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Research paper

Novel cajaninstilbene acid derivatives as antibacterial agents

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ABSTRACT

Discovery of novel antibacterial agents with new structural scaffolds that combat drug-resistant pathogens is an urgent task. Cajaninstilbene acid, which is isolated from pigeonpea leaves, has shown antibacterial activity. In this study, a series of cajaninstilbene acid derivatives were designed and synthesized. The antibacterial activities of these compounds against gram-negative and gram-positive bacteria, as well as nine strains of methicillin-resistant staphylococcus aureus (MRSA) bacteria are evaluated , and the related structure-activity relationships are discussed. Assays suggest that some of the synthetic cajaninstilbene acid derivatives exhibit potent antibacterial activity against gram-positive bacterial strains and MRSA. Among these compounds, **5b**, **5c**, **5j** and **5k** show better antibacterial activity than the positive control compounds. The results of MTT assays illustrate the low cytotoxicity of the active compounds.

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1. Introduction

With the expansion of the use and abuse of antibiotics, increasing numbers of drug-resistant strains have emerged in the clinic [1]. One of the prototypical “superbugs” caused by antibiotic use and overuse is methicillin-resistant *Staphylococcus aureus* (MRSA) [2], against which almost all antibiotics are ineffective. As a consequence, novel antibacterial agents with new structural scaffolds are urgently needed to overcome the growing problem of drug resistance. Natural products are an important source of new drugs, and searching for novel antibacterial agents from natural products and their derivatives is an important approach to the discovery of antibacterial agents that can overcome drug resistance.

It has been reported that cajaninstilbene acid (**CSA**, Fig. 1), isolated from pigeonpea leaves [3], shows antibacterial activity against gram-positive bacteria. The minimal inhibitory concentrations (MIC) of **CSA** for *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* are 13, 25, and 25 μg/mL respectively. Its antibacterial activity against *Staphylococcus aureus* is equal to that of the erythromycin positive group, through it is lower than that of penicillin or chloramphenicol [4]. As a natural active compound extracted from edible beans, **CSA** is only mildly toxic [5], so it and its derivatives may have good potential as drugs. **CSA** may have promising activity, but there is still a large gap between its antibacterial effect and that of several existing drugs such as penicillin, norfloxacin and linezolid. In this study, a series of **CSA** derivatives were designed and synthesized in a search for compounds with improved antibacterial activity, and their antibacterial activity against gram-negative, gram-positive, and MRSA bacteria were evaluated, and the structure-activity relationships (SAR) were elucidated and discussed. Among these compounds, several new compounds with low toxicity and promising antibacterial activity were identified.

2. Chemistry

In an effort to develop novel antibacterial agents, a variety of **CSA** derivatives have been designed and synthesized. As shown in Fig. 1, the drug design strategies include: (a) removal of the isoprenyl group of **CSA**, to obtain compounds **3a**–**3k**, **6a**, **6c** and **6f**; (b) esterification of the carboxyl group, to synthesize **4a**–**4k**; (c) cleavage of the cinnamenyl group, giving compound **10**; and (d) introduction of substituents into the phenyl ring B, to obtain **CSA** derivatives **5b**–**5k**.

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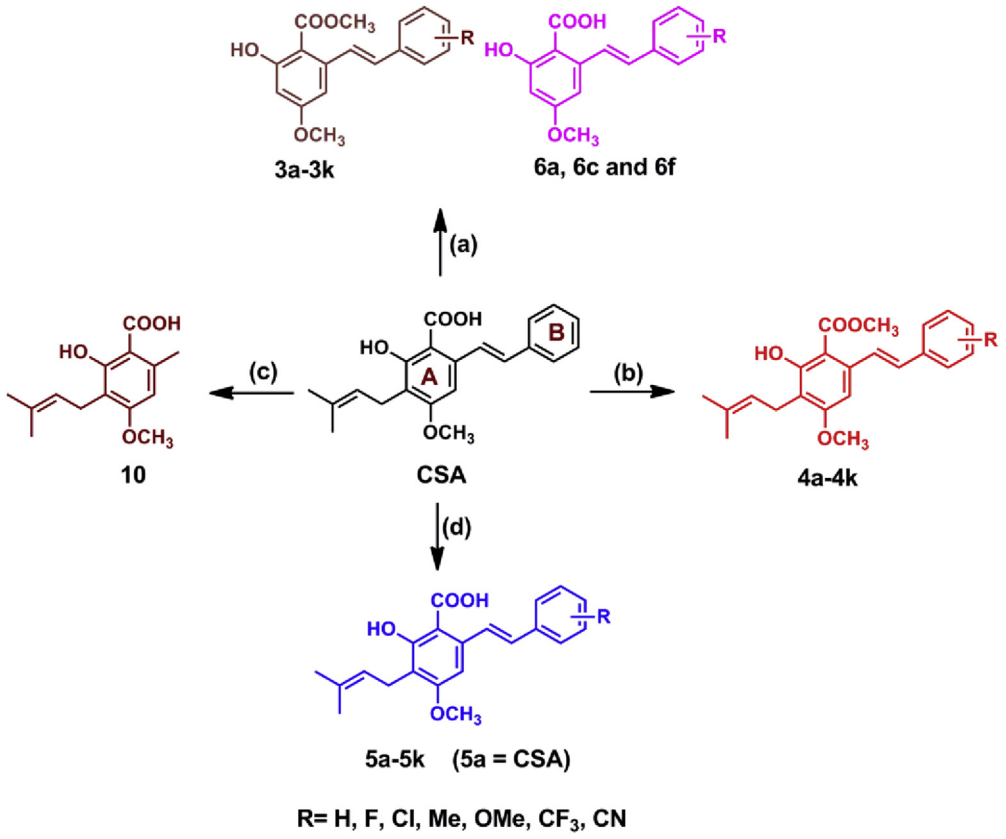


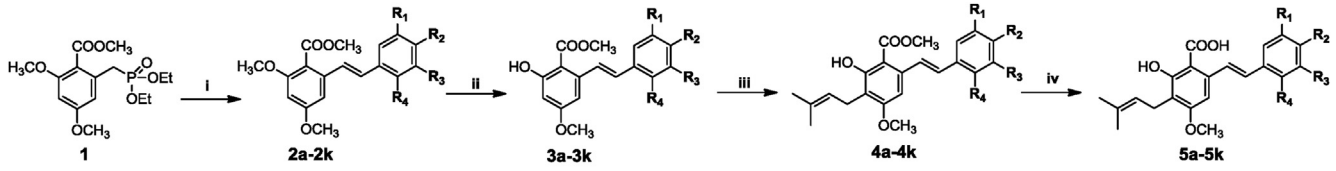
Fig. 1. Design of novel cajaninstilbene acid (**CSA**) derivatives.

The synthetic routes to the compounds in Fig. 1 are depicted in Schemes 1–3. Intermediate **1** was prepared according to a published method [6]. Compounds **2a**–**2k** were synthesized by reacting **1** with different benzaldehydes under the conditions of the Horner–Wadsworth–Emmons reaction [7]. During this reaction, the *E*-configuration products can be isolated in high yield and high selectivity when a catalytic amount of 15-crown-5 (the cyclic pentamer of ethylene oxide) is used [8]. Compounds **3a**–**3k** were obtained from selective demethylation of the 2-OMe group of **2a**–**2k**. Compounds **3a**–**3k** were then treated with prenyl bromide and NaH to give **4a**–**4k**. The yield of **4a**–**4k** depends on the amount of NaH and prenyl bromide and the optimum ratio (1.2 equiv) was determined in a number of experiments [9]. Finally, cajaninstilbene acid and its derivatives **5a**–**5k** were obtained by hydrolysis of the ester group of **4a**–**4k** (Scheme 1). Another series (**6a**, **6c** and **6f**) was conveniently prepared by hydrolysis of **3a**, **3c** and **3f** (Scheme 2). In addition, as shown in Scheme 3, intermediate **8** was prepared from **7** by oxidative aromatization using iodine in methanol [10]. Compound **10** was synthesized by coupling the isoprenyl group and ester hydrolysis.

3. Results and discussion

3.1. Evaluation of antibacterial activity

The antibacterial activities of all synthesized compounds were



2a, 3a, 4a, 5a(CA): R_1=H, R_2=H, R_3=H, R_4=H

2b, 3b, 4b, 5b: R_1=H, R_2=H, R_3=H, R_4=F

2c, 3c, 4c, 5c: R_1=H, R_2=H, R_3=F, R_4=H

2d, 3d, 4d, 5d: R_1=H, R_2=F, R_3=H, R_4=H

2e, 3e, 4e, 5e: R_1=H, R_2=Cl, R_3=H, R_4=H

2f, 3f, 4f, 5f: R_1=H, R_2=CH_3O, R_3=H, R_4=H

2g, 3g, 4g, 5g: R_1=H, R_2=CH_3, R_3=H, R_4=H

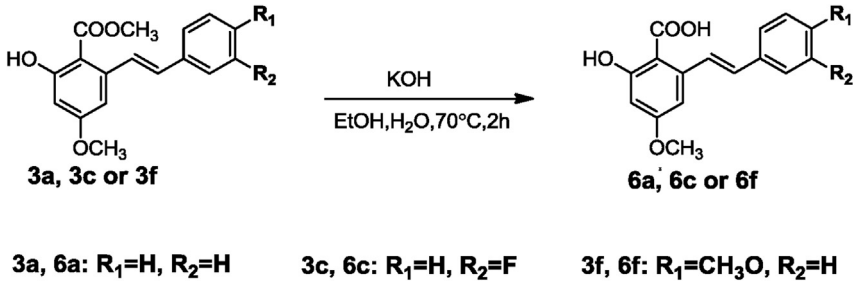
2h, 3h, 4h, 5h: R_1=R_2=R_3=CH_3O, R_4=H

2i, 3i, 4i, 5i: R_1=H, R_2=H, R_3=CN, R_4=H

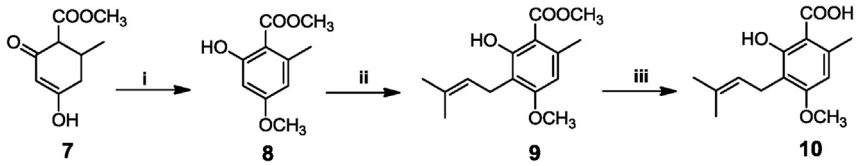
2j, 3j, 4j, 5j: R_1=H, R_2=CF_3, R_3=H, R_4=H

2k, 3k, 4k, 5k: R_1=H, R_2=F, R_3=H, R_4=F

Scheme 1. General synthetic route of cajaninstilbene acid derivatives **3a**–**3k**, **4a**–**4k** and **5a**–**5k**. Reagents and conditions: (i) aromatic aldehyde, 15-crown-5, NaH, THF, −2 °C, 2 h; (ii) BCl_3, CH_2Cl_2, −30 °C, 2 h; (iii) prenyl bromide, NaH, 78 °C, 2 h; (iv) KOH, EtOH, H_2O, 70 °C, 2 h.



Scheme 2. Synthetic route of compounds **6a**, **6c** and **6f**.



Scheme 3. Synthetic route of compound **10**. Reagents and conditions: (i) I_2, CH_3OH, reflux, 4 h; (ii) prenyl bromide, 78 °C, 2 h; (iii) KOH, EtOH, H_2O, 70 °C, 2 h.

evaluated *in vitro* by a serial dilution method to obtain their minimum inhibitory concentration (MIC) values against a variety of different strains, including gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*), gram-negative (*Eschemeerichia coli*, *Proteus vulgaris*, *Pesudomonas aeruginosa*) and nine strains of methicillin-resistant *Staphylococcus aureus* bacteria. The MIC is defined as the lowest concentration of antibacterial agent at which no growth of the strain can be observed [11]. Penicillin, norfloxacin were used as positive controls in the assays. The MIC values are summarized in Tables 1–3.

3.1.1. Cajaninstilbene acid derivatives lacking the isoprenyl group (*3a*–*3k*, *6a*, *6c* and *6f*)

The isoprenyl group is considered to be an active essential group of many natural products [12], such as prenylated stilbene, which is an effective agent for the treatment of tapeworm infection [13]. In order to investigate whether the isoprenyl group of **CSA** is also necessary for its antibacterial activity, we synthesized a series of compounds lacking an isoprenyl group (**3a**–**3k**). A preliminary test of bioactivity showed that **3a**–**3k** exhibited no antibacterial activity; their minimal inhibitory concentration (MIC) values were greater than 128 μg/mL against all tested strains (Table 1). We then synthesized **6a**, **6c** and **6f**, which lacks an isoprenyl group but, like **CSA** has a free carboxyl group, to determine if the loss of antibacterial activity is caused by the loss of the isoprenyl group. The MIC results showed that **6a**, **6c** and **6f** also have no antibacterial activity. (Table 1, MIC: $\geq$ 64 μg/mL), indicating that the isoprenyl group is necessary for the antibacterial activity of **CSA**.

3.1.2. Cajaninstilbene acid ester derivatives (*4a*–*4k*)

Carboxylic acid esters can be absorbed *in vivo* quickly and completely, and have been widely used to improve the bioavailability of drugs, such as cefuroxime ester [14]. Thus a series of cajaninstilbene acid ester derivatives were synthesized in this study, in which the carboxyl group of cajaninstilbene acid (**CSA**) on the A ring was esterified by methanol (**4a**–**4k**) to investigate whether the esterification of **CSA** will improve its activity or druggability. The results demonstrated that cajaninstilbene acid esters have no significant antibacterial property, as shown in Table 2. The MIC values of the compounds against the tested bacterial strains were all greater than 64 μg/mL, and most of them were even greater than 128 μg/mL. Compared with the **CSA**, whose MIC values were mostly between 8 and 32 μg/mL for the tested bacterial strains, the antibacterial activities of ester derivatives were significantly decreased, indicating that the free carboxyl group of **CSA** is also necessary for its antibacterial activity.

3.1.3. Cajaninstilbene acid derivatives with no cinnamenyl group (*10*)

A new compound (**10**), which does not contain the cinnamenyl group, was synthesized to determine whether the cinnamenyl group is essential for the antibacterial activity of **CSA**. Assays (Table 2) revealed that the antibacterial activity of **10** (MIC: 32–64 μg/mL) is much less than that of **CSA** (MIC: 8–32 μg/mL). This result indicates that the cinnamenyl group is also necessary for the antibacterial activity. Combination of all the above results, leads to the conclusion that the isopentenyl, the free carboxyl, as well as the cinnamenyl group of **CSA** are all required for the antibacterial activity of **CSA**. Therefore, the subsequent design was focused on derivatization of the stilbene acid.

3.1.4. Cajaninstilbene acid derivatives with different substituents on phenyl ring B (*5b*–*5k*)

In order to explore the impact of the substituents on the phenyl ring B on the antibacterial activity and to develop improved antibacterial agents, a variety of cajaninstilbene acid derivatives with different substituents on benzene ring B (**5b**–**5k**) were synthesized. Since trifluoromethyl and fluorine are well known to improve the efficiency of many drugs [15–17] trifluoromethyl (**5j**) and fluorine (**5b**, **5c**, **5d** and **5k**) were introduced into the benzene ring B. The MIC results (Table 3) shown that all the compounds with a fluorine atom (**5b**–**5d**, **5j** and **5k**) exhibit strong inhibitory activities towards all the three wild gram-negative bacterial strains (*S.a*, *S.e*, *B.u*) and nine methicillin resistant *staphylococcus aureus* bacteria with MIC values in the range of 0.5–8 μg/mL, and were therefore significantly superior to **CSA** (8–32 μg/mL). For example, the inhibitory activity of compound **5j** was 2–64 times greater than that of **CSA**. Since both trifluoromethyl and fluorine are electron withdrawing groups, we further investigated whether other electron withdrawing substituent groups on the benzene ring B, such as chlorine (**5e**) and cyano (**5i**), can also enhance the antibacterial activity. The results show that the minimal inhibitory concentrations of **5e** and **5i** against *S.a*, *S.e*, *B.s* and MRSA were 0.5–8 μg/mL, and thus **5e** and **5i** are also superior to **CSA**. On the other hand, we

Table 1
In vitro antibacterial activity of compounds **3a**–**3k**, **6a**, **6c** and **6f**.

Compound	MIC[a] (μg/mL)														
	S.a[b]	*S.e*[c]	*B.s*[d]	*E.c*[e]	*P.v*[f]	*P.a*[g]	43300[h]	523512[i]	425055[j]	513045[k]	62202[l]	51033[m]	52056[n]	515992[o]	510019[p]
3a	>128	128	128	>128	>128	128	>128	>128	128	>128	>128	128	>128	>128	>128
3b	>128	128	128	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	>128
3c	>128	128	128	128	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	128
3d	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128
3e	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128
3f	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	128
3g	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	128	>128	>128	>128
3h	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	64	>128
3i	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	64	>128
3j	>128	128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128
3k	>128	>128	>128	>128	>128	>128	128	>128	128	>128	>128	128	>128	>128	>128
6a	>128	>128	64	>128	>128	>128	>128	>128	>128	>128	128	>128	128	>128	>128
6c	>128	64	>128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	>128	>128
6f	>128	>128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
CSA(**5a**)	16	16	32	>128	>64	>64	16	32	16	16	8	16	16	32	32
Penicillin	2	2	2	64	>16	16	32	32	32	32	32	32	64	32	32
Norfloxacin	2	2	2	32	16	16	4	8	4	4	8	4	8	16	8

[a] MIC is defined as the minimum concentration of a compound that inhibits growth by 99%. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section.
[b] *Staphylococcus aureus* ATCC25923.
[c] *Staphylococcus epidermidis* ATCC12228.
[d] *Bacillus subtilis* ATCC6633.
[e] *EScheмeerichia coli* ATCC25922.
[f] *Proteus vulgaris* ATCC49101.
[g] *Pesudomonas aeruginosa* ATCC2785.
[h] MRSA ATCC43300.
[i] MRSA ATCC 523512.
[j] MRSA ATCC425055.
[k] MRSA ATCC513045.
[l] MRSA ATCC62202.
[m] MRSA ATCC51033.
[n] MRSA ATCC52056.
[o] MRSA ATCC515992.
[p] MRSA ATCC510019.

also investigated the effects of introducing of electron donating group into the phenyl ring B. The results (Table 3) showed that both a methoxy group (**5f**, **5h**) and a methyl group (**5g**) reduce the antibacterial activity (MIC: 16–64 μg/mL). Our results clearly show therefore that introduction of electron withdrawing group into the benzene ring B can increase the antibacterial activity while introduction of electron donating groups reduces the antibacterial activity. Additionally, it can be noted that there is no significant difference among the activities of **5b**, **5c** and **5k** (MIC 0.5–8 μg/mL), which indicates that the number of substituents has no influence on the activity.

3.2. Antibacterial activity summary

Antibacterial assay results showed that an overwhelming majority of compounds have no activity against gram-negative bacteria, while some compounds exhibited strong antibacterial activity on gram-positive bacteria, as well as methicillin-resistant *Staphylococcus aureus* (MRSA). In particular, the antibacterial activities of compounds **5b**, **5c**, **5j** and **5k** (MIC: 0.5–8 μg/mL), were much better than those of the natural product cajaninstilbene acid (MIC: 8–32 μg/mL). These results illustrated that the modification of **CSA** in this study has greatly improved its antibacterial activity. It should be noted that compounds **5b**, **5c**, **5j** and **5k** also have good antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA); the MIC values of these compounds for certain MRSA strains are 0.5 μg/mL, 16-fold superior to that of **CSA** (MIC: 8–32 μg/mL), 64–128-fold superior to penicillin (MIC: 32–64 μg/mL), and 8–32-fold than norfloxacin (MIC: 4–16 μg/mL). These results indicate that compounds **5b**, **5c**, **5j** and **5k** are promising antibacterial candidates for the treatment of multidrug-resistant pathogens.

3.3. Cytotoxicity assay

The *in vitro* cytotoxicity of compounds **5b**, **5c**, **5j** and **5k** were further examined in mouse macrophage cell lines (RAW 264.7) in terms of a maximum tolerated test (MTT) to determine their selectivity between bacterial and normal cells [18]. As shown in Table 4, Compounds **5b**, **5c**, **5j** and **5k** all had no obvious cytotoxicity in the RAW 264.7 cells at concentration of 8 μg/mL (the largest of the MIC values), and the relative cell viabilities of the treated cells were all more than 90%. The cytotoxic concentrations for normal cells, indicated by the IC_{50} were much higher than their MIC values, and their selectivity index (SI) was good (Table 4). The low cytotoxicity for normal cells and good selective index values of these compounds indicate their potential usefulness in the development of drugs for infectious diseases.

3.4. Structure-activity relationships summary

Based on our results, the following structure-activity relationships (SARs) of the **CSA** derivatives can be summarized (Fig. 2). First, the activities of compounds **5a**–**5k** were significantly better than those of compounds **4a**–**4k**, which indicates that the free carboxyl on the benzene ring A was necessary for the activity. Second, compounds**3a**–**3k**, **6a**, **6c** and **6f** had no antibacterial activity, indicating that the loss of the isoprenyl group leads to the significant decrease of activity. Third, the activity of **10** was significantly lower than that of compounds **5a**–**5k**, which indicates that

Table 2
In vitro antibacterial activity of compounds **4a**–**4k** and **10**.

Compound	MIC[a] (μg/mL)														
	S.a[b]	*S.e*[c]	*B.s*[d]	*E.c*[e]	*P.v*[f]	*P.a*[g]	43300[h]	523512[i]	425055[j]	513045[k]	62202[l]	51033[m]	52056[n]	515992[o]	510019[p]
4a	>128	>128	>128	>128	>128	>128	128	>128	128	128	128	>128	>128	>128	>128
4b	>128	>128	>128	>128	>128	>128	128	>128	128	128	128	>128	>128	>128	>128
4c	64	>128	128	>128	>128	>128	>128	64	>128	128	128	128	>128	>128	128
4d	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	128	128	>128
4e	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128
4f	128	>128	>128	>128	>128	>128	128	128	128	128	>128	>128	128	>128	128
4g	>128	64	128	>128	>128	>128	128	>128	128	>128	>128	128	>128	>128	>128
4h	>128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	64	>128
4i	>128	128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	>128	>128
4j	64	64	128	>128	>128	>128	>128	64	64	128	128	>128	128	>128	>128
4k	>128	64	128	>128	>128	>128	>128	64	128	>128	64	128	128	>128	>128
10	32	64	64	>128	>128	>128	32	32	32	32	32	32	64	32	64
CSA(**5a**)	16	16	32	>128	>64	>64	16	32	16	16	8	16	16	32	32
Penicillin	2	2	2	64	>16	16	32	32	32	32	32	32	64	32	32
Norfloxacin	2	2	2	32	16	16	4	8	4	4	8	4	8	16	8

[a] MIC is defined as the minimum concentration of a compound that inhibits growth by 99%. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section.
[b] *Staphylococcus aureus* ATCC25923.
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[l] MRSA ATCC62202.
[m] MRSA ATCC51033.
[n] MRSA ATCC52056.
[o] MRSA ATCC515992.
[p] MRSA ATCC510019.

Table 3
In vitro antibacterial activity of compounds **5b**–**5k**.

Compound	MIC[a] (μg/mL)														
	S.a[b]	*S.e*[c]	*B.s*[d]	*E.c*[e]	*P.v*[f]	*P.a*[g]	43300[h]	523512[i]	425055[j]	513045[k]	62202[l]	51033[m]	52056[n]	515992[o]	510019[p]
5b	4	1	0.5	64	>64	>64	1	2	0.5	2	0.5	1	2	2	2
5c	4	2	0.5	>128	>64	>64	1	2	2	2	0.5	1	0.5	1	1
5d	8	4	1	>128	>64	>64	4	4	2	8	1	1	1	8	1
5e	8	4	2	128	>64	>64	2	4	4	4	2	0.5	2	4	1
5f	32	32	16	>128	>64	>64	16	16	8	16	16	32	16	16	16
5g	16	32	16	>128	>64	>64	32	32	16	8	32	16	16	32	32
5h	64	32	16	>128	>64	>64	16	16	16	16	32	64	32	16	16
5i	4	2	16	>128	>64	>64	8	2	1	4	0.5	2	4	2	2
5j	2	2	1	>128	>64	>64	2	0.5	0.5	8	0.5	1	4	0.5	2
5k	1	0.5	2	>128	>64	>64	2	2	0.5	2	1	1	2	1	2
CSA(**5a**)	16	16	32	>128	>64	>64	16	32	16	16	8	16	16	32	32
Penicillin	2	2	2	64	>16	16	32	32	32	32	32	32	64	32	32
Norfloxacin	2	2	2	32	16	16	4	8	4	4	8	4	8	16	8

[a] MIC is defined as the minimum concentration of a compound that inhibits growth by 99%. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section.
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[o] MRSA ATCC515992.
[p] MRSA ATCC510019.

Table 4
IC_{50} (μg/mL) and selectivity index (SI) values of active compounds (**5b**, **5c**, **5j** and **5k**) against mouse macrophage cell lines (RAW 264.7[a]).

Compound	MIC[b] (μg/mL)	% Cell viability at 8 μg/mL	IC_{50}[c](μg/mL)	SI value[c,d]
5b	0.5–4	92.68 ± 1.24	24.69 ± 2.75	6.17–41.38
5c	0.5–4	91.72 ± 0.78	20.17 ± 3.60	5.04–40.34
5j	0.5–2	90.56 ± 1.28	25.71 ± 0.87	12.86–51.42
5k	0.5–2	94.36 ± 0.45	46.54 ± 2.82	23.27–93.08

[a] RAW264.7 monolayers were used as an *in vitro* model of cervicovaginal epithelium for testing the cytotoxicity of the new compounds.
[b] Minimum inhibitory concentration against *S.a*, *S.e*, *B.s*, MRSA43300, MRSA523512, MRSA425055, MRSA513045, MRSA62202l, MRSA51033, MRSA52056, MRSA515992, MRSA510019.
[c] IC_{50} is defined as the concentration at which 50% growth is inhibited.
[d] Selectivity index (*in vitro*): IC_{50} in RAW 264.7 cells/MIC.

cinnamenyl group is very important for the activity. Fourth, the introduction of electron withdrawing groups such as trifluoromethyl, fluorine, and chlorine into the phenyl ring B can increase the activity, especially in the case of fluorine. The number and position of the fluorine atoms have no obvious effect on the activity since the activity of **5k** is similar to that of **5b** and **5c**. In contrast, when the benzene ring B was substituted with electron donating group, a decrease in activity is observed.

4. Conclusion

A series of cajaninstilbene acid derivatives were designed and synthesized for the first time. Some of the derivatives were found to have better antibacterial activities than positive controls and cajaninstilbene acid against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, especially MRSA. The MIC values of compounds **5b**, **5c**, **5j** and **5k** against MRSA are up to 0.5 μg/mL, 8–32-fold superior to that of norfloxacin. The cytoxic assay exhibits a good selective index between bacterial and normal cells for the active compounds **5b**, **5c**, **5j** and **5k**. These findings indicate the potential usefulness of **5b**, **5c**, **5j** and **5k** in drug development. Moreover, some structure-activity relationships of **CSA** derivatives were determined and will be useful in the future to guide the design and modification of new candidate **CSA** analogues as antibacterial agents.

5. Experimental protocols

5.1. Chemistry

Intermediate **1** was synthesized according to the literature [6]. Compound **2** and the major chemical reagents were purchased from Alfa Aesar or Sigma Aldrich Co. The synthetic routes to the cajaninstilbene acid derivatives **2a**–**2k**, **3a**–**3k**, **4a**–**4k**, **5a**–**5k**, **6a**, **6c**, **6f** and **10** are depicted in Schemes 1–3. All the compounds were fully analyzed and characterized by ^1H and ^{13}C-nuclear magnetic resonance (NMR), mass spectrometry (MS) and high resolution mass spectrometry (ESI-HRMS) before biological screening.

5.1.1. General procedure for the synthesis of compounds *2a*–*2k*

15-crown-5 (40 mg) and NaH (100 mg, 2.5 mmol, 60% in mineral oil) were dissolved in dry THF (20 mL) followed by the addition of the solution of compound **1** (1.38 g, 2 mmol) and aromatic aldehydes (0.025 mol) at −2 °C. After 20 min, the solution was warmed to room temperature and reacted for 2 h, after which most of the THF was evaporated, ice H_2O was added and the solution was extracted with ether. The combined ether layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue, which was purified over silica gel (petroleum ether: EtOAc = 8:1) to afford compound **2a**–**2k** as a colorless solid.

*5.1.1.1. (E)-Methyl 2,4-dimethoxy-6-styrylbenzoate (**2a**).* Yield 88%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.2 Hz, 2H), 7.38 (m, 3H), 7.28 (d, *J* = 16.2, 2H), 6.79 (d, *J* = 2.4 Hz, 1H), 6.78 (d, *J* = 16.2 Hz, 1H), 6.42 (d, *J* = 2.4 Hz, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 161.5, 158.3, 137.6, 136.9, 131.8, 128.7, 128.1, 126.8, 125.5, 116.0, 101.5, 98.1, 56.0, 55.5, 52.3.

*5.1.1.2. (E)-Methyl –2.4-dimethoxy-6- styrylbenzoate (**2b**).* Yield 92%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (td, *J* = 7.7, 1.7 Hz, 1H), 7.25 (dt, *J* = 4.5, 3.9 Hz, 1H), 7.20 (d, *J* = 1.7 Hz, 2H), 7.17–7.02 (m, 2H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.43 (d, *J* = 2.1 Hz, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 161.6, 158.3, 137.5, 129.4, 129.3, 127.7, 127.7, 127.4, 127.3, 124.8, 124.7, 124.3, 124.3, 124.0, 123.9, 116.0, 116.0, 115.7, 101.5, 98.3, 56.0, 55.5, 52.4; ESI-LRMS *m/z*: 317.1 $[M+H]^+$. ESI-HRMS *m/z*: 317.1188 $[M+H]^+$, calcd for $C_{18}H_{17}FO_4$ 317.1184.

*5.1.1.3. (E)-Methyl-2-(3-fluorostyryl)-4,6-dimethoxybenzoate (**2c**).* Yield 88%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (td, *J* = 7.9, 5.9 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.19–7.13 (m, 1H), 7.12–6.91 (m, 3H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.42 (d, *J* = 2.1 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 158.3, 139.3, 139.2, 137.1, 130.6, 130.5, 130.2, 130.1, 126.8, 122.7, 122.7, 116.0, 115.0, 114.7, 113.3, 113.0, 101.6, 98.3, 56.0, 55.5, 52.4; ESI-LRMS *m/z*: 317.2 $[M+H]^+$. ESI-HRMS *m/z*: 317.1184 $[M+H]^+$, calcd for $C_{18}H_{17}FO_4$ 317.1184.

*5.1.1.4. (E)-Methyl 2-(4-fluorostyryl)-4,6-dimethoxybenzoate (**2d**).* Yield 89%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52–7.34 (m, 2H), 7.13–6.83 (m, 4H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.40 (d, *J* = 2.1 Hz, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$)

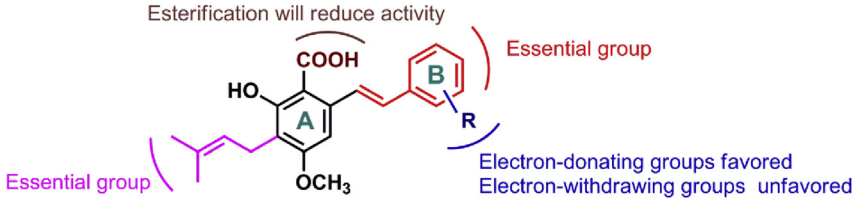

Fig. 2. Structure-activity relationships of cajaninstilbene acid (**CSA**) derivatives.

δ 168.5, 161.6, 158.3, 137.4, 133.1, 133.1, 130.5, 128.4, 128.3, 125.2, 125.1, 115.9, 115.7, 115.5, 101.5, 98.0, 55.9, 55.4, 52.3; ESI-LRMS *m/z*: 317.1 $[M+H]^+$. ESI-HRMS *m/z*: 317.1188 $[M+H]^+$, calcd for $C_{18}H_{17}FO_4$ 317.1184.

*5.1.1.5. (E)-Methyl 2-(4-chlorostyryl)-4,6-dimethoxybenzoate (**2e**).* Yield 93%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.36 (m, 1H), 7.35–7.28 (m, 1H), 7.07 (d, *J* = 16.1 Hz, 1H), 6.97 (d, *J* = 16.1 Hz, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.42 (d, *J* = 2.1 Hz, 1H), 3.92 (s, 1H), 3.87 (s, 1H), 3.83 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 161.6, 158.3, 137.3, 135.4, 133.7, 130.4, 128.9, 128.0, 126.0, 115.9, 101.6, 98.2, 56.0, 55.5, 52.4; ESI-LRMS *m/z*: 333.3 $[M+H]^+$. ESI-HRMS *m/z*: 333.0889 $[M+H]^+$, calcd for $C_{18}H_{18}ClO_4$ 333.0888.

*5.1.1.6. (E)-Methyl 2,4-dimethoxy-6-(4-methoxystyryl)benzoate (**2f**).* Yield 85%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.38 (m, 2H), 7.03 (d, *J* = 16.1 Hz, 1H), 6.96 (d, *J* = 16.2 Hz, 1H), 6.93–6.86 (m, 2H), 6.77 (d, *J* = 2.1 Hz, 1H), 6.41 (d, *J* = 2.1 Hz, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 161.5, 159.7, 158.2, 137.9, 131.3, 129.7, 128.1, 123.2, 115.8, 114.1, 101.3, 97.7, 56.0, 55.5, 55.3, 52.3; ESI-LRMS *m/z*: 329.4 $[M+H]^+$. ESI-LRMS *m/z*: 333.3 $[M+H]^+$. ESI-HRMS *m/z*: 329.1385 $[M+H]^+$, calcd for $C_{19}H_{20}O_5$ 329.1384.

*5.1.1.7. (E)-Methyl 2,4-dimethoxy-6-(4-methylstyryl)benzoate (**2g**).* Yield 72%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.1 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.11–6.96 (t, 2H), 6.76 (d, *J* = 2.1 Hz, 1H), 6.40 (d, *J* = 2.1 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H), 2.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 161.5, 158.2, 138.1, 137.7, 134.1, 131.7, 129.4, 126.7, 124.4, 115.9, 101.4, 97.9, 56.0, 55.5, 52.3, 21.3; ESI-LRMS *m/z*: 313.3 $[M+H]^+$. ESI-HRMS *m/z*: 313.1434 $[M+H]^+$, calcd for $C_{19}H_{20}O_4$ 313.1434.

*5.1.1.8. (E)-Methyl 2,4-dimethoxy-6-(3,4,5-trimethoxystyryl)benzoate (**2h**).* Yield 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (d, *J* = 16.1 Hz, 1H), 6.91 (d, *J* = 16.1 Hz, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.66 (s, 2H), 6.36 (d, *J* = 2.1 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 6H), 3.83 (s, 3H), 3.82 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 161.6, 158.3, 153.4, 138.3, 137.5, 132.7, 131.7, 125.0, 115.7, 103.9, 101.6, 98.0, 60.9, 56.1, 56.0, 55.4, 52.2; ESI-LRMS *m/z*: 411.7 $[M+Na]^+$. ESI-HRMS *m/z*: 389.1597 $[M+H]^+$, calcd for $C_{21}H_{24}O_7$ 389.1595.

*5.1.1.9. (E)-Methyl 2-(3-cyanostyryl)-4,6-dimethoxybenzoate (**2i**).* Yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (dd, *J* = 7.4, 6.2 Hz, 2H), 7.54 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 16.2 Hz, 1H), 6.98 (d, *J* = 16.1 Hz, 1H), 6.73 (d, *J* = 2.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 161.7, 158.5, 138.1, 136.8, 131.2, 130.7, 130.3, 129.5, 129.3, 128.2, 118.4, 116.1, 113.0, 101.8, 98.7, 56.1, 55.6, 52.5; ESI-LRMS *m/z*: 324.3 $[M+H]^+$. ESI-HRMS *m/z*: 324.1233 $[M+H]^+$, calcd for $C_{19}H_{17}NO_4$ 324.123.

*5.1.1.10. (E)-Methyl 2,4-dimethoxy-6-(4-(trifluoromethyl)styryl)benzoate (**2j**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (q, *J* = 8.6 Hz, 4H), 7.18 (d, *J* = 16.1 Hz, 1H), 7.03 (d, *J* = 16.1 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.43 (d, *J* = 2.1 Hz, 1H), 3.93 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 161.7, 158.4, 140.4, 137.0, 130.2, 128.0, 126.9, 125.7, 125.6, 125.6, 125.5, 116.1, 101.8, 98.5, 56.0, 55.5, 52.4; ESI-LRMS *m/z*: 367.1 $[M+H]^+$. ESI-HRMS *m/z*: 367.1154 $[M+H]^+$, calcd for $C_{19}H_{17}F_3O_4$ 367.1152.

*5.1.1.11. (E)-Methyl 2-(2,4-difluorostyryl)-4,6-dimethoxybenzoate (**2k**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.43 (m, 1H), 7.10 (s, 2H), 6.92–6.77 (m, 2H), 6.75 (d, *J* = 2.0 Hz, 1H), 6.42 (d, *J* = 2.0 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 161.3, 158.0, 137.4, 128.2, 127.3, 122.9, 122.7, 115.9, 111.8, 111.8, 111.5, 104.5, 104.1, 101.4, 98.3, 56.0, 55.2, 52.0; ESI-LRMS *m/z*: 335.3 $[M+H]^+$. ESI-HRMS *m/z*: 335.1100 $[M+H]^+$, calcd for $C_{18}H_{16}F_2O_4$ 335.1089.

*5.1.2. General procedure for the synthesis of compounds **3a**-**3k***

Compound **2a**-**2k** (0.02 mol) was dissolved in dry CH_2Cl_2 (100 mL) and BCl_3 (2 mL, 1 mol/L) was added at −30 °C. The reaction mixture was stirred for 2 h at −20 °C after which it was quenched by the addition of iced H_2O. The organic layer was separated and dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue, which was purified over silica gel (petroleum ether: Et_2O = 20:1) to give compound **3a**-**3k** as a white solid.

*5.1.2.1. (E)-Methyl 2-hydroxy-4-methoxy-6-styrylbenzoate (**3a**).* ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1H), 7.73 (d, *J* = 16.2 Hz, 1H), 7.50–7.38 (m, 2H), 7.43 – 7.34 (m, 2H), 7.31 (d, *J* = 16.2 Hz , 1H) , 6.83 (d, *J* = 16.2 Hz, 1H) , 6.78 (d, *J* = 2.1 Hz , 1H) , 6.45 (d, *J* = 2.1 Hz, 1H) , 3.95 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 165.2, 164.2, 142.9, 137.4, 130.9, 129.9, 128.8, 127.9, 126.7, 108.0, 104.0, 100.2, 55.5, 52.3.

*5.1.2.2. (E)-Methyl 2-(2-fluorostyryl)-6-hydroxy-4-methoxybenzoate (**3b**).* Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.72 (s, 1H), 7.80 (d, *J* = 16.1 Hz, 1H), 7.57 (td, *J* = 7.6, 1.7 Hz, 1H), 7.32–7.22 (m, 1H), 7.13 (m, 2H), 6.95 (d, *J* = 16.1 Hz, 1H), 6.66 (d, *J* = 2.6 Hz, 1H), 6.47 (d, *J* = 2.6 Hz, 1H), 3.97 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 165.2, 164.2, 142.8, 132.4, 132.4, 129.1, 129.0, 127.6, 127.6, 125.3, 125.1, 124.3, 124.2, 123.3, 123.3, 116.1, 115.8, 108.1, 104.0, 100.4, 55.5, 52.3; ESI-LRMS *m/z*: 303.3 $[M+H]^+$. ESI-HRMS *m/z*: 303.1028 $[M+H]^+$, calcd for $C_{17}H_{15}FO_4$ 303.1027.

*5.1.2.3. (E)-Methyl 2-(3-fluorostyryl)-6-hydroxy-4-methoxybenzoate (**3c**).* Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.72 (s, 1H), 7.80 (d, *J* = 16.1 Hz, 1H), 7.57 (td, *J* = 7.6, 1.7 Hz, 1H), 7.32–7.22 (m, 1H), 7.16–7.25 (m, 2H), 6.95 (d, *J* = 16.1 Hz, 1H), 6.66 (d, *J* = 2.6 Hz, 1H), 6.47 (d, *J* = 2.6 Hz, 1H), 3.97 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 165.2, 164.2, 142.3, 139.8, 139.7, 131.2, 130.3, 130.1, 129.6, 129.6, 122.7, 122.7, 114.8, 114.5, 113.0, 112.7, 108.2, 103.9, 100.4, 55.5, 52.4; ESI-LRMS *m/z*: 303.3 $[M+Na]^+$. ESI-HRMS *m/z*: 303.1028 $[M+H]^+$, calcd for $C_{17}H_{15}FO_4$ 303.1027.

*5.1.2.4. (E)-Methyl 2-(4-fluorostyryl)-6-hydroxy-4-methoxybenzoate (**3d**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, 1H), 7.60 (d, *J* = 15.9 Hz, 1H), 7.51–7.39 (m, 2H), 7.06 (t, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 15.9 Hz, 1H), 6.60 (d, *J* = 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 165.2, 164.2, 142.7, 133.6, 129.7, 128.2, 128.1, 115.8, 115.6, 108.0, 103.9, 100.2, 55.5, 52.3; ESI-LRMS *m/z*: 303.3 $[M+H]^+$. ESI-HRMS *m/z*: 303.1028 $[M+H]^+$, calcd for $C_{17}H_{15}FO_4$ 303.1027.

*5.1.2.5. (E)-Methyl 2-(4-chlorostyryl)-6-hydroxy-4-methoxybenzoate (**3e**).* Yield 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.66 (s, 1H), 7.65 (d, *J* = 16.0 Hz, 1H), 7.46–7.38 (m, 2H), 7.37–7.29 (m, 2H), 6.74 (d, *J* = 15.9 Hz, 1H), 6.60 (d, *J* = 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 165.2, 164.2, 142.5, 135.9, 133.4, 130.5, 129.5, 128.9, 127.8, 108.1, 103.9, 100.3, 55.5, 52.3; ESI-LRMS *m/z*: 319.3 $[M+H]^+$. ESI-HRMS *m/z*: 319.0732 $[M+H]^+$, calcd for $C_{17}H_{16}ClO_4$ 319.0732.

*5.1.2.6. (E)-Methyl 2-hydroxy-4-methoxy-6-(4-methoxystyryl)benzoate (**3f**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.69 (s, 1H), 7.57 (d, *J* = 15.9 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 15.9 Hz, 1H), 6.62 (d, *J* = 2.6 Hz, 1H), 6.42 (d, *J* = 2.6 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$)

δ 171.8, 165.1, 164.1, 159.5, 143.2, 130.5, 130.2, 127.9, 127.7, 114.2, 107.7, 104.0, 100.0, 55.5, 55.4, 52.2; ESI-LRMS *m/z*: 337.2 $[M+Na]^+$. ESI-HRMS *m/z*: 315.1226 $[M+H]^+$, calcd for $C_{17}H_{16}O_5$ 315.1227.

*5.1.2.7. (E)-Methyl 2-hydroxy-4-methoxy-6-(4-methylstyryl)benzoate (**3g**).* Yield 95%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.71 (s, 1H), 7.65 (d, J = 15.9 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 15.9 Hz, 1H), 6.64 (d, J = 2.5 Hz, 1H), 6.44 (d, J = 2.6 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 165.2, 164.2, 143.1, 137.8, 134.7, 130.8, 129.5, 128.9, 126.6, 107.8, 104.0, 100.1, 55.5, 52.2, 21.3; ESI-LRMS *m/z*: 299.1 $[M+H]^+$. ESI-HRMS *m/z*: 299.1278 $[M+H]^+$, calcd for $C_{18}H_{18}O_4$ 299.1278.

*5.1.2.8. (E)-Methyl 2-hydroxy-4-methoxy-6-(3,4,5-trimethoxystyryl) benzoate (**3h**).* Yield 90%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.65 (s, 1H), 7.56 (d, J = 15.9 Hz, 1H), 6.70 (s, 2H), 6.68 (d, J = 15.9 Hz, 1H) 6.58 (d, J = 2.5 Hz, 1H), 6.40 (d, J = 2.6 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 6H), 3.86 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 165.2, 164.2, 153.4, 142.7, 138.1, 133.2, 130.7, 129.4, 107.8, 103.9, 103.8, 100.2, 61.0, 56.1, 55.4, 52.2; ESI-LRMS *m/z*: 375.3 $[M+H]^+$. ESI-HRMS *m/z*: 375.1439 $[M+H]^+$, calcd for $C_{20}H_{22}O_7$ 375.1438.

*5.1.2.9. (E)-Methyl 2-(3-cyanostyryl)-6-hydroxy-4-methoxybenzoate (**3i**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.64 (s, 1H), 7.80–7.63 (m, 3H), 7.54 (dt, J = 7.6, 1.3 Hz, 1H), 7.47 (d, J = 7.7 Hz, 1H), 6.73 (d, J = 16.0 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.45 (d, J = 2.6 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 165.2, 164.2, 141.8, 138.7, 132.5, 130.9, 130.8, 130.0, 129.6, 128.4, 118.8, 113.0, 108.4, 103.9, 100.7, 55.6, 52.4; ESI-LRMS *m/z*: 310.3 $[M+H]^+$. ESI-HRMS *m/z*: 310.1074 $[M+H]^+$, calcd for $C_{18}H_{15}NO_4$ 310.1074.

*5.1.2.10. (E)-Methyl-2-hydroxy-4-methoxy-6-(4-(trifluoromethyl) styryl)benzoate (**3j**).* Yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.71 (s, 1H), 7.63 (d, J = 15.9 Hz, 1H), 7.47 (m, 1H), 7.08 (t, J = 8.7 Hz, 2H), 6.78 (d, J = 15.9 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 6.46 (d, J = 2.6 Hz, 1H), 3.97 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 165.2, 164.2, 160.8, 142.7, 133.6, 129.7, 128.2, 128.1, 115.9, 115.6, 108.0, 103.9, 100.2, 55.5, 52.3; ESI-LRMS *m/z*: 351.3 $[M-H]^+$. ESI-HRMS *m/z*: 353.0993 $[M+H]^+$, calcd for $C_{18}H_{15}F_3O_4$ 353.0995.

*5.1.2.11. (E)-Methyl-2-(2,4-difluorostyryl)-6-hydroxy-4-methoxybenzoate (**3k**).* Yield 95%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1H), 7.72 (d, J = 16.1 Hz, 1H), 7.53 (td, J = 8.6, 6.5 Hz, 1H), 6.97 (d, J = 16.1 Hz, 1H), 6.96–6.77 (m, 2H), 6.63 (d, J = 2.5 Hz, 1H), 6.47 (d, J = 2.6 Hz, 1H), 3.96 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 165.1, 164.1, 142.5, 131.9, 128.5, 128.3, 122.3, 111.5, 108.2, 104.5, 104.2, 103.8, 100.4, 100.3, 55.4, 52.1; ESI-LRMS *m/z*: 319.3 $[M-H]^+$. ESI-HRMS *m/z*: 321.0934 $[M+H]^+$, calcd for $C_{17}H_{14}F_2O_4$ 321.0933.

*5.1.3. Procedure for the synthesis of compounds **8***

To a solution of compound **7** (10 g, 54 mmol) in MeOH (100 mL) was added I_2 (27.6 g, 108 mmol). The mixture was heated at reflux for 4 h. Excess MeOH was evaporated, then the mixture was extracted with EtOAc (300 mL × 3), and washed with H_2O (50 mL × 2) and brine (50 mL), dried with Na_2SO_4, and filtered. The filtrate was concentrated to give compound **8** (5.7 g) as a white solid, yield 54%.^{1}H NMR (300 MHz, $CDCl_3$) δ 11.77 (s, 1H), 6.30 (d, J = 2.6 Hz, 1H), 6.25 (d, J = 2.6 Hz, 1H), 3.90 (s, 3H), 3.77 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 165.5, 163.9, 143.1, 111.1, 105.2, 98.7, 55.2, 51.8, 24.3.

*5.1.4. General procedure for the synthesis of compounds **4a**–**4k** and **9***

Compound **3a**–**3k** or **8** (7.0 mmol) was dissolved in dry toluene (50 mL) and NaH (8.4 mmol, 60% in mineral oil) was added at 45 °C. The reaction mixture was stirred at 45 °C for 0.5 h after which the prenyl bromide (8.4 mmol) was added at room temperature. The mixture was stirred for 2 h at 78 °C, then quenched by the addition of saturated NaCl and the organic layer was separated and dried over anhydrous Na_2SO_4. It was then filtered and concentrated to give a residue, which was purified over silica gel (petroleum ether: Et_2O = 100:1) to give compound **4a**–**4k** or **9** as a white solid separately.

*5.1.4.1. (E)-Methyl 2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-styrylbenzoate (**4a**).* Yield 41%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.74 (s,1H), 7.55 (m,3H), 7.39 (t, J = 7.2 Hz, 2H), 7.28 (t, J = 7.2 Hz, 1H), 7.08 (d, J = 16.2 Hz, 1H), 6.85 (s,1H), 5.27 (t, J = 7.2 Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.43 (d, J = 7.2 Hz, 2H), 1.84 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 161.5, 161.4, 140.3, 137.5, 131.9, 130.7, 130.1, 128.8, 127.7, 126.6, 122.1, 116.7, 104.6, 102.9, 55.7, 52.2, 25.8, 22.1, 17.8.

*5.1.4.2. (E)-Methyl-2-hydroxy-3-(3-methylbut-2-enyl)-4-dimethoxy-6-styrylbenzoate (**4b**).* Yield 44%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 16.2 Hz, 1H), 7.57 (td, J = 7.6, 1.6 Hz, 1H), 7.25 (m, 1H), 7.20–7.04 (m, 2H), 6.92 (d, J = 16.1 Hz, 1H), 6.62 (s, 1H), 5.29–5.13 (m, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.39 (d, J = 7.0 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 162.1, 161.5, 161.4, 140.2, 133.1, 133.1, 131.9, 129.0, 128.9, 127.5, 127.4, 125.4, 125.3, 124.3, 124.3, 122.4, 122.0, 116.9, 116.1, 115.8, 104.5, 102.9, 55.7, 52.2, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 371.2 $[M+H]^+$; ESI-HRMS *m/z*: 371.1655 $[M+H]^+$, calcd for $C_{22}H_{24}FO_4$ 371.1653.

*5.1.4.3. (E)-Methyl-6-(3-fluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)- benzoate (**4c**).* Yield 40%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.72 (s, 1H), 7.74 (d, J = 15.9 Hz, 1H), 7.44–7.15 (m, 3H), 7.06–6.92 (m, 1H), 6.75 (d, J = 15.9 Hz, 1H), 6.61 (s, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.41 (d, J = 7.1 Hz, 2H), 1.82 (s, 3H), 1.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 161.5, 161.4, 139.9, 139.9, 139.8, 132.0, 132.0, 130.3, 130.1, 128.8, 122.6, 122.5, 122.0, 117.0, 114.7, 114.4, 113.0, 112.7, 104.5, 102.9, 55.7, 52.4, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 371.4 $[M+H]^+$; ESI-HRMS *m/z*: 371.1643 $[M+H]^+$, calcd for $C_{22}H_{24}FO_4$ 371.1653.

*5.1.4.4. (E)-Methyl-6-(4-fluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoate (**4d**).* Yield 38%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, 1H), 7.63 (d, J = 15.9 Hz, 1H), 7.53–7.41 (m, 2H), 7.07 (t, J = 8.7 Hz, 2H), 6.74 (d, J = 15.9 Hz, 1H), 6.59 (s, 1H), 5.22 (m, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.38 (d, J = 7.0 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 161.5, 161.4, 140.1, 133.8, 131.9, 130.5, 130.5, 128.9, 128.1, 128.0, 122.1, 116.8, 115.8, 115.6, 104.5, 102.8, 55.7, 52.3, 25.8, 22.2, 17.8; ESI-LRMS *m/z*: 371.4 $[M+H]^+$. ESI-HRMS *m/z*: 371.1643 $[M+H]^+$, calcd for $C_{22}H_{24}FO_4$ 371.1653.

*5.1.4.5. (E)-Methy6-(4-chlorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoate (**4e**).* Yield 43.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.68 (s, 1H), 7.68 (d, J = 15.9 Hz, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.5 Hz, 2H), 6.72 (d, J = 15.9 Hz, 1H), 6.58 (s, 1H), 5.22 (t, J = 7.0 Hz, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.38 (d, J = 7.0 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 161.5, 161.4, 139.9, 136.0, 133.3, 132.0, 131.3, 129.0, 128.8, 127.8, 122.0, 116.9, 104.5, 102.9, 55.7, 52.3, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 409.3 $[M+Na]^+$. ESI-HRMS *m/z*: 387.1358 $[M+H]^+$, calcd for $C_{22}H_{24}FO_4$ 387.1358.

*5.1.4.6. (E)-Methyl-2-hydroxy-4-methoxy-6-(4-methoxystyryl)-3-(3-methylstyryl)benzoate (**4f**).* Yield 36%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1H), 7.59 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 15.9 Hz, 1H), 6.60 (s, 1H), 5.22 (m, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.38 (d, J = 7.0 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 161.4, 161.3, 159.4, 140.6, 131.8, 130.3, 129.6, 128.5, 127.8, 122.2, 116.3, 114.2, 104.5, 102.6, 55.7, 55.4, 52.2, 25.9, 22.2, 17.9; ESI-LRMS *m/z*: 383.2 $[M+H]^+$; ESI-HRMS *m/z*: 383.1834 $[M+H]^+$, calcd for $C_{23}H_{27}O_5$ 383.1853.

*5.1.4.7. (E)-Methyl-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-(4-methylstyryl)- benzoate (**4g**).* Yield 35%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.71 (s, 1H), 7.68 (d, J = 15.9 Hz, 2H), 7.41 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 15.9 Hz, 2H), 6.61 (s, 1H), 5.32–5.14 (m, 1H), 3.93 (s, 3H), 3.93 (s, 3H), 3.39 (d, J = 7.1 Hz, 2H), 2.38 (s, 3H), 1.81 (s, 3H), 1.70 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 161.5, 161.3, 140.5, 137.7, 134.8, 131.9, 130.0, 129.7, 129.5, 126.5, 122.1, 116.5, 104.5, 102.8, 55.7, 52.3, 25.9, 22.2, 21.3, 17.9; ESI-LRMS *m/z*: 389.4 $[M+Na]^+$. ESI-HRMS *m/z*: 367.1818 $[M+H]^+$, calcd for $C_{23}H_{27}O_4$ 367.1904.

*5.1.4.8. (E)-Methyl-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-(3,4,5-trimethoxystyryl)benzoate (**4h**).* Yield 35%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.32 (s, 1H), 7.17 (d, J = 16.4 Hz, 1H), 6.66 (s, 2H), 6.45 (s, 1H), 6.22 (d, J = 16.4 Hz, 1H), 5.08 (t, J = 6.6 Hz, 1H), 3.90 (s, 6H), 3.88 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.32 (d, J = 6.4 Hz, 2H), 1.68 (s, 3H), 1.61 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 162.8, 162.6, 153.4, 140.8, 137.7, 133.5, 131.6, 130.7, 127.7, 123.8, 121.6, 104.6, 103.2, 98.3, 61.0, 56.1, 55.7, 52.1, 26.9, 26.1, 25.8, 18.1; ESI-LRMS *m/z*: 443.3 $[M+H]^+$; ESI-HRMS *m/z*: 443.2064 $[M+H]^+$, calcd for $C_{25}H_{31}O_7$ 443.2064.

*5.1.4.9. (E)-Methyl-6-(3-cyanostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)- benzoate (**4i**).* Yield 36%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.66 (s, 1H), 7.72 (d, J = 16.0 Hz, 1H), 7.60 (m, 2H), 7.55 (dd, J = 6.4, 1.3 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 6.73 (d, J = 16.0 Hz, 1H), 6.57 (s, 1H), 5.27–5.15 (m, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.38 (d, J = 7.1 Hz, 2H), 1.79 (s, 3H), 1.68 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 161.5, 161.4, 139.3, 138.8, 133.4, 132.1, 130.9, 130.6, 130.0, 129.6, 127.7, 121.8, 118.8, 117.4, 113.0, 104.6, 103.0, 55.7, 52.5, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 378.0 $[M+H]^+$. ESI-HRMS *m/z*: 378.1691 $[M+H]^+$, calcd for $C_{23}H_{24}NO_4$ 378.1700.

*5.1.4.10. (E)-Methyl-6-(3-cyanostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)- benzoate (**4j**).* Yield 34%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, 1H), 7.80 (d, J = 15.9 Hz, 1H), 7.67–7.55 (m, 4H), 6.78 (d, J = 16.0 Hz, 1H), 6.60 (s, 1H), 5.22 (m, 1H), 3.93 (s, 6H), 3.39 (d, J = 7.1 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 161.5, 161.4, 139.6, 133.2, 132.0, 128.5, 126.7, 125.7, 125.7, 121.9, 117.3, 104.6, 103.0, 55.7, 52.3, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 421.4 $[M+H]^+$. ESI-HRMS *m/z*: 421.1610 $[M+H]^+$, calcd for $C_{23}H_{24}F_3O_4$ 421.1621.

*5.1.4.11. (E)-Methyl 6-(2,4-difluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoate (**4k**).* Yield 34%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, 1H), 7.72 (d, J = 16.1 Hz, 1H), 7.56–7.49 (m, 1H), 6.98–6.8 (m, 2H), 6.83 (d, J = 16.1 Hz, 1H), 6.59 (s, 1H),5.24–5.20 (m, 1H), 3.93 (s, 6H), 3.38 (d, J = 7.1 Hz, 2H), 1.80 (s, 3H), 1.68 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 161.5, 161.4, 140.1, 132.8, 128.3, 128.1, 122.0, 121.5, 118.2, 111.8, 111.5, 104.6, 104.5, 104.2, 103.9, 102.9, 55.7, 52.3, 25.9, 22.2, 17.8; ESI-LRMS *m/z*: 389.5 $[M+H]^+$. ESI-HRMS *m/z*: 389.1558 $[M+H]^+$, calcd for $C_{22}H_{22}F_2O_4$ 389.1559.

*5.1.4.12. Methyl-2-hydroxy-4-methoxy-6-methyl-3-(3-methylbut-2-en-1-yl)benzoate (**9**).* Yield 42.3%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.80 (s, 1H), 6.28 (s, 1H), 5.43–4.90 (m, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.33 (d, J = 7.0 Hz, 2H), 2.52 (s, 3H), 1.78 (s, 3H), 1.67 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 161.9, 161.1, 140.6, 131.6, 122.5, 114.7, 106.1, 105.7, 55.5, 51.8, 25.8, 24.7, 21.9, 17.8; ESI-LRMS *m/z*: 265.3 $[M+H]^+$. ESI-HRMS *m/z*: 265.1441 $[M+H]^+$, calcd for $C_{17}H_{15}FO_4$ 265.1434.

*5.1.5. General procedure for the synthesis of compounds **5a** (**CSA**)–**5k**, **10**, **6a**, **6c** and **6f***

The compound **4a**–**4k** (1.42 mmol) was dissolved in EtOH, H_2O (10 mL: 2 mL) and KOH (4.26 mmol) were added. The mixture was heated to 90 °C for 2 h. Then the pH of the mixture was adjusted to 2 with 10% HCl followed by extraction with EtOAc (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue, which was purified by column chromatography (petroleum ether:Me_2CO = 10:1) to give **5a** (**CSA**)–**5k** as white solid respectively. In addition, **10**, **6a**, **6c** and **6f** were synthesized from compounds **9**, **3a**, **3c** and **3e** by this method.

*5.1.5.1. (E)-2-Hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-styrylbenzoic acid (**CSA** or **5a**).* ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.53 (s, 1H), 7.86 (d, J = 16.2 Hz, 1H), 7.55 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.34 (t, J = 7.2 Hz, 1H), 6.82 (d, J = 16.2 Hz, 1H), 6.69 (s, 1H), 5.25 (t, J = 6.8 Hz, 1H), 3.98 (s,3H), 3.40 (d, J = 6.8 Hz, 2H), 1.83 (s, 3H), 1.72 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 165.2, 164.2, 142.9, 137.3, 132.0, 130.9, 129.9, 128.7, 127.9, 126.7, 121.9, 116.8, 108.0, 103.9, 55.5, 52.3.

*5.1.5.2. (E)-6-(2-Fluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoic acid (**5b**).* Yield 92.5%; ^{1}H NMR (300 MHz, Me_2CO-d_6): δ 8.10 (d, J = 16.2 Hz, 1H), 7.73 (td, J = 7.7, 1.7 Hz, 1H), 7.40–7.30 (m, 1H), 7.28–7.13 (m, 2H), 7.08 (d, J = 16.2 Hz, 1H), 6.86 (s, 1H), 5.38–5.07 (m, 1H), 4.01 (s, 3H), 3.37 (d, J = 7.2 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6): δ 173.2, 162.1, 161.6, 140.7, 133.1, 133.1, 130.7, 129.3, 129.2, 127.7, 127.6, 125.4, 125.3, 124.6, 124.5, 122.3, 122.2, 122.1, 116.3, 115.8, 115.5, 104.0, 102.7, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 357.3 $[M+H]^+$. ESI-HRMS *m/z*: 357.1494 $[M+H]^+$, calcd for $C_{21}H_{22}FO_4$ 357.1497.

*5.1.5.3. (E)-6-(3-Fluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl) benzoic acid (**5c**).* Yield 94%; ^{1}H NMR (300 MHz, Me_2CO-d_6): δ 8.08 (d, J = 16.1 Hz, 1H), 7.54–7.26 (m, 3H), 7.16–6.95 (m, 2H), 6.88 (s, 1H), 5.30–5.13 (m, 1H), 3.99 (s, 3H), 3.36 (d, J = 7.3 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6): δ 173.2, 162.0, 161.5, 140.6, 140.4, 131.9, 130.7, 130.5, 130.4, 128.9, 122.8, 122.8, 122.3, 116.2, 114.3, 114.0, 112.8, 112.5, 104.2, 102.6, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 357.3 $[M+H]^+$; ESI-HRMS *m/z*: 357.1499 $[M+H]^+$, calcd for $C_{21}H_{22}FO_4$ 357.1497.

*5.1.5.4. (E)-6-(4-Fluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl) benzoic acid (**5d**).* Yield 95%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 7.96 (d, J = 16.1 Hz, 1H), 7.81–7.54 (m, 2H), 7.16 (t, J = 8.8 Hz, 2H), 7.01 (d, J = 16.1 Hz, 1H), 6.86 (s, 1H), 5.31–5.10 (m, 1H), 3.99 (s, 3H), 3.36 (d, J = 7.2 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.3, 162.1, 161.5, 140.8, 134.3, 134.3, 130.7, 130.3, 130.3, 129.0, 128.5, 128.4, 122.3, 116.0, 115.6, 115.3, 104.0, 102.5, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 357.2 $[M+H]^+$. ESI-HRMS *m/z*: 357.1504 $[M+H]^+$, calcd for $C_{21}H_{22}FO_4$ 357.1497.

*5.1.5.5. (E)-6-(4-Chlorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoic acid (**5e**).* Yield 92%; ^{1}H NMR

(300 MHz, Me_2CO-d_6): δ 12.29 (s, 1H), 8.01 (d, J = 16.1 Hz, 1H), 7.59 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 16.0 Hz, 1H), 6.86 (s, 1H), 5.22 (t, J = 7.1 Hz, 1H), 3.98 (s, 3H), 3.36 (d, J = 7.1 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.2, 162.1, 161.5, 140.6, 136.7, 132.7, 131.2, 130.7, 128.9, 128.7, 128.1, 122.3, 116.2, 103.9, 102.6, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 395.3 $[M+Na]^+$; ESI-HRMS *m/z*: 373.1174 $[M+H]^+$, calcd for $C_{21}H_{22}ClO_4$ 373.1201.

*5.1.5.6. (E)-2-Hydroxy-4-methoxy-6-(4-methoxystyryl)-3-(3-methylbut-2-en-1-yl)benzoic acid (**5f**).* Yield 92%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 7.89 (d, J = 16.0 Hz, 1H), 7.52 (d, J = 8.7 Hz, 2H), 6.97 (m, 2H), 6.86 (s, 1H), 5.22 (m, 1H), 3.99 (s, 3H), 3.83 (s, 3H), 3.35 (d, J = 7.2 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.5, 162.0, 161.5, 159.6, 141.3, 130.6, 130.4, 130.0, 128.0, 127.9, 122.4, 115.6, 114.1, 103.9, 102.2, 55.3, 54.7, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 369.4 $[M+H]^+$. ESI-HRMS *m/z*: 369.1672 $[M+H]^+$, calcd for $C_{22}H_{25}O_5$ 369.1697.

*5.1.5.7. (E)-2-Hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-(4-methylstyryl)benzoic acid (**5g**).* Yield 93%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 7.98 (d, J = 16.0 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 16.0 Hz, 1H), 6.86 (s, 1H), 5.22 (dd, J = 7.9, 6.6 Hz, 1H), 3.99 (s, 3H), 3.36 (d, J = 7.2 Hz, 2H), 2.34 (s, 3H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.4, 162.1, 161.5, 141.1, 137.4, 135.1, 130.6, 130.3, 129.3, 126.6, 122.4, 115.8, 103.9, 102.4, 55.3, 25.0, 21.8, 20.3, 17.0; ESI-LRMS *m/z*: 375.3 $[M+Na]^+$. ESI-HRMS *m/z*: 353.1762 $[M+H]^+$, calcd for $C_{22}H_{25}O_4$ 353.1747.

*5.1.5.8. (E)-2-Hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-(3,4,5-trimethoxystyryl)benzoic acid (**5h**).* Yield 95%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 12.32 (s, 1H), 7.94 (d, J = 16.0 Hz, 1H), 6.96 (d, J = 16.1 Hz, 1H), 6.89 (s, 2H), 6.84 (s, 1H), 5.22 (t, J = 7.2 Hz, 1H), 3.99 (s, 3H), 3.87 (s, 6H), 3.75 (s, 3H), 3.36 (d, J = 7.3 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.4, 162.0, 161.5, 153.7, 141.0, 138.4, 133.5, 130.7, 130.4, 129.8, 122.3, 115.8, 104.2, 102.3, 59.7, 55.5, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 429.4 $[M+H]^+$; ESI-HRMS *m/z*: 429.1879 $[M+H]^+$, calcd for $C_{24}H_{29}O_7$ 429.1908.

*5.1.5.9. (E)-6-(3-Cyanostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoic acid (**5i**).* Yield 91%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 8.16 (d, J = 16.1 Hz, 1H), 8.05–7.85 (m, 3H), 7.65 (m, 2H), 7.06 (d, J = 16.1 Hz, 1H), 6.88 (s, 1H), 5.22 (m, 1H), 3.99 (s, 3H), 3.36 (d, J = 7.2 Hz, 3H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.2, 162.0, 161.5, 140.2, 139.2, 133.0, 130.8, 130.7, 130.0, 129.9, 127.9, 122.2, 118.4, 116.5, 112.8, 104.3, 102.7, 55.3, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 364.3 $[M+H]^+$. ESI-HRMS *m/z*: 364.1539 $[M+H]^+$, calcd for $C_{22}H_{22}NO_4$ 364.1543.

*5.1.5.10. (E)-2-Hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)-6-(4-(trifluoromethyl)styryl)benzoic acid (**5j**).* Yield 93%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.51 (s, 1H), 7.92 (d, J = 16.0 Hz, 1H), 7.74–7.56 (m, 4H), 6.83 (d, J = 15.9 Hz, 1H), 6.64 (s, 1H), 5.20 (t, J = 7.1 Hz, 1H), 3.96 (s, 3H), 3.38 (d, J = 7.1 Hz, 2H), 1.79 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 162.5, 162.3, 141.1, 132.9, 132.1, 129.2, 126.9, 125.7, 121.7, 117.4, 103.5, 103.0, 55.8, 25.8, 22.1, 17.8; ESI-LRMS *m/z*: 429.3 $[M+Na]^+$. ESI-HRMS *m/z*: 407.1442 $[M+H]^+$, calcd for $C_{22}H_{22}F_3O_4$ 407.1465.

*5.1.5.11. (E)-6-(2,4-Difluorostyryl)-2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)benzoic acid (**5k**).* Yield 92%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 8.05 (d, J = 16.2 Hz, 1H), 7.85–7.71 (m, 1H), 7.13–7.06 (m, 2H), 7.02 (d, J = 16.1 Hz, 1H), 6.85 (s, 1H), 5.41–5.08 (m, 1H), 4.00 (s, 3H), 3.36 (d, J = 7.2 Hz, 2H), 1.78 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 205.3, 173.2, 162.1, 161.6, 140.3, 133.0, 130.7, 130.4, 129.0, 128.4, 122.0, 121.9, 116.0, 112.0, 111.3, 104.0, 102.7, 55.1, 25.0, 21.8, 17.0; ESI-LRMS *m/z*: 473.4 $[M+H]^+$. ESI-HRMS *m/z*: 375.1401 $[M+H]^+$, calcd for $C_{21}H_{20}F_2O_4$ 375.1402.

*5.1.5.12. 2-Hydroxy-4-Methoxy-6-methyl-3-(3-methylbut-2-en-1-yl) benzoic acid (**10**).* Yield 95%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 6.49 (s, 1H), 5.33–5.07 (m, 1H), 3.90 (s, 3H), 3.30 (d, J = 7.2 Hz, 2H), 2.59 (s, 3H), 1.75 (s, 3H), 1.63 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.8, 162.5, 161.3, 141.3, 130.3, 122.7, 114.0, 106.1, 105.1, 55.1, 25.0, 23.8, 21.6, 16.9; ESI-LRMS *m/z*: 251.3 $[M+H]^+$; ESI-HRMS *m/z*: 251.1271 $[M+H]^+$, calcd for $C_{14}H_{19}O_4$ 251.1278.

*5.1.5.13. (E)-2-Hydroxy-4-methoxy-6-styrylbenzoic acid (**6a**).* Yield 95%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 7.88 (d, J = 16.0 Hz, 1H), 7.54–7.48 (m, 2H), 7.23–7.38 (m, 2H), 7.30–7.22 (m, 1H), 6.87 (d, J = 16.0 Hz, 1H), 6.68 (d, J = 2.4 Hz, 1H), 6.42 (d, J = 2.5 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.0, 164.9, 164.0, 143.0, 137.6, 130.3, 129.5, 128.3, 127.3, 126.3, 106.3, 104.3, 99.8, 54.6; ESI-LRMS *m/z*: 271.4.3 $[M+H]^+$. ESI-HRMS *m/z*: 271.0964 $[M+H]^+$, calcd for $C_{16}H_{15}O_4$ 271.0965.

*5.1.5.14. (E)-2-(3-Fluorostyryl)-6-hydroxy-4-methoxybenzoic acid (**6c**).* Yield 93%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 12.15 (s, 1H), 8.02 (d, J = 16.0 Hz, 1H), 7.43 (m,2H), 7.40–7.29 (m, 1H), 7.11–7.03 (m, 1H), 6.99 (d, J = 16.0 Hz, 1H), 6.75 (d, J = 2.5 Hz, 1H), 6.48 (d, J = 2.6 Hz, 1H), 3.91 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 172.7, 165.9, 164.8, 164.5, 161.6, 142.8, 140.3, 140.2, 131.3, 130.5, 130.4, 129.4, 122.9, 122.8, 114.4, 114.1, 112.9, 112.6, 107.2, 103.6, 100.4, 55.1; ESI-LRMS *m/z*: 289.3 $[M+H]^+$. ESI-HRMS *m/z*: 289.0873 $[M+H]^+$, calcd for $C_{17}H_{15}FO_4$ 289.0871.

*5.1.5.15. (E)-2-Hydroxy-4-methoxy-6-(4-methoxystyryl)benzoic acid (**6f**).* Yield 95%; ^{1}H NMR (300 MHz, Me_2CO-d_6) δ 12.01 (s, 1H), 7.85 (d, J = 16.1 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 6.96 (m, 3H), 6.74 (d, J = 2.5 Hz, 1H), 6.43 (d, J = 2.6 Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (75 MHz, Me_2CO-d_6) δ 173.0, 165.8, 164.4, 159.7, 143.7, 130.5, 130.3, 128.0, 127.3, 114.1, 106.6, 103.5, 99.9, 55.1, 54.7; ESI-LRMS *m/z*: 301.3 $[M+H]^+$. ESI-HRMS *m/z*: 301.1072 $[M+H]^+$, calcd for $C_{17}H_{16}O_5$ 301.1071.

5.2. Biological assays

5.2.1. In vitro antibacterial activity evaluation by MIC assay

The MIC values were determined using a broth dilution method [19]. The starting concentration of the compounds tested was 256 μg/mL. The solution of the compound in DMSO (15 μL) was added to 285 μL of bacterial culture (5×10^5 cells/mL) at the first well of a flat-bottomed 96-well tissue culture plate. The solution was then double diluted. Bacterial culture solution containing appropriate compound (150 μL) was discarded at the last well in order to ensure 150 μL volume of bacterial culture in every well. The plates were incubated at 37 °C for 24 h in electro-heating standing-temperature cultivator and were read visually. The minimum concentration of the sample showing no turbidity was recorded as the MIC.

5.2.2. Cytotoxicity assay

The active compounds (**5b**, **5c**, **5j** and **5k**) were further examined for cytotoxicity in mouse macrophage cell lines (RAW 264.7) at concentration gradients [20]. The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine and 100 units/mL penicillin/streptomycin. Cell cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were passaged at preconfluent densities using a solution containing 0.25% trypsin and 0.5 mM EDTA. Cells were seeded at a

density of 10,000 cell/well in a 96 well plate for 12 h with 10% FBS, 1% penicillin and streptomycin, and 1% L-glutamine resulting in 80% confluency. Each dose was prepared in 1% FBS medium by 1000 × dilution of the drug which was prepared in DMSO solution to ensure DMSO concentration less than 0.1%. Control experiments showed that 0.1% DMSO had no effect on cytotoxicity. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product with DMSO for 45 min. Absorbance was detected by a Gen5 Reader at 562 nm. The experiment was performed in 4 replicate wells for each compound or concentration with at least three experimental runs (N = 12).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.008.

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