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Biological activity evaluation and structure–activity relationships analysis of ferulic acid and caffeic acid derivatives for anticancer

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ABSTRACT

The anticancer activities of alkyl esters and NO-donors of ferulic acid (FA) and caffeic acid (CA) were assessed by a high-throughout screening (HTS) method, and the structure–activity relationships were described. CA alkyl esters had better anticancer activities than FA alkyl esters with the same alkyl substituent. Mono-nitrates and phenylfuroxan nitrates were more potent than the dual nitrates. Phenylsulfonylfuroxan nitrates of FA, especially compounds **8b**–**8d**, exhibited more potent activities in anticancer.

Despite of global efforts to limit the incident of cancer, it has become the leading cause of death in the last 50 years.[1] Lung cancer remains the number one cancer killer in both men and women.[2,3] Breast cancer is the most common cancer in females worldwide, and mortality from breast cancer is consistently due to tumor metastasis.[4] Cervical cancer is the second most common cancer of women worldwide, accounting for an estimated 11,070 new cases and 3870 deaths in USA for 2008.[5] Head and neck cancer is among the 10th most common cancer worldwide, there are about 780,000 new cases per year over all the world.[6] Besides that, melanoma is the most serious type of skin cancer as a malignant tumor of melanocytes.[7] With the incidence of these cancers rapidly rising in the developed and developing countries, there is an urgent need to develop more effective drugs.

Aromatic acids in the plant kingdom are now recognized as promising chemopreventive agents. They exhibit a wide spectrum of pharmacological activities, such as anticancer.[8] Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA), a characteristic aromatic acid, was active against the lung carcinogenesis by benzopyrene.[9] Additionally, it could decrease incidences of azoxymethane-induced large bowel neoplasms, suggesting it has a potential as a chemopreventive agent for rice germ on colonic neoplasia.[10,11] Caffeic aicd (3,4-dihydroxycinnamic acid, CA), another bioactive aromatic acid, had a potent inhibitory effect on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion and TPA-induced formation of 5-hydroxymethyl-2-deoxyuridine in DNA of mouse skin as well as an inhibitory effect on the synthesis of DNA, RNA and protein in cultured HeLa cells.[12] Furthermore, dietary with rich CA and FA, such as fruits and vegetables, may play a role in the body's defense against carcinogenesis by inhibiting the formation of N-nitroso compounds.[13] Earlier studies indicated that FA and CA could suppress benzo(a)pyrene-induced forestomach carcinogenesis in mice,[14] inhibit the tumor promotion in mouse skin induced by TPA or 7,12-dimethylbenz[*a*]anthracene,[15,16] significantly reduce the incidences of tongue neoplasms (squamous cell papilloma and carcinoma) and preneoplastic lesions (hyperplasia and dysplasia).[8]

However, they were rapidly absorbed with low bioavailabilities after single oral administration, which limited their clinical use.[17,18] Besides that, FA and CA are insoluble in water and oil, which also limits their applications.[19] Therefore, in order to improve their liposolubility and achieve more potent anticancer agents, it is necessary to modify the structures of FA and CA.

Esterification is one way of modifying the physical properties of FA and CA.[19,20] Previously, we have shown that higher yields of alkyl esters of FA (**1a**–**1h**) could be obtained under microwave irradiation, which is not only faster than using conventional heating methods, but also potentially more efficient, clean, and safe.[21] CA alkyl esters (**2a**–**2h**) could also be obtained under the same reaction condition (Scheme 1).[22]

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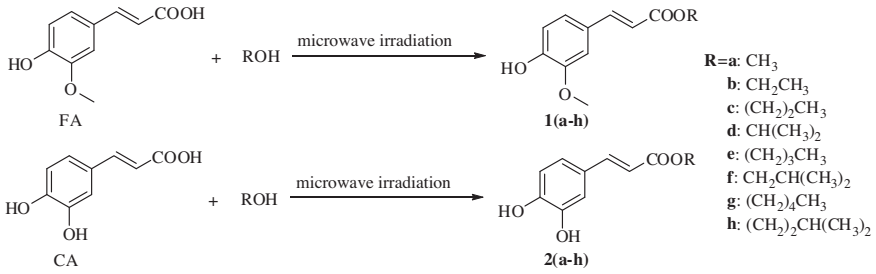


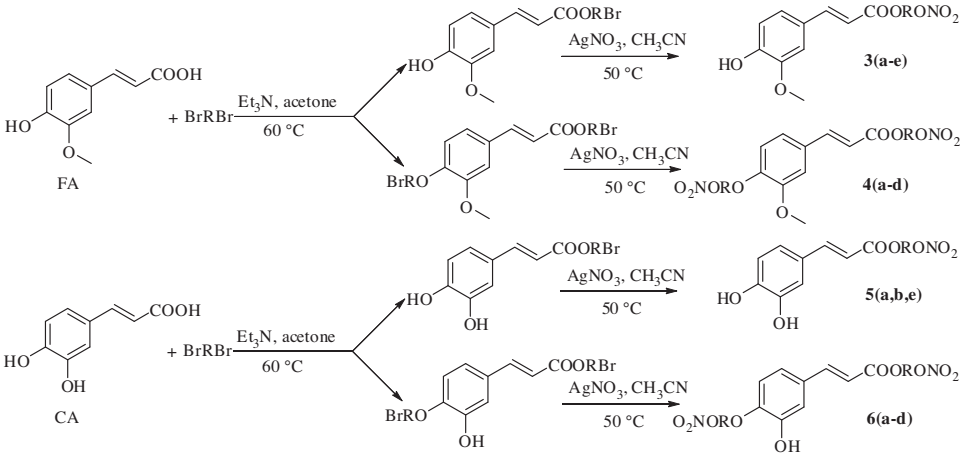
Scheme 1. Synthesis of alkyl esters of FA (**1a**–**1h**) and CA (**2a**–**2h**).

Nitric oxide (NO) is a key mediator involved in many physiological and pathological processes.[23] High levels of NO and its metabolic derivatives can modify functional proteins by S-nitrosylation, nitration, and disulfide formation, leading to bioregulation, inactivation, and cytotoxicity, particularly in tumor cells.[24,25] Indeed, some synthesized NO-releasing compounds have shown cytotoxic activity against human colon carcinoma cells and human hepatocellular carcinoma cells in vitro, inhibiting the growth and metastasis of cancers in vivo.[26,27] Furoxan (1,2,5-oxadiazole-2-oxides) derivatives are biologically active compounds that are capable of releasing high levels of NO in the presence of thiols and a lack of tolerance.[28,29] Hybrid NO-donor furoxan-based drugs are a novel type of drug that retains the pharmacological activity of the parent compound but also has the biological actions of NO.[30] In this study, we synthesized novel NO-donor–FA hybrids and NO-donor–CA hybrids according to our previous research.[31,32] FA was first treated with dibromoalkanes bearing three to six carbons in the presence of Et_3N and acetone at 50 °C, and then was further converted to the nitrates **3a**–**3d** or **4a**–**4d**, respectively, with $AgNO_3$ in THF/CH_3CN (Scheme 2). However, when FA was treated with (*E*)-1,4-dibromobut-2-ene, and then was further converted to the nitrate **3e** with $AgNO_3$ in THF/CH_3CN. Nitric ester–CA hybrids **5a**–**5c** and **6a**–**6d** were obtained through the same reaction condition but CA was the lead compound (Scheme 2). Furthermore, five 4-hydroxyl-3-phenylfuroxan–FA hybrids (**7a**–**7e**) and four 4-hydroxymethyl-3-phenylsulfonylfuroxan–FA hybrids (**8a**–**8d**) were synthesized through modifying the carboxyl group of FA with phenylfuroxan or phenylsulfonylfuroxan. 4-hydroxyl-3-phenylfuroxan–CA hybrid (**9**) was synthesized through modifying the carboxyl group of CA with phenylfuroxan (Scheme 3). The yield of every synthetic compound was shown in Table 1.

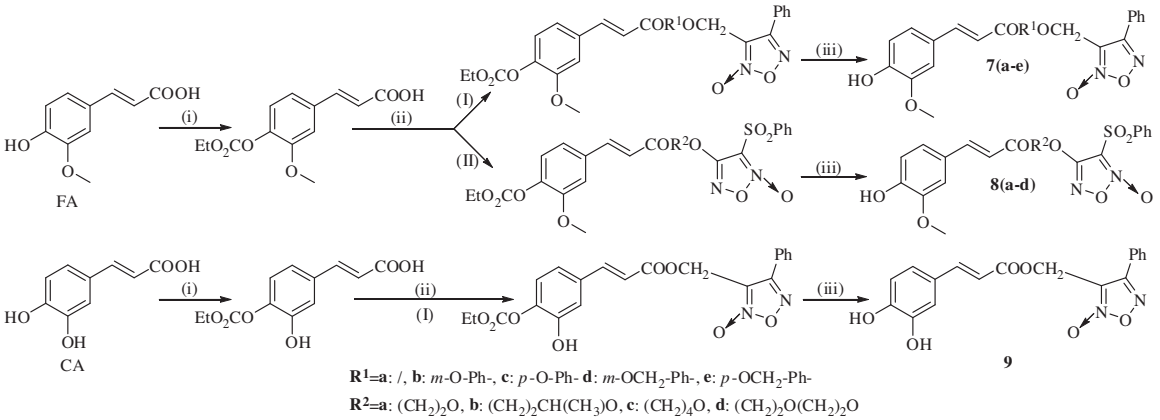
In the present study, in order to explore the potential anticancer activity of 16 alkyl esters and 26 NO-donors of FA and CA, their biological activities were determined in an in vitro human disease-oriented cancer cell line screening panel using high-throughout screening (HTS) method,[33] including human lung cancers, melanoma, cervical, neck and head, and human breast cancer cells. HTS, driven by the great progress in automation technology and combinatorial chemistry, has been widely implemented in drug discovery since the early 1990s and rapidly became one of the major sources of drug leads.[34] The results of anticancer activities of 42 FA and CA derivatives against the growth of 14 human cancer cell lines were shown in Table 1.

As illustrated in Table 1, almost all FA and CA derivatives exhibited different potent anticancer activities against the growth of different human cancer cell lines. About the nine kinds of lung cancer cells, most compounds except **1a**, **1d**, **1g**, **4a**–**4d**, **6b**–**6d**, **7b**, **7d**, **7e** and **8a**–**8d** had better activities on A549, H157 and 1299 cells; had weaker activities on H460 and Calu 1 cells; had the weakest activities on 1792, H266, Hop62 and 292G cells. Most compounds had better activities on LOX-IMVI cell than on M14 cell. Almost all compounds had significant cytotoxic against Hela cells with small IC_{50} values, indicating that they were probably good agents for the treatment of human cervical cancer.

The anticancer activity of most NO-donors including mono-nitrates **3** and **5**, phenylfuroxan nitrates **7** and **9**, and phenylsulfonylfuroxan nitrates **8**, in general but not always, were superior to the alkyl esters **1** and **2**. On the other hand, the NO-donors had a slightly higher anticancer activity in this test system. In the series of alkyl esters, CA derivatives (**2a–2h**) had better anticancer activities than FA (**1a**–**1h**) with the same alkyl substituent, such as compounds **2a** versus **1a** and **2g** versus **1g**. This result was consistent with the



Scheme 2. Synthesis of nitric ester–FA hybrids (**3a**–**3e**, **4a**–**4d**) and nitric ester–CA hybrids (**5a**, **5b**, **5e**, **6a**–**6d**).



Scheme 3. Synthesis of 4-hydroxyl-3-phenylfuroxa–FA hybrids (**7a–7e**), 4-hydroxymethyl-3-phenylsulfonylfuroxa–FA hybrids (**8a–8d**) and 4-hydroxyl-3-phenylfuroxa–CA hybrid (**9**). Reagents and conditions: (i) $ClCO_2Et$, 1 N NaOH, 50 °C; (ii) DCC, DMAP, CH_2Cl_2; (iii) $NH_2(CH_2)_2OH$, 95%EtOH; (I) 4-hydroxyl-3-phenylfuroxan; (II) 4-hydroxymethyl-3-phenylsul-fonylfuroxan.

Table 1
The yield (%) and in vitro anticancer activities in human cancer cell lines (IC_{50} in μM) of alkyl esters of FA and CA, NO-donors–FA hybrids and 4-hydroxyl-3-phenylfuroxa-CA hybrid

No.	Yield (%)	Lung cancer									Melanoma		Cervical	Neck & head	Breast
		A549	H157	H460	1792	H266	Hop62	1299	292G	Calu1	LOX-IMVI	M14	Hela	M4E	SKBR
1a	79.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	5.55	>50.0	>50.0
1b	81.0	12.5	32.3	29.1	25.6	28.1	26.8	36.8	32.0	>50.0	>50.0	>50.0	2.13	33.4	24.9
1c	77.0	15.6	15.2	25.2	34.1	20.4	22.4	40.3	35.9	48.9	15.2	28.1	5.62	18.5	24.5
1d	69.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	31.1	>50.0	>50.0
1e	73.0	8.23	9.03	19.4	15.0	18.3	14.9	8.23	22.9	17.2	19.3	18.1	10.3	15.0	13.2
1f	63.0	34.6	28.3	>50.0	>50.0	35.7	>50.0	38.0	>50.0	>50.0	>50.0	>50.0	28.7	>50.0	44.8
1g	58.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0
1h	46.0	43.6	40.2	45.0	37.7	48.1	31.2	31.5	48.4	32.2	48.4	45.8	>50.0	30.5	41.5
2a	75.0	7.54	6.25	14.8	6.09	>50.0	3.86	3.53	3.11	5.50	21.8	8.86	3.77	8.89	5.15
2b	73.0	7.22	7.62	13.1	18.4	>50.0	12.5	4.93	5.25	17.2	6.21	9.82	1.96	11.2	4.16
2c	72.0	4.39	7.16	7.01	22.1	34.4	7.81	5.85	7.58	17.7	7.50	13.5	1.51	12.4	3.87
2d	71.0	>50.0	11.0	10.5	>50.0	>50.0	>50.0	30.8	>50.0	>50.0	>50.0	>50.0	9.65	>50.0	31.5
2e	68.0	3.51	2.62	5.62	5.75	11.3	5.59	5.81	6.46	11.7	9.54	11.1	0.59	8.66	2.17
2f	64.0	13.6	8.55	16.0	>50.0	>50.0	>50.0	37.7	37.5	>50.0	>50.0	>50.0	4.70	36.8	16.2
2g	70.0	5.75	2.28	1.91	6.35	7.24	5.11	4.69	7.73	8.57	3.38	7.48	2.02	6.80	2.96
2h	69.0	8.76	8.25	16.5	31.9	33.8	15.7	17.9	27.6	20.2	8.03	30.6	9.39	29.1	13.1
3a	29.9	13.2	20.5	29.0	28.5	44.0	19.4	28.3	39.4	>50.0	29.5	42.6	6.70	18.4	17.2
3b	91.7	8.82	12.9	19.5	20.8	24.8	13.1	4.51	28.4	30.1	20.4	32.5	6.35	20.3	8.92
3c	83.0	6.39	13.4	12.3	13.0	21.2	12.5	20.8	24.9	26.3	>50.0	24.5	13.4	21.8	8.06
3d	89.4	37.4	32.1	>50.0	48.7	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	16.3	>50.0	15.2
3e	46.2	5.95	4.29	4.98	4.86	9.79	5.13	2.34	21.9	2.48	2.36	3.89	1.00	9.38	6.53
4a	22.2	15.8	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	1.42	>50.0	>50.0	3.62	>50.0	>50.0
4b	99.3	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	0.74	>50.0	>50.0
4c	86.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0
4d	17.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	27.8	>50.0	>50.0	>50.0	>50.0	>50.0
5a	87.0	10.8	4.34	3.31	18.1	>50.0	10.0	8.03	13.5	10.7	18.9	24.8	3.11	20.0	5.77
5b	90.0	22.6	5.19	3.52	15.4	23.3	10.0	3.14	16.7	21.7	>50.0	19.9	2.25	17.5	4.55
5e	55.3	0.40	1.36	2.90	0.41	10.3	4.65	0.41	7.95	0.42	0.43	1.49	0.40	4.31	0.41
6a	82.0	0.69	5.71	9.58	6.61	>50.0	10.0	>50.0	4.98	12.5	6.94	8.96	>50.0	14.1	4.81
6b	79.0	0.41	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	3.93	>50.0	3.23
6c	95.7	0.45	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	7.23	>50.0	5.73	>50.0	2.71
6d	83.4	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	1.35	>50.0	39.7
7a	89.1	2.06	4.53	7.03	11.1	13.4	8.66	12.9	16.5	22.9	13.8	14.0	7.82	3.37	4.98
7b	63.9	0.72	24.7	11.6	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	49.4	>50.0	>50.0	44.6	14.2
7c	78.9	3.39	43.1	15.8	8.15	>50.0	30.4	28.5	31.0	28.8	>50.0	>50.0	12.1	>50.0	11.0
7d	73.0	>50.0	>50.0	29.4	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	5.64	>50.0	4.18
7e	91.4	17.2	>50.0	44.2	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	11.7
8a	82.3	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0
8b	97.7	0.42	0.41	0.42	0.40	0.43	0.42	0.41	1.77	0.45	0.41	0.41	0.40	1.18	0.40
8c	92.0	0.40	0.42	0.45	0.42	0.40	0.41	0.42	1.93	0.41	0.44	0.40	0.46	0.84	0.42
8d	86.6	0.41	0.44	0.43	1.07	0.42	0.48	0.49	2.88	0.45	0.42	0.41	0.43	1.12	0.41
9	44.0	2.71	>50.0	2.54	3.78	14.2	6.12	>50.0	9.17	7.68	13.3	12.5	1.23	12.9	0.70

Note: >50.0 means that the data were not applicable.

literature in which it was reported that CA derivatives (**2a–2h**) showed stronger activities than FA (**1a–1h**) with the same alkyl substituent on colon-HCT 116, breast-MCF-7 and lung-NCI H460 cells.[35] In the series of FA alkyl esters **1**, **1e** showed the highest anticancer activity. However, **1a**, **1d**, **1f** and **1g** had the anticancer activity almost at the highest concentration (IC_{50} 50 μM). In the

series of CA alkyl esters **2**, all compounds except **2d** and **2f** showed significant anticancer activities. Generally, the results also showed that compounds with straight-chain substituent had better anticancer activities than those with branched-chain substituent, such as compounds **1b** versus **1d** and **2a** versus **2f**.

In the series of NO-donors, phenylsulfonylfuroxan nitrates **8b**–**8d** had the very potent anticancer activity against all the human cancer cells. Their IC_{50} values were all less than 10 μM, which were approximately 70 times more active than compound **1g** (IC_{50} >700 μM). The results indicated that phenylsulfonylfuroxan nitrates of FA were the most potent compounds among the FA and CA derivatives tested. It was probably because the phenylsulfonylfuroxan group can produce high levels of NO.[28–30] Generally, mononitrates **3** and **5** and phenylfuroxan nitrates **7** and **9** were more potent than the dual nitrates **4** and **6**, suggesting the FA or CA phenolic hydroxyl group was required for anticancer activity. For the series of mono-nitrates **3** and **5**, the unsaturated nitrates **3e** and **5e** showed higher anticancer activities than the saturated ones. Furthermore, the results showed that, in the series of mono-nitrates **3**, the anticancer activity increased as the number of atoms in the nitric esters increased, such as compounds **3a**–**3c**; but decreased significantly when the number of atoms in the nitric esters was six (compound **3d**). In the series of phenylfuroxan nitrates **7** and **9**, phenylfuroxan–CA **9** showed a much higher anticancer activity than phenylfuroxan–FA **7**. No substituent between FA and phenylfuroxan **7a** showed a much higher anticancer activity than analogues with benzene ring substituent **7b**–**7e**. Besides that, benzene ring substituent without methylene between FA and phenylfuroxan showed higher activity than the benzene ring with methylene substituent, such as **7b**, **7c** and **7d**, **7e**, indicating that the NO release activity decreased with the chain between FA and phenylfuroxan became much longer. For the series of phenylsulfonylfuroxan nitrates **8**, the butyl ether derivatives (**8b** and **8c**) and dual diethyl ether derivative **8d** had a much higher anticancer activity than the mono-diethyl ether derivative **8a**.

In summary, the present study clearly described the structure–activity relationships between the alkyl esters and NO-donors of FA and CA in anticancer. Both the substituent group and the level of releasing NO were essential for potent activity. Alkyl esters and NO-donors of FA and CA possessed activities on the growth of human cancer cell lines, and most of them produced notable selective cytotoxicity against Hela cells. The NO-donors of FA and CA had a slightly higher anticancer activity in this test system. More importantly, the results suggest that phenylsulfonylfuroxan nitrates of FA, especially compounds **8b**–**8d**, may be considered to be promising anticancer agent for further studies.

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33. Human lung cancers (A549, H157, H460, 1792, H266, Hop62, 1299, 292G and Calu1), melanoma (LOX-IMVI and M14), cervical (Hela), neck and head (M4E) and human breast cancer (SKBR) were from American Type Culture Collection (ATCC, USA) grown in RPMI 1640 containing 10% foetal calf serum (FCS), 100 UI/mL penicillin G and 100 mg/mL streptomycin. Dimethyl sulphoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis. MO), Alamar blue were from Promega. Cells were seeded into 384-well plates (Costar# 3712) (800–1000 cell/well or 20–25 cells/μL, 45 μL medium/well) using a Liquid dispenser (Thermo Fisher Multidrop Combi) in a bio-safety cabinet. Plates were placed in an incubator overnight to allow for attachment and recovery. Compound plates were utilized and prepared to yield 10 mM of compound in DMSO (neat) by Robot (Sciclone software), to generate 8 concentrations with series dilution, wells were reserved on each plate for background and vehicle control (0.5% DMSO). Using the liquid handling system, the following day the cells were treated with drug for 72 h, the final concentrations used in the assay are 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 μM in triplicate. A volume of 5 μL/well Alamar blue was transferred into the assay plates for a final concentration of 10% Alamar blue. The plates were exposed to an excitation wavelength of 530 nm, and the emission at 560 nm was recorded to determine whether any of the test compounds fluoresce at the emission wavelength and thus interfere with the assay. Plates were returned to incubator and the fluorescence was read at 4 h. The percent viability was expressed as fluorescence counts in the presence of test compound as a percentage of that in the vehicle control. The mean value and standard error for each treatment was determined and the percentage of cell viability relative to control (0.5% DMSO) was calculated. The IC_{50} was defined as the concentration of drug that killed 50% of the total cell population as compared to control cells at the end of the incubation period.
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