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RESEARCH ARTICLE

Synthesis and antioxidant, cytotoxicity and antimicrobial activities of novel curcumin mimics

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Abstract

Claisen-Schmidt condensation of 3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2,4,5- trimethoxybenzaldehyde **3** and various aromatic, heterocyclic and alicyclic amides of 3- aminoacetophenone **6**(**a–s**) afforded novel curcumin mimics. All the synthesized compounds were characterized by IR, ^{1}H NMR, Mass spectroscopy and evaluated for antioxidant, cytotoxicity and antimicrobial activity. Out of the 20 compounds screened, compounds **7i**, **7l**, **7q**, and **7n** have shown excellent radical scavenging activity, compounds **7o**, **7t**, **7f**, and **7r** have shown significant xanthine oxidase inhibition, and compounds **7a**, **7k** and **7l** were found to be potent inhibitors of selected cancer cell lines. Compounds **7h**, **7t**, **7l**, **7i**, and **7e** have shown good antibacterial activity, whereas compounds **7j**, **7f**, **7o**, **7h**, and **7t** exhibited significant antifungal activity.

Keywords: Curcumin mimics, antioxidant, xanthine oxidase, cytotoxicity, antimicrobial

Introduction

Reactive oxygen species (ROS), including the hydroxyl radical, superoxide anion, hydrogen peroxide and peroxynitric species are continuously generated in the process of respiration as natural byproduct of oxygen metabolism[1]. These species are sufficiently reactive to cause injury to cells through the destruction of components such as proteins, lipids, sugars and nucleotides[2,3]. Under normal circumstances, cells are able to defend against ROS damage through the use of enzymes such as superoxide dismutase and catalase[4–6]. Small molecule antioxidants such as ascorbic acid, uric acid and glutathione also play an important role as cellular antioxidants[7–9]. Therefore, under normal condition, ROS are maintained at constant level in the body by the antioxidant defence mechanism. However, imbalances between the formation and detoxification of ROS can result in significant damage to cells, a situation known as oxidative stress. Oxidative stress leads to the initiation or progression of various diseases such as cancer[10], ischemia-reperfusion injury[11], atherosclerosis[12], cardiovascular[13], inflammation[14], and neurodegenerative disorders such as Alzheimer's and Parkinson[15,16].

Xanthine oxidase (XO) is a key enzyme that catalyzes the oxidation of xanthine and hypoxanthine into uric acid and plays a vital role in causing hyperuricemia and gout[17]. It is considered to be a main source of oxidative stress and destructive free radicals in ischemia-reperfusion injury associated with heart attack, stroke and spinal cord injury, as well as being a destructive force myocardial or renal hypoxia and infarction[18–20]. Because of this, there is a growing interest in the discovery of natural and unnatural antioxidants that attenuate oxidative stress and as a result serve as protective agents against these diseases[21].

Curcumin is a β-diketone constituent of the turmeric that is obtained from the powdered rhizome of *Curcuma longa*. Curcumin has a wide range of interesting biological activities such as anti-inflammatory, antioxidant, antiviral, cutaneous wound healing, hypocholesterolemic effects in diabetic patients, anti-angiogenic and stimulatory response to stress-induced biological activity[22,23].

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Curcumin has been demonstrated to possess preventative activity against Aβ-aggregation in Alzheimer's model[24]. Several studies have shown that curcumin manifests anti-proliferative activity against various cancers, including leukemia, colon, liver, breast and prostrate cancers[23,25–27]. Although curcumin is non-toxic and has promising biological activities, preclinical and clinical studies have indicated that poor bioavailability and pharmacokinetic profile are poor due to its instability under physiological condition which has limited its application in anticancer therapies[28–30]. Evidences from both *in vitro* and *in vivo* studies show that β-diketone moiety is responsible for instability and weak pharmacokinetic profiles of curcumin. During last decade, synthetic modifications of curcumin, which were aimed at enhancing its bioactivities, suggested that the stability and metabolic profile of curcumin could be enhanced by deleting β-diketone moiety. Recent studies from several independent groups demonstrated that curcumin analogues without β-diketone either retained or increased various biological activities such as anticancer[31,32], antibacterial[33] and anti-inflammatory[34]. In continuation of our studies in synthesizing various biologically active compounds[35], in the present study we have synthesized and characterized the novel curcumin mimics and evaluated these for antioxidant, cytotoxic and antimicrobial activity.

Chemistry

Although curcumin is non-toxic and has promising biological activities, clinical studies indicate its poor bioavailability and pharmacokinetic profile. To overcome these barriers several research groups have synthesized curcumin analogues. Robinson et al[31]. have synthesized enone and dienone analogues, and Woo et al[36]. have synthesized the curcumin mimics which were shown to possess increased anti-angiogenic activity. Flavopiridol (NSC 649890) [cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-1-benzopyran-4-one] is a synthetic flavone that has been shown to possess anti-tumour activity against various tumour cell lines such as human lung carcinoma and breast carcinoma[37]. Olefin analogues of flavopiridol have been synthesized and shown to be potent inhibitor of CDK[38] and glycogen phosphorylase[39] Figure 1. We herein report on the synthesis of the novel curcumin mimic incorporating olefin as well as aromatic, alicyclic or heteroaromatic amide moities. The title compounds were prepared using straight forward chemistry (Scheme 1). Compound **2** (olefin) was prepared by reacting 1, 3, 5-trimethoxy benzene with 1-methyl-4-piperidone in presence of hydrogen chloride gas in glacial acetic acid[40]. Compound **2** on Vilsmier-Hack formylation gave 3-(1,2,3,6-tetrahydro-1-methylpyridine-4-yl)-2,4,6-trimethoxybenzaldehyde **3**. Compounds **6a–s** were prepared by acylation of 3-aminoacetophenone with different acylchlorides in basic medium, except compound **6r** and **6s** which were prepared using an earlier reported method[41]. Compounds **6a–s** on Claisen-Schmidt condensation with compound **3** under basic media afforded a residue, which on purification by column chromatography with 1% ammonia and 0.5–1% methanol in chloroform as eluting solvent furnished title compounds in good yields (Table 1). All the synthesized curcumin mimics were characterized by IR, ^{1}H NMR and Mass spectral analysis. All the synthesized curcumin mimics **7**(**a–s**) and synthetic intermediate **7t** were evaluated for antioxidant, cytotoxicity and antimicrobial activity.

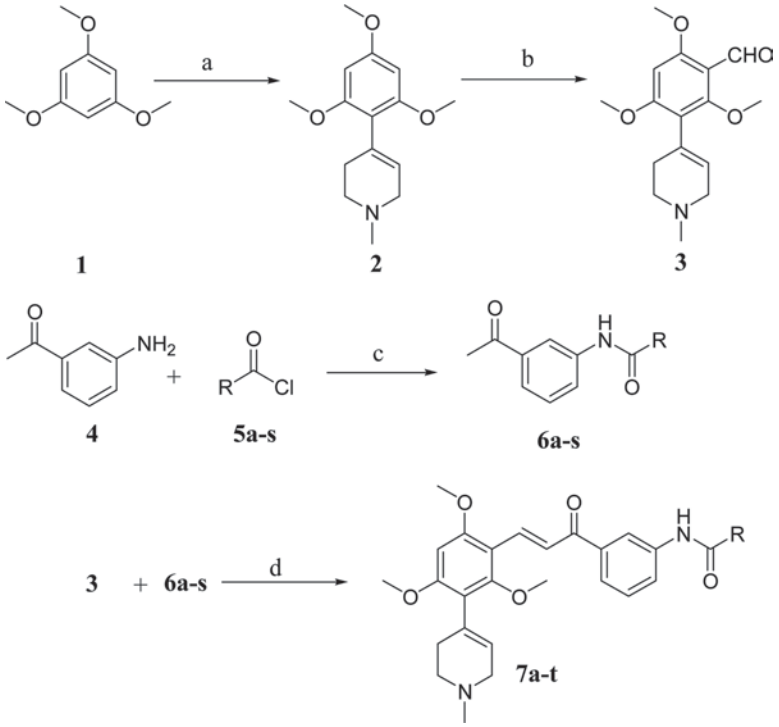
Results and discussion

Taking into the account of multifactorial character of oxidative stress which is involved in many pathological states, we have evaluated antioxidant activity of curcumin mimics against DPPH stable free radical. Free radical scavenging activity was measured in terms of % DPPH inhibition and results are presented in Table 2. All the synthesized compounds have shown excellent to moderate radical scavenging activity. Compounds **7i**, **7l**, **7q**, **7f**, **7r**, **7j**, **7e**, **7o**, **7t** and **7c** have shown excellent radical scavenging activity (90–80%) as compared to standard trolox (80%), compounds **7k**, **7s** have shown good radical scavenging activity (77–75%), whereas the rest of the compounds have shown moderate radical scavenging activity. Structure activity relationship (SAR) study of a curcumin mimics demonstrated that the presence of electron donating substituents on the amide ring increases antioxidant activity, whereas the presence of electron withdrawing substituents on the amide ring decreases the antioxidant activity except in the case of compound **7e** and **7o**. Butylated compounds are excellent inhibitors of reactive oxygen species (ROS[42]); however, in the present case, compound **7m** has shown moderate antioxidant activity. This may be ascribed to the absence of hydroxyl group ortho to butyl group on aromatic ring. Compound **7n** containing cinnamide moiety shows good antioxidant activity which is as expected since cinnamic derivatives have been known to be excellent ROS scavenger[43]. A comparison of the antioxidant activity of compounds **7a**, **7k**, **7l** reveal that the replacement of aromatic amide moiety (**7a**) with heterocyclic amide moiety such as, thiphen (**7k**) or furan (**7l**) results in increased antioxidant activity, particularly in the latter case. Similarly, incorporation of alicyclic amide moiety (**7r**) also showed increased activity compared to (**7a**) which contains aromatic amide moiety.

Xanthine Oxidase (XO) is a cytosolic enzyme that is an important source of oxygen free radicals. Results of XO inhibition by curcumin mimics are presented in Table 2. These results indicate that all the synthesized compounds have excellent to moderate XO inhibition. Among all synthesized compounds, **7o**, **7t**, **7f**, **7r**, **7l**, **7i**, **7k** and **7q** were excellent inhibitors of XO (94–90%) as compared to standard allopurinol (90%), compounds **7d**, **7n**, **7m**, **7c**, and **7h** were good inhibitors of XO (89–80%), whereas the rest of the compounds showed moderate inhibition. SAR study revealed that compounds containing electron

withdrawing substituents on amide ring are excellent inhibitors of XO compared to their counterparts containing electron donating groups with the exception **7i** and **7q**. Interestingly, compound **7t** an intermediate of curcumin mimics, has shown excellent XO inhibition. Changing the position of fluorine on amide ring from para position (**7f**) to meta position (**7d**) showed a decrease in activity. There is further decrease in the activity as the position of fluorine is changed from meta (**7d**) to ortho (**7b**). This suggests that a substituent at para or meta position is favourable for enzyme inhibition. In addition it was found that heteroaromatic amides such as furan amide (**7l**), thiophen amide (**7k**) are better inhibitors than the aromatic amide (**7a**). Similarly, curcumin mimics containing alicyclic cyclopropyl amide moiety (**7r**) shows excellent XO inhibition as compared to one containing aromatic amide moiety (**7a**).

Results shown in Table 3 indicate that all synthesized compounds have shown cytotoxicity against all selected cancer cell lines. Compounds **7a**, **7k**, and **7l** completely inhibited selected ACHN (human renal carcinoma), Panc 1 (human pancreatic carcinoma), Calu 1 (human non-small cell lung carcinoma), H460 (human non cell lung carcinoma) and HCT 116 (human colon carcinoma) cancer cell lines (92–100%) at 10 μM as compared to standard flavopiridol (68–73%) and gemcitabine (78–84%) at 700 and 500 nM, respectively. Compounds **7e**, **7g**, and **7s** have moderate cytotoxicity. Of the 20 compounds synthesized, compound **7a** and **7k** are cytotoxic to all selected five cancer cell lines studied. The cytotoxicity of these compounds are in compliance with the literature[36]. Compound **7l** has preferential cytotoxicity against pancreas, lung and non-small cell lung carcinoma cancer cell lines. Similarly, compound **7g** shows cytotoxicity against non-small cell lung carcinoma, human colon and renal cancer cell lines. In general, the following trend was observed: (i) electron withdrawing groups on the amide ring are more cytotoxic than their counterparts containing electron donating groups, (ii) curcumin mimics containing heteroaromatic amide moiety showed highest cytotoxicity, (iii) the order of cytotoxicity with respect to



Scheme 1. Reagents and conditions: (a) N-methyl- 4-piperidone, AcOH, HCl, 85-90 0C, 3.5h; (b) DMF, POCl3, rt, 2 h; (C) NaOH (d) NaOH, EtOH, rt, 24 h

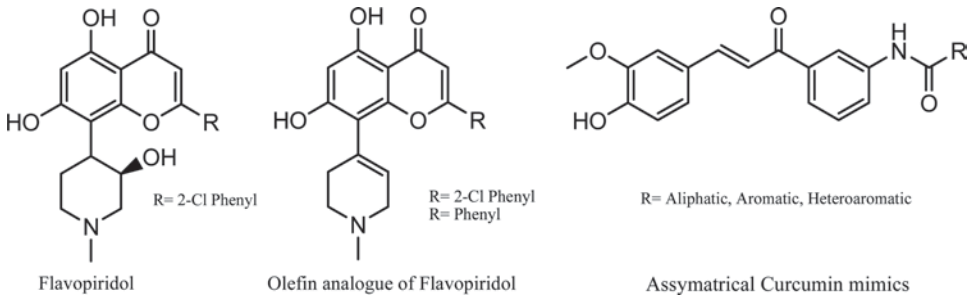


Figure 1. Flavopiridol and curcumin mimics.

position of substituents on the amide moiety was found to be meta > para > ortho.

All synthesized compounds were subjected to antimicrobial study by *in vitro* disk diffusion method against *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacilus subtilis*, *Penicillium chrysogenum*, *Trichoderma viridae*, *Candida albicans*, *Microsporum cannis* and *Fusarium moniliformae*. Nystatin and tetracycline used as standards against fungi and bacteria, respectively. The results are reported as a mean of triplicate measurements. Antibacterial and antifungal activities of compounds are shown in Table 4. Results show that all the synthesized compounds possess good antimicrobial activity. Compounds **7h**, **7t**, **7l**, **7i**, **7e**, **7j**, **7o**, **7c**, and **7r** show antibacterial activity more or less equal to that of standard at tested concentration; however, the rest of the compounds displayed weak antibacterial activity. It is observed that of the synthesized 20 compounds screened against three gram negative strains *S. typhi*, *P. vulgaris*, *P. aeruginosa* and two gram positive strain *S. aureus*, *B. subtilis,* almost all the compounds are active against gram positive strains, whereas some of the compounds are inactive against gram negative strain. In addition, it was observed that substituents on amide ring at meta and para positions showed higher antibacterial activity as compared to the respective ortho derivatives with the exception of **7h**. Interestingly, compound **7t**, an intermediate of curcumin mimic, has shown equal antibacterial activity as that of curcumin mimics. Curcumin mimics containing heterocyclic and alicyclic amides moieties have shown promising antibacterial activity. Compounds **7j**, **7f**, **7o**, **7h**, **7t**, **7c**, **7e**, and **7r** showed strong antifungal activity as compared to nystatin at tested concentration. Some of the compounds are inactive against fungal strains especially against *C. albicans*. Structure activity relationship study indicates that compounds containing electron withdrawing groups on the amide moiety have shown higher antifungal activity than their counterparts containing electron donating groups except **7j**. Alicyclic cyclopropyl amide **7r** showed higher antifungal activity than the aromatic amide **7a**. Compounds having substituents at para and meta position of amide ring have higher antifungal activity than the ortho substituted counterparts.

Conclusion

In this study, we have synthesized a series of novel curcumin mimics and evaluated for antioxidant, cytotoxicity and antimicrobial activity. Compounds **7i**, **7l**, **7q**, and **7n** showed excellent antioxidant activity. The compounds **7o**, **7t**, **7f**, and **7r** have shown promising xanthine oxidase inhibition. The presence of substituents at meta and para position of amide was found to be essential for antioxidant activity. Compounds **7a**, **7k**, and **7l** have shown potent cytotoxicity against selected human cancer cell lines. The presence of substituents at meta position was found to be necessary for cytotoxicity. Compounds **7h**, **7t**, **7l**, **7i**, and **7e** showed good antibacterial activity and compounds **7j**, **7f**, **7o**, **7h**, and **7t** exhibited antifungal activity against all the strains tested. Overall compounds **7l**, **7t**, **7i**, **7o**, and **7r** need to be screened further for other cancer cell lines and *in vivo* study and toxicology. These molecules can be considered as lead molecules for novel cytotoxic and antioxidant agent.

Table 1. Synthesis of curcumin mimics.

Entry	R	Product	Yield[a] (%)	MP
1	C_6H_5	7a	88	89–91°C
2	o-F C_6H_4	7b	79	86–88°C
3	m-Cl C_6H_4	7c	85	108–110°C
4	m-F C_6H_4	7d	91	75–77°C
5	m-CF_3 C_6H_4	7e	86	120–122°C
6	p-F C_6H_4	7f	90	118–120°C
7	m-Br C_6H_4	7g	76	190–192°C
8	o-CF_3 C_6H_4	7h	70	105–107°C
9	m-CH_3 C_6H_4	7i	84	102–104°C
10	p-OCH_3 C_6H_4	7j	93	125–127°C
11	C_4H_4S	7k	72	108–110°C
12	C_4H_4O	7l	81	104–106°C
13	p-$C(CH_3)_3$ C_6H_4	7m	75	155–157°C
14	P-C_2H_2 C_6H_4	7n	81	81–83°C
15	p-Cl C_6H_4	7o	94	100–102°C
16	o-Cl C_6H_4	7p	83	92–94°C
17	p-CH_3 C_6H_4	7q	89	95–97°C
18	C_3H_5	7r	69	75–77°C
19	m-CN C_6H_4	7s	65	113–115°C
20	RCO = H	7t	85	216–218°C

[a]Isolated yield.

Table 2. Antioxidant activity of curcumin mimics.

Compound	% inhibition by DPPH 50 μg/mL	Xanthine oxidase inhibition (%) 100 μg/mL
7a	50.20 ± 1.8	62.13 ± 1.2
7b	65.23 ± 2.6	56.23 ± 1.3
7c	80.42 ± 1.2	85.46 ± 3.2
7d	60.85 ± 2.3	89.22 ± 1.6
7e	82.13 ± 3.2	70.52 ± 4.2
7f	85.12 ± 3.5	92.50 ± 2.1
7g	60.45 ± 1.2	75.10 ± 1.8
7h	56.12 ± 1.5	80.95 ± 3.1
7i	90.12 ± 2.1	92.00 ± 1.8
7j	82.90 ± 1.8	85.21 ± 4.2
7k	77.52 ± 1.9	90.15 ± 1.5
7l	87.16 ± 1.6	92.11 ± 2.5
7m	68.85 ± 2.2	86.45 ± 1.3
7n	85.43 ± 4.3	88.15 ± 2.6
7o	81.19 ± 2.8	94.50 ± 2.8
7p	58.12 ± 1.6	65.42 ± 1.6
7q	86.15 ± 2.4	90.12 ± 2.3
7r	83.20 ± 1.8	92.45 ± 1.4
7s	75.20 ± 1.6	92.10 ± 3.2
7t	80.45 ± 1.4	94.11 ± 1.2
Trolox[a]	80.20 ± 2.5	—
Allopurinol[a]	—	90.12 ± 1.6

[a]Standard; data represent mean of three replicates.

Experimental

Chemistry

All the chemicals were purchased from Aldrich Chemical Co. (Nanded, Maharashtra, India). Melting points were determined with digital thermometer and were uncorrected. IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer and ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ using tetramethyl silane as an internal standard. Mass spectra were obtained with a Shimadzu LCMS-2010 EV. Chromatographic purification was performed with silica gel (100–200 Mesh). Thin layer chromatography was performed on pre-coated silica plates (Merks kiesegel 60F254, 0.2 mm thickness) sheet. The spots could be visualized easily under UV light.

Synthesis of 3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2,4,5-trimethoxybenzaldehyde (3)

Olefin **2** (1.0 g, 4 mmol) was dissolved in dry DMF (13.3 mL) under anhydrous condition. It was cooled to 0°C, $POCl_3$ (7.2 mL) was added drop wise for 30 min and stirring continued for 2 h at 25°C. After completion of reaction (TLC), reaction mass was poured over crushed ice (50 g) and basified with Na_2CO_3, extracted with chloroform, dried over anhydrous Na_2SO_4, purified through silica gel column using 0.5–1% methanol + 1% liquor ammonia in chloroform as eluting solvent afforded product **3** (0.6 g) in 51% yield.

General procedure for the synthesis of curcumin mimics (7a-s)

Amides **6a–s** (1 mmol) were dissolved in ethanol (15 mL), NaOH (20%) was added to it and stirred for 5 min. 3-(1,2,3,6-tetrahydro-1-methylpyridine-4-yl)-2,4,6-trimethoxybenzaldehyde **3** (1 mmol) was added and stirring continued for 24 h at room temperature. After completion of reaction (TLC), reaction mixture was poured over crushed ice, extracted with chloroform, organic extract was washed with water, dried (anhydrous Na_2SO_4) and concentrated. Purification was carried using silica gel column using mixture of 0.5–1% methanol + 1% liquor ammonia in chloroform as an eluent to obtain the title compounds.

N-(3-((2E)-3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2,4,6-trimethoxyphenyl)acryloyl)phenyl)benzamide (7a):

IR (KBr) cm^{-1}: 3470, 3170, 2986, 2852, 1649, 1617, 1590, 1570, 1510, 1420, 1020, 945, 844, 763. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.50 (s, 1H, -NH), 8.48 (s, 1H), 8.08 (d, 1H, *J* = 8 Hz), 7.95 (m, 2H), 7.85 (d, 1H, *J* = 15.6 Hz), 7.72 (d, 1H, *J* = 7.6 Hz), 7.45 (m, 4H), 7.40 (m, 1H), 6.30 (s, 1H), 5.58 (1H), 3.98 (s, 3H), 3.86 (s, 3H), 3.77 (s, 3H), 3.12 (s, 2H), 2.64 (s, 2H), 2.42 (s, 3H), 2.35 (s, 2H). MS: *m/z* 513 [M+1].

2-fluoro-N-(3-((2E)-3-(3-(1,2,3,6-tetrahydro-1-methylpyridin-4-yl)-2,4,6-trimethoxyphenyl)acryloyl)phenyl)benzamide(7b):

IR (KBr) cm^{-1}: 3410, 3115, 2984, 1660, 1603, 1429, 1139, 1016, 982, 828, 760. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.67 (s, 1H, -NH), 8.44 (s, 1H), 8.04 (m, 3H), 7.95 (d, 1H, *J* = 16 Hz), 7.79 (d, 1H, *J* = 7.08 Hz), 7.53 (m, 4H), 6.30 (s, 1H), 5.53 (s, 1H), 3.99 (s, 3H), 3.80 (s, 3H), 3.70 (s, 3H), 3.27 (s, 2H), 2.88 (s, 2H), 2.53 (s, 3H), 2.46 (s, 2H). MS: *m/z* 531 [M+1].

Biological activities

DPPH antioxidant assay

The DPPH radical scavenging activity of curcumin mimics were measured according to the procedure

Table 3. Cytotoxicity profile of curcumin mimics against selected cancer cell lines.

Compound	Conc in μM	Panc	H460	Calu	HCT 116	ACHN
7a	10	97	99	97	97	92
7b	10	8	43	0	32	26
7c	10	12	25	38	22	16
7d	10	58	61	55	69	65
7e	10	78	60	82	67	80
7f	10	0	22	18	9	15
7g	10	61	77	54	80	82
7h	10	25	71	0	84	0
7i	10	20	7	0	15	35
7j	10	23	23	11	12	29
7k	10	98	100	98	98	97
7l	10	91	51	89	79	72
7m	10	4	0	10	2	8
7n	10	20	15	13	7	0
7o	10	40	32	28	18	39
7p	10	0	2	9	10	9
7q	10	12	0	21	6	15
7r	10	37	24	0	13	18
7s	10	62	78	52	59	55
7t	10	42	37	29	21	14
Flavopiridol	700 nM	68	73	82	83	73
Gemcitabine	500 nM	78	79	87	86	84

Panc1: pancreas, H460: non-small cell lung carcinoma, Calu-1: Lung, hct116: Colon cancer, ACHN: Renal.

described by Bandgar et al. with minor modification[44]. A purple-coloured (DPPH˙) is a stable free radical, which is reduced to DPPH (yellow coloured) by reacting with an antioxidant. The reaction mixture consisted of 100 μM DPPH in absolute ethanol with different concentrations (0–1000 μM) of test compounds. A negative control with the same DPPH concentration in ethanol without sample was used. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 10 min. The decrease in absorbance of resulting solution was then measured spectrophotometrically at 517 nm against ethanol. All measurements were made in triplicate and averaged. Trolox was used as standard.

Xanthine oxidase inhibition

Xanthine oxidase inhibitory activity was assayed spectrophotometrically at 295 nm under aerobic condition[45]. The reaction mixture contained 200 mM sodium pyrophosphate buffer (pH 7.5), 100 μm xanthine, and 0.4 U of XO. The absorption rate at 295 nm indicate the formation of uric acid at 25°C. Samples were dissolved directly in the buffer and incorporated with enzyme assay to assess the inhibitory activity. The experiments were carried out with three sets of apparatus testing XO inhibitory activity. XO activity was expressed as % inhibition of XO, calculated as % inhibition = (1-B/A) × 100 where A is the change in absorbance of the assay without the curcumin mimics and B is the change in absorbance of the assay with curcumin mimics. The enzyme kinetics was similar to XO assay method.

Cytotoxicity assay

The selected cancer cell lines such as ACHN (human renal carcinoma), Panc 1 (human pancreatic carcinoma), Calu-1 (human non-small cell lung carcinoma), H460 (human non cell lung carcinoma) and HCT 116 (human colon carcinoma) were used for evaluation of cytotoxicity of test compounds. The propidium iodide florescence assay was performed for the demonstration of cytotoxic effect of test compound. Seed cells of 3000–7500 cells/well of individual cell line were placed in 2000 μL of tissue culture grade 96-well plates and allowed them to recover for 24 h in humidified 5% CO_2 incubator at 37°C. After culturing for 24 h, individual test compounds (10 μM in 0.3% DMSO) were added onto triplicate wells. DMSO (0.3%) alone was used as control. After 48 h in humidified 5% CO_2 incubator at 37°C condition, the medium was removed and treated with 25 μL of propidium iodide (50 μg/mL in water/medium) per well. The plates were frozen at –80°C for 24 h then thawed and allowed it to room temperature, and the plate absorbance was read on fluorometer (polarstar BMG Tech), using 530 nm excitation and 620 nm emission wavelengths. Percent cytotoxicity of the test compounds were calculated by using following formula.

$$\text{Cytotoxicity}\,(\%) = 1 - T/C \times 100$$

T = OD of treated cells

C = OD of control

Flavopiridol (700 nM) and Gemcitabine (500 nM) were used as standard anticancer drugs for comparison[46].

Table 4. Antimicrobial activity of curcumin mimics (zone of inhibition in mm).

Compound	Bacteria 50 μg/mL ST	PV	PA	SA	BS	Fungi 100 μg/mL PC	TV	CA	MC	FM
7a	ND	ND	10	ND	10	12	ND	ND	ND	ND
7b	12	ND	15	10	15	15	ND	ND	ND	13
7c	20	22	20	22	20	17	18	20	22	20
7d	10	ND	ND	12	ND	12	ND	ND	ND	ND
7e	20	22	20	22	20	17	18	20	22	20
7f	18	22	21	22	20	19	19	20	20	20
7g	ND	ND	ND	12	10	ND	10	ND	ND	ND
7h	24	22	24	20	24	18	18	20	20	20
7i	22	22	22	22	25	17	18	19	20	18
7j	20	22	20	22	24	20	22	20	20	20
7k	20	19	22	21	25	18	20	18	18	18
7l	22	22	24	24	20	17	18	19	19	20
7m	12	ND	ND	ND	15	ND	ND	ND	ND	ND
7n	19	19	20	20	22	17	18	18	18	18
7o	22	24	20	22	22	20	22	20	18	18
7p	14	ND	ND	12	10	12	10	ND	ND	ND
7q	18	20	20	20	20	17	20	20	21	19
7r	20	22	20	22	20	17	18	20	22	20
7s	14	ND	ND	2	10	12	ND	ND	ND	12
7t	22	24	24	21	23	18	19	20	22	20
Tetracyclin	18	20	20	20	20	—	—	—	—	—
Nystatin	—	—	—	—	—	16	17	18	20	20

Data represent mean of three replicates.

ND, not determined; ST, *Salmonella typhi*; PV, *Proteus vulgaris*; PA, *Pseudomonas aeruginosa*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*; PC, *Penicillium chrysogenum*; TV, *Trichoderma viridae*; CA, *Candida albicans*; MC, *Microsporum cannis*; FM, *Fusarium moniliformae*.

Antimicrobial activity

Antimicrobial activities of all the synthesized compounds were determined by agar diffusion method[35]. All the bacteria and fungal species used were procured from Institute of Microbial Type Culture Collection (IMTCC), Chandigarh, India, and National Collection of Industrial Microorganisms (NCIM), Pune, India, namely, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *P. chrysogenum, T. viridae, C. albicans, M. cannis* and *F. moniliformae.* All the synthesized compounds were dissolved to prepare a stock solution of 1 mg/mL using DMSO. Stock solution was aseptically transferred and suitably diluted to have solutions of concentration ranging 50–100 µg. For antifungal activity, different fungal spore suspension in sterile distilled water was adjusted to give a final concentration of 106 cfu/mL. Inoculum of 0.1 mL spore suspension of each fungus was spread on Sabouraud's Dextrose agar plates (HiMedia). For antibacterial activity, Muller Hinton agar was used (HiMedia) seeded with 0.1 mL of respective bacterial strains suspension prepared in sterile saline (0.85%) of 105 cfu/mL dilution. The wells of 6 mm diameter were filled with 0.1 mL each test compound separately for each fungi and bacterial strain. The DMSO alone was used as control. The antibiotics tetracycline (10 µg/mL) and nystatin (30 µg/mL) were used as reference for antibacterial and antifungal, respectively. Inoculated plates in duplicate were then incubated at 37 ± 0.5°C for antibacterial activity for 24 h and at 28 ± 0.2°C for antifungal activity for 48 h. After incubation, the antimicrobial activity was measured in terms of the zone of inhibition in mm.

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Declaration of interest

The authors report no conflicts of interest.

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