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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, antimicrobial, anticancer evaluation, and QSAR studies of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones

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Abstract A series of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones (**1**–**17**) was synthesized and tested in vitro for its antimicrobial and anticancer potentials. The biological screening results indicated that compounds having *m*-chloro substituent on benzaldehyde portion showed antimicrobial potential, whereas compounds having chloro, methoxy, and hydroxyl groups showed anticancer potential. The quantitative structure activity relationship (QSAR) studies indicated the importance of topological parameter, valence first order molecular connectivity index in describing antifungal activity. The developed QSAR models, however, were statistically insignificant with reference to anticancer activity of the synthesized compounds.

Keywords 4-Aminoantipyrine derivatives · Antimicrobial · Anticancer · QSAR

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Introduction

Extensive use of antibiotics has led to the development of multidrug-resistant microbial pathogens. This highlights the incessant need for the development of new classes of antimicrobial agents as well as the alteration of known drugs in such a way as to reduce their resistance to pathogens, while retaining their physiological action (Guzeldemirci *et al*., 2010).

Undoubtedly, cancer ranks high among human diseases and is still in dire need of effective therapy. Lack of a wide array of anticancer drugs to capitalize on new discoveries regarding tumor genesis, coupled with the unique growth patterns of diverse repertoires of cancer, have driven the attention of researchers toward the discovery of novel anticancer agents (Abdel-Jalil *et al*., 2010).

Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) was the first pyrazolone derivative used in the management of pain and inflammation (Bondock *et al*., 2008). Review of the literature in the last decade has revealed that antipyrine and 4-aminoantipyrine derivatives possess a number of biological activities viz. antimicrobial (Bayrak *et al*., 2010; Rostom *et al*., 2009), antiviral (Rusinov *et al*., 2005), antioxidant (Santos *et al*., 2010; Bashkatova *et al*., 2005), anticancer (Rosu *et al*., 2010), antipyretic (Shchegolkov *et al*., 2006), analgesic, and anti-inflammatory (Mahle *et al*., 2010; Rubtsov *et al*., 2002) activities.

Quantitative structure activity relationship (QSAR) study provides medicinal chemists valuable information that is useful for drug design and prediction of drug activity. QSAR models are mathematical equations which construct a relationship between chemical structures and their biological activities as a linear regression model in the form $y = Xb + e$. This equation may be used to describe a

set of predictor variables (X) with a predicted variable (y) by means of a regression vector (b) (Sabet *et al*., 2010).

Schiff bases are considered to be the most important group of compounds in medicinal chemistry due to their preparative accessibility, structural variety, and wide biological profile (Rosu *et al*., 2010). With this in view and in continuation of our study on exploring the biological profile of schiff bases (Kumar *et al*., 2010a, b, 2011; Judge *et al*., 2011a, b; Narang *et al*., 2011a, b), we hereby report the synthesis, antimicrobial, anticancer evaluation, and QSAR studies of 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones (**1**–**17**).

Experimental

Melting points were determined in open capillary tubes on a sonar melting point apparatus and were uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel–G). ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsiliane (internal standard). Infrared (IR) spectra were recorded on Perkin Elmer FTIR spectrometer using KBr pellets.

General procedure for synthesis of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones (**1**–**17**)

A solution of 0.05 mol of appropriate benzaldehyde in ethanol was added to a solution of 0.05 mol of 4-amino-antipyrine in 50 ml ethanol, and the mixture was refluxed for 4–5 h. The reaction mixture was then allowed to cool at room temperature, and the precipitate obtained was filtered, dried, and recrystallized from ethanol.

Compound ***1***

Mp(°C) 220–222; Yield—81.45%; IR (KBr pellets) cm^{-1} 1,647 (C=O str., Ar.), 1,608 (C=N str., N=CH), 1,579 (C=C skeletal str., phenyl), 1,487 (C=C str., antipyrine), 1,369 (C–N str., aryl 3^0 amine) 1,310 (C–N str., aryl 3^0 amine); ^{1}H NMR (MEOD): 2.48 (s, 3H, –NCH$_3$), 6.79–7.71 (m, 9H, ArH), 9.37 (s, 1H, –N=CH), 3.21 (s, 6H, –N(CH$_3$)$_2$).

Compound ***2***

Mp(°C) 171–173; Yield—92.76%; IR (KBr pellets) cm^{-1} 1,629 (C=O str., Ar.), 1,595 (C=N str., N=CH), 1,575 (C=C skeletal str., phenyl), 1,484 (C=C str., antipyrine); 1,210 (OH in plane bending), 1,150(C–O–C str.); ^{1}H NMR (MEOD): 2.43 (s, 3H, –NCH$_3$), 6.99–7.80 (m, 8H, ArH), 9.48 (s, 1H, –N=CH).

Compound ***3***

Mp(°C) 86–88; Yield—83.26%; IR (KBr pellets) cm^{-1} 1,643 (C=O str., Ar.), 1,592 (C=N str., N=CH), 1,578 (C=C skeletal str., phenyl), 1,486 (C=C str., antipyrine); 1,135(C–O–C str.); ^{1}H NMR (MEOD): 6.88–7.67 (m, 9H, ArH), 9.68 (s, 1H, –N=CH), 2.51 (s, 3H, –NCH$_3$), 3.25(s, 3H, –OCH$_3$), 2.53 (s, 3H, –NCH$_3$).

Compound ***4***

Mp(°C) 208–210; Yield—81.64%; IR (KBr pellets) cm^{-1} 1,615 (C=O str., Ar.), 1,590 (C=N str., N=CH), 1,556 (C=C skeletal str., phenyl), 1,487 (C=C str., antipyrine); 1,245 (OH in plane bending); ^{1}H NMR (MEOD): 6.75–7.64 (m, 9H, ArH), 9.54 (s, 1H, –N=CH), 2.55 (s, 3H, –NCH$_3$).

Compound ***5***

Mp(°C) 216–218; Yield—87.48%; IR (KBr pellets) cm^{-1} 1,645 (C=O str., Ar.), 1,588 (C=N str., N=CH), 1,564 (C=C skeletal str., phenyl), 1,485 (C=C str., antipyrine) 750 (C–Cl str.); ^{1}H NMR (MEOD): 2.55 (s, 3H, –NCH$_3$), 7.39–8.24 (m, 9H, ArH), 10.12 (s, 1H, –N=CH), 3.30(s, 3H, –OCH$_3$).

Compound ***6***

Mp(°C) 176–178; Yield—77.52%; IR (KBr pellets) cm^{-1} 1,647 (C=O str., Ar.), 1,608 (C=N str., N=CH), 1,574 (C=C skeletal str., phenyl), 1,484 (C=C str., antipyrine); 1,128 (C–O–C str.); ^{1}H NMR (MEOD): 2.34 (s, 3H, –NCH$_3$), 6.96–7.73(m, 9H, ArH), 9.53 (s, 1H, –N=CH).

Compound ***7***

Mp(°C) 256–258; Yield—94.66%; IR (KBr pellets) cm^{-1} 1,646 (C=O str., Ar.), 1,594 (C=N str., N=CH), 1,573 (C=C skeletal str., phenyl), 1,487 (C=C str., antipyrine); 1,136 (C–O–C str.), ^{1}H NMR (MEOD): 2.33 (s, 3H, –NCH$_3$), 6.90–7.68 (m, 8H, ArH), 9.42 (s, 1H, –N=CH), 3.47(s, 6H, –OCH$_3$).

Compound ***8***

Mp(°C) 211–213; Yield—88.44%; IR (KBr pellets) cm^{-1} 1,625 (C=O str., Ar.), 1,578 (C=N str., N=CH), 1,517 (C=C skeletal str., phenyl), 1,486 (C=C str., antipyrine); 1,256 (OH in plane bending), 1,130(C–O–C str.); ^{1}H NMR (MEOD): 2.50 (s, 3H, –NCH$_3$), 6.86–7.59 (m, 8H, ArH),

9.42 (s, 1H, –N=CH), 3.33(s, 3H, $–OCH_3$); 4.88 (s, 1H –OH).

Compound ***9***

Mp(°C) 192–194; Yield—84.75%; IR (KBr pellets) cm^{-1} 1,648 (C=O str., Ar.), 1,591 (C=N str., N=CH), 1,572 (C=C skeletal str., phenyl), 1,483 (C=C str., antipyrine), 1,136 (C–O–C str.); ^{1}H NMR (MEOD): 2.52 (s, 3H, $–NCH_3$), 7.00–8.09 (m, 9H, ArH), 9.92 (s, 1H, –N=CH), 3.33 (s, 3H, $–OCH_3$).

Compound ***10***

Mp(°C) 195–197; Yield—88.30%; IR (KBr pellets) cm^{-1} 1,645 (C=O str., Ar.), 1,592 (C=N str., N=CH), 1,568 (C=C skeletal str., phenyl), 1,489 (C=C str., antipyrine); 1,522 (NO_2 str.), ^{1}H NMR (MEOD): 2.66 (s, 3H, $–NCH_3$), 7.46–8.74 (m, 9H, ArH), 9.54 (s, 1H, –N=CH).

Compound ***11***

Mp(°C) 231–233; Yield—76.87%; IR (KBr pellets) cm^{-1} 1,648 (C=O str., Ar.), 1,594 (C=N str., N=CH), 1,563 (C=C skeletal str., phenyl), 1,481 (C=C str., antipyrine); ^{1}H NMR (MEOD): 2.52 (s, 3H, $–NCH_3$), 7.42–7.85 (m, 10H, ArH), 9.56 (s, 1H, N=CH), 3.25 (s, 6H, $–N(CH_3)_2$).

Compound ***12***

Mp(°C) 195–197; Yield—73.28%; IR (KBr pellets) cm^{-1} 1,662 (C=O str., Ar.), 1,589 (C=N str., N=CH), 1,486 (C=C str., antipyrine); 1,246 (OH in plane bending), 1,137(C–O–C str.); ^{1}H NMR (MEOD): 3.46 (s, 3H, $–OCH_3$), 7.42–7.84 (m, 8H, ArH), 9.55 (s, 1H, –N=CH), 4.96 (s, 1H, –OH).

Compound ***13***

Mp(°C) 135–137; Yield—90.10%; IR (KBr pellets) cm^{-1} 1,647 (C=O str., Ar.), 1,592 (C=N str., N=CH), 1,571 (C=C skeletal str., phenyl), 1,482 (C=C str., antipyrine) 749(C–Cl str.); ^{1}H NMR(MEOD): 7.41–8.23 (m, 9H, ArH), 9.95 (s, 1H, –N=CH), 2.57 (s, 3H, $–NCH_3$).

Compound ***14***

Mp(°C) 274–276; Yield—92.80%; IR (KBr pellets) cm^{-1} 1,643 (C=O str., Ar.), 1,581 (C=N str., N=CH), 1,562 (C=C skeletal str., phenyl), 1,487 (C=C str., antipyrine) 748 (C–Cl str.); ^{1}H NMR (MEOD): 7.26–7.58 (m, 8H, ArH), 9.49 (s, 1H, –N=CH), 2.51 (s, 3H, $–NCH_3$).

Compound ***15***

Mp(°C) 179–181; Yield—84.54%; IR (KBr pellets) cm^{-1} 1,602 (C=O str., Ar.), 1,576 (C=N str., N=CH), 1,528 (C=C skeletal str., phenyl), 1,491 (C=C str., antipyrine); 1,238 (OH in plane bending); ^{1}H NMR (MEOD): 2.22 (s, 3H, $–NCH_3$), 6.71–7.79 (m, 9H, ArH), 4.62(s, 1H, –OH).

Compound ***16***

Mp(°C) 210–212; Yield—78.24%; IR (KBr pellets) cm^{-1} 1,643 (C=O str., Ar.), 1,590 (C=N str., N=CH), 1,565 (C=C skeletal str., phenyl), 1,484 (C=C str., antipyrine) 747 (C–Cl str.); ^{1}H NMR (MEOD): 2.33 (s, 3H, $–NCH_3$), 7.60–7.84 (m, 9H, H of ArH), 9.91 (s, 1H, N=CH).

Compound ***17***

Mp(°C) 230–232; Yield—76.34%; IR (KBr pellets) cm^{-1} 1,649 (C=O str., Ar.), 1,577 (C=N str., N=CH), 1,502 (C=C skeletal str., phenyl), 1,488 (C=C str., antipyrine), 1,123 (C–O–C str.); ^{1}H NMR (MEOD): 2.50 (s, 3H, $–NCH_3$), 6.86–7.59 (m, 7H, ArH), 9.42 (s, 1H, –N=CH), 3.33(s, 9H, $–OCH_3$).

Evaluation of antimicrobial activity

Determination of MIC

The antimicrobial activity of the synthesized compounds was tested against Gram-positive bacteria: *Staphylcococcus aureus* MTCC 2901, *Bacillus sublitis* MTCC 2063, Gram-negative bacterium: *Escherichia coli* MTCC 1652 and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 8189 using 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 days (*A. niger*), and at 37°C for 48 h (*C. albicans*), and the results were recorded in terms of MIC.

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 a compound that produces a 99.9% end point reduction (Rodriguez-Arguelles *et al*., 2005).

QSAR studies

The structures of 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones were first pre-optimized using Molecular Mechanics Force Field (MM+) procedure included in Hyperchem 6.03 (1993), and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). A gradient norm limit of 0.01 kcal/Å was used for the geometry optimization. The lowest energy structure of each molecule was used 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).

Determination of anticancer activity

Human colorectal DLD-1 (ATCC CCL-221), HT29 (ATCC HTB-38), HCT116 (ATCC CCL-247), and murine leukemia, P388 (ATCC TIB 63) 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 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 (Mosmann, 1983).

Results and discussion

Chemistry

A series of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones (**1**–**17**) were synthesized through reaction of the corresponding aromatic aldehydes with 4-aminoantipyrine (Scheme 1). The physicochemical characteristics of the synthesized compounds are presented in Table 1.

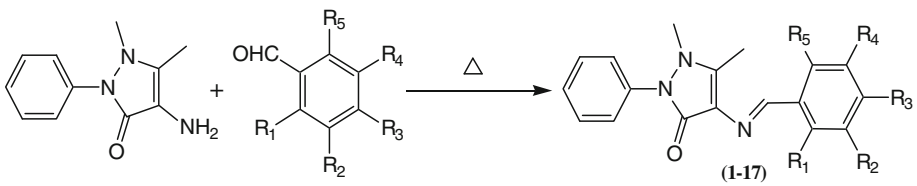
The structures of the synthesized compounds (**1**–**17**) were ascertained on the basis of their consistent IR and NMR spectral characteristics. The formation of Schiff bases was confirmed by the presence of stretching bands in the range of 1,608–1,576 cm^{-1}. The presence of C=O functional group of antipyrine was marked by the appearance of stretching bands ranging from 1,662 to 1,602 cm^{-1}. The presence of C=C skeletal stretching bands in the region of 1,589–1,502 cm^{-1} indicated the presence of phenyl nucleus. The C–Cl stretching bands at 750–747 cm^{-1} depicted the presence of chloro functional group in compounds **5**, **13**, **14**, and **16**. The appearance of aromatic stretching bands in the range of 1,137–1,128 cm^{-1} in compounds **3**, **6**, **7**, **8**, **9**, and **17** indicated the presence of methoxy group in their structures. The appearance of stretching band around 1,256–1,210 cm^{-1} indicated the presence of hydroxyl group in compounds **2**, **4**, **8**, and **15**. The presence of aromatic nitro stretching band at 1,522 cm^{-1} (asymmetric NO_2 stretching) depicted the presence of nitro functional group in compound **10**.

The formation of Schiff bases was confirmed by the appearance of singlet signal around δ 9.37–10.12 ppm. The appearance of singlet signal in the range of δ 2.22–2.66 ppm confirmed the presence of *N*-CH_3 group of 4-amino antipyrine. The appearance of singlet signal in the range of δ 3.25–3.47 ppm in compounds **3**, **6**, **7**, **8**, **9**, and **17** indicated the presence of methoxy group in their structures. The appearance of multiplet signal around δ 6.71–8.74 ppm depicted the presence of aromatic protons.

Antimicrobial activity

The synthesized compounds were evaluated for their in vitro antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and antifungal activity against *C. albicans* and *A. niger* by tube dilution method using norfloxacin (antibacterial) and fluconazole (antifungal) as reference standards, and the results are presented in Table 2.

Among the synthesized compounds, 4-[(3-chloro-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (**16**) was found to be effective against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans* with pMIC values of 1.72, 1.72, 2.02, and 2.02, respectively (Table 2).



Scheme 1 Scheme for the synthesis of 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones

Table 1 Physicochemical characteristics of the synthesized 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones

Comp.	R_1	R_2	R_3	R_4	R_5	M. formula	M. wt.	m.p.	Rf value[a]	% Yield
1	H	H	$N(CH_3)_2$	H	H	$C_{20}H_{22}N_4O$	334	231–233	0.80	76.87
2	H	OC_2H_5	OH	H	H	$C_{20}H_{21}N_3O_3$	351	220–222	0.62	81.45
3	H	H	OCH_3	H	H	$C_{19}H_{19}N_3O_2$	321	171–173	0.62	92.76
4	H	OH	H	H	H	$C_{18}H_{17}N_3O_2$	307	86–88	0.84	83.26
5	Cl	H	H	H	H	$C_{18}H_{16}ClN_3O$	326	195–197	0.84	73.28
6	H	OCH_3	H	H	H	$C_{19}H_{19}N_3O_2$	321	135–137	0.64	90.10
7	H	OCH_3	OCH_3	H	H	$C_{20}H_{21}N_3O_3$	351	274–276	0.80	92.80
8	H	OCH_3	OH	H	H	$C_{19}H_{19}N_3O_3$	337	208–210	0.76	81.64
9	OCH_3	H	H	H	H	$C_{19}H_{19}N_3O_2$	321	216–218	0.80	87.48
10	H	NO_2	H	H	H	$C_{18}H_{16}N_4O_3$	336	176–178	0.90	77.52
11	H	H	H	H	H	$C_{18}H_{17}N_3O$	291	179–181	0.70	84.54
12	OH	OCH_3	H	H	H	$C_{19}H_{19}N_3O_3$	337	210–212	0.60	78.24
13	H	H	Cl	H	H	$C_{18}H_{16}ClN_3O$	326	256–258	0.70	94.66
14	Cl	H	Cl	H	H	$C_{18}H_{15}Cl_2N_3O$	360	211–213	0.80	88.44
15	H	H	OH	H	H	$C_{18}H_{17}N_3O_2$	307	230–232	0.80	76.34
16	H	Cl	H	H	H	$C_{18}H_{16}ClN_3O$	326	192–194	0.96	84.75
17	H	OCH_3	OCH_3	OCH_3	H	$C_{21}H_{23}N_3O_4$	381	195–197	0.82	88.30

[a] TLC mobile phase—Toluene:Chloroform (7:3)

Table 2 Antimicrobial activity (pMIC in μM/ml) of the synthesized 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones

Comp.	$pMIC_{sa}$	$pMIC_{bs}$	$pMIC_{ec}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
1	1.13	1.13	1.43	1.43	1.43	1.23	1.43	1.31
2	1.15	1.15	1.45	1.45	1.45	1.25	1.45	1.33
3	1.11	1.11	1.41	1.41	1.41	1.21	1.41	1.29
4	1.09	1.39	1.39	1.39	1.39	1.29	1.39	1.33
5	1.12	1.42	1.42	1.42	1.42	1.32	1.42	1.36
6	1.11	1.11	1.41	1.41	1.41	1.21	1.41	1.29
7	1.15	1.15	1.45	1.45	1.45	1.25	1.45	1.33
8	1.13	1.13	1.43	1.43	1.43	1.23	1.43	1.31
9	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41
10	1.13	1.43	1.43	1.43	1.43	1.33	1.43	1.37
11	1.07	1.07	1.37	1.37	1.37	1.17	1.37	1.25
12[a]	1.13	1.13	1.43	1.43	1.13	1.23	1.28	1.25
13	1.12	1.12	1.12	1.42	1.42	1.12	1.42	1.24
14	1.16	1.16	1.46	1.46	1.46	1.26	1.46	1.34
15[a]	1.39	1.39	1.39	1.39	1.69	1.39	1.54	1.45
16[a]	1.72	1.72	2.02	2.02	1.42	1.82	1.72	1.78
17	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48
S.D.	0.18	0.19	0.17	0.15	0.10	0.16	0.09	0.13
Std.	2.61[b]	2.61[b]	2.61[b]	2.64[c]	2.64[c]	–	–	–

[a] Outliers

[b] Norfloxacin

[c] Fluconazole

4-[(4-Hydroxy-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1, 2-dihydro-pyrazol-3-one (**15**) was effective against *A. niger* (pMIC = 1.69). The results of MBC/MFC studies (Table 3) revealed that the synthesized compounds were bacteriostatic and fungistatic in action as their MFC and MBC values were threefold higher than their MIC values (Emami *et al.*, 2004).

Anticancer activity

The anticancer activity of the synthesized 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones was determined against human colorectal DLD-1, HT29, HCT116, and murine leukemia, P388 cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,

Table 3 MBF/MFC of the synthesized 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones

Comp.	Minimum bactericidal/fungicidal concentration (μM/ml)				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	>0.15	>0.15	>0.15	>0.15	0.15
2	>0.14	>0.14	>0.14	>0.14	>0.14
3	>0.16	>0.16	>0.16	>0.16	>0.16
4	>0.16	>0.16	>0.16	>0.16	>0.16
5	>0.15	>0.15	>0.15	>0.15	>0.15
6	>0.16	>0.16	>0.16	>0.16	0.16
7	>0.14	>0.14	>0.14	>0.14	>0.14
8	>0.15	>0.15	>0.15	>0.15	>0.15
9	>0.16	>0.16	>0.16	>0.16	>0.16
10	>0.15	>0.15	>0.15	>0.15	>0.15
11	>0.17	>0.17	>0.17	>0.17	>0.17
12	>0.15	>0.15	>0.15	>0.15	>0.15
13	>0.15	>0.15	>0.15	>0.15	0.15
14	>0.14	>0.14	>0.14	>0.14	>0.14
15	>0.16	>0.16	>0.16	>0.16	>0.16
16	>0.15	>0.15	>0.15	>0.15	>0.15
17	>0.13	>0.13	>0.13	>0.13	0.13
Standard	0.019[a]	0.019[a]	0.019[a]	0.040[b]	0.040[b]

[a] Norfloxacin

[b] Fluconazole

Table 4 Cytotoxicity (pIC_{50}) of the synthesized 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones against human colon and murine leukemia cancer cell lines

Compound	pIC_{50} (μM/ml)			
	P388	DLD-1	HCT116	HT29
1	0.33	−0.06	0.08	0.48
2	0.53	0.14	−0.03	0.53
3	0.73	0.01	0.24	0.23
4	−0.36	−0.43	0.21	0.78
5	−0.49	−0.38	0.07	0.51
6	0.98	−0.08	0.03	0.81
7	0.85	−0.17	0.24	0.22
8	0.50	−0.12	0.33	0.27
9	0.64	0.03	0.49	1.15
10	0.31	0.31	0.44	0.13
11	0.62	−0.03	0.23	0.07
12	−0.07	NA	1.05	0.62
13	−0.19	0.20	0.23	0.51
14	−0.44	0.90	0.57	0.75
15	0.27	−0.19	0.19	0.41
16	0.26	−0.14	0.04	0.57
17	0.30	−0.30	0.28	0.56
5-FU	1.66	2.03	1.34	1.07

Data represent mean values of three replicates

NA Not able to obtain IC_{50} after three independent tests

5-diphenyl tetrazolium bromide (MTT) assay, and the results are presented in Table 4.

4-[(3-Methoxy-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (**6**) showed potent activity against the P388 cancer cell line (pIC_{50} = 0.98 μM/ml, Table 4). Against the DLD-1 cancer cell line, 4-[(2, 4-dichloro-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1, 2-dihydro-pyrazol-3-one (**14**) emerged as the most effective one with a pIC_{50} value of 0.90 μM/ml. 4-[(2-Hydroxy-3-methoxy-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1, 2-dihydro-pyrazol-3-one (**12**) showed potent activity against HCT116 cancer cell line (pIC_{50} = 1.05 μM/ml). Compound, 4-[(2-methoxy-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (**9**) exhibited potential anticancer activity against HT29 cancer cell line (pIC_{50} = 1.15 μM/ml). All the synthesized compounds, however, were less active in comparison with the standard drug 5-fluorouracil (5-FU) except compound **9** (pIC_{50} = 1.15 μM/ml) which was almost equivalent to that of 5-FU (pIC_{50} = 1.07 μM/ml) against HT29. This indicated that compound **9** be selected as a lead compound for the development of novel anticancer agents.

Structure–activity relationship

1. Compound **16**, that possess electron-withdrawing *m*-chloro substituent on aromatic aldehyde portion emerged as the most potent candidate against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*. The role of electron-withdrawing group in improving antimicrobial activity is supported by the studies of Sharma *et al*., (2004). It is important to note that incubation temperature was the same for all three bacterial species as well as *C. albicans* (a fungus). This similarity in the incubation conditions may be the reason for the higher activity of compound **16** against *C. albicans* and the bacterial species.
2. In the case of antifungal activity against *A. niger*, compound **15** with *p*-hydroxy group on benzaldehyde portion was found to be the most active one. Furthermore, it is important to note that the presence of OH group may be effective in forming hydrogen bonding at the target site. The role of electron releasing group in improving antifungal activity is supported by the studies of Emami *et al*., (2008).
3. In the case of anticancer activity against DLD-1 cell line, compound **14** with 2,4-dichloro moiety proved to be the most active one among the synthesized

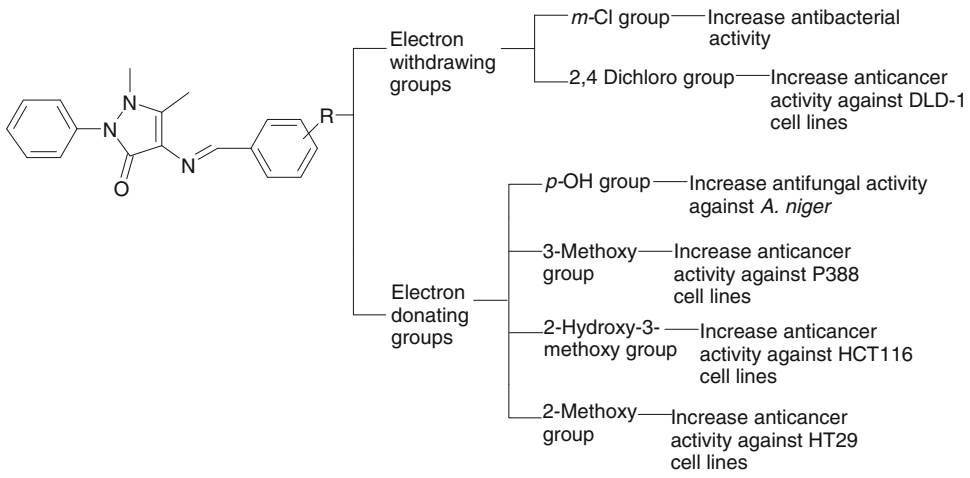


Fig. 1 Structural requirements for the antimicrobial and anticancer activities of the synthesized 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones

antipyrine derivatives. Compounds **6** and **9** that have *m*- and *o*-methoxy groups, respectively, were found to have higher anticancer activity against P388 and HT29 cell lines, respectively, whereas compound **12** having 2-hydroxy, 3-methoxy group emerged as the most effective against the HCT116 cell line. This showed that different structural requirements exhibit selectivity against different cancer cell lines.

The aforementioned findings are summarized in Fig. 1.

QSAR study for antimicrobial activity

In order to identify the substituent effect on antimicrobial activity, QSAR study was undertaken using linear free energy relationship model (LFER) as described by Hansch and Fujita (Hansch and Fujita, 1964). Biological activity (MIC values) was first transformed into pMIC values (i.e., –log MIC) and then used as dependent variable in QSAR study. The different molecular descriptors and values of selected descriptors used in the present study are depicted in Tables 5 and 6, respectively.

Our earlier studies (Kumar *et al*., 2011, Judge *et al*., 2011a, b; Narang *et al*., 2011a, b) indicated that the multi-target QSAR (*mt*-QSAR) models are better than the one-target QSAR (*ot*-QSAR) models in describing anti-microbial activity. In the present study, therefore, we developed *mt*-QSAR models to describe the antimicrobial activity of synthesized 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones.

According to *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 *ot*-QSAR models, however, is not practical to be used, when results of each compound is predicted for more than one target. In these cases one *ot*-QSAR for each target must be developed. This subsequently led to an increased interest in the development of *mt*-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 in describing the antibacterial and antifungal activities

Table 5 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	Nuclear Energy (*Nu. E*)	Electronic
21	Molar refractivity (MR)	Steric

(Gonzalez-Diaz *et al*., 2008; Cruz-Monteagudo *et al*., 2007; Gonzalez-Diaz *et al*., 2007; Gonzalez-Diaz and Prado-Prado, 2008).

In the light of the above, we have attempted to develop three different *mt*-QSAR models, viz., *mt*-QSAR model that describes antibacterial and antifungal activities as well as a common *mt*-QSAR model that describes antimicrobial (overall antibacterial and antifungal) activity of the synthesized compounds.

In order to develop *mt*-QSAR models, we have calculated the average antibacterial, antifungal, and antimicrobial activities of antipyrine derivatives which are presented in Table 2. These average activity values were correlated with molecular descriptors of the synthesized compounds (Table 7).

In the present study, compounds **12**, **15**, and **16** were identified as outliers as their presence resulted in a loss of correlation ($r = 0$, Eq. 1) with $^{1}\chi^{v}$ which never entered the model, whereas their removal improved the r value significantly with $^{1}\chi^{v}$ ($r = 0.942$, Eq. 2) during regression analysis. 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) are not the same as in the (rest of the) training data.
2. *X* outliers are substances molecular descriptors of which 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 the outliers (**12**, **15**, and **16**) when compared to other antipyrine

Table 6 Values of selected parameters used in QSAR studies of 4-(substituted benzylidene-amino)-1, 5-dimethyl-2-phenyl-1, 2-dihydropyrazol-3-ones

Comp.	log *P*	$^{1}\chi^{v}$	R	*J*	W	*Nu. E*	I.P.	LUMO	HOMO	μ
1	2.64	8.14	11.99	1.38	1628.00	25410.60	8.02	−0.61	−8.02	3.03
2	2.18	8.36	12.52	1.37	1778.00	27521.50	8.36	−0.76	−8.36	2.68
3	2.23	7.64	11.61	1.38	1439.00	23669.60	8.39	−0.71	−8.39	4.07
4	1.97	7.25	11.08	1.39	1236.00	22102.80	8.60	−0.83	−8.60	3.98
5	2.92	7.63	11.09	1.43	1220.00	22310.90	8.68	−0.71	−8.68	2.42
6	2.23	7.64	11.61	1.37	1407.00	23758.70	8.56	−0.76	−8.56	3.62
7	2.10	8.17	12.56	1.40	1774.00	27595.50	8.31	−0.75	−8.31	4.08
8	1.84	7.78	12.02	1.40	1578.00	25758.10	8.38	−0.78	−8.38	3.02
9	2.23	7.64	11.63	1.44	1375.00	24372.10	8.56	−0.50	−8.56	3.24
10	2.08	7.61	11.99	1.37	1580.00	25096.60	8.87	−1.23	−8.87	9.26
11	2.36	7.11	10.68	1.41	1088.00	20434.00	8.55	−0.75	−8.55	2.99
12[a]	1.84	7.78	12.04	1.41	1546.00	26129.00	8.51	−0.86	−8.51	1.54
13	2.92	7.62	11.08	1.40	1252.00	21860.70	8.62	−0.90	−8.62	3.89
14	3.47	8.14	11.49	1.43	1388.00	23831.60	8.74	−0.85	−8.74	3.42
15[a]	1.97	7.62	11.08	1.41	1236.00	21936.00	8.66	−0.88	−8.66	4.48
16[a]	2.92	8.70	13.51	1.43	2129.00	31999.10	8.52	−0.87	−8.52	4.97
17	1.98	8.70	13.51	1.43	2129.00	31999.10	8.52	−0.87	−8.52	4.97

[a] Outliers

Table 7 Correlation matrix for the antibacterial, antifungal, and antimicrobial activities of synthesized compounds with selected molecular descriptors

	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$	log *P*	$^{1}\chi^{v}$	*J*	*Nu. E*	I.P.	LUMO
$pMIC_{ab}$	1.000	0.436	0.986	−0.279	0.410	0.472	0.595	0.206	−0.007
$pMIC_{af}$		1.000	0.579	0.107	0.942	0.139	0.835	−0.104	−0.202
$pMIC_{am}$			1.000	−0.233	0.544	0.453	0.692	0.168	−0.044
log *P*				1.000	0.030	0.314	−0.403	0.238	0.068
$^{1}\chi^{v}$					1.000	0.083	0.887	−0.364	0.026
J						1.000	0.025	0.246	0.370
Nu. E							1.000	−0.323	−0.080
I.P.								1.000	−0.601
LUMO									1.000

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) (Furusjo *et al.*, 2006).

The antifungal activity of the antipyrine derivatives is best described by the topological parameter, valence first-order molecular connectivity index ($^1\chi^v$) ($r = 0.942$, Table 7, Eq. 2)

LR *mt*-QSAR model for antifungal activity

$$\begin{aligned} &\mathrm{pMIC_{af}} = 1.441, \\ &\quad n = 17,\ r = 0,\ q^2 = -0.131,\ s = 0.089,\ F \\ &\quad = -1,\ \mathrm{SSE} = 0.127,\ \mathrm{PRESS} = 0.144,\ r^2 = 0 \end{aligned} \tag{1}$$

$$\begin{aligned} &\mathrm{pMIC_{af}} = 0.062\,{}^1\chi^v + 0.941, \\ &\quad n = 14,\ r = 0.942,\ q^2 = 0.849,\ s = 0.009,\ F \\ &\quad = 94.86,\ \mathrm{SSE} = 0.001,\ \mathrm{PRESS} = 0.002,\ r^2 = 0.888 \end{aligned} \tag{2}$$

were and hereafter, n is the number of data points; r is the correlation coefficient; q^2 is the cross validated r^2 obtained by leave one out method; s is the standard error of the estimate; and F is the Fischer statistics. SSE is the sum of squared errors of prediction, PRESS is the predicted residual error sum of squares, and r^2 is the squared correlation coefficient.

The antifungal activity of the synthesized compounds is positively correlated to valence first-order molecular connectivity index ($^1\chi^v$) i.e., activity of the synthesized compounds will increase with increase in value of valence first-order molecular connectivity index ($^1\chi^v$). This is evident from the antifungal activity data of the synthesized compounds (Table 2) and their $^1\chi^v$ values (Table 6). Compound **17** which has a high $^1\chi^v$ value of 8.70 (Table 6) exhibited maximum antifungal activity ($\mathrm{pMIC_{af}} = 1.48$, Table 2), whereas compound **11** with a minimum $^1\chi^v$ value of 7.11 (Table 6), showed minimum antifungal activity ($\mathrm{pMIC_{af}} = 1.37$, Table 2). It was also evident that the outliers (**12**, **15**, and **16**) did not demonstrate a linear relationship with their $^1\chi^v$ and antifungal activity values.

The molecular connectivity index, an adjacency-based topological index proposed by Randic, is denoted by χ and is defined as the sum over all the edges (ij) as per the following:

$$\chi \sum_{i=1}^{n} (V_i V_j)^{-1/2}$$

where V_i and V_j are the degrees of adjacent vertices i and j, respectively, and n is the number of vertices in a hydrogen-suppressed molecular structure (Lather and Madan, 2005).

The topological index, χ, signifies the degree of branching, connectivity of atoms, and unsaturation in the molecule which accounts for variation in activity (Gupta *et al.*, 2003).

From the correlation matrix (Table 7), it is observed that the electronic parameter nuclear energy (*Nu. E*) was effective in describing antibacterial activity of the synthesized compounds (Eq. 3).

LR *mt*-QSAR model for antibacterial activity

$$\begin{aligned} &\mathrm{pMIC_{ab}} = 0.00002 Nu.\ E + 0.802, \\ &\quad n = 14,\ r = 0.594,\ q^2 = 0.187,\ s = 0.078,\ F \\ &\quad = 6.56,\ \mathrm{SSE} = 0.073,\ \mathrm{PRESS} = 0.135,\ r^2 = 0.353 \end{aligned} \tag{3}$$

In the case of antifungal activity, the coefficient of nuclear energy in Eq. 3 is positive which signifies that the antibacterial activity of the synthesized compounds positively correlated to nuclear energy. This is evident from the antibacterial activity data of the synthesized antipyrine derivatives (Table 2), and their nuclear energy values (Table 6).

The addition of topological parameter, Balaban index (J) to electronic parameter, nuclear energy (*Nu. E*) significantly improved the correlation coefficient from 0.594 to 0.750 (Eq. 4).

MLR *mt*-QSAR model for antibacterial activity

$$\begin{aligned} &\mathrm{pMIC_{ab}} = 1.783J + 0.00002 Nu.\ E - 1.685, \\ &\quad n = 14,\ r = 0.750,\ q^2 = 0.296,\ s = 0.067,\ F \\ &\quad = 7.08,\ \mathrm{SSE} = 0.049,\ \mathrm{PRESS} = 0.080,\ r^2 = 0.563 \end{aligned} \tag{4}$$

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

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

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 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 (Balaban, 1982).

$\mu = \mathrm{M} - \mathrm{N} + 1.$

The *mt*-QSAR model of antimicrobial activity (Eq. 5) depicted the importance of electronic parameter, nuclear energy (*Nu*. *E*), in describing antimicrobial activity of the synthesized compounds.

LR *mt*-QSAR model for antimicrobial activity

$$\mathrm{pMIC_{am}} = 0.00002Nu.\,E + 0.972,$$
$$n = 14,\ r = 0.692,\ q^2 = 0.219,\ s = 0.046,\ F = 11.04,\ \mathrm{SSE} = 0.026,\ \mathrm{PRESS} = 0.039,\ r^2 = 0.479 \quad (5)$$

Further, in search of a better QSAR model, topological parameter, Balaban topological index (*J*), was coupled with electronic parameter, nuclear energy (*Nu*. *E*). This change resulted in a significant improvement of the *r* value ($r = 0.818$, Eq. 6) as well as its q^2 value ($q^2 = 0.475$) which was almost equal to the qualifying value i.e., $q^2 = 0.5$.

MLR *mt*-QSAR model for antimicrobial activity

$$\mathrm{pMIC_{am}} = 1.125J + 0.00001Nu.\,E - 0.598,$$
$$n = 14,\ r = 0.818,\ q^2 = 0.475,\ s = 0.038,\ F = 11.15,\ \mathrm{SSE} = 0.016,\ \mathrm{PRESS} = 0.026,\ r^2 = 0.670 \quad (6)$$

The QSAR model expressed by Eq. 2 was cross-validated by its high q^2 values ($q^2 = 0.849$) obtained by leave one out (LOO) method. The value of q^2 greater than 0.5 is the basic requirement for a QSAR model to be considered valid (Golbraikh and Tropsha, 2002). As the observed and predicted values are close to each other (Table 8), the *mt*-QSAR model for antifungal activity (Eq. 2) is the valid one. The plot of predicted $\mathrm{pMIC_{af}}$ against observed $\mathrm{pMIC_{af}}$ (Fig. 2) also favors the developed model expressed by Eq. 2. The low PRESS and SSE values also supported the validity of Eq. 2. Further, the plot of observed $\mathrm{pMIC_{af}}$ versus residual $\mathrm{pMIC_{af}}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of residuals was observed on both sides of zero (Kumar *et al*., 2007).

Even though the low residual values were observed in the case of antibacterial and antimicrobial activity predictions using Eqs. 4 and 6, respectively (Table 8), the q^2 values derived for these equations by leave one out method were less than 0.5, which rendered these models invalid. In summary, the *mt*-QSAR models [Eqs. 1–6] indicated that antifungal activity of the synthesized antipyrine derivatives is governed by the topological parameter, valence first-order molecular connectivity index ($^1\chi^v$).

In general, calculation of predictive r^2 (r^2_{pred}) is a useful statistical parameter for the validation of QSAR models,

Table 8 Observed and predicted antimicrobial activity obtained by *mt*-QSAR models

Comp.	$\mathrm{pMIC_{ab}}$			$\mathrm{pMIC_{af}}$			$\mathrm{pMIC_{am}}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.23	1.24	−0.01	1.43	1.45	−0.02	1.31	1.32	−0.01
2	1.25	1.27	−0.02	1.45	1.46	−0.01	1.33	1.34	−0.01
3	1.21	1.22	−0.01	1.41	1.41	0.00	1.29	1.30	−0.01
4	1.29	1.21	0.08	1.39	1.39	0.00	1.33	1.29	0.04
5	1.32	1.28	0.04	1.42	1.41	0.01	1.36	1.33	0.03
6	1.21	1.20	0.01	1.41	1.41	0.00	1.29	1.29	0.00
7	1.25	1.33	−0.08	1.45	1.45	0.00	1.33	1.38	−0.05
8	1.23	1.29	−0.06	1.43	1.42	0.01	1.31	1.35	−0.04
9	1.41	1.33	0.08	1.41	1.41	0.00	1.41	1.37	0.04
10	1.33	1.22	0.11	1.43	1.41	0.02	1.37	1.30	0.07
11	1.17	1.20	−0.03	1.37	1.38	−0.01	1.25	1.28	−0.03
12[a]	1.23	1.35	−0.12	1.28	1.42	−0.14	1.25	1.35	−0.10
13	1.12	1.21	−0.09	1.42	1.41	0.01	1.24	1.29	−0.05
14	1.26	1.30	−0.04	1.46	1.45	0.01	1.34	1.35	−0.01
15[a]	1.39	1.26	0.13	1.54	1.41	0.13	1.45	1.29	0.16
16[a]	1.82	1.50	0.32	1.72	1.48	0.24	1.78	1.46	0.32
17	1.48	1.45	0.03	1.48	1.48	0.00	1.48	1.47	0.01

[a] Outliers

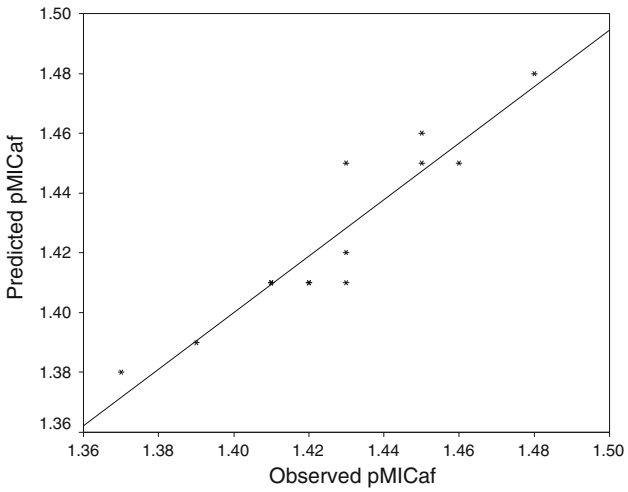


Fig. 2 Plot of observed $\mathrm{pMIC_{af}}$ against predicted $\mathrm{pMIC_{af}}$ by Eq. 2

but the application was not possible in the present study as the compounds were not divided into test and training sets. The predictive r^2 can be calculated by using the formula (Roy *et al*., 2009).

$$r^2_{\mathrm{pred}} = 1 - \left[\Sigma(Y_{\mathrm{pred(test)}} - Y_{\mathrm{test}})^2 / \Sigma(Y_{\mathrm{test}} - Y_{\mathrm{Mean(training)}})^2\right]$$

In general, for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. In the present study, however, the range of antimicrobial activities of the synthesized compounds is within one order of

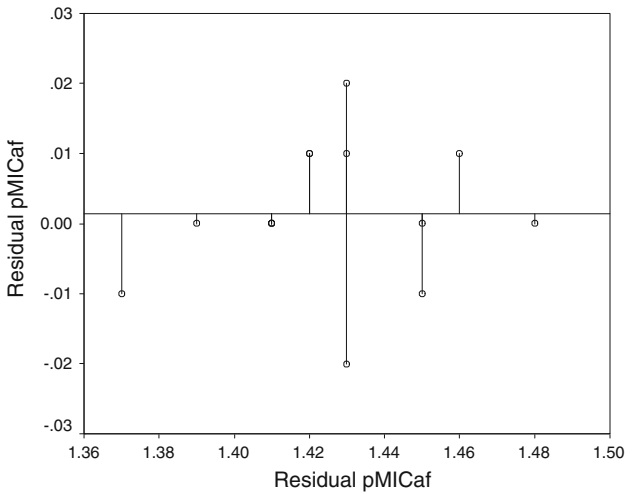


Fig. 3 Plot of observed $pMIC_{af}$ against residual $pMIC_{af}$ by Eq. 2

Table 9 Correlation between different molecular descriptors with anticancer activity of the synthesized compounds

Parameter	pIC_{50} (μM/mL)				
	P388	DLD-1	HCT116	HT29	Avg
log *P*	0.597	0.576	0.185	0.227	−0.001
$^{0}\chi$	0.267	0.008	−0.086	−0.137	0.119
$^{0}\chi^{v}$	0.122	0.123	−0.084	−0.015	0.127
$^{1}\chi$	0.338	−0.043	−0.120	−0.139	0.136
$^{1}\chi^{v}$	0.069	0.200	−0.133	0.010	0.123
$^{2}\chi$	0.142	0.118	−0.053	−0.146	0.086
$^{2}\chi^{v}$	−0.267	0.405	0.022	0.074	0.049
$^{3}\chi$	−0.245	0.354	0.119	−0.105	−0.019
$^{3}\chi^{v}$	−0.544	0.525	0.154	0.123	−0.040
κ_1	0.298	−0.012	−0.103	−0.140	0.126
κ_2	0.435	−0.102	−0.179	−0.150	0.157
κ_3	0.388	0.013	−0.212	−0.194	0.150
$\kappa\alpha_1$	0.217	0.049	−0.086	−0.092	0.123
$\kappa\alpha_2$	0.323	−0.017	−0.156	−0.083	0.154
$\kappa\alpha_3$	0.238	0.130	−0.177	−0.102	0.150
R	0.338	−0.043	−0.120	−0.139	0.136
J	−0.349	−0.004	0.422	0.333	0.006
W	0.314	−0.028	−0.145	−0.184	0.098
Te	−0.141	−0.117	−0.039	0.121	−0.121
I.P.	−0.447	0.331	0.475	0.126	0.018
LUMO	0.242	−0.317	−0.227	0.442	0.167
HOMO	0.447	−0.331	−0.475	−0.126	−0.018
μ	0.044	0.216	0.369	−0.337	0.084

magnitude. This is in accordance with results suggested by Bajaj *et al*. (2005) who stated that the reliability of the QSAR model lies in its predictive ability, even though the activity data are narrow in range. 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 (Narasimhan *et al*., 2007). The minimum standard deviation (Table 2) observed in the antimicrobial activity justifies its use in QSAR studies.

QSAR study for anticancer activity

In order to identify the substituent effect on anticancer activity of the synthesized compounds, QSAR study was undertaken using linear free energy relationship model (LFER) as described by Hansch and Fujita (Hansch and Fujita, 1964). Biological activity data determined as IC_{50} values were first transformed into pIC_{50} values on molar basis and then used as dependent variable in QSAR study. Preliminary analysis was carried out by finding out the correlation between anticancer activities of the anti-pyrine derivatives with their molecular descriptors (Table 9).

None of the parameters showed appreciable correlation with anticancer activity of the synthesized compounds (Table 9). We attempted both linear and multiple linear regressions in the development of valid QSAR models to predict the anticancer potential of the synthesized compounds. However, we were unable to develop the valid QSAR models, as none of the derived *ot*-QSAR models was statistically significant (Eqs. 7–10).

The poor correlation of different molecular descriptors with anticancer activity prevented the development of *mt*-QSAR model using the average anticancer activity.

ot-QSAR model for anticancer activity against P388 cell lines

$$pIC_{50(P388)} = -0.637 \log P + 1.814, \quad n = 14,\ r = 0.597,\ q^2 = 0.171,\ s = 0.408,\ F = 6.65,\ SSE = 1.999,\ PRESS = 2.570,\ r^2 = 0.356 \tag{7}$$

ot-QSAR model for anticancer activity against DLD-1 cell lines

$$pIC_{50(DLD-1)} = 0.410 \log P - 0.961, \quad n = 14,\ r = 0.575,\ q^2 = 0.389,\ s = 0.277,\ F = 5.95,\ SSE = 0.926,\ PRESS = 1.924,\ r^2 = 0.331 \tag{8}$$

ot-QSAR model for anticancer activity against HCT-116 cell lines

$$pIC_{50(HCT116)} = 0.396\text{I.P.} - 3.135, \quad n = 14,\ r = 0.475,\ q^2 = 0.0002,\ s = 0.159,\ F = 3.49,\ SSE = 0.305,\ PRESS = 0.394,\ r^2 = 0.225 \tag{9}$$

ot-QSAR model for anticancer activity against HT-29 cell lines

$$pIC_{50(HT29)} = 0.816\,LUMO + 1.131,\quad n = 14,\ r = 0.442,\ q^2 = 0.185,\ s = 0.282,\ F = 2.91,\ SSE = 0.960,\ PRESS = 1.414,\ r^2 = 0.195 \quad (10)$$

Conclusion

A series of 4-(substituted benzylidene-amino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-ones (**1**–**17**) was synthesized and screened in vitro for its antimicrobial and anticancer activities. The antimicrobial results indicated that 4-[(3-chloro-benzylidene)-amino]-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one (**16**) was the most effective antimicrobial agent among the synthesized compounds. The anticancer screening results indicated that compound **9** ($pIC_{50} = 1.15$ μM/ml) which was effective at a concentration closer to that of the reference drug, 5-FU ($pIC_{50} = 1.07$ μM/ml) against HT29 cell line, may be used as a lead compound for the development of novel anticancer agents. The QSAR studies for antimicrobial activity demonstrated the importance of topological parameter, valence first-order molecular connectivity index ($^1\chi^v$), in describing antifungal activity of the synthesized compounds. The poor correlation of molecular descriptors with anticancer activity prevented the development of valid QSAR model for its prediction.

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