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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, anticancer activity, and QSAR-studies of some new tetrahydropyrimidines

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Abstract Several new substituted 1,2,3,4 tetrahydropyrimidine derivatives have been synthesized and evaluated for their in vitro anticancer activity on various cell lines. Some of the molecules have exhibited significantly potent inhibition on several cell lines. To find out inter correlation between anticancer activity and molecular descriptors, QSAR study was carried out. Molecular descriptors used for the study were ClogP (lipophilic), CMR (steric), and polarity (electronic). Anticancer activity is expressed in the form of $LogGI_{50}^{+}$. Activity on different cell lines was independently correlated with molecular descriptors. On the basis of the results, significant correlation between anticancer activity on some of the cell lines and molecular descriptor was observed.

Keywords 1,2,3,4 Tetrahydropyrimidine · Anticancer activity · Linear free energy relationship · QSAR

Introduction

In the past decades, the pyrimidines and their derivatives have attracted increasing interest in the realms of natural and synthetic organic chemistry because of their diverse therapeutic and pharmacological properties (Kappe, 1993). These non planer heterocyclic compounds have emerged as an integral backbone of calcium channel modulators (Rovnyak *et al*., 1995), antihypertensive agents (Kappe, 2002), α1a-adrenergic receptor antagonists (Barrow *et al*., 2000), neuropeptide Y (NPY) antagonist (Sun *et al*., 2009), mitotic kinesis inhibitors (Kappe *et al*., 2000), hepatitis B virus replication inhibitors (Deres *et al*., 2003), and several marine derived natural products such as crambine and betzelladine β (potent HIVgp-120 CD4 inhibitors).

Ptilomycalin alkaloids have been reported to contain DHPMs and THPMs moiety (Snider and Shi, 1993; Patil *et al*., 1995). Among tetrahydropyrimidines (THPMs) with other types of bioactivity, Cerebrocrast (Klusa, 1995) has been introduced as a neuroprotectant and cognition enhancer. In addition, antiaggregatory activity in a number of THPMs has also been discovered (Cooper *et al*., 1992). The biological as well as medicinal importance of 1,2,3,4-THPM prompted us for further modification in the heterocyclic frame work for synthesis of new THPMs. While doing literature survey we have observed that not a single article is published on the anticancer screening of title compounds. And therefore, it is worth to undertake the synthesis and anticancer screening of 1,2,3,4-THPMs. In the present communication, we report herein the synthesis, anticancer activity, and QSAR studies of the title compounds.

A simple and direct method, first reported by Biginelli 1893, involves a three component, one-pot condensation of an aldehyde, a β–ketoester, and urea or thiourea under strongly acidic condition. This has led to the development of multi-step strategies that produce overall higher yield, but lack the simplicity of Biginelli synthesis. As a result, many improved procedures for the preparation of THPMs have been recently reported (Birgit *et al*., 2000; Kappe, 1998; Armido *et al*., 1997; Yadav *et al*., 2001; Zheng *et al*., 2006; Reddy *et al*., 2004; Han *et al*., 2005). In this study,

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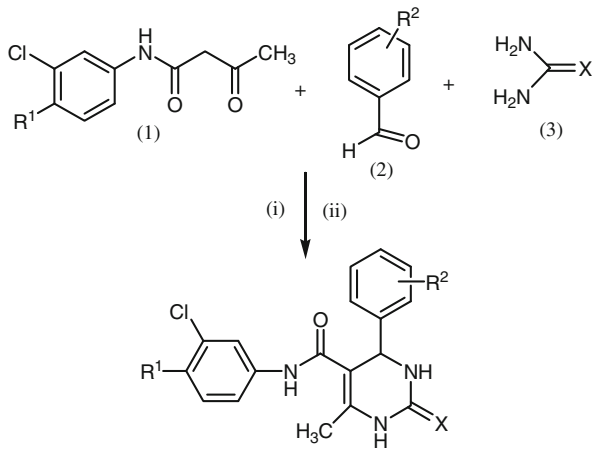
various derivatives of *N*-(3,4-dihelophenyl)-6-methyl-4-aryl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamides **4a–l** were prepared by cyclo condensation of different *N*-(substituted phenyl)-3-oxo butanamides, substituted aromatic aldehydes and urea/thiourea in the presence of concentrated HCl (Scheme 1).

Results and discussion

Best fitting equations obtained from simple and multiple regression analysis are represented in Table 1. In all the cases, best correlation was observed when all the three descriptors are correlated. Poor correlation was observed between physicochemical parameters and anticancer activity on leukemia, lung, and colon cancer cell lines, Eqs. 1, 2, and 6 with correlation coefficient (r) 0.38, 0.38, and 0.48, respectively. Significantly good correlation between anticancer activity on breast cancer cell line and descriptor was observed $r = 0.87$, Eqs. 3. Study suggests less lipophilic, less bulky, and electron withdrawing substituent may increase the potency. Positive influence of lipophilic and electronic parameters and negative influence of steric parameter was observed, when descriptors are correlated with anticancer activity on prostate cancer cell line ($r = 0.92$). Identical correlation between molecular



Comp.	-R^1	-X	-R^2
4a to 4d	Cl	S	(a) 2-Cl, (b) 3-OC_6H_5 (c) 3-OCH_3, (d) 3-OH
4e to 4f	Cl	O	(e) 2-OH, (f) 3-Cl
4g to 4k	F	S	(g) 2-Cl, (h) 3-Cl (i) 3-OC_6H_5 (j) 3-OCH_3, (k) 3-OH
4l	F	O	(l) 3-OC_6H_5

Scheme 1 Synthetic route of the title compounds. *i* Con. HCl and ethanol, *ii* reflux

Table 1 Equation for regression analysis

Sr. no.	Equations	Statistics			
		N	*r*	*s*	*F*
1	$(\log GI_{50}^{+})_{Le} = -0.144(\pm0.18)ClogP + 0.194(\pm0.18)CMR + 0.012(\pm0.09)D - 6.164(\pm1.50)$	12	0.38	0.297	0.44
2	$(\log GI_{50}^{+})_{Lu} = -0.117(\pm0.32)ClogP + 0.012(\pm0.32)CMR + 0.111(\pm0.16)D - 4.203(\pm2.59)$.	12	0.38	0.512	0.45
3	$(\log GI_{50}^{+})_{B} = -0.007(\pm0.00)ClogP - 0.004(\pm0.006)CMR + 0.0098(\pm0.003)D - 4.74(\pm0.054)$.	12	0.87	0.01	8.09
4	$(\log GI_{50}^{+})_{P} = 0.149(\pm0.055)ClogP - 0.263(\pm0.059)CMR + 0.034(\pm0.036)D - 2.526(\pm0.51)$.	12	0.92	0.09	13.85
5	$(\log GI_{50}^{+})_{R} = -0.138(\pm0.082)ClogP - 0.098(\pm0.084)CMR + 0.089(\pm0.041)D - 2.622(\pm0.679)$.	12	0.91	0.13	12.64
6	$(\log GI_{50}^{+})_{CO} = -0.231(\pm0.18)ClogP - 0.146(\pm0.18)CMR - 0.026(\pm0.09)D - 5.50(\pm1.49)$.	12	0.46	0.29	0.79
7	$(\log GI_{50}^{+})_{O} = -0.155(\pm0.05)ClogP - 0.0076(\pm0.051)CMR - 0.037(\pm0.025)D - 3.86(\pm0.416)$.	12	0.93	0.08	16.27
8	$(\log GI_{50}^{+})_{M} = -0.00329(\pm0.026)ClogP - 0.062(\pm0.026)CMR - 0.0344(\pm0.013)D - 4.174(\pm0.212)$.	12	0.89	0.04	10.45
9	$(\log GI_{50}^{+})_{CN} = -0.18(\pm0.049)ClogP - 0.024(\pm0.05)CMR - 0.039(\pm0.025)D - 4.381(\pm0.41)$.	12	0.93	0.08	16.08

N No. of compounds, *r* correlation coefficient, *s* standard deviation, *F* statistical, *Le* leukemia, *Lu* lung, *B* breast, *P* prostate, *R* renal, *CO* colon, *O* ovarian, *M* melanoma, *CN* CNS. GI_{50}^{+} = molar concentration of the compound that inhibits 50% net cell growth

descriptor and anticancer activity on renal, ovarian, and melanoma cell lines were observed, Eqs. 5, 7, and 8 with correlation coefficient (r) 0.91, 0.93, and 0.89, respectively. Study suggests that the incorporation of less lipophilic, less bulky electron donating group enhances the potency (Table 2).

Correlation between activity on CNS cancer cell line and molecular descriptors suggest that by decreasing the lipophilicity and electron density with increase in bulk of substituent will increase potency, Eqs. 9.

Significance of correlation between anticancer activity and physicochemical parameters was checked by F (Fischer's) test. Significance of correlation coefficients of parameters was checked by Student's t test. Correlations between observed values (Eq. 1) and calculated values (Table 3) are represented by Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9 in the form of graphical representation.

Experimental

QSAR study

To obtain relationship between physicochemical parameters and anticancer activity for the present series, we have carried out QSAR study by Hansch's LFER method (Tiwari *et al*., 2006; Hansch and Leo, 1995). $LogGI_{50}{}^{+}$ is used as dependent variable. Molecular descriptors representing lipophilicity (ClogP), steric (CMR), and electronic (dipole) were correlated with anticancer activity. Activities on different cancer cell line were correlated separately. Simple and multiple regression analysis were carried out. Equations representing best correlation are represented in (Table 1). Regression analysis was carried out by PC based QSAR software (Coburn, 1976). ClogP and CMR of all the compounds were calculated by using Bio-Loom software (http://www.biobyte.com) and dipole of all the compounds were calculated by using Chem Draw Ultra (http://www.cambridgesoft.com).

Anticancer activity

All the synthesized compounds were screened for their anticancer activity at National Institute of Health, National Cancer Institute, Maryland, USA (Anne *et al*., 1991; Weislow *et al*., 1989). Each compound was tested at five different concentrations against 60 cell lines of nine types of human cancer, namely leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer. A 48 h continuous drug exposure protocol was used, and a

Table 2 Correlation matrices of anticancer activity

Sr. no.		A.c.a	ClogP	CMR	*D*
1	Leukemia cancer cell line				
	A.c.a.	1.000			
	ClogP	0.083	1.000		
	CMR	0.261	0.852	1.000	
	D	−0.124	0.33	−0.210	1.000
2	Lung cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.273	1.000		
	CMR	0.301	0.852	1.000	
	D	−0.257	0.033	−0.210	1.000
3	Breast cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.552	1.000		
	CMR	−0.658	0.852	1.000	
	D	0.628	0.033	−0.210	1.000
4	Prostate cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.424	1.000		
	CMR	−0.780	0.852	1.000	
	D	0.581	−0.189	−0.414	1.000
5	Renal cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.851	1.000		
	CMR	−0.743	0.852	1.000	
	D	−0.295	0.033	−0.210	1.000
6	Colon cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.389	1.000		
	CMR	−0.209	0.852	1.000	
	D	−0.040	0.033	−0.210	1.000
7	Ovarian cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.904	1.000		
	CMR	−0.731	0.852	1.000	
	D	−0.234	0.033	−0.210	1.000
8	Melanoma cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.741	1.000		
	CMR	−0.658	0.852	1.000	
	D	−0.115	0.033	−0.210	1.000
9	CNS cancer cell line				
	A.c.a.	1.000			
	ClogP	−0.902	1.000		
	CMR	−0.787	0.852	1.000	
	D	0.172	0.033	−0.210	1.000

A.c.a anticancer activity

Table 3 Calculated anticancer activity of compound **4a–l**

Sr. no.	Leukemia HL-60 (TB)	Lung HOP-92	Breast NCI/ADP-RES	Prostate PC-3	Renal CKCI-1	Colon COLO-205	Ovarian IGRO-V1	Melanoma SK-MEL-5	CNS SF-268
4a	−4.706	−4.728	−4.797	−4.626	−4.678	−5.032	−4.865	−4.975	−4.995
4b	−4.492	−5.001	−4.823	−5.021	−5.016	−5.055	−5.07	−5.089	−5.222
4c	−4.563	−4.598	−4.789	−4.769	−4.612	−4.822	−4.757	−4.992	−4.836
4d	−4.57	−4.547	−4.785	−4.743	−4.467	−4.758	−4.654	−4.954	−4.749
4e	−4.689	−4.308	−4.762	−4.482	−4.521	−4.795	−4.687	−4.963	−4.665
4f	−4.831	−4.503	−4.776	−4.365	−4.735	−5.08	−4.904	−4.983	−4.922
4g	−4.734	−4.666	−4.792	−4.569	−4.571	−4.998	−4.793	−4.944	−4.924
4h	−4.743	−4.747	−4.799	−4.594	−4.506	−5.017	−4.766	−4.919	−4.952
4i	−4.508	−4.828	−4.808	−4.927	−4.999	−4.994	−5.034	−5.093	−5.112
4j	−4.608	−4.691	−4.797	−4.756	−4–382	−4.823	−4.634	−4.914	−4.819
4k	−4.598	−4.485	−4.78	−4.683	−4.36	−4.36	−4.582	−4.923	−4.678
4l	−4.629	−4.469	−4.783	−4.72	−5.083	−5.083	−5.084	−5.112	−5.028

Calculated anticancer activity on different cell lines is expressed in the form of Log GI_{50}^{+}, where GI_{50}^{+} = molar concentration of the compound that inhibits 50% net cell growth

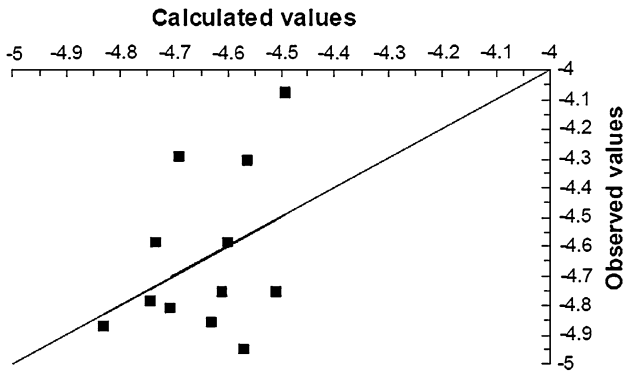


Fig. 1 Correlation between observed and calculated values is represented for leukemia cancer cell line. *Line* represents ratio of observed and calculated result

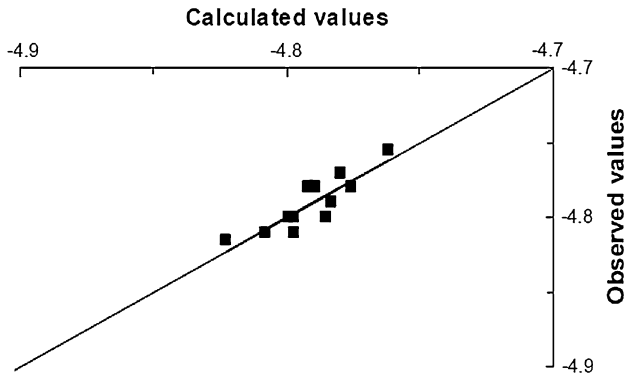


Fig. 3 Correlation between observed and calculated values is represented for breast cancer cell line. *Line* represents ratio of observed and calculated result

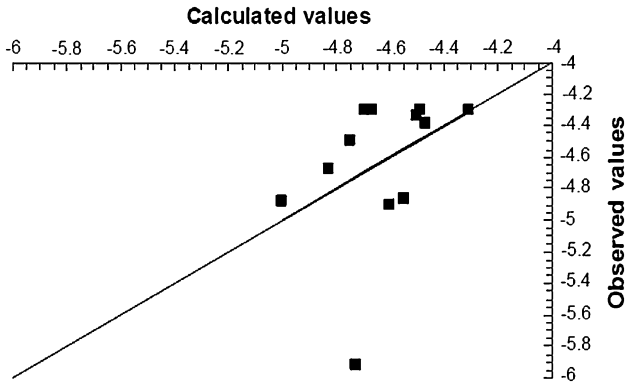


Fig. 2 Correlation between observed and calculated values is represented for lung cancer cell line. *Line* represents ratio of observed and calculated result

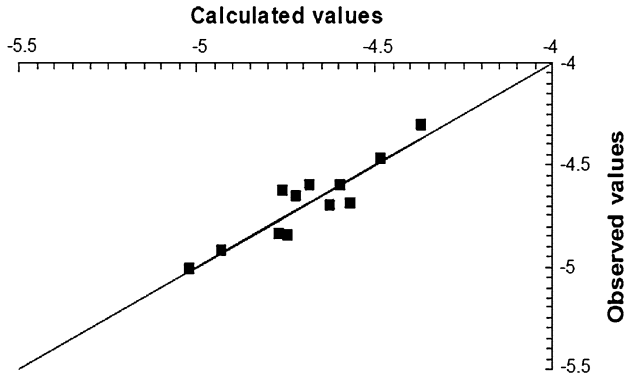


Fig. 4 Correlation between observed and calculated values is represented for prostate cancer cell line. *Line* represents ratio of observed and calculated result

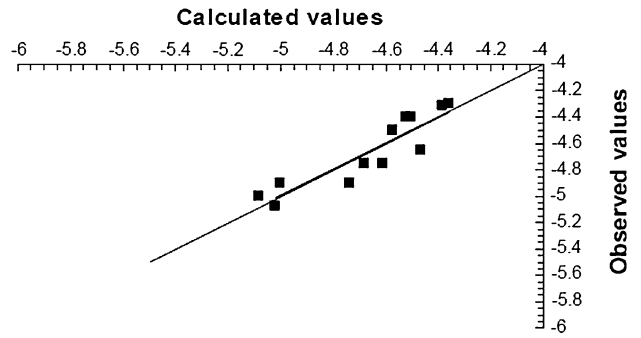


Fig. 5 Correlation between observed and calculated values is represented for renal cancer cell line. *Line* represents ratio of observed and calculated result

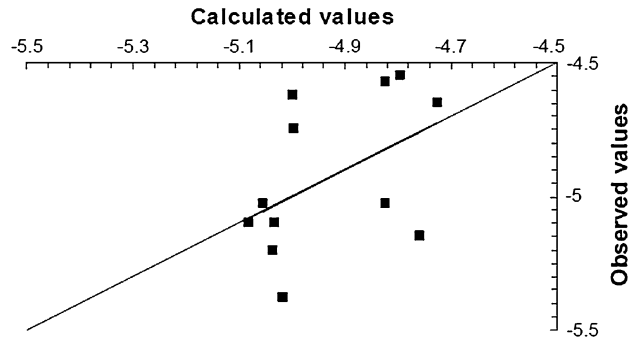


Fig. 6 Correlation between observed and calculated values is represented for colon cancer cell line. *Line* represents ratio of observed and calculated result

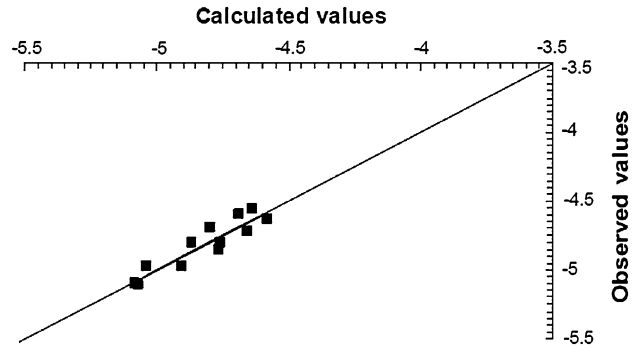


Fig. 7 Correlation between observed and calculated values is represented for ovarian cancer cell line. *Line* represents ratio of observed and calculated result

sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The methodology for the SRB assay is described in this article briefly. Adherent cell cultures are fixed in situ by adding 50 μl of cold 50% (wt/vol) trichloroacetic acid (TCA) (final concentration, 10% TCA) and incubating for 60 min at 4°C. The supernatant is then discarded, and the plates are washed five times with deionized water and dried. One hundred microliters of SRB solution (0.4% wt/vol in 1% acetic acid) is added to each

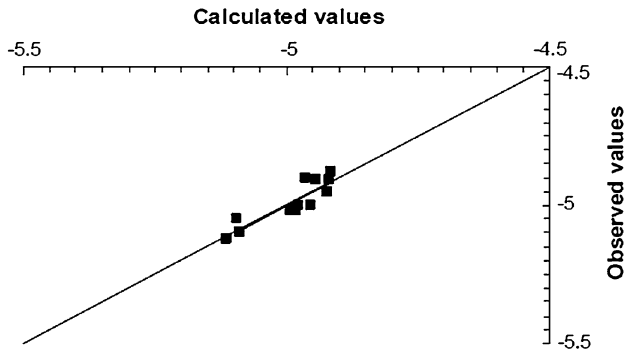


Fig. 8 Correlation between observed and calculated values is represented for melanoma cancer cell line. *Line* represents ratio of observed and calculated result

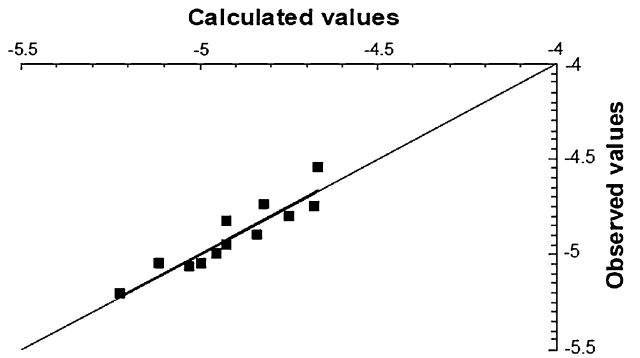


Fig. 9 Correlation between observed and calculated values is represented for CNS cancer cell line. *Line* represents ratio of observed and calculated result

microtiter well, and the culture is incubated for 10 min at room temperature. Unbound SRB is removed by washing five times with 1% acetic acid. Then the plates are air-dried. Bound stain is solubilized with tris buffer, and the optical densities are read on an automated spectrophotometric plate reader at a single wavelength of 515 nm.

For suspensions of cell cultures (e.g., leukemia), the method is the same, except that at the end of the drug-incubation period, the settled cells are fixed to the bottom of the microtiter well by gently adding 50 μl of 80% cold TCA (final concentration, 16% TCA).

With the SRB assay, a measure is also made of the cell population density at time zero (the time at which drugs are added) from two extra reference plates of inoculated cells fixed with TCA just before drug addition to the test plates. Thus, we have three measurements: control optical density (C), test optical density (T), and optical density at time zero (T_0).

Using these measurements, cellular responses can be calculated for growth stimulation, for no drug effect, and for growth inhibition. If T is greater than or equal to T_0, the calculation is $100 \times [(T-T_0)/(C-T_0)]$. If T is less than T_0, cell killing has occurred and can be calculated from

Table 4 Anticancer activity of the compound **4a–l**

Sr. no.	Leukemia HL-60 (TB)	Lung HOP-92	Breast NCI/ADP-RES	Prostate PC-3	Renal CKCI-1	Colon COLO-205	Ovarian IGRO-V1	Melanoma SK-MEL-5	CNS SF-268
4a	−4.81	−5.92	−4.80	−4.70	−4.75	−5.10	−4.80	−5.00	−5.05
4b	−4.08	−4.88	−4.81	−5.01	−5.07	−5.03	−5.11	−5.10	−5.21
4c	−4.31	−4.90	−4.78	−4.84	−4.75	−5.03	−4.80	−5.02	−4.90
4d	−4.95	−4.87	−4.80	−4.85	−4.65	−5.15	−4.72	−5.00	−4.80
4e	−4.30	−4.30	−4.75	−4.47	−4.40	−4.55	−4.60	−4.90	−4.55
4f	−4.87	−4.34	−4.78	−4.30	−4.90	−5.10	−4.98	−5.02	−4.95
4g	−4.59	−4.30	−4.78	−4.69	−4.50	−4.62	−4.75	−4.91	−4.83
4h	−4.79	−4.49	−4.80	−4.60	−4.40	−5.38	−4.85	−5.08	−5.00
4i	−4.76	−4.68	−4.81	−4.92	−4.90	−4.75	−4.97	−5.05	−5.05
4j	−4.76	−4.30	−4.81	−4.63	−4.31	−4.57	−4.56	−4.88	−4.74
4k	−4.59	−4.30	−4.77	−4.60	−4.30	−4.65	−4.64	−4.95	−4.75
4l	−4.86	−4.39	−4.79	−4.65	−5.00	−5.20	−5.10	−5.12	−5.07

$100 \times [(T-T_0)/T_0]$. Growth inhibition of 50% (GI_{50}) is calculated from $100 \times [(T-T_0)/(C-T_0)] = 50$, which is the drug concentration causing a 50% reduction in the net protein increase in control cells during the drug incubation. Results were expressed as $\log GI_{50}^{+}$, which indicates molar concentration of compound that inhibits 50% net cell growth. Test compounds have exhibited inhibition at significantly low concentration (Table 4).

Chemistry

Melting points were taken in open capillaries using paraffin bath and are uncorrected. IR spectra were recorded on FTIR-SIMADZU-8201pc (υ_{max} in cm^{-1}); ^1H NMR spectra were recorded on BRUKER AVANCE 300 FT–NMR spectrometer using $CDCl_3$ as a solvent and Mass spectra carried out on Applied Bio system QTrap LC-Mass Spectrometer. Elemental analysis of all the compounds was performed on Heracus CHN-Rapid Analyzer and the results were within ±0.4% of theoretical values. Purity was checked by TLC using TLC aluminum sheet coated with silica gel G 60, supplied by E. Merck. The spots were located by exposing the plates to iodine vapor. All the chemicals were supplied by S D Fine chemicals (India) Ltd., National Chemicals (India), and E. Merck Inc. and were used without any further purification. Physicochemical parameters are expressed in the form of ClogP, CMR, and Polar along with their values as mentioned below.

*4-(2-Chlorophenyl)-N-(3,4-dichlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4a**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 2-chlorobenzaldehyde (0.01 mol) and thiourea (0.01 mol) was placed in the round bottom flask, 20 ml of ethanol and 0.8 ml of HCl was added to it and the reaction mixture was refluxed for 6 h. The reaction mixture was poured onto crushed ice. The solid obtained was filtered, dried, and then recrystallized from ethanol (99%). m.p. 242–243°C, yield is 65%. IR (KBr): 3383 cm^{-1} (>NH medium, pyrimidine ring), 3263 and 1640 cm^{-1} (–CO–NH medium, amide), 3097 cm^{-1} (–C–H str., aromatic), 2973 cm^{-1} ($–CH_3$ str.), 1508 cm^{-1} (>NH weak), 1438 cm^{-1} (>CH medium, aromatic ring), 1020 cm^{-1} (–C–Cl str., aromatic), 1130 cm^{-1} (>C=S, str.), 640, 610 cm^{-1} (str., trisubstituted aromatic), ^1H NMR ($CDCl_3$): δ ppm 2.20 (s, 3H, $–C–CH_3$), 5.59 (s, 1H, –CH), 7.0–7.93 (m, 7H, Ar–H), 9.73 (s, 1H, –NH), 9.84 (s, 1H, –NH), 10.4 (s, 1H, –CONH). LC–MS: m/z 428.99 with 36% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{14}C_{l3}N_3OS$: C, 50.66; H, 3.31; N, 9.85; Found C, 50.47; H, 3.26; N, 9.74%. ^{13}C NMR: δ ppm 15.1, 50.1, 106.4, 123.0, 126.7, 128.2, 128.3, 128.6, 129.0, 29.9, 132.2, 133.9, 142.8, 159.1, 163.1, 180.3. ClogP (5.40), CMR (11.36), Polar (2.24).

*N-(3,4-Dichlorophenyl)-6-methyl-4-(3-phenoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4b**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 3-phenoxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 230–232°C, yield is 56%. IR (KBr): 3382 cm^{-1} (>NH medium, pyrimidine ring), 3260 cm^{-1} (–CO–NH medium, amide), 3099 cm^{-1} (–C–H str., aromatic), 2972 cm^{-1} ($–CH_3$ str.), 1508 cm^{-1} (>NH weak), 1438 cm^{-1} (>CH medium, aromatic ring), 1201 cm^{-1} (C–O–C str., aromatic), 1037 cm^{-1} (–C–Cl str., aromatic),1130 cm^{-1} (>C=S, str.). ^1H NMR ($CDCl_3$): δ ppm 2.18 (s, 3H, $–C–CH_3$), 5.54 (s, 1H, –CH), 6.73–7.93 (m, 12H, Ar–H), 9.71 (s, 1H, –NH), 9.82 (s, 1H, –NH), 10.35 (s, 1H, –CONH). LC–MS: m/z 484.09

with 27% relative intensity [M^+]. Anal. Calcd. For $C_{24}H_{19}C_{l2}N_3O_2S$: C, 59.51; H, 3.95; N, 8.67; Found C, 59.40; H, 3.86; N, 8.58%. ^{13}C NMR: δ ppm 15.1, 55.5, 106.4, 114.1, 115.5, 117.5, 120.0, 121.9, 123.0, 128.3, 128.5, 129.0, 129.9, 133.9, 142.9, 156.8, 157.0, 159.1, 163.1, 180.3. ClogP (6.79), CMR (13.54), Polar (1.49).

*N-(3,4-Dichlorophenyl)-4-(3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4c**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 3-methoxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 190–191°C, yield is 54%. IR (KBr): 3386 cm^{-1} (>NH medium, pyrimidine ring), 3266 cm^{-1} (–CO–NH medium, amide), 3094 cm^{-1} (–C–H str., aromatic), 2977 cm^{-1} (–CH_3 str.), 1503 cm^{-1} (>NH weak), 1436 cm^{-1} (>CH medium, aromatic ring), 1210 cm^{-1} (C–O–C str., aromatic), 1035 cm^{-1} (–C–Cl str., aromatic),1120 cm^{-1} (>C=S, str.). ^{1}H NMR ($CDCl_3$): δ ppm 2.19 (s, 3H, –C–CH_3), 3.71 (s, 3H, –O–CH_3), 5.57 (s, 1H, –CH), 6.58–7.90 (m, 7H, Ar–H), 9.75 (s, 1H, –NH), 9.84 (s, 1H, –NH), 10.38 (s, 1H, –CONH). LC–MS: m/z 422.05 with 21% relative intensity [M^+]. Anal. Calcd. for $C_{19}H_{17}C_{l2}N_3O_2S$: C, 54.03; H, 4.06; N, 9.95; Found C, 53.94; H, 4.01; N, 9.87%. ^{13}C NMR: δ ppm 15.1, 55.5, 55.8, 106.4, 110.9, 112.3, 119.2, 123.0, 129.0, 129.6, 129.9, 133.9, 144.2, 159.1, 160.4, 163.1, 180.3. ClogP (4.6), CMR (11.49), Polar (2.59).

*N-(3,4-Dichlorophenyl)-4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-carboxamide (**4d**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 3-hydroxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 239–241°C, yield is 58% IR (KBr): 3388 cm^{-1} (–OH variable, aromatic ring), 3385 cm^{-1} (>NH medium, pyrimidine ring), 3263 cm^{-1} (–CO–NH medium, amide), 3095 cm^{-1} (–C–H str., aromatic), 2972 cm^{-1} (–CH_3 str.), 1501 cm^{-1} (>NH weak), 1438 cm^{-1} (>CH medium, aromatic ring), 1021 cm^{-1} (–C–Cl str., aromatic),1125 cm^{-1} (>C=S, str.). ^{1}H NMR ($CDCl_3$): δ ppm 2.17 (s, 3H, –C–CH_3), 5.55 (s, 1H, –CH), 6.53–7.85 (m, 7H, Ar–H), 9.75 (s, 1H, –NH), 9.81 (s, 1H, –OH), 9.84 (s, 1H, –NH), 10.38 (s, 1H, –CONH). LC–MS: m/z 409.02 with 68% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{15}C_{l2}N_3O_2S$: C, 52.95; H, 3.70; N, 10.29; Found C, 52.76; H, 3.59; N, 10.15%. ^{13}C NMR: δ ppm 15.1, 55.5, 106.4, 112.5, 113.9, 119.5, 123.0, 129.0, 129.9, 130.0, 133.9, 144.6, 158.3, 159.1, 163.1, 180.3. ClogP (4.02), CMR (11.03), Polar (2.38).

*N-(3,4-Dichlorophenyl)-4-(2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4e**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 2-hydroxybenzaldehyde (0.01 mol) and urea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 235–236°C, yield is 58%. IR (KBr): 3386 cm^{-1} (–OH variable, aromatic ring), 3381 cm^{-1} (>NH medium, pyrimidine ring), 3244 cm^{-1} (–CO–NH medium, amide), 3102 cm^{-1} (–C–H str., aromatic),2935 cm^{-1} (–CH_3 str.), 1710 (>C=0 str.), 1503 cm^{-1} (>NH weak), 1458 cm^{-1} (>CH medium, aromatic ring), 1028 cm^{-1} (–C–Cl str., aromatic). ^{1}H NMR ($CDCl_3$): δ ppm 2.20 (s, 3H, –C–CH_3), 5.49 (s, 1H, –CH), 6.63–7.85 (m, 7H, Ar–H), 9.77 (s, 1H, –NH), 9.83 (s,1H, –OH), 9.86 (s, 1H, –NH), 10.42 (s, 1H, –CONH). LC–MS: m/z 393.19 with 43% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{15}C_{l2}N_3O_3$: C, 55.12; H, 3.85; N, 10.71; Found C, 55.03; H, 3.71; N, 10.59%. ^{13}C NMR: δ ppm 14.4, 43.9, 108.6, 115.7, 121.2, 122.8, 123.0, 128.2, 128.3, 129.0, 129.9, 133.9, 146.1, 150.2, 154.0, 163.1. ClogP (3.84), CMR (10.17), Polar (4.22).

*4-(3-Chlorophenyl)-N-(3,4-dichlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4f**)*

A mixture of *N*-(3,4-dichlorophenyl)-3-oxobutanamide (0.01 mol), 3-chlorobenzaldehyde (0.01 mol) and urea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 231–232°C, yield is 58%. IR (KBr): 3382 cm^{-1} (>NH medium, pyrimidine ring), 3246 cm^{-1} (–CO–NH medium, amide), 3101 cm^{-1} (–C–H str., aromatic), 2933 cm^{-1} (–CH_3 str.), 1701 cm^{-1} (>C=0 str.), 1500 cm^{-1} (>NH weak), 1458 cm^{-1} (>CH medium, aromatic ring), 1028 cm^{-1} (–C–Cl str., aromatic). ^{1}H NMR ($CDCl_3$): δ ppm 2.15 (s, 3H, C–CH_3), 5.51 (s, 1H, –CH), 7.27–7.89(m, 7H, Ar–H,), 7.61 (s, 1H, –NH), 9.46 (s, 1H, –NH), 10.42 (s, 1H, –CONH). LC–MS: m/z 411.03 with 79% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{14}C_{l3}N_3O_2$: C, 52.64; H, 3.44; N, 10.23; Found C, 52.50; H, 3.32; N, 10.11%. ^{13}C NMR: δ ppm 14.4, 49.6, 108.6, 123.0, 125.0, 126.7, 126.8, 129.0, 129.9, 130.0, 133.9, 134.1, 144.6, 146.1, 150.2, 163.1. ClogP (5.27), CMR (10.51), Polar (4.03).

*N-(3-Chloro-4-fluorophenyl)-4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4g**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 2-chlorobenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 215–217°C, yield is 55%. IR (KBr): 3336 cm^{-1} (>NH medium, pyrimidine ring), 3207 cm^{-1} (–CO–NH medium, amide), 3097 cm^{-1} (–C–H str., aromatic), 2975 cm^{-1} (–CH_3 str.), 1506 cm^{-1} (>NH weak), 1437 cm^{-1} (>CH

medium, aromatic ring), 1033 cm^{-1} (–C–Cl str., aromatic), 1177 cm^{-1} (>C=S, str.). ^1H NMR ($CDCl_3$): δ ppm 2.13 (s, 3H, –C–CH_3), 4.59 (s, 1H, –CH), 7.01–7.85 (m, 7H, Ar–H), 9.71 (s, 1H, –NH), 9.80 (s, 1H, –NH), 10.44 (s, 1H, –CONH). LC–MS: m/z 411.02 with 68% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{14}C_{l2}FN_3OS$: C, 52.69; H, 3.44; N, 10.24; Found C, 55.53; H, 3.31; N, 10.09%. ^{13}C NMR: δ ppm 15.1, 50.1, 106.4, 123.0, 126.7, 128.2, 128.3, 128.6, 129.0, 132.2, 129.9, 133.9, 142.8, 159.1, 163.1, 180.3. ClogP (4.95), CMR (10.88), Polar (2.27).

*N-(3-Chloro-4-fluorophenyl)-4-(3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4h**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 3-chlorobenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 224–225°C, yield is 56%. IR (KBr): 3335 cm^{-1} (>NH medium, pyrimidine ring), 3206 cm^{-1} (–CO–NH medium, amide), 3097 cm^{-1} (–C–H str., aromatic), 2975 cm^{-1} (–CH_3 str.), 1506 cm^{-1} (>NH weak), 1437 cm^{-1} (>CH medium, aromatic ring), 1038 cm^{-1} (–C–Cl str., aromatic), 1177 cm^{-1} (>C=S, str.). ^1H NMR ($CDCl_3$): δ ppm 2.15 (s, 3H, –C–CH_3), 4.57 (s, 1H, –CH), 7.11–7.85 (m, 7H, Ar–H), 9.73(s, 1H, –NH), 9.81 (s, 1H, –NH), 10.41 (s, 1H, –CONH). LC–MS: m/z 411.03 with 29% relative intensity [M^+]. Anal. Calcd. for $C_{18}H_{14}C_{l2}FN_3OS$: C, 52.69; H, 3.44; N, 10.24; Found C, 55.59; H, 3.32; N, 10.14%. ^{13}C NMR: δ ppm 15.1, 54.7, 106.4, 123.0, 125.0, 126.7, 126.8, 129.0, 129.9, 130.0, 133.9, 134.1, 144.6, 159.1, 163.1, 180.3. ClogP (4.95), CMR (10.88), Polar (1.54).

*N-(3-Chloro-4-fluorophenyl)-6-methyl-4-(3-phenoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4i**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 3-phenoxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 220–222°C, yield is 46%. IR (KBr): 3337 cm^{-1} (>NH medium, pyrimidine ring), 3207 cm^{-1} (–CO–NH medium, amide), 3097 cm^{-1} (–C–H str., aromatic), 2976 cm^{-1} (–CH_3 str.), 1507 cm^{-1} (>NH weak),1436 cm^{-1} (>CH medium, aromatic ring), 1210 cm^{-1} (C–O–C str., aromatic), 1034 cm^{-1} (–C–Cl str., aromatic), 1175 cm^{-1} (>C=S, str.). ^1H NMR ($CDCl_3$): δ ppm 2.15 (s, 3H, –C–CH_3),4.54 (s, 1H, –CH), 6.72–7.85 (m, 12H, Ar–H), 9.68 (s, 1H, –NH), 9.83 (s, 1H, –NH), 10.42 (s, 1H, –CONH). LC–MS: m/z 432.09 with 32% relative intensity [M+]. Anal.Calcd. for $C_{24}H_{19}ClFN_3O_2S$: C, 61.60; H, 4.09; N, 8.98; Found C, 61.42; H, 4.01; N, 8.89%. ^{13}C NMR: δ ppm 15.1, 55.5, 106.4, 114.1, 115.5, 117.5, 120.0, 121.9, 123.0, 128.3, 128.5, 129.0, 129.9, 133.9, 142.9, 156.8., 157.0, 159.1, 163.1, 180.3. ClogP (6.34), CMR (13.06), Polar (2.52).

*N-(3-Chloro-4-fluorophenyl)-4-(3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4j**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 3-methoxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 226–227°C, yield is 54%. IR (KBr): 3333 cm^{-1} (>NH medium, pyrimidine ring), 3202 cm^{-1} (–CO–NH medium, amide), 3095 cm^{-1} (–C–H str., aromatic), 2977 cm^{-1} (–CH_3 str.), 1504 cm^{-1} (>NH weak),1436 cm^{-1} (>CH medium, aromatic ring), 1201 cm^{-1} (C–O–C str., aromatic), 1033 cm^{-1} (–C–Cl str., aromatic), 1175 cm^{-1} (>C=S, str.). ^1H NMR ($CDCl_3$): δ ppm 2.16 (s, 3H, –C–CH_3), 3.73 (s, 3H, –O–CH_3), 5.57 (s, 1H, –CH), 6.68–7.98 (m, 7H, Ar–H), 9.73 (s, 1H, –NH), 9.84 (s, 1H, –NH), 10.51 (s, 1H, –CONH). LC–MS: m/z 406.07 with 26% relativeintensity [M^+]. Anal. Calcd. for $C_{19}H_{17}ClFN_3O_2S$: C, 56.23; H, 4.22; N, 10.35; Found C, 56.10; H, 4.15; N, 10.23%. ^{13}C NMR: δ ppm 15.1, 55.5, 55.8, 106.4, 110.0, 1122.3, 119.2, 123.0, 129.0, 129.6, 129.9, 133.9, 144.2, 159.1, 160.4, 163.1, 180.3. ClogP (4.16), CMR (11.01), Polar (1.23).

*N-(3-Chloro-4-fluorophenyl)-4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4k**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 3-hydroxybenzaldehyde (0.01 mol) and thiourea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 235–236°C, yield is 51%. IR (KBr): 3384 cm^{-1} (–OH variable, aromatic ring), 3335 cm^{-1} (>NH medium, pyrimidine ring), 3203 cm^{-1} (–CO–NH medium, amide), 3095 cm^{-1} (–C–H str., aromatic), 2973 cm^{-1} (–CH_3 str.), 1508 cm^{-1} (>NH weak), 1435 cm^{-1} (>CH medium, aromatic ring), 1036 cm^{-1} (–C–Cl str., aromatic), 1173 cm^{-1} (>C=S, str.) ^1H NMR ($CDCl_3$): δ ppm 2.20 (s, 3H, –C–CH_3), 5.39 (s, 1H, –CH), 6.60–8.10 (m, 7H, Ar–H), 9.73 (s, 1H, –NH), 9.82 (s, 1H, –OH), 9.86 (s, 1H, –NH), 10.42 (s, 1H, –CONH). LC–MS: m/z 393.05 with 56% relative intensity [M^+].Anal. Calcd. for $C_{18}H_{15}ClFN_3O_2S$: C, 55.17; H, 3.86; N, 10.72; Found C, 55.01; H, 3.75; N, 10.60%. ^{13}C NMR: δ ppm 15.1, 55.8, 106.4, 112.5, 113.9, 119.5, 123.0, 129.0, 129.9, 133.0, 133.9, 144.6, 158.3, 159.1, 163.1, 180.3. ClogP (3.57), CMR (10.55), Polar (2.41).

*N-(3-Chloro-4-fluorophenyl)-6-methyl-2-oxo-4-(3-phenoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (**4l**)*

A mixture of *N*-(3-chloro-4-fluorophenyl)-3-oxobutanamide (0.01 mol), 3-phenoxybenzaldehyde (0.01 mol) and urea (0.01 mol) was treated as in the synthesis of **(4a)**, m.p. 229–230°C, yield is 45%. IR (KBr): 3448 cm^{-1} (>NH medium, pyrimidine ring), 3276 cm^{-1} (–CO–NH medium, amide), 3101 cm^{-1} (–C–H str., aromatic), 2965 cm^{-1} ($–CH_3$ str.),1710 cm^{-1} (>C=0 str.),1500 cm^{-1} (>NH weak), 1455 cm^{-1} (>CH medium, aromatic ring), 1220 cm^{-1} (C–O–C str., aromatic), 1030 cm^{-1} (–C–Cl str., aromatic). ^{1}H NMR ($CDCl_3$): δ ppm 1.82 (s, 3H, $–C–CH_3$), 5.56 (s, 1H, –CH), 6.72–8.05 (m, 12H, Ar–H), 7.61 (s, 1H, –NH), 9.43 (s, 1H, –NH), 10.45 (s, 1H, –CONH). LC–MS: m/z 452.19 with 29% relative intensity [M^+]. Anal.Calcd. for $C_{24}H_{19}ClFN_3O_3$: C, 63.79; H, 4.24; N, 9.30; Found C, 63.65; H, 4.11; N, 9.12%. ^{13}C NMR: δ ppm 14.4, 50.4, 108.6, 114.1, 115.5, 117.5, 120.0, 121.9, 123.0, 128.3, 128.5, 129.0, 129.9, 130.0, 133.9, 142.9, 146.1, 150.2, 156.8, 157.0, 163.1, 180.3. ClogP (6.21), CMR (12.21), Polar (4.61).

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