes are important members of the pyrimidine family, known to possess a wide range of biological activities. Recently, a new series of 5-(3-(1*H*-indol-3-yl)-substituted phenyl allylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)triones were synthesized by Biradar et al. using simple and efficient condensation of chalcones with barbituric acid. The synthesized compounds were screened for their antioxidant (free radical scavenging, total antioxidant capacity and ferric reducing antioxidant power) and DNA cleavage activities. Some of the synthesized compounds especially 5-((*E*)-3-(1*H*-indol-3-yl)-1-*p*-tolylallylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)trione exhibited excellent DNA cleavage activity [14]. Akue-Gedu et al. synthesized a series of amino-pyridyl indole derivatives and tested

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them for their *in vitro* antiproliferative activities toward a human fibroblast primary culture and two human solid cancer cell lines (MCF-7 and PA 1). Among the synthesized compounds 4-(1*H*-Indol-3-yl)-5-(3-aminophenyl)pyrimidin-2-amine reported to have potential anticancer activity [15].

It is envisaged that the combination of two pharmacophores *viz*. combination of indole and tehydropyrimidine (Scheme 1) would generate novel molecular templates, which are likely to exhibit interesting biological properties. Motivated by the above facts and in continuation of our research efforts in the field of synthesis, antimicrobial and QSAR studies [16,17], we hereby report the synthesis, antimicrobial, anticancer and QSAR studies of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

2. Results and discussion

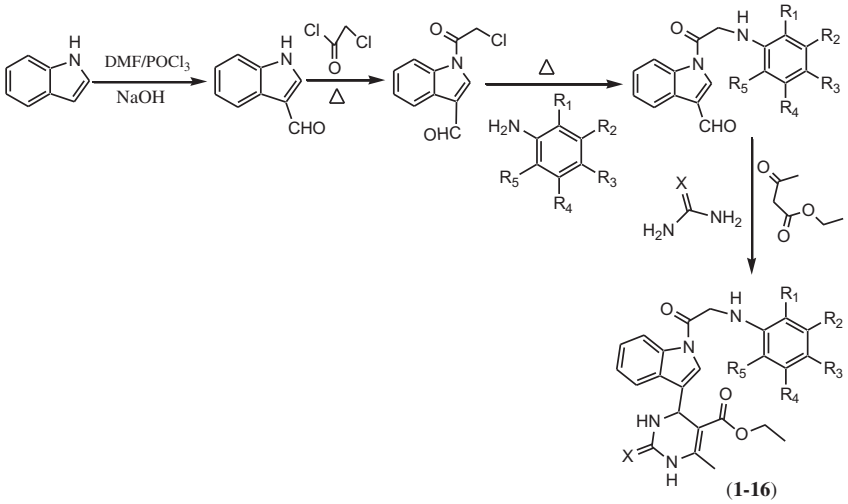
2.1. Chemistry

Synthesis of the target compounds (**1**–**16**) was carried out as outlined in Scheme 1. All the compounds were obtained in appreciable yield and their physicochemical characteristics are presented in Table 1. The structures of the synthesized compounds (**1**–**16**) were ascertained on the basis of their consistent IR and NMR spectral characteristics. The IR stretching bands in the region 1694–1612 cm^{-1} indicated the formation of tertiary amide linkage. IR bands ranging from 1343 to 1202 cm^{-1} indicated the presence of secondary amine in the synthesized compounds. Further the presence of C=O stretching bands in the range 1457–1321 cm^{-1} confirmed the formation of ester as well as the target compounds. Presence of tetrahydropyrimidine and indole nucleus in the target compounds was indicated by IR stretching bands in the range 3098–2860 cm^{-1} and 3475–3353 cm^{-1} respectively. IR bands in

Table 1
Physicochemical characteristics of synthesized 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

Comp.	Mol. formula	M.wt.	Mp (°C)	R_f value[a]	% Yield
1	$C_{24}H_{24}N_4O_3S$	448	412–414	0.60	84.24
2	$C_{24}H_{23}ClN_4O_3S$	483	398–400	0.57	81.00
3	$C_{24}H_{22}ClN_5O_5S$	528	420–422	0.48	78.20
4	$C_{24}H_{22}ClN_5O_5$	512	408–410	0.54	80.10
5	$C_{25}H_{25}N_5O_6$	491	402–404	0.49	76.40
6	$C_{25}H_{25}N_5O_5S$	508	428–430	0.51	82.33
7	$C_{24}H_{23}N_5O_6$	477	386–388	0.53	78.25
8	$C_{24}H_{23}N_5O_5S$	494	412–414	0.41	79.00
9	$C_{24}H_{23}N_5O_5S$	494	417–419	0.45	82.50
10	$C_{24}H_{23}N_5O_6$	477	424–426	0.52	83.40
11	$C_{24}H_{23}N_5O_6$	477	406–408	0.47	82.55
12	$C_{24}H_{25}N_5O_4$	447	392–394	0.48	81.30
13	$C_{24}H_{23}ClN_4O_3S$	483	416–418	0.68	83.28
14	$C_{24}H_{23}ClN_4O_4$	467	378–380	0.47	81.78
15	$C_{24}H_{23}N_5O_5S$	494	410–412	0.54	84.20
16	$C_{24}H_{25}N_5O_3S$	464	422–424	0.51	78.00

[a] TLC mobile phase = benzene.



Comp.	R_1	R_2	R_3	R_4	R_5	X	Comp.	R_1	R_2	R_3	R_4	R_5	X
1	H	H	H	H	H	S	**9**	H	NO_2	H	H	H	S
2	Cl	H	H	H	H	S	**10**	H	NO_2	H	H	H	O
3	H	Cl	NO_2	H	H	S	**11**	H	H	NO_2	H	H	O
4	H	Cl	NO_2	H	H	O	**12**	NH_2	H	H	H	H	O
5	CH_3	H	NO_2	H	H	O	**13**	H	H	Cl	H	H	S
6	CH_3	H	NO_2	H	H	S	**14**	H	H	Cl	H	H	O
7	NO_2	H	H	H	H	O	**15**	H	H	NO_2	H	H	S
8	NO_2	H	H	H	H	S	**16**	NH_2	H	H	H	H	S

Scheme 1. Synthetic route followed for the synthesis of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine -5-carboxylic acid ethyl esters.

the range 1615–1597 and 1458–1420 cm^{-1} indicated the presence of oxo or thioxo group in the synthesized compounds. IR bands in the region 1561–1458 cm^{-1}, confirmed presence of aromatic ring in the target compounds.

The appearance of triplet signal in the range of δ 1.18–1.61 and quadrate signal at δ 2.01–4.25 indicated the ester formation in synthesized compounds. Further, the appearance of singlet signal at δ 3.66–4.89 indicated the presence of secondary amine and confirmed the formation of target compounds. The appearance of multiplet signal around δ 6.57–8.06 depicts the presence of protons of aromatic nucleus and signal at δ 1.63–2.19 indicated the presence of tetrahydropyrimidine nucleus in the synthesized compounds. The elemental analysis results were within ±0.4% of the theoretical values.

2.2. Antimicrobial activity

The synthesized compounds (**1**–**16**) were screened *in vitro* for their antimicrobial activity by tube dilution method using norfloxacin and fluconazole as reference drugs for antibacterial and antifungal activities, respectively. The antimicrobial activity result is presented in Table 2.

Antimicrobial screening demonstrated compounds **10** and **11** to possess antibacterial activity against *Staphylococcus aureus* ($pMIC_{sa}$ = 1.88 μM/mL each). In the case of *Bacillus subtilis*, compound **4** emerged as most effective antibacterial agent with $pMIC_{bs}$ value 1.61 μM/mL. Among the synthesized compounds **13** and **15** ($pMIC_{ec}$ values 2.19 and 2.50 μM/mL respectively) emerged as the most active candidates against the Gram-negative bacterium *Escherichia coli*. Compound **15** ($pMIC_{ca}$ = 1.90 μM/mL) and compound **3** ($pMIC_{an}$ = 1.63 μM/mL) emerged as the most effective antifungal agent against *Candida albicans* and *Aspergillus niger* respectively. The antibacterial results depicted that the synthesized indole derivatives have better antibacterial potential against Gram-negative bacterium *E. coli* [compound **15** being nearly equipotent to the standard drug norfloxacin ($pMIC_{ec}$ = 2.61 μM/mL)] than Gram-positive bacteria. Results from the antifungal study indicated that the synthesized compounds were less effective than the standard drug, fluconazole ($pMIC_{af}$ = 2.64 μM/mL).

In general, the results of MBC/MFC (Table 3) revealed that the synthesized compounds were bacteriostatic and fungistatic in action as their MFC and MBC values were 3-fold higher than their MIC values (a drug is considered to be bacteriostatic/fungistatic when its MFC and MBC values are 3-fold higher than its MIC values) [18].

2.3. 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 (LFER) model described by Hansch and Fujita [19]. Biological activity data determined as MIC values was first transformed into pMIC values (*i.e.* −log MIC) and used as dependent variable in the QSAR study. The different molecular descriptors (independent variables) selected for the present study are listed in Table 4. The values of selected molecular descriptors used in the QSAR study are presented in Table 5.

Our earlier studies [16,17] indicated that the multi-target QSAR (*mt*-QSAR) models are better than one-target QSAR (*ot*-QSAR) models in describing the antimicrobial activity. In the present study therefore, we developed multi-target QSAR models to describe the antimicrobial activity of the synthesized 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

According to the *ot*-QSAR models one should use five different equations with different errors to predict the activity of a new compound against the five microbial species. The utilization of *ot*-QSAR models, which are almost in the whole literature however, was not practical when we had to predict each compound results for more than one target. In those cases we had to develop one *ot*-QSAR for each target. However, very recently the interest has increased in the development of multi-target QSAR (*mt*-QSAR) models. As opposed to *ot*-QSAR, the *mt*-QSAR model is a single equation that considers the nature of molecular descriptors, which are common and essential for describing the antibacterial and antifungal activities [20–23].

In the present study, we attempted to develop three different types of *mt*-QSAR models *viz. mt*-QSAR model to describe antibacterial activity of the synthesized compounds against *S. aureus*,

Table 2
Antimicrobial activity (μM/mL) of synthesized 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	1.55	1.25	1.55	1.55	1.55	1.45	1.55	1.49
2	1.29	1.29	1.59	1.59	1.59	1.39	1.59	1.47
3	1.63	1.32	1.63	1.63	1.63	1.53	1.63	1.57
4	1.61	1.61	1.61	1.61	1.61	1.61	1.61	1.61
5	1.59	1.29	1.59	1.59	1.59	1.49	1.59	1.53
6	1.61	1.61	1.61	1.61	1.61	1.61	1.61	1.61
7	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
8	1.30	1.60	1.60	1.60	1.60	1.50	1.60	1.54
9	1.60	1.60	1.90	1.60	1.60	1.70	1.60	1.66
10	1.88	1.58	1.88	1.58	1.58	1.78	1.58	1.70
11	1.88	1.58	1.88	1.58	1.58	1.78	1.58	1.70
12	1.55	1.55	1.85	1.55	1.55	1.65	1.55	1.61
13[a]	1.59	1.59	2.19	1.59	1.59	1.79	1.59	1.71
14[a]	1.57	1.27	1.27	1.57	1.57	1.37	1.57	1.45
15[a]	1.60	1.60	2.50	1.90	1.60	1.90	1.75	1.84
16[a]	1.87	1.57	2.17	1.57	1.57	1.87	1.57	1.75
S.D	0.17	0.15	0.31	0.08	0.02	0.17	0.04	0.11
Std.	2.61[b]	2.61[b]	2.61[b]	2.64[c]	2.64[c]	–	–	–

[a] Outliers.
[b] Norfloxacin.
[c] Fluconazole.

Table 3
MBF/MFC of synthesized 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

Comp.	Minimum bactericidal/fungicidal concentration (μM/mL)				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	>0.11	>0.11	>0.11	>0.11	>0.11
2	>0.10	>0.10	>0.10	>0.10	>0.10
3	>0.09	>0.09	>0.09	>0.09	>0.09
4	>0.10	>0.10	>0.10	>0.10	>0.10
5	>0.10	>0.10	>0.10	>0.10	>0.10
6	>0.10	>0.10	>0.10	>0.10	>0.10
7	>0.10	>0.10	>0.10	>0.10	>0.10
8	>0.10	>0.10	>0.10	>0.10	>0.10
9	>0.10	>0.10	0.10	>0.10	>0.10
10	0.10	>0.10	0.10	>0.10	>0.10
11	0.10	>0.10	0.10	>0.10	>0.10
12	>0.11	>0.11	0.11	>0.11	>0.11
13	>0.10	>0.10	0.05	>0.10	>0.10
14	>0.11	>0.11	>0.11	>0.11	>0.11
15	>0.10	>0.10	0.03	0.10	>0.10
16	0.11	>0.11	0.05	>0.11	0.11
Standard	0.019[a]	0.019[a]	0.019[a]	0.040[b]	0.040[b]

[a] Norfloxacin.
[b] Fluconazole.

Table 4
QSAR descriptors used in the study.

S.No.	QSAR descriptor	Type
1	log *P*	Lipophilic
2	Zero order molecular connectivity indices ($^0\chi$)	Topological
3	First order molecular connectivity indices ($^1\chi$)	Topological
4	Second order molecular connectivity indices ($^2\chi$)	Topological
5	Valence zero order molecular connectivity indices ($^0\chi^v$)	Topological
6	Valence first order molecular connectivity indices ($^1\chi^v$)	Topological
7	Valence second order molecular connectivity indices ($^2\chi^v$)	Topological
8	Kier's alpha first order shape indice ($\kappa\alpha_1$)	Topological
9	Kier's alpha second order shape indice ($\kappa\alpha_2$)	Topological
10	Kier's first order shape indice (κ_1)	Topological
11	Randic topological index	Topological
12	Balaban topological index	Topological
13	Wiener's topological index	Topological
14	Kier's second order shape indice (κ_2)	Topological
15	Ionization potential	Electronic
16	Dipole moment (μ)	Electronic
17	Energy of highest occupied molecular orbital (HOMO)	Electronic
18	Energy of lowest unoccupied molecular orbital (LUMO)	Electronic
19	Total energy (Te)	Electronic
20	Molar refractivity (MR)	Steric

B. subtilis and *E. coli*, *mt*-QSAR model to describe antifungal activity of the synthesized compounds against *C. albicans* and *A. niger* as well as a common *mt*-QSAR model to describe the antimicrobial (overall antibacterial and antifungal) activity of the synthesized indole derivatives against all of the above mentioned microorganisms.

In order to develop *mt*-QSAR models, initially we calculated the average antibacterial, antifungal and antimicrobial activities values of indole derivatives (Table 2). These average activity values were correlated with the molecular descriptors of the synthesized compounds (Table 6). In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between valence second order molecular connectivity index ($^2\chi^v$) and valence third order molecular connectivity index ($^3\chi^v$) ($r = 0.994$), and low interrelationship was observed for lipophillic parameter log *P* and Balaban topological index, *J* ($r = 0.057$).

In the present study, among the synthesized 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters (**1**–**16**), four molecules (**13**–**16**) were identified as outliers because their presence resulted in a loss of correlation ($r = 0.439$, Eq. (1)), whereas their removal improved the *r* value significantly with Balaban index, *J* ($r = 0.705$, Eq. (2)) during regression analysis. In multivariate statistics, it is common to define three types of outliers [24].

1. *X*/*Y* relation outliers are substances for which the relationship between the descriptors (*X* variables) and the dependent variables (*Y* variables) are not the same as in the (rest of the) training data.
2. *X* outliers are substances whose molecular descriptors 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.

There was no difference in the activity (Table 2) as well as the molecular descriptor range (Table 6) of these outliers (**13**–**16**) when compared to the other indole derivatives. This indicated a fact that the outliers belong to the category of *Y* outliers (substances for which the reference value of response is invalid).

From the correlation matrix (Table 6), it was observed that the topological parameter Balaban index (*J*) was found to dominate description of the antibacterial activity of the synthesized compounds (Eq. (2)).

2.3.1. LR-mt-QSAR model for antibacterial activity

$$\begin{aligned} &\mathrm{pMIC_{ab}} = -3.329J + 6.105 \\ &n = 16 \quad r = 0.439 \quad q^2 = 0.135 \quad s = 0.154 \quad F = 3.34 \end{aligned} \tag{1}$$

2.3.2. LR-mt-QSAR model for antibacterial activity

$$\begin{aligned} &\mathrm{pMIC_{ab}} = -4.141J + 7.159 \\ &n = 12 \quad r = 0.705 \quad q^2 = 0.284 \quad s = 0.092 \quad F = 9.91 \end{aligned} \tag{2}$$

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.

The topological parameters signify the degree of branching, connectivity of atoms and the unsaturation in the molecule, that accounts for variation in activity. The topological parameter, Balaban index $J = J(G)$ of G is defined as

Table 5
Values of selected parameters used in QSAR studies of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

Comp.	$^2\chi^v$	$^3\chi^v$	$\kappa\alpha_2$	$\kappa\alpha_3$	*J*	LUMO	HOMO	μ	log *P*	MR
1	7.82	0.90	9.74	4.68	1.36	−0.88	−8.95	2.37	2.92	127.99
2	8.37	1.07	10.15	4.87	1.37	−0.90	−8.96	3.84	3.44	132.79
3	8.81	1.18	10.95	5.41	1.34	−1.16	−9.21	8.43	3.40	140.12
4	8.37	1.07	10.68	5.25	1.34	−1.12	−9.21	7.96	1.99	132.13
5	8.27	1.04	10.50	5.14	1.34	−1.07	−9.12	6.42	1.94	132.36
6	8.71	1.15	10.76	5.30	1.34	−1.08	−9.15	6.32	3.34	140.35
7	7.78	0.89	10.27	4.91	1.37	−0.92	−9.09	6.71	1.48	127.32
8	8.23	1.00	10.54	5.06	1.37	−1.04	−9.12	6.54	2.88	135.31
9	8.26	1.02	10.54	5.21	1.31	−1.12	−9.11	6.55	2.88	135.31
10	7.82	0.91	10.27	5.05	1.31	−1.08	−9.09	6.98	1.48	127.32
11	7.81	0.91	10.27	5.05	1.32	−1.07	−9.13	6.49	1.48	127.32
12	7.60	0.87	9.67	4.60	1.37	−0.53	−8.34	0.87	0.74	124.70
13[a]	8.43	1.10	10.15	5.02	1.35	−0.92	−8.99	2.85	3.44	132.79
14[a]	7.99	0.99	9.88	4.86	1.35	−0.58	−8.97	1.85	2.04	124.80
15[a]	8.25	1.02	10.54	5.21	1.32	−1.08	−9.15	6.34	2.88	135.31
16[a]	8.04	0.98	9.94	4.75	1.37	−0.88	−8.36	1.91	2.14	132.69

[a] Outliers.

Table 6
Correlation matrix for the antibacterial, antifungal and antimicrobial activity of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$	$^2\chi^v$	$^3\chi^v$	$\kappa\alpha_2$	*J*	log *P*	MR
$pMIC_{ab}$	1.000	−0.059	0.992	−0.349	−0.349	0.048	−0.705	−0.570	−0.291
$pMIC_{af}$		1.000	0.067	0.894	0.869	0.971	−0.278	0.556	0.862
$pMIC_{am}$			1.000	−0.236	−0.240	0.170	−0.740	−0.499	−0.182
$^2\chi^v$				1.000	0.994	0.833	−0.131	0.780	0.959
$^3\chi^v$					1.000	0.802	−0.136	0.746	0.931
$\kappa\alpha_2$						1.000	−0.363	0.459	0.825
J							1.000	0.057	−0.131
log P								1.000	0.815
MR									1.000

$$J = M/(\mu + 1) \sum_{\text{Bonds}} (\text{d}i \cdot \text{d}j) - 0.5$$

where *M* is the number of bonds in *G*, μ is the cyclomatic number of *G*, and di (i = 1,2,3, *N*; *N* is the number of vertices in *G*) is the distance sum. The cyclomatic number $\mu = \mu(G)$ of a cyclic graph *G* is equal to the minimum number of edges necessary to be erased from *G* in order to transform it into the related acyclic graph. In case of monocyclic graph $\mu = 1$ otherwise it is calculated by means of the following expression [25].

$$\mu = M - N + 1.$$

Coupling of topological parameter Balaban index (*J*) with lipophillic parameter, log *P* significantly improved the correlation coefficient from 0.705 to 0.882 (Eq. (3)). Further low correlation coefficient between *J* and log *P* ($r = 0.057$) justifies their coupling.

2.3.3. MLR-mt-QSAR model for antibacterial activity

$$\begin{aligned} pMIC_{ab} &= -3.963\,J - 0.717 \log P + 7.086 \\ n &= 12 \quad r = 0.882 \quad q^2 = 0.674 \quad s = 0.064 \quad F = 15.83 \end{aligned} \tag{3}$$

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 [26].

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 [27].

The developed QSAR model (Eq. (3)) was cross validated by q^2 value ($q^2 = 0.674$) obtained by leave one out (LOO) method. Each compound is eliminated once, a model is derived from the remaining compounds and the eliminated compounds are predicted from this model. The same procedure is repeated after elimination of another compound until all the compounds have been eliminated once [28]. The value of q^2 more than 0.5 indicated that the model developed is a valid one. According to the recommendations of Golbraikh and Tropsha, the only way to estimate the true predictive power of a model is to test their ability to predict accurately the biological activities of compounds. As the observed and predicted values are close to each other (Table 7), the *mt*-QSAR model for antibacterial activity (Eq. (3)) is a valid one [29]. The plot of predicted $pMIC_{ab}$ against observed $pMIC_{ab}$ (Fig. 1) also favors the developed model expressed by Eq. (3). Further, the plot of observed $pMIC_{ab}$ *vs* residual $pMIC_{ab}$ (Fig. 2) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero [30].

Topological parameter Kier's alpha second order shape index ($\kappa\alpha_2$) was found to be most effective in describing antifungal activity of the synthesized compounds (Eq. (4)).

2.3.4. LR-mt-QSAR model for antifungal activity

$$\begin{aligned} pMIC_{af} &= 0.060\,k\alpha_2 + 0.971 \\ n &= 12 \quad r = 0.971 \quad q^2 = 0.923 \quad s = 0.006 \quad F = 167.81 \end{aligned} \tag{4}$$

Antifungal activity of the synthesized compounds is positively correlated with Kier's alpha second order shape indice ($\kappa\alpha_2$). Compounds **3** and **15** having high $\kappa\alpha_2$ values (10.95 and 10.54 respectively) displayed high antifungal activity ($pMIC_{af} = 1.63$ and 1.75 respectively). Similarly compounds **1** and **12** having low $\kappa\alpha_2$ values (9.74 and 9.67 respectively) exhibited low antifungal potential ($pMIC_{af} = 1.55$ each).

A set of very useful topological indices of the second generation is composed by the kappa indices of molecular shape and flexibility [31]. According to Kier, the shape of a molecule may be partitioned into attributes, each describable by the count of bonds of various path lengths. The basis for devising a relative index of shape is given by the relationship of the number of path of length *l* in the molecule *i*, ^lP_i, to some reference values based on molecules with a given number of atoms, *n*, in which the values of ^lP are maximum and minimum, $^lP_{max}$ and $^lP_{min}$.

Table 7
Comparison of observed and predicted antimicrobial activity obtained by *mt*-QSAR model.

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.45	1.49	−0.04	1.55	1.55	0.00	1.49	1.53	−0.04
2	1.39	1.43	−0.04	1.59	1.58	0.01	1.47	1.50	−0.03
3	1.53	1.53	0.00	1.63	1.62	0.01	1.57	1.56	0.01
4	1.61	1.63	−0.02	1.61	1.61	0.00	1.61	1.61	0.00
5	1.49	1.64	−0.15	1.59	1.60	−0.01	1.53	1.62	−0.09
6	1.61	1.54	0.07	1.61	1.61	0.00	1.61	1.56	0.05
7	1.58	1.55	0.03	1.58	1.58	0.00	1.58	1.56	0.02
8	1.50	1.45	0.05	1.60	1.60	0.00	1.54	1.50	0.04
9	1.70	1.68	0.02	1.60	1.60	0.00	1.66	1.65	0.01
10	1.78	1.78	0.00	1.58	1.58	0.00	1.70	1.70	0.00
11	1.78	1.74	0.04	1.58	1.58	0.00	1.70	1.68	0.02
12	1.65	1.62	0.03	1.55	1.55	0.00	1.61	1.60	0.01
13[a]	1.79	1.68	0.11	1.59	1.58	0.01	1.71	1.54	0.17
14[a]	1.37	1.68	−0.31	1.57	1.56	0.01	1.45	1.59	−0.14
15[a]	1.90	1.77	0.13	1.75	1.60	0.15	1.84	1.62	0.22
16[a]	1.87	1.60	0.27	1.57	1.57	0.00	1.75	1.54	0.21

[a] Outliers.

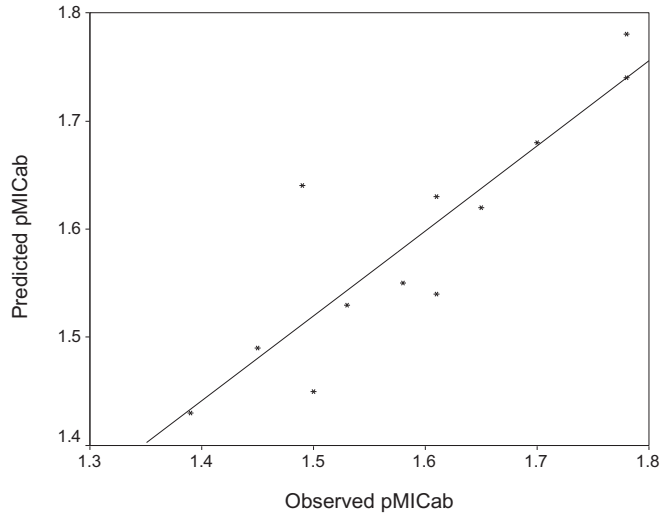


Fig. 1. Plot of predicted $pMIC_{ab}$ values against observed $pMIC_{ab}$ values for linear regression developed model by Eq. (2).

The modified kappa shape indices are given by:

$$k\alpha_1 = (n+\alpha)(n+\alpha-1)^2 \Big/ \left({}^1P_i+\alpha\right)^2$$

$$k\alpha_2 = (n+\alpha-1)(n+\alpha-2)^2 \Big/ \left({}^2P_i+\alpha\right)^2$$

$$k\alpha_3 = (n+\alpha-1)(n+\alpha-3)^2 \Big/ \left({}^3P_i+\alpha\right)^2 n \text{ is odd}$$

$$k\alpha_3 = (n+\alpha-3)(n+\alpha-2)2 \Big/ \left({}^3P_i+\alpha\right)^2 n \text{ is even.}$$

The *mt*-QSAR model for antimicrobial activity (Eq. (5)) depicted the importance of Balaban topological index (J) in describing antimicrobial activity of the synthesized compounds.

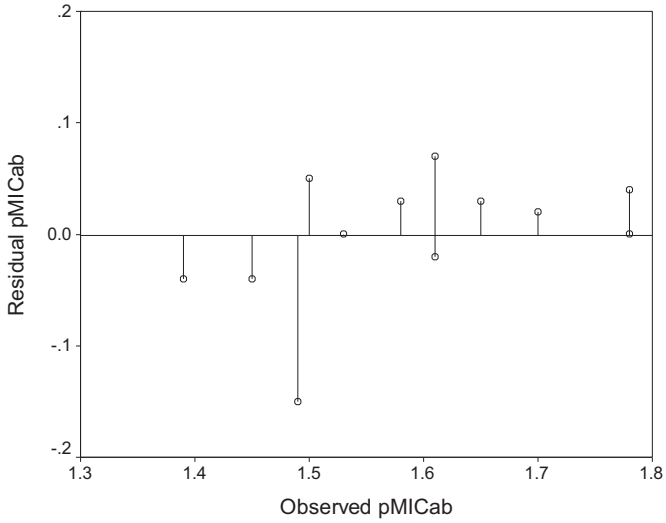


Fig. 2. Plot of residual $pMIC_{ab}$ values against observed $pMIC_{ab}$ values for linear regression developed model by Eq. (2).

2.3.5. LR-mt-QSAR model for antimicrobial activity

$$\begin{aligned} &\mathrm{pMIC_{am}} = -2.608J + 5.097 \\ &n = 12 \quad r = 0.740 \quad q^2 = 0.361 \quad s = 0.053 \quad F = 12.12 \end{aligned} \tag{5}$$

In search of a better QSAR model, we coupled Balaban topological index (J) with lipophillic parameter, log P and this change resulted in improvement of correlation coefficient from 0.740 to 0.870 (Eq. (6)).

2.3.6. MLR-mt-QSAR model for antimicrobial activity

$$\begin{aligned} &\mathrm{pMIC_{am}} = -2.516J - 0.037\log P + 5.059 \\ &n = 12 \quad r = 0.870 \quad q^2 = 0.642 \quad s = 0.040 \quad F = 14.03 \end{aligned} \tag{6}$$

The validity and predictability of the QSAR models *i.e.* Eqs. (4) and (6) are indicated by high values of their correlation coefficient (r) as well as the low residual values (Table 7). It is important to note a fact here that the removal of compounds **13**–**16** as outliers was justified from the high residual values observed in their case (Table 7). It can be concluded from *mt*-QSAR models [Eqs. (2)–(6)] that the antibacterial, antifungal and the overall antimicrobial activities of the synthesized indole derivatives are governed by the topological parameter, Balaban index (J) followed by lipophillic parameter, log P.

Generally, for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. However, in the present study the range of antimicrobial activities of the synthesized compounds was within one order of magnitude. This is in accordance with results suggested by Bajaj et al. [32] who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data were in the narrow range. When biological activity data lies in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies [33]. The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies its use in QSAR studies.

2.4. The anticancer activity

The anticancer activity of the synthesized 6-methyl-4-[1-(2-substituted- phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters (**1**–**16**) was determined against breast cancer (MCF 7) and colon cancer (HCT 116) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the results are presented in Table 8. 4-{1-[2-(2-Amino-phenylamino)-acetyl]-1*H*-indol-3-yl}-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**12**) was found to be the most effective against MCF-7 cancer cell lines with an IC_{50} value of 2.5 μg/mL (Table 8). Against HCT-116 cancer cell lines, 4-{1-[2-(2-chloro-4-nitro-phenylamino)-acetyl]-1*H*-indol-3-yl}-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**4**) emerged as the most effective one with an IC_{50} value of 5 μg/mL. In general, all the synthesized compounds were less active than the standard drug 5-fluorouracil (5-FU), but compound **4** was more potent than 5-FU against HCT-116 cell lines, which indicates that this compound may be taken as a lead compound to develop novel anticancer agents.

2.5. Structure–activity relationship

The structure–activity relationship deduced from the antimicrobial and anticancer results can be summarized as follows:

Table 8
Cytotoxicity (IC_{50}) of synthesized 6-methyl-4-[1-(2-substituted- phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters against human colon cell line HCT 116 and breast cancer cell line MCF 7.[a]

Comp.	MCF 7	HCT 116
	IC_{50} (μg/mL)	IC_{50} (μg/mL)
1	52	450
2	ND	ND
3	50	29
4	50	5
5	50	600
6	48	>1000
7	40	250
8	550	>1000
9	>1000	130
10	ND	ND
11	>1000	NA
12	2.5	300
13	80	NA
14	200	NA
15	>1000	NA
16	100	NA
5-FU	0.67	6

NA – Not able to obtain IC_{50} after three independent tests, ND – Not detected.
[a] Data represents mean values of three replicates.

- Antimicrobial screening results indicated that the presence of electron withdrawing groups on phenylamino moiety enhances antimicrobial activity of the synthesized compounds against all bacterial and fungal strains tested and electron-releasing groups did not significantly improve antimicrobial activity. The role of electron withdrawing groups in improving antimicrobial activities is supported by Laxmi et al. in their study on synthesis and antimicrobial evaluation of 1-(2-oxo-2-phenyl-ethyl)-2-phenyl-1*H*-indole-3yl)methylene) semi-carbazone derivatives [34].
- In the case of antibacterial activity against *E. coli*, electron withdrawing nitro group on phenylamino moiety enhances activity greater than electron withdrawing chloro group. Further electron withdrawing nitro and chloro groups on phenylamino moiety produced synergistic action in improving antimicrobial activity of the synthesized compounds against *B. subtilis* and *C. albicans*.
- Presence of thioxo moiety on 2-position of tetrahydropyrimidine ring improved antimicrobial activity against Gram (–ve) bacterium and fungal species, whereas oxo moiety enhanced antimicrobial against Gram (+ve) bacterial species.
- Anticancer study indicated that the presence of electron withdrawing group on phenylamino moiety enhanced anticancer activity of the synthesized compounds against HCT-116 cancer cell lines, whereas electron-releasing group increases anticancer activity against MCF-7 cancer cell lines.

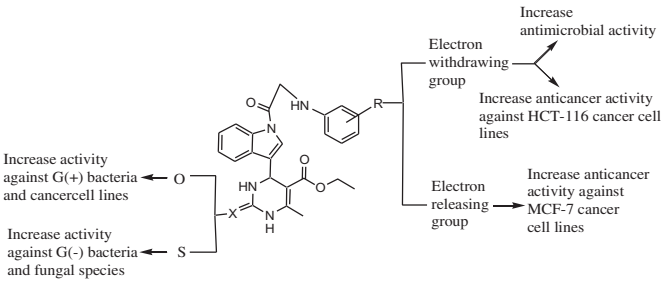


Fig. 3. Structural requirements for the antimicrobial and anticancer activities of 6-methyl-4-[1-(2-substituted- phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters.

- Presence of oxo moiety on 2-position of tetrahydropyrimidine ring improved anticancer activity against both the cancer cell lines tested and thiooxo moiety did not significantly improve anticancer activity. This is in accordance with the findings of Biradar et al. [14]. The aforementioned findings are summarized in Fig. 3.

3. Conclusion

In the present study, a series of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters (**1**–**16**) were synthesized and evaluated for their *in vitro* antimicrobial and anticancer potentials. The results from the antimicrobial studies indicated that the synthesized compounds were less effective antifungal agents than the standard drug, fluconazole ($pMIC_{af} = 2.64$ μM/mL). 6-Methyl-4-{1-[2-(4-nitro-phenylamino)-acetyl]-1*H*-indol-3-yl}-2-thioxo-1,2,3, 4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**15**, $pMIC_{ec} = 2.50$ μM/mL) was found to be almost equipotent to standard drug, norfloxacin ($pMIC_{ec} = 2.61$ μM/mL) against *E. coli* and emerged as most potent antimicrobial agent ($pMIC_{am} = 1.84$ μM/mL).The results from the QSAR studies demonstrated the importance of topological parameters Balaban index (*J*) followed by lipophillic parameter, log *P* in describing the antimicrobial activity of the synthesized compounds. The anticancer screening results indicated that the synthesized compounds were less active than the standard drug 5-fluorouracil (5-FU) but 4-{1-[2-(2-chloro-4-nitro-phenylamino)-acetyl]-1*H*-indol-3-yl}-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester (**4,** $IC_{50} = 5$ μg/mL) was more potent than 5-FU ($IC_{50} = 6$ μg/mL) against HCT-116 and has the potential to be taken as a lead compound for the development novel anticancer agents.

4. Experimental

Starting materials were obtained from commercial sources and were used without further purification. Reaction progress was observed by thin layer chromatography making use of commercial silica gel plates (Merck), Silica gel F254 on aluminum sheets. Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined by Bruker Avance II 400 NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (d, ppm) downfield from tetramethylsilane (internal standard) NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on a Varian Resolutions Pro FTIR spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer.

*4.1. General procedure for the synthesis 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 (**1**–**16**)*

4.1.1. General procedure for the synthesis of 1H-indole-3-carbaldehyde

A solution of indole (0.21 mol) in 100 mL dimethylformamide was prepared and kept aside. A formylation complex was also prepared by cooling 80 mL dimethylformamide in an external ice bath (internal temperature about 12 °C), followed by the addition of 20 mL phosphorus oxychloride dropwise over the course of 30 min. This formylation mixture was then warmed to 25 °C and added the solution of indole in dimethylformamide dropwise (with continued stirring) over a period of 30 min. Stirring was continued for yet another 45 min, during which time the temperature was raised to

40 °C. The reaction mixture was then poured onto chipped ice which produced a clear red solution. This was made basic with the addition of 200 mL of 5 N sodium hydroxide which allowed the separation of a yellow solid. This was diluted by the addition of 200 mL hot water and, after cooling the product was removed by filtration and washed with cold water. The product was recrystallized from aqueous dimethylformamide to yield 1*H*-indole-3-carbaldehyde as faint orange needles.

4.1.2. General procedure for the synthesis of 1-(2-chloro-acetyl)-1H-indole-3-carbaldehyde

A mixture of 1*H*-indole-3-carbaldehyde (0.1 mol), choloroacetyl chloride (0.1 mol), in ethanol was heated under reflux for 10–12 h. TLC was used to monitor the progress of the reaction. After completion of reaction, the reaction mixture was poured on crushed ice and filtered under suction; the precipitate thus obtained was washed with water and recrystallized from ethanol.

4.1.3. General procedure for the synthesis of 1-(2-substituted-phenylamino-acetyl)-1H-indole-3-carbaldehyde

A mixture of 1-(2-chloro-acetyl)-1*H*-indole-3-carbaldehyde (0.1 mol) and corresponding aniline (0.1 mol), in ethanol was heated under reflux for 12–15 h. TLC was used to monitor the progress of the reaction. After completion of reaction, the reaction mixture was poured on crushed ice and filtered under suction, the precipitate thus obtained was washed with water and recrystallized from ethanol.

*4.1.4. General procedure for the synthesis 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 (**1**–**16**)*

A mixture of 1-(2-substituted-phenylamino-acetyl)-1*H*-indole-3-carbaldehyde (0.1 mol), urea or thiourea (0.2 mol), ethyl-acetoacetate (0.1 mol) in ethanol was heated under reflux for 12–15 h in the presence of hydrochloric acid. TLC was used to monitor the progress of the reaction. After completion of reaction, the reaction mixture was poured on crushed ice and filtered under suction, the precipitate was washed with water. The pure product was obtained by recrystallization from ethanol.

Compound **1**: Mp (°C) 412–414; Yield – 84.24%; IR (KBr pellets) cm^{-1} 3398 (NH str., indole), 1612 (C=O str., 3^0 amide), 1268 (C–N str., aryl 2^0 amine), 1420 (C=S str., thioxo), 2939 (CH str., tetrahydropyrimidine), 1322 (C–O–C str.), 1236 (CH in plane bending, Ar.), 1521 (C=C skeletal str., Ar.); ^{1}H NMR (MEOD): δ 1.27–1.308 (t, 3H, CH_3 of OC_2H_5), 3.92–4.07 (m, CH_2 of OCH_2CH_3), 2.19 (s, 2H –NH pyrimidine), 4.03 (s, 1H, NH 2^0 amine); Anal. Calculated for $C_{24}H_{24}N_4O_3S$: C, 64.27; H, 5.39; N, 12.49; Found: C, 64.30; H, 5.35; N, 12.48.

Compound **2**: Mp (°C) 398–400; Yield – 81.00%; IR (KBr pellets) cm^{-1} 3397 (NH str., indole), 1613 (C=O str., 3^0 amide), 1269 (C–N str., aryl 2^0 amine), 1458 (C=S str., thioxo), 1322 (C–O–C str.), 1236 (CH in plane bending, Ar.), 1523 (C=C skeletal str., Ar.) 742 (C–Cl); ^{1}H NMR (MEOD): δ 6.90–7.88 (m, 9H, ArH), 1.25–1.29 (t, 3H, CH_3 of OC_2H_5), 3.33–3.64 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{23}ClN_4O_3S$: C, 59.68; H, 4.80; N, 11.60; Found: C, 59.60; H, 4.84; N, 11.58.

Compound **3**: Mp (°C) 420–422; Yield – 78.20%; IR (KBr pellets) cm^{-1} 3474 (NH str., indole), 1636 (C=O str., 3^0 amide), 1268 (C–N str., aryl 2^0 amine), 1420 (C=S str., thioxo), 3091 (CH str., tetrahydropyrimidine), 1340 (C–O–C str., ester), 1247 (CH in plane bending, Ar.), 1561 (C=C skeletal str., Ar.), 743 (C–Cl),1502(N–O str. NO_2); ^{1}H NMR (MEOD): δ 6.99–8.04 (m, 9H, ArH), 1.30 (t, 3H, CH_3 of OC_2H_5), 2.05 (s, 2H, NH pyrimidine), 3.66 (s, 1H, NH 2^0 amine), 3.28–3.33 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{22}ClN_5O_5S$: C, 54.60; H, 4.20; N, 13.26; Found: C, 54.63; H, 4.19; N, 13.25.

Compound **4**: Mp (°C) 408–410; Yield – 80.10%; IR (KBr pellets) cm^{-1} 3475 (NH str., indole), 1637 (C=O str., 3^0 amide), 1296 (C–N str., aryl 2^0 amine), 1603(C=O str., oxo), 3098 (CH str., tetrahydropyrimidine), 1340(C–O–C str., ester), 1247 (CH in plane bending, Ar.), 1561 (C=C skeletal str., Ar.) 744 (C–Cl), 1502(N–O str. NO_2); ^{1}H NMR (MEOD): δ 6.99–8.05 (m, 9H, ArH), 1.30 (t, 3H, CH_3 of OC_2H_5), 3.66 (s, 1H, NH 2^0 amine), 3.65–3.33 (m, CH_2 of OCH_2CH_3) Anal. Calculated for $C_{24}H_{22}ClN_5O_6$: C, 56.31; H, 4.33; N, 13.68; Found: C, 56.26; H, 4.34; N, 13.75.

Compound **5**: Mp (°C) 402–404; Yield – 76.40%; IR (KBr pellets) cm^{-1} 1658 (C=O str., 3^0 amide) 1335 (C–O–C str., ester), 1516 (C=C skeletal str., Ar.); ^{1}H NMR (MEOD): δ 7.99–8.06 (m, 9H, ArH) 1.30 (t, 3H, CH_3 of OC_2H_5), 2.24 (s, 3H, CH_3 Ar), 4.09 (s, 1H, NH 2^0 amine), 3.46–3.65 (m, CH_2 of OCH_2CH_3) Anal. Calculated for $C_{25}H_{25}N_5O_6$: C, 61.09; H, 5.13; N, 14.25; Found: C, 6.01; H, 5.16; N, 14.28.

Compound **6**: Mp (°C) 428–430; Yield – 82.33%; IR (KBr pellets) cm^{-1} 1612 (C=O str., 3^0 amide), 1205 (C–N str., aryl 2^0 amine), 1335(C–O–C str., ester), 1521 (C=C skeletal str., Ar.); ^{1}H NMR (MEOD): δ 7.24–7.99 (m, 9H, ArH), 1.30 (t, 3H, CH_3 of OC_2H_5), 2.23 (s, 3H, CH_3 Ar.), 4.24–4.25 (m, 2H, CH2 of OC_2H_5), 4.24 (s, 1H, NH 2^0 amine); Anal. Calculated for $C_{25}H_{25}N_5O_5S$: C, 59.16; H, 4.96; N, 13.80; Found: C, 59.21; H, 4.98; N, 13.88.

Compound **7**: Mp (°C) 386–388; Yield – 78.25%; IR (KBr pellets) cm^{-1} 3391 (NH str., indole), 1655 (C=O str., 3^0 amide), 1202 (C–N str., aryl 2^0 amine), 1615 (C=O str., oxo), 1341 (C–O–C str., ester), 1458 (C=C skeletal str., Ar.); ^{1}H NMR (MEOD): δ 6.65–8.04 (m, 10H, ArH), 2.86–3.32 (m, CH_2 of OCH_2CH_3), 4.89 (s, 1H, NH 2^0 amine); Anal. Calculated for $C_{24}H_{23}N_5O_6$: C, 60.37; H, 4.86; N, 14.67; Found: C, 60.38; H, 3.84; N, 14.74.

Compound **8**: Mp (°C) 412–414; Yield – 79.00%; IR (KBr pellets) cm^{-1} 1615 (C=O str., 3^0 amide) 1457 (C=S str., thioxo), 1345 (C–O–C str., ester); ^{1}H NMR (MEOD): δ 6.65–8.57 (m, 10H, ArH), 1.32–1.54 (t, 3H, CH_3 of OC_2H_5), 2.68–3.02 (m, CH_2 of OCH_2CH_3) Anal. Calculated for $C_{24}H_{23}N_5O_5S$: C, 58.41; H, 4.70; N, 14.19; Found: C, 58.38; H, 4.74; N, 14.28.

Compound **9**: Mp (°C) 417–419; Yield – 82.50%; IR (KBr pellets) cm^{-1} 3394 (NH str., indole), 1614 (C=O str., 3^0 amide), 1257 (C–N str., aryl 2^0 amine), 1425(C=S str., thioxo), 3061(CH str., tetrahydropyrimidine), 1339(C–O–C str., ester), 1257 (CH in plane bending, Ar.), 1552 (C=C skeletal str., Ar.), 1525 (N–O str. NO_2); ^{1}H NMR (MEOD): δ 6.81–8.36 (m, 10H, ArH), 1.28–1.56 (t, 3H, CH_3 of OC_2H_5), 2.24–3.02 (m, CH_2 of OCH_2CH_3) Anal. Calculated for $C_{24}H_{23}N_5O_5S$: C, 58.41; H, 4.70; N, 14.19; Found: C, 58.48; H, 4.71; N, 14.18.

Compound **10**: Mp (°C) 424–426; Yield – 83.40%; IR (KBr pellets) cm^{-1} 3394 (NH str., indole), 1617 (C=O str., 3^0 amide), 1235 (C–N str., aryl 2^0 amine), 1599 (C=O str., oxo), 3175 (CH str., tetrahydropyrimidine), 1344 (C–O–C str., ester), 1235 (CH in plane bending, Ar.), 1551 (C=C skeletal str., Ar.) 1524 (N–O str. NO_2); ^{1}H NMR (MEOD): δ 6.88–8.90 (m, 10H, ArH), 1.24–1.42 (t, 3H, CH_3 of OC_2H_5), 2.01–2.88 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{23}N_5O_6$: C, 60.37; H, 4.86; N, 14.67; Found: C, 60.33; H, 4.84; N, 14.70.

Compound **11**: Mp (°C) 406–408; Yield – 82.55%; IR (KBr pellets) cm^{-1} 3353 (NH str., indole), 1618 (C=O str., 3^0 amide), 1304 (C–N str., aryl 2^0 amine), 1597 (C=O str., oxo), 3053 (CH str., tetrahydropyrimidine), 1329 (C–O–C str., ester), 1180 (CH in plane bending, Ar.), 1597 (C=C skeletal str., Ar.) 1524 (N–O str. NO_2); ^{1}H NMR (MEOD): δ 6.83–8.03 (m, 10H, ArH), 1.29–1.56 (t, 3H, CH_3 of OC_2H_5), 2.87–3.50 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{23}N_5O_6$: C, 60.37; H, 4.86; N, 14.67; Found: C, 60.39; H, 4.84; N, 14.68.

Compound **12**: Mp (°C) 392–394; Yield – 81.30%; IR (KBr pellets) cm^{-1} 3395 (NH str., indole), 1694 (C=O str., 3^0 amide), 1237 (C–N str., aryl 2^0 amine), 1617(C=O str., oxo), 1342(C–O–C str., ester), 1237 (CH in plane bending, Ar.), 1548 (C=C skeletal str., Ar.); ^{1}H

NMR (MEOD): δ 7.50–7.58 (m, 9H, ArH), 1.18–1.20 (t, 3H, CH_3 of OC_2H_5), 1.61 (s, CH_3 of tetrahydropyrimidine); Anal. Calculated for $C_{24}H_{25}N_5O_4$: C, 64.27; H, 5.39; N, 12.49; Found: C, 64.34; H, 5.35; N, 12.53.

Compound **13**: Mp (°C) 416–418; Yield – 83.28%; IR (KBr pellets) cm^{-1} 3394 (NH str., indole), 1612 (C=O str., 3^0 amide), 1236 (C–N str., aryl 2^0 amine), 1458 (C=S str., thioxo), 1321 (C–O–C str., ester), 1236 (CH in plane bending, Ar.), 1519 (C=C skeletal str., Ar.), 743 (C–Cl); Anal. Calculated for $C_{24}H_{23}ClN_4O_3S$: C, 64.27; H, 5.39; N, 12.49; Found: C, 64.32; H, 5.40; N, 12.45.

Compound **14**: Mp (°C) 378–380; Yield – 81.78%; IR (KBr pellets) cm^{-1} 3389 (NH str., indole), 1616 (C=O str., 3^0 amide), 1338 (C–N str., aryl 2^0 amine), 1696 (C=O str., oxo), 3058 (CH str., tetrahydropyrimidine), 1338 (C–O–C str., ester), 1198 (CH in plane bending, Ar.), 1548 (C=C skeletal str., Ar.) 689 (C–Cl); ^1H NMR (MEOD): δ 6.99–8.04 (m, 10H, ArH), 1.30 (t, 3H, CH_3 of OC_2H_5), 1.61 (s, 3H, CH_3 pyrimidine), 3.66 (s, 1H, NH 2^0 amine), 3.33–3.65 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{23}ClN_4O_4$: C, 61.74; H, 4.97; N, 12.00; Found: C, 61.66; H, 4.94; N, 11.94.

Compound **15**: Mp (°C) 410–412; Yield – 84.20%; IR (KBr pellets) cm^{-1} 1614 (C=O str., 3^0 amide), 1343 (C–N str., aryl 2^0 amine), 1423(C=S str., thioxo), 1457(C–O–C str., ester), 1199 (CH in plane bending, Ar.), 1525 (C=C skeletal str., Ar.); ^1H NMR (MEOD): δ 6.67–8.01 (m, 10H, ArH), 1.26–2.01 (t, 3H, CH_3 of OC_2H_5), 2.71–3.01 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{23}N_5O_5S$: C, 58.41; H, 4.70; N, 14.19; Found: C, 58.46; H, 3.64; N, 14.18.

Compound **16**: Mp (°C) 422–424; Yield – 78.00%; IR (KBr pellets) cm^{-1} 1614 (C=O str., 3^0 amide), 1340 (C–N str., aryl 2^0 amine), 1429 (C=S str., Thioxo), 2860 (CH str., tetrahydropyrimidine), 1521 (C=C skeletal str., Ar.); ^1H NMR (MEOD): δ 7.50–7.73 (m, 10H, ArH), 1.18–1.61 (t, 3H, CH_3 of OC_2H_5), 2.29–3.18 (m, CH_2 of OCH_2CH_3); Anal. Calculated for $C_{24}H_{25}N_5O_3S$: C, 62.18; H, 5.44; N, 15.11; Found: C, 62.20; H, 5.46; N, 15.16.

4.2. Evaluation of antimicrobial activity

4.2.1. Determination of MIC

The antimicrobial activity of synthesized compounds was performed against Gram-positive bacteria: *S. aureus* MTCC 2901, *B. subtilis* MTCC 2063, Gram-negative bacterium: *E. coli* MTCC 1652 and fungal strains: *C. albicans* MTCC 227 and *A. niger* MTCC 8189 using tube dilution method [35]. Dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) [36]. 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 MIC.

4.2.2. Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were determined by subculturing 100 μL of culture from each tube (which remained clear in the MIC determination) into fresh medium. MBC and MFC values represent the lowest concentration of compound that produces a 99.9% end point reduction [37].

4.3. QSAR studies

The structures of 6-methyl-4-[1-(2-substituted-phenylamino-acetyl)-1*H*-indol-3-yl]-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl esters were first pre-optimized with the Molecular Mechanics Force Field (MM^+) procedure included in Hyperchem 6.03 (Hyperchem 6.0, 1993) and the resulting geometries were further refined by means of the semiempirical 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 [38] (TSAR 3D Version 3.3, 2000). Further, the regression analysis was performed using the SPSS software package [39].

4.4. Evaluation of anticancer activity

Human colon (HCT 116) and breast (MCF 7) 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. Cytotoxicity of the synthesized compounds at various concentrations was assessed using 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 cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments [40].

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