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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of combretastatin analogs: evaluation of in vitro anticancer activity and molecular docking studies

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Abstract This study is based on the synthesis of a series of combretastatin analogs with different substitutions on one aryl moiety and a carboxylic group in connecting chain. Cis-configuration with respect to aryl groups was established by X-ray crystal analysis. All the synthesized compounds were evaluated for anticancer activity against a panel of cell lines. Six compounds **1a**, **1b**, **1c**, **1k**, **1n**, and **1p** showed marked anticancer activity against human colon (colo-205), lung (A549), ovary (IGROV-1), prostrate (PC-3), CNS (SF-295), leukemia (THP-1), and breast (MCF-7) cell lines. Out of these, **1b** showed remarkable inhibitory activity comparable to paclitaxel against lung cancer cell line with IC50 3.9 μM. Importance of carboxylic group in the synthesized compounds was studied by flexible docking study of **1b** which showed the importance of carboxylic group interactions with colchicine-binding site of $\alpha\beta$-tubulin.

Keywords Anticancer · Combretastatin · Molecular docking · Phenyl acetic acid · Tubulin inhibitor

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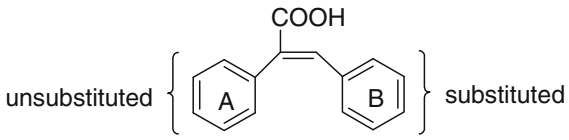
Introduction

Cancer is one of the major causes of death throughout the world. In the U.S., the number of deaths caused by cancer is second highest and next to that from the cardiovascular diseases (Shewach and Kuchta, 2009). Tubulin, a validated target in cancer chemotherapy plays an important role in the formation of the mitotic spindle, which provides the structural framework for the physical segregation of the chromosomes during the mitosis (Hadfield *et al*., 2003). Combretastatin, binds to β-tubulin and strongly inhibit tubulin polymerization by binding to the colchicine site (Ouyang *et al*., 2006; Tron *et al*., 2006; Woods *et al*., 1995) and disrupts the normal mitotic spindle function (McGown and Fox, 1989), and its action may be through endothelial-cadherin signaling pathway (Vincent *et al*., 2005). Combretastatin was first isolated from the bark of African willow tree *Combretum caffrum* (Pettit *et al*., 1987). Two synthetic combretastatin analogs viz., D-24851 and ABT-75 are in advanced phased clinical trials (Ouyang *et al*., 2006; Stokvis *et al*., 2004). Plenty of combretastatin analogs (Fig. 1) have been synthesized (Liou *et al*., 2004, 2008) till date with different substituents on aryl rings. The structure–activity relationship of these analogs reveals that methoxy substituents in ring B are required for biological activity. Furthermore, free hydroxyl group or other equivalent hydrogen bonding donors at ring B are also essential for activity (Furst *et al*., 2009). Combretastatin has low aqueous solubility and *Z*-configured C–C double bond prone to isomerization to *E*-form during storage and administration, which results in dramatic reduction of activity (Bellina *et al*., 2006). To overcome this isomerization problem when heterocyclic rings viz., isoxazole, imidazole, triazole, azetidinones, etc. were introduced in place of the ethene bridge, however this also reduces activity because molecule

becomes planar, as molecular modeling study confirms that combretastatin with ethene bridge has twisted geometries preferred for binding to the colchicines binding site (Lee *et al*., 2008; Kaffy *et al*., 2006). The structure-based modeling studies have been reported to address important structure–activity relationship for the combretastatins (Brown *et al*., 1995; Nandy *et al*., 1991; Kong *et al*., 2005). Recently, Ducki *et al*. (2009) described a docking model for tubulin–combretastatin interactions and compared with colchicine. These studies suggested that the combretastatins show similar binding mode as colchicine and fit well at the colchicine-binding site of tubulin and interaction energies of the compounds with colchicine-binding sites were also calculated (Bellina *et al*., 2006). The structure-based modeling studies have been reported to address important structure–activity relationship for the combretastatins (Brown *et al*., 1995; Nandy *et al*., 1991; Kong *et al*., 2005). A key structural feature is the presence of double bond forcing the two aromatic rings to stay within an appropriate distance is therefore responsible for tubulin affinity (Kaffy *et al*., 2006).

With this background, we herein wish to report the synthesis and anticancer activity of combretastatin analogs of the following general structure:



Results and discussion

Chemistry

Sixteen combretastatin analogs (**1a**–**1p**) were synthesized by condensation of phenyl acetic acid (1) with different substituted aldehydes (**2**) using triethyl amine and acetic anhydride as reported previously (Cushman *et al*., 1995) (Scheme 1). The products showed that the two phenyl rings are *cis* to each other which is in tune with the classical Pschorr reaction (The Merck Index, 2006) and it was further confirmed by X-ray crystal analysis (Fig. 2). The reaction was monitored by TLC. The compounds were purified (yield 46–63%) by column chromatography using silica gel #60–120 and characterized by spectral data (IR, NMR, and MS). All compounds showed IR absorption bands at around 3300–3400 for C–H str. 1680–1700 for carbonyl, 1600 for C=C (phenyl) and about 1200 cm^{-1} for C–O stretching. In 1H NMR proton at C-3 as a singlet is

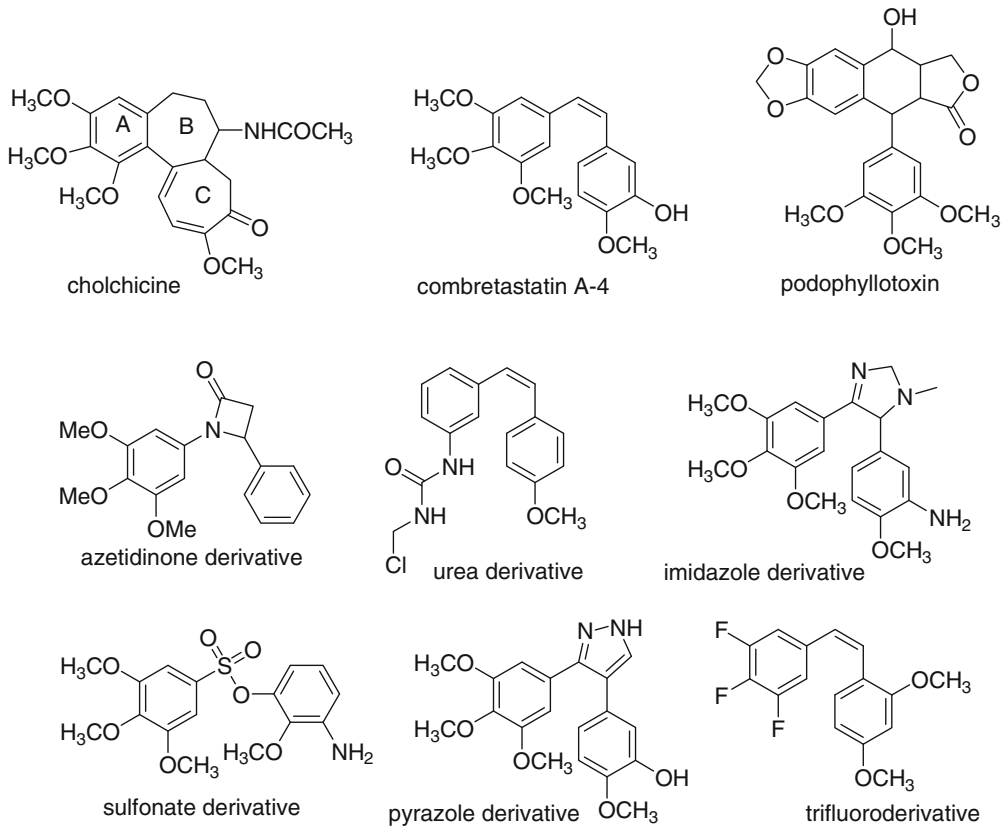
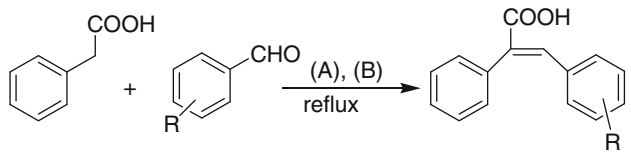


Fig. 1 Combretastatin and its other synthetic analogs

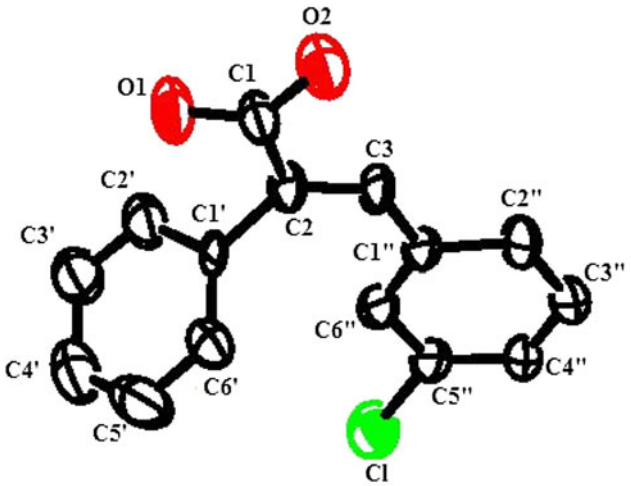


1 **2** **3**

A, acetic anhydride; B, triethyl amine

R= different substitution/s

Scheme 1 Synthesis of combretastatin analogs



1m

Fig. 2 ORTEP derived from a single crystal X-ray analysis of **1m** (non-hydrogen atoms have been labeled using a crystallographic numbering scheme)

highly deshielded because of being β to carbonyl with δ-value 7.8–8.2. Phenyl protons show signal around 7.2–7.7. 13C NMR carbonyl shows signal at around 165–170. Phenyl carbons show signals at around 115–132. 13C assignments have been corroborated by DEPT studies.

Biological (anticancer) activity

All compounds were evaluated for their anticancer activity (Table 1) against human colon (colo-205), lung (A549), ovary (IGROV-1), prostrate (PC-3), CNS (SF-295), leukemia (THP-1), and breast (MCF-7) cell lines. Out of these **1a**, **1b**, **1c**, **1k**, **1n**, and **1p** showed anticancer activity against colo-205 (colon), A-549 (lung), and MCF-7 (breast) cell lines. Compounds **1b** and **1n** showed marked activity comparable to paclitaxel against lung cancer cell line. A comparison of the cytotoxicity (IC50) of compounds **1a**–**1p** with their corresponding calculated Log*P* (Clog*P*) values (Table 1) found no correlation, indicating that the activity observed is not based on the lipophilicity alone.

Molecular docking

To get better interpretation of the observed activity of the synthesized combretastatin analogs, a flexible docking study of most potent compound **1b** was performed on colchicine-binding site of tubulin (PDB entry: 1SA0) (Ravelli *et al.*, 2004). All docking runs were performed using GOLD software. To validate that the selected docking procedure for the prediction of the correct binding mode of ligands binding to the colchicine-binding site, colchicine was extracted from the original X-ray structure (1SA0) and re-docked using GOLD software (Gold *et al.*, 2009). The highest scoring conformation was selected and compared with crystal structure with most stable conformation. The docked conformation of colchicine using GOLD was found to be similar to that found in the original X-ray structure (RMSD = 0.72 Å between the best scored conformers from docking and X-ray structure).

The GOLD scores and visual inspection allowed us to select the most probable binding conformation of **1b** (Fig. 3). The docking study showed that **1b** fits well at the colchicine-binding site. The dimethoxy phenyl ring (ring B) gets positioned in a hydrophobic cavity created by Leu248β, Leu255β, and Val318β. Ring B of inhibitor adopts similar orientation as the trimethoxy ring of colchicine in close proximity to Cys241β. The two methoxy groups at C2 and C3 are 5.2 and 3.8 Å away from the Cys241β, respectively. The three methoxy groups of colchicine also interact with the same residue. The methoxy function at C-2 is involved in a hydrophobic interaction with side chain of Ala250β (Fig. 3). The ring A gets positioned in a hydrophobic cleft formed by Ala180α and Val181α and oriented almost similar as ring C of colchicine. The carbonyl function of carboxylic group of **1b** is involved in hydrogen bond interaction with free $-NH_2$ of Asn258β. The amino acid residue is seen in interaction with OH of tropolone ring of colchicines and the involvement of residue in hydrophobic cleft as well as in hydrophilic region with involvement of H-bond are important indication for the involvement of colchicines binding site for this type of compounds.

Conclusion

A series of combretastatin analogs have been synthesized by condensation of phenyl acetic acid and different substituted aldehydes. Structures were assigned with the help of elemental analysis and spectral techniques like IR,

Table 1 Anticancer activity data of compounds **1a–1p**

Compound code	Conc. (mol)	% Growth inhibition							CLog*P*[a]
		PC-3 Prostrate	IGR-OV-1 Ovary	SF-295 CNS	A-549 Lung	MCF-7 Breast	COLO-205 Colon	THP-1 Leukemia	
1a	5×10^{-5}	0	0	14	52	29	–	–	3.59
	1×10^{-4}	4	0	25	54	41	–	–	
1b	5×10^{-5}	2	6	8	60	25	–	–	3.25
	1×10^{-4}	4	11	24	89	52	–	–	
1c	5×10^{-5}	10	0	13	21	54	–	–	2.89
	1×10^{-4}	22	1	25	65	63	–	–	
1d	5×10^{-5}	11	0	15	0	32	–	–	3.55
	1×10^{-4}	39	32	38	45	39	–	–	
1e	5×10^{-5}	12	8	0	0	14	–	–	2.77
	1×10^{-4}	23	17	12	20	24	–	–	
1f	5×10^{-5}	4	–	–	0	–	5	0	3.24
	1×10^{-4}	28	–	–	20	–	16	21	
1g	5×10^{-5}	10	–	–	15	–	32	0	3.33
	1×10^{-4}	22	–	–	25	–	60	0	
1h	5×10^{-5}	0	–	–	10	–	15	8	4.04
	1×10^{-4}	9	–	–	43	–	44	35	
1i	5×10^{-5}	0	–	–	0	–	0	0	3.60
	1×10^{-4}	0	–	–	10	–	0	4	
1j	5×10^{-5}	0	–	–	0	–	0	0	2.89
	1×10^{-4}	7	–	–	0	–	0	0	
1k	5×10^{-5}	26	–	–	0	–	51	0	4.76
	1×10^{-4}	35	–	–	0	–	70	22	
1l	5×10^{-5}	36	32	–	12	5	–	–	4.30
	1×10^{-4}	52	42	–	14	10	–	–	
1m	5×10^{-5}	30	0	–	32	0	–	–	4.30
	1×10^{-4}	68	38	–	40	0	–	–	
1n	5×10^{-5}	14	0	–	61	10	–	–	4.30
	1×10^{-4}	42	0	–	74	22	–	–	
1o	5×10^{-5}	53	6	–	10	6	–	–	2.92
	1×10^{-4}	60	18	–	20	20	–	–	
1p	5×10^{-5}	31	15	–	52	14	–	–	2.32
	1×10^{-4}	74	31	–	82	26	–	–	
Adriamycin	1×10^{-6}	–	–	87	–	77	–	–	
Mitomycin	1×10^{-5}	58	–	–	–	65	–	–	
Paclitaxel	1×10^{-5}	–	66	–	67	–	–	–	
5-Fluorouracil	2×10^{-5}	–	–	–	–	–	74	69	

IC50 of only active compounds **1a**, **1b**, **1c**, **1k**, **1n**, and **1p** were calculated on the basis of 5 different concentrations viz., 1×10^{-5}, 3×10^{-5}, 5×10^{-5}, 7×10^{-5}, and 1×10^{-4} mol, found to be 85.4, 3.9, 88.0, >100, 34.0, and 96.5 μM, respectively

[a] Clog*P* values were calculated using ChemDraw Ultra V.8.0 (Cambridge Soft Corporation)

MS, NMR (1H & 13C). All the synthesized compounds were evaluated for anticancer activity against various cell lines and some of them were found to be active. The favorable binding conformation of **1b** suggests its prevailing role as microtubule polymerization inhibitors and anticancer agents.

Experimental

The reagents were purchased from Sigma-Aldrich, Loba and CDH, India and used without further purification. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples

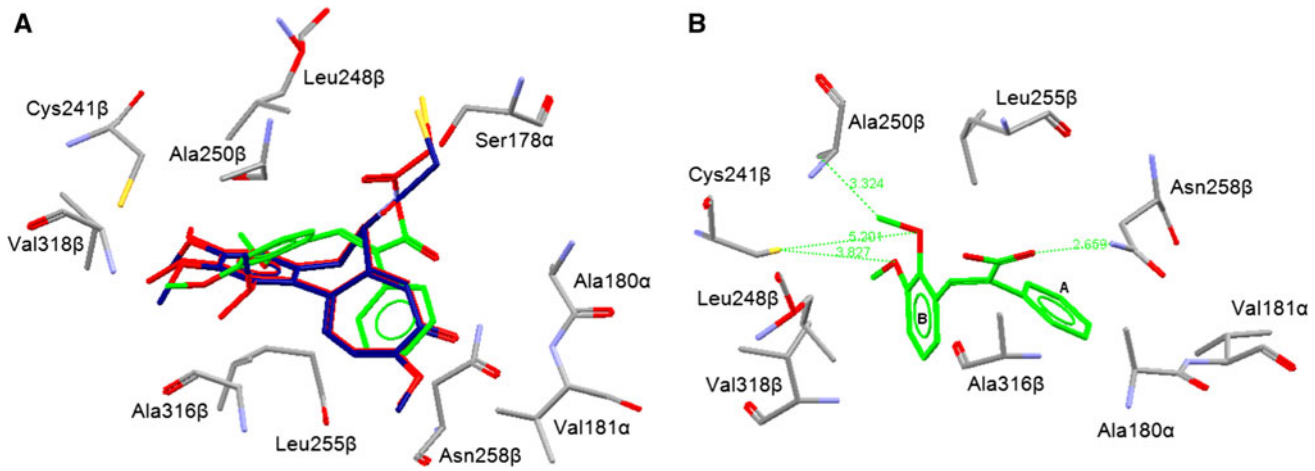


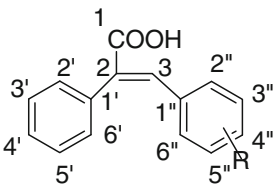
Fig. 3 **a** Overlay of colchicine (*blue/dark*) and **1b** at the colchicine-binding site. **b** Docking conformation of **1b** (Color figure online)

and by spectroscopic data (IR, 1H NMR, and 13C NMR spectra). The spectra were measured in $CDCl_3$ relative to TMS (0.00 ppm). IR (KBr pallets) spectra were recorded on a Fourier transform infrared (FT-IR) Thermo spectrophotometer. Melting points were determined in open capillaries and were found to be uncorrected.

General procedure for synthesis of compounds (**1a**–**1p**) (Table 2)

A mixture of phenyl acetic acid (7.36 mmol), different substituted benzaldehydes (7.36 mmol) and triethylamine (2 ml) in Ac_2O (4 ml) was refluxed for 4–9 h. After completion of reaction (monitored by TLC), the reaction mixture was cooled and acidified with 35% aqueous HCl (6 ml) and kept at room temperature overnight. The precipitate was collected by filtration and purified by column chromatography using silica gel 60–120 and 30% ethyl acetate in hexane as mobile phase to afford the pure product.

Spectral analyses



*(Z)-2,3-Diphenyl-prop-2-enoic acid (**1a**)*

M.p. 145–147°C; IR ν_{max} cm^{-1} (KBr): 3450, 1674, 1615, 1269; 1HNMR ($CDCl_3$) δ; 7.3–7.0 (10H, m, 5xAr–H, 5xAr′–H), 7.95 (1H, s, H-3); 13C NMR ($CDCl_3$): 116–128.7, 132.0, 143.7, 166.5; MS (ESI) $m/z = 246.8$ $(M+Na)^+$; Anal. ($C_{15}H_{12}O_2$) C, H.

*(Z)-3-(2,3-Dimethoxyphenyl)-2-phenyl-prop-2-enoic acid (**1b**)*

M.p. 147–149; IR ν_{max} cm^{-1} (KBr): 3418, 1672, 1638, 1260; 1HNMR ($CDCl_3$) δ: 3.8 (3H, s, H-2″-OCH_3), 3.9 (3H, s, H-3″-OCH_3), 6.1 (1H, d, $j = 7.7$ Hz, Ar–H), 7.3–7.2 (5H, m, 5xAr–H), 8.2 (1H, s, H-3); 13C NMR ($CDCl_3$): 52.6 (C-2″-OCH_3), 53.8 (C-3″-OCH_3), 119.0, 121.0, 123.0, 128.4, 129.6, 130.0, 132.0, 133.9, 136.2, 138.0, 139.7, 140.0, 141.1, 146.1, 166.8; MS (ESI) $m/z = 307$ $(M+Na)^+$; Anal. ($C_{17}H_{16}O_4$) C, H.

*(Z)-3-(3,4,5-Trimethoxyphenyl)-2-pheny-prop-2-enoic acid (**1c**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 3411, 1670, 1617, 1579, 1506, 1421; 1HNMR ($CDCl_3$) δ: 3.5 (6H, s, 2xAr-OCH_3), 3.8 (3H, s, Ar-OCH_3), 6.3 (2H, s, Ar–H), 7.4–7.2 (5H, m, 5xAr′-H), 7.8 (1H, s, H-3); 13C NMR ($CDCl_3$): 52.3, 53.0, 55.6, 121.1, 126.6, 128.0, 128.2, 128.9, 129.8, 142.2, 151.7, 152.6, 156.9, 159.7, 164.5, 174.8; MS (ESI) $m/z = 337$ $(M+Na)^+$; Anal. ($C_{18}H_{18}O_5$) C, H.

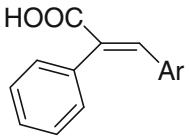
*(Z)-3-(3,4-Methylenedioxyphenyl)-2-phenyl-prop-2-enoic acid (**1d**)*

M.p. 188–189; IR ν_{max} cm^{-1} (KBr): 3417, 1667, 1485, 1423, 1184, 1004; 1HNMR ($CDCl_3$) δ: 5.9 (2H, s, $-OCH_2O-$), 6.6 (3H, m, Ar″–H), 7.4–7.2 (5H, m, 5xAr′-H), 7.8 (1H, s, H-3); 13C NMR ($CDCl_3$): 101.6, 114.0, 119.0, 122.0, 132.4, 136.0, 166.6; MS (ESI) $m/z = 291$ $(M+Na)^+$; Anal. ($C_{16}H_{12}O_4$) C, H.

*(Z)-3-(3-Hydroxy-4-methoxypheny)-2-phenyl-prop-2-enoic acid (**1e**)*

M.p. 202–202; IR ν_{max} cm^{-1} (KBr): 3511, 1670, 1607, 1459, 1267, 1135, 1005, 802, 709; 1HNMR ($CDCl_3$)

Table 2 Reaction time, yield, and elemental analysis data of the synthetics



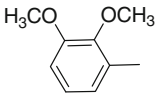
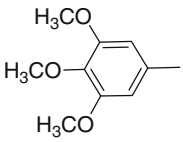
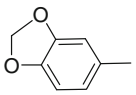
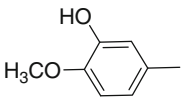
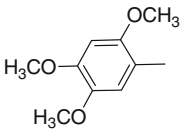
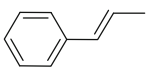
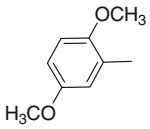
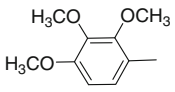
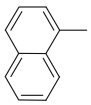
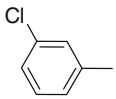
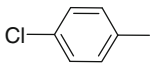
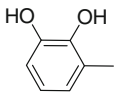
S. No.	Compound code	Ar	Reaction time (h)	% Yield	Elemental analysis (cal./found)		
					C	H	N
1	**1a**		5	58	80.34/80.24	5.39/5.26	–
2	**1b**		7	60	71.82/71.79	5.62/5.42	–
3	**1c**		6	55	68.78/68.56	5.77/5.67	–
4	**1d**		6	55	71.82/71.62	4.51/4.35	–
5	**1e**		5	63	71.10/71.16	5.22/5.56	–
5	**1f**		6	49	68.78/68.56	5.77/5.82	–
7	**1g**		4	58	66.91/66.64	4.12/4.05	5.20/5.24
8	**1h**		8	54	81.58/81.45	5.64/5.45	–

Table 2 continued

S. No.	Compound code	Ar	Reaction time (h)	% Yield	Elemental analysis (cal./found)		
					C	H	N
9	**1i**		6	59	71.82/71.79	5.62/5.22	–
10	**1j**		7	58	68.78/68.56	5.77/5.67	–
11	**1k**		9	53	83.19/83.34	5.14/5.11	–
12	**1l**		5	56	69.64/69.61	4.29/4.46	–
13	**1m**		6	54	69.64/69.51	4.29/4.42	–
14	**1n**		7	60	69.64/69.51	4.29/4.42	–
15	**1o**		6	48	72.33/72.23	5.0/5.12	–
16	**1p**		6	46	70.31/70.34	4.72/4.75	–

δ: 3.0 (Ar-OH), 3.7 (3H, s, Ar-OCH_3), 6.5 (1H, s, Ar–H), 6.6 (1H, s, Ar′–H), 7.3–7.1 (6H, m, 1xAr″-H, 5xAr′-H), 7.7 (1H, s, H-3); 13C NMR ($CDCl_3$): 43.7, 56.2, 113.2, 115.6, 120.0, 126.4, 128.0, 128.7, 132.6, 132.4, 166.5; MS (ESI) $m/z = 315$ $(M+Na)^+$; Anal. ($C_{16}H_{14}O_4$) C, H.

*(Z)-3-(2,4,5-Trimethoxyphenyl)-2-phenyl-prop-2-enoic acid (**1f**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 3477, 1668, 1603, 1506, 1264; 1HNMR ($CDCl_3$) δ: 3.18 (3H, s, Ar-OCH_3), 3.87 (6H, s, Ar-OCH_3), 6.26 (H, s, Ar″–H), 6.43 (H, s, Ar″–H) 7.3–7.4 (5H, m, 5xAr′-H), 8.3 (1H, s, H-3); 13C NMR ($CDCl_3$): 52.3, 53.0, 55.6, 121.1, 126.6, 128.0, 128.2, 128.9, 129.8, 142.2, 151.7, 152.6, 156.9, 159.7, 164.5, 174.8; MS (ESI) $m/z = 337$ $(M+Na)^+$; Anal. ($C_{18}H_{18}O_5$) C, H.

*(Z)-3-(2-Nitrophenyl)-2-phenyl-prop-2-enoic acid (**1g**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 3411, 1670, 1617, 1579, 1506, 1421; 1HNMR ($CDCl_3$) δ: 6.3–6.9 (4H, m, Ar″–H), 7.6–7.2 (5H, m, 5xAr′-H), 8.16 (1H, s, H-3); 13C NMR ($CDCl_3$): 123.3, 124.8, 126.6, 128.0, 128.3, 128.8, 129.1, 130.2, 130.5, 133.5, 140.4, 167.9; MS (ESI) $m/z = 292$ $(M+Na)^+$; Anal. ($C_{15}H_{11}NO_4$) C, H, N.

*(2Z,4E)-2,5-Diphenylpenta-2,4-dienoic acid (**1h**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 3446, 1678, 1621, 1579, 1346; 1HNMR ($CDCl_3$) δ: 5.59 (1H, d, $j = 15.2$, $= CH_2$), 5.96 (1H, dd, $j = 15.2$ & $j = 14.8$, $= CH_2$), 6.8 (1H, d, $j = 15.2$, $= CH_2$), 7.4–7.2 (5H, m, 5xAr′-H), 7.7–7.5 (5H, m, 5xAr′-H) 7.8 (1H, s, H-3); 13C NMR ($CDCl_3$): 120.2, 122.1, 122.8, 123.0, 123.3, 124.2, 126.6, 128.0, 128.2, 128.9, 129.8, 135.5, 135.9, 144.2, 164.5; MS (ESI) $m/z = 273$ $(M+Na)^+$; Anal. ($C_{17}H_{14}O_2$) C, H.

*(Z)-3-(2,5-Dimethoxyphenyl)-2-phenyl-prop-2-enoic acid (**1i**)*

M.p. 147–149; IR ν_{max} cm^{-1} (KBr): 3437, 1666, 1580, 1260; 1HNMR ($CDCl_3$) δ: 3.2 (3H, s, OCH_3), 3.9 (3H, s, –OCH_3), 6.2 (1H, d, $j = 7.9$ Hz, Ar–H), 6.75 (1H, d, $j = 7.9$ Hz, Ar–H) 7.3–7.2 (5H, m, Ar′–H), 8.3 (1H, s, H-3); 13C NMR ($CDCl_3$): 50.6, 54.3, 119.0, 121.0, 125.0, 127.5, 129.6, 130.0, 132.0, 133.8, 135.2, 138, 139.7, 139.0, 145.1, 146.7, 167.6; MS (ESI) $m/z = 307$ $(M+Na)^+$; Anal. ($C_{17}H_{16}O_4$) C, H.

*(Z)-3-(2,3,4-Trimethoxyphenyl)-2-pheny-prop-2enoic acid (**1j**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 3411, 1667, 1491, 1258; 1HNMR ($CDCl_3$) δ: 3.7 (3H, s, Ar-OCH_3), 3.8 (3H, s, Ar-OCH_3), 3.9 (3H, s, Ar-OCH_3), 6.3 (1H, d, $j = 8.8$, Ar″–H), 6.4 (1H, d, $j = 8.8$, Ar″–H), 7.4–7.2 (5H, m, Ar′–H), 8.2 (1H, s, H-3); 13C NMR ($CDCl_3$): 52.9, 53.8, 55.6, 121.1, 126.6, 128.0, 128.2, 128.9, 129.8, 140.2, 151.7, 152.6, 156.9, 159.7, 164.5, 174.5; MS (ESI) $m/z = 337$ $(M+Na)^+$; Anal. ($C_{18}H_{18}O_5$) C, H.

*(Z)-3-(Naphthalene-5yl)-2-phenyl-prop-2-enoic acid (**1k**)*

M.p. 182–184; IR ν_{max} cm^{-1} (KBr): 1677, 1614, 1579, 1506, 1260; 1HNMR ($CDCl_3$) δ: 7.4–7.2 (5H, m, Ar–H), 8.4–8.1 (7H, m, 5xAr′-H), 8.5 (1H, s, = CH); 13C NMR ($CDCl_3$): 120, 123, 124.9, 125.3, 127.3, 127.9, 128.6, 129.3, 130, 130.4, 131.2, 131.5, 131.8, 133.5, 142.2, 146.3, 169.1; MS (ESI) $m/z = 297$ $(M+Na)^+$; Anal. ($C_{19}H_{14}O_2$) C, H.

*(Z)-3-(2-Chlorophenyl)-2-phenyl acrylic acid (**1l**)*

M.p. 148–151; IR ν_{max} cm^{-1} (KBr): 3421, 1663, 1619, 1589, 1516, 1411; 1HNMR ($CDCl_3$) δ: 7.0–6.8 (4H, m, Ar″–H), 7.6–7.1 (5H, m, 5xAr′-H), 8.2 (1H, s, H-3); 13C NMR ($CDCl_3$): 120.3, 129.8, 126.6, 128.0, 128.3, 128.8, 129.1, 130.2, 130.5, 133.5, 140.4, 167.9; MS (ESI) $m/z = 281$ $(M+Na)^+$; Anal. ($C_{15}H_{11}ClO_2$) C, H.

*(Z)-3-(3-Chlorophenyl)-2-phenyl-prop-2enoic acid (**1m**)*

M.p. 131–133; IR ν_{max} cm^{-1} (KBr): 3421, 1663, 1619, 1589, 1516, 1411; 1HNMR ($CDCl_3$) δ: 7.0–6.8 (4H, m, Ar″–H), 7.6–7.1 (5H, m, 5xAr′-H), 8.2 (1H, s, H-3); 13C NMR ($CDCl_3$): 120.3, 129.8, 126.6, 128.0, 128.3, 128.8, 129.1, 130.2, 130.5, 133.5, 140.4, 167.9; MS (ESI) $m/z = 281$ $(M+Na)^+$; Anal. ($C_{15}H_{11}ClO_2$) C, H.

*(Z)-3-(4-Chlorophenyl)-2-phenyl-prop-2enoic acid (**1n**)*

M.p. 121–123; IR ν_{max} cm^{-1} (KBr): 3451, 1643, 1624, 1585, 1516, 1421; 1HNMR ($CDCl_3$) δ: 6.4 (2H, d, $J = 8.4$, H-3″ & 5″)6.9 (2H, d, $J = 8.4$, H-2″ & 6″), 7.75–7.32 (5H, m, 5xAr′-H), 7.89 (1H, s, H-3); 13C NMR ($CDCl_3$): 119, 129.8, 126.6, 128.0, 128.3, 129.8, 126.6, 130.8, 130.5, 133.5, 145.4, 168.5; MS (ESI) $m/z = 281$ $(M+Na)^+$; Anal. ($C_{15}H_{11}ClO_2$) C, H.

*(Z)-3-(2-Acetoxyphenyl)-2-phenyl-prop-2enoic acid (**1o**)*

M.p. 178–180; IR ν_{max} cm^{-1} (KBr): 3396, 1643, 1589, 1411; 1HNMR ($CDCl_3$) δ: 3.47 (3H, S, $OCOCH_3$) 7.8–7.6 (4H, m, Ar″–H), 7.5–7.3 (5H, m, 5xAr′-H), 8.26 (1H, s, H-3); 13C NMR ($CDCl_3$): 123.3, 129.8, 126.6, 128.0, 128.3, 124.8, 129.1, 130.2, 132.5, 133.5, 140, 167.1; MS (ESI) $m/z = 305$ $(M+Na)^+$; Anal. ($C_{17}H_{14}O_4$) C, H.

*(Z)-3-(2,3-Dihydroxyphenyl)-2-phenyl-prop-2enoic acid (**1p**)*

M.p. 191–192; IR ν_{max} cm^{-1} (KBr): 3475, 3354, 1633, 1589, 1411; 1HNMR ($CDCl_3$) δ: 3.77 (2H, S, OH, D_2O

exchangeable) 7.2–7.0 (3H, m, Ar″–H), 7.5–7.3 (5H, m, 5xAr′-H), 8.21 (1H, s, H-3); 13C NMR ($CDCl_3$): 123.3, 126.6, 128.0, 128.8, 124.8, 129.1, 130.2, 132.5, 133.5, 136.0, 166.0; MS (ESI) $m/z = 266$ $(M+Na)^+$; Anal. (C17H14O4) C, H.

Materials and methods of docking study

The coordinates of colchicine were obtained from protein data bank (PDB entry: 1sa0) (Ravelli *et al*., 2004). The structure of **1b** was drawn in ChemDraw and subjected to energy minimization in the MOPAC module, using the AM1 procedure for closed shell systems, implemented in the CS Chem3D Ultra (ChemDraw Ultra 6.0, 2000). The ligands were docked at the colchicine-binding site of tubulin using the GOLD 4.0.1 (Gold *et al*., 2009). Gold performs genetic algorithm based ligand docking to optimize the conformation of ligand at the receptor binding site. It utilizes Gold Score fitness function to evaluate the various conformations of ligand at the binding site and comprises of four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals (vdw) energy, ligand internal vdw energy, and ligand torsional strain energy.

Anticancer activity protocol

In vitro cytotoxicity against seven human cancer cell lines was determined using 96-well tissue culture plate (Monks *et al*., 1991). The cells were allowed to grow in carbon dioxide incubator (37°C) for 24 h. Test materials in complete growth medium (100 µl) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h in a carbon dioxide incubator. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 µl) on top of the medium in all the wells. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites, serum proteins, etc. and air-dried. The plates were stained with sulforhodamine B dye (0.4% in 1% acetic acid, 100 µl) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried (Skehan *et al*., 1990). The adsorbed dye was dissolved in Tris–HCl Buffer (100 µl, 0.01 M, pH 10.4) and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in the presence of test material was calculated considering the growth in the absence of any test material as 100% and in turn percent growth inhibition in the presence of test material was calculated. IC50 of only active compounds were calculated on the basis of 5 different concentrations viz., 1×10^{-5}, 3×10^{-5}, 5×10^{-5}, 7×10^{-5}, and 1×10^{-4} mol.

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