Bioorganic & Medicinal Chemistry Letters 25 (2015) 791–794

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design of novel CSA analogues as potential safeners and fungicides

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ARTICLE INFO

Article history:
Received 10 November 2014
Revised 14 December 2014
Accepted 26 December 2014
Available online 6 January 2015

Keywords:
Herbicide safeners
CSA analogues
Synthesis
SAR
Antifungal activity

ABSTRACT

Study of safeners has been seldom reported in literature. In this work, a series of novel acylsulfamoylbenzamide analogues was designed and synthesized with newly developed safener cyprosulfamide (**CSA**) as the leading compound. The activity assay against the herbicide thiencarbazone-methyl (**TCM**) on maize revealed that fifteen compounds showed better protective effect than **CSA** on the fresh weight of aerial parts, twelve compounds exhibited better activity on the dry weight of aerial parts. Remarkably, two compounds (**6Ih**, **7Il**) had protective effect on the four aspects of **TCM** treated maize. Further antifungal assay showed their excellent activity against *Physollospora piricola*. The structure–activity relationships of **CSA** analogues as safeners and fungicides were discussed and it might be valuable for further molecular modification of new **CSA** analogues.

Herbicides are not always sufficiently compatible with some important crops such as corn, rice or cereals, and their use is strictly limited.[1] In some crops, they cannot be used or only be used at so low application rates that the desired broad herbicidal activity against harmful weeds is not ensured. Specifically, many herbicides cannot be used entirely selectively against harmful weeds in corn, rice, cereal, sugarcane and other crops.[2,3] To overcome these disadvantages, it is considerably necessary to employ safeners in combination with herbicides. As it is well known that safeners can improve selectivity of herbicide between crop and weed species, also it can be applied either as a mixture with the herbicide or as a seed-treatment to the crop seed before sowing.[4,5]

Since last century, several compounds have been developed as safeners and have been used in agricultural field including classes of dichloroacetanilides, carboxylic acid analogues, oxime esters and so on. The first commercial synthetic herbicide safener Naphthalic anhydride (NA) was discovered by Hoffman in 1969. It was versatile and could protect various crops from a great variety of herbicides.[6,7] After Dichlormid[8] (R-25788) was developed as a safener and Eradicane[9] composed of the herbicide and Dichlormid was on sale, safeners have played an important role in protecting crops from the damage of herbicides.

In particular, the continued requirements of environmental friendly crop protection and the resistance of herbicides are dependent on fundamental knowledge of herbicide metabolism between target and non-target plants. Safeners, with their unique modes of action, are vital tools in the acquisition of such knowledge. Therefore, finding new safeners is always meaningful to agriculture.

Recently, some new herbicides have been developed, however, few safeners were provided to meet the needs of the herbicide market[10] until a novel type and important safener cyprosulfamide (**CSA**, Fig. 1)[1,11–13] was invented by Bayer Crop Science in 2005. This safener has been co-developed with herbicides benzoylpyrazole[11] and thiencarbazone-methyl[14] (**TCM**, Fig. 1) and was demonstrated to have excellent activity as a safener combined with **TCM** in 2007.[15] Since **TCM** is a very important herbicide for the selective control of grasses and broadleaf weeds primarily in corn and has now been registered in some important corn producing countries of Europe and other parts of the world. Many new herbicide compositions, such as Corvus and Adengo 465 SC[16] comprised of **TCM** and **CSA** have been registered in different countries since 2007.

CSA belongs to the chemical class of aromatic sulfonamides. It can reliably protect corns from the damage of **TCM** through enhancing herbicide metabolism via gene activation. Using herbicides composed of **TCM** and **CSA** is a successful path for weed control in conventional and herbicide-tolerant corn production systems and the herbicidal activity is maintained in the presence of **CSA**.[17]

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http://dx.doi.org/10.1016/j.bmcl.2014.12.085
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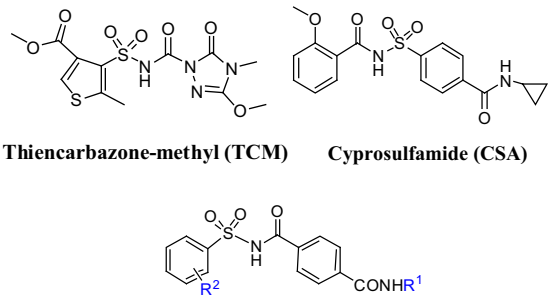


Figure 1. Structures of **TCM**, **CSA** and title compounds.

However, structure–activity relationships (SAR) study of **CSA** and its analogues beneficial to new safener development have been rarely reported.[18] Owing to the excellent bioactivity of **CSA** as one of the best sellers and its unclear mechanism, a series of novel acylsulfamoylbenzamide analogues (Fig. 1, Scheme 1) was designed and synthesized for the first time with **CSA** as the leading compound based on molecular similarity. Their activity assay as safeners on protecting the maize from the damage of **TCM** was evaluated.

The preparation of target compounds contains four steps (Scheme 1), the carboxyl group in starting material **1** was firstly activated to the mixed anhydride group, following amination by three different amines to give intermediates **2**. Oxidation of methyl group of **2** by $KMnO_4$ in water afforded acids **3**, which was activated to give mixed anhydrides similar with the first step. However, amination of anhydrides with sulfanilamides were unsuccessful, therefore, the final condensation of anhydrous **3** with sulfanilamides (**4** and **5**) was fulfilled by EDCI and DMAP to give title compounds **6** and **7**, respectively.

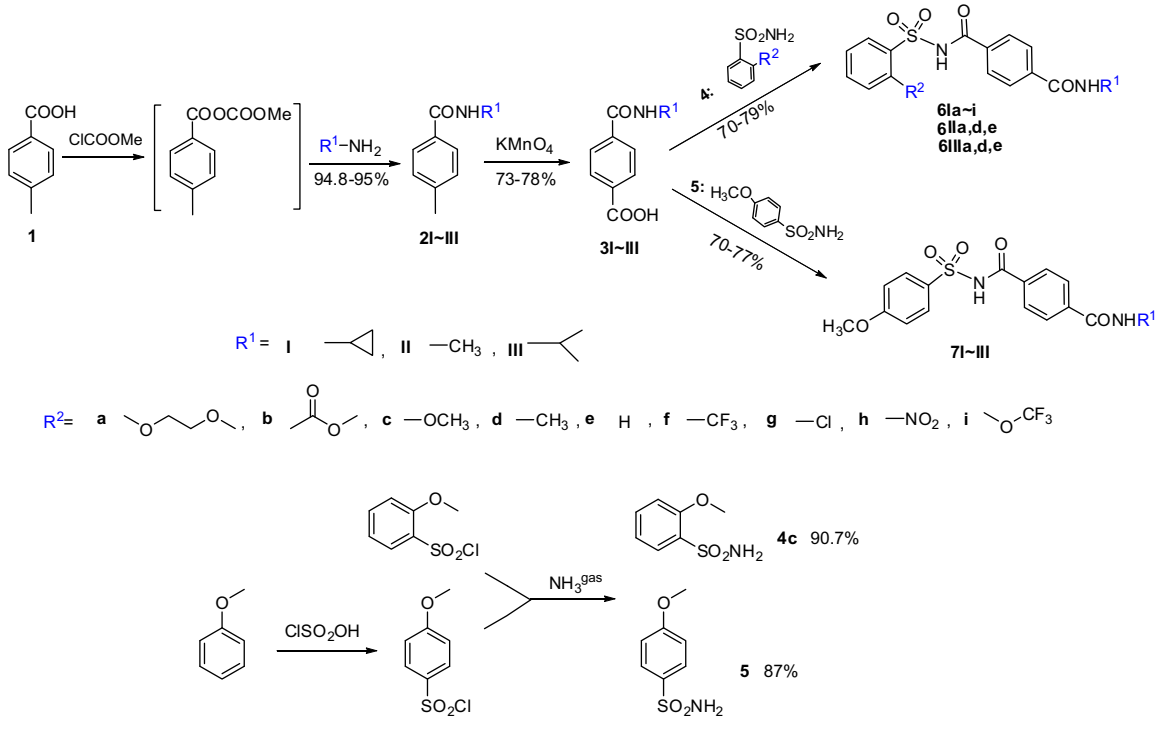
Safener activity: In the bioassay of *Oryzeae*, *Echinochloa*, wheat and maize are always used to evaluate the toxicity of potential compounds because of low compounds usage and rapid screening.[19,20] Maize seedlings of Zhengdan 958 (from Henan Agricultural Science without treatments) were used to evaluated the protective effect of **CSA** analogues[21] (20 g per unit area, 666.7 m^2). Results of repeated experiments (five seedlings for each group, twice) were summarized in Table 1 and Table 2.

When exposed to thiencarbazone-methyl (2.5 g per unit area, 666.7 m^2), growth of maize seedlings was strongly arrested, its main performance was a loss of fresh and dry weight of aerial parts. This effect was largely reversed in the presence of **CSA** (20 g per unit area, 666.7 m^2). All title compounds except **6IIa**, **6IIe**, **6IId** exhibited better activity to plant height of maize than **CSA** (−7.3%). The inhibition rate of **TCM** to the fresh weight of aerial parts of maize was 12.9% (Table 1, entry 2), which was reduced to 8.0% after the addition of **CSA** (Table 1, entry 3). It was found that for the inhibition of fresh weight of aerial parts, ten compounds (**6Ia**, **6Ib**, **6Ic**, **6Id**, **6Ie**, **6If**, **6Ih**, **6Ii**, **6IId**, **7II**) could protect maize from the damage of **TCM** at a certain extent (−11.6%, −6.7%, −2.8%, −11.2%, −8.5%, −10.3%, −14.9%, −3.4%, −5.8%, −18.6%, respectively). The inhibition rate of **TCM** to the dry weight of aerial parts of maize was 14.7% (Table 1, entry 2), which was reduced to 13.4% after the addition of **CSA** (entry 3), obvious protective effect was displayed by five compounds (**6Ia**, **6Id**, **6If**, **6Ih**, **7II**) at a certain extent (−0.7%, −6.4%, −10.1%, −13.5%, −7.1%).

For the dry weight of underground parts, most of title compounds exhibited obvious inhibition (Table 2). While three compounds (**6Ie**, **6Ih**, **7II**) could protect the root of maize seedling at a certain extent (−0.6%, −3.9%, −6.7%, respectively).

For all compounds tested, **6Ih** and **7II** showed protective effect to all the four aspects of maize growth.

Structure–activity relationship: For the plant height of maize seedlings, difference among compounds with cyclopropyl (**6Ia**, **6Id**, **6Ie**), methyl (**6IIa** ,**6IId**, **6IIe**) and isopropyl group (**6IIIa**, **6IIId**, **6IIIe**) showed that compounds with the cyclopropyl group exhibited stronger protective effect (−25.4%, −22%, −24.2% for **6Ia**, **6Id**, **6Ie**, respectively) than compounds with methyl group (−1.6%, −18.9%, 6.1% for **6IIa** ,**6IId**, **6IIe**, respectively) and



Scheme 1. General synthetic routes for title compounds.

Table 1
Protective effect of **CSA** analogues to aerial parts of **TCM** treated maize seedling

Entry	Compd no	Plant height				Fresh weight of aerial parts				Dry weight of aerial parts			
		$\bar{x}$ (cm)	Significance of difference		Inhibition rate (%)	Five plants (g)	Significance of difference		Inhibition rate (%)	Five plants (g)	Significance of difference		Inhibition rate (%)
			5%	1%			5%	1%			5%	1%	
1	Control*	49.2	de	BC	0	30.04	ab	A	0	7.03	ab	A	0
2	**TCM**	50.4	bcde	ABC	−2.4	26.17	ab	A	12.9	6	ab	A	14.7
3	**TCM+CSA**	52.8	abcde	ABC	−7.3	27.64	ab	A	8	6.09	ab	A	13.4
4	**TCM+6Ia**	61.7	a	AB	−25.4	33.53	a	A	−11.6	7.08	ab	A	−0.7
5	**TCM+6Ib**	59.5	abc	ABC	−20.9	32.04	ab	A	−6.7	6.76	ab	A	3.8
6	**TCM+6Ic**	53.2	abcde	ABC	−8.1	30.89	ab	A	−2.8	6.69	ab	A	4.8
7	**TCM+6Id**	60	ab	AB	−22	33.39	a	A	−11.2	7.48	a	A	−6.4
8	**TCM+6Ie**	61.1	a	AB	−24.2	32.59	a	A	−8.5	6.36	ab	A	9.5
9	**TCM+6If**	62	a	A	−26	33.12	a	A	−10.3	7.74	a	A	−10.1
10	**TCM+6Ig**	53.8	abcde	ABC	−9.3	29.97	ab	A	0.2	6.65	ab	A	5.4
11	**TCM+6Ih**	55.3	abcde	ABC	−12.4	34.53	a	A	−14.9	7.98	a	A	−13.5
12	**TCM+6Ii**	54.5	abcde	ABC	−10.8	31.05	ab	A	−3.4	6.87	ab	A	2.3
13	**TCM+6IIa**	50	cde	ABC	−1.6	25.65	ab	A	14.6	5.64	ab	A	19.8
14	**TCM+6IId**	58.5	abcd	ABC	−18.9	31.79	ab	A	−5.8	6.76	ab	A	3.8
15	**TCM+6IIe**	46.2	e	C	6.1	19.08	b	A	36.5	3.61	b	A	48.6
16	**TCM+6IIIa**	56.1	abcd	ABC	−14	27.95	ab	A	7	5.74	ab	A	18.3
17	**TCM+6IIId**	52.2	abcde	ABC	−6.1	25.89	ab	A	13.8	5.47	ab	A	22.2
18	**TCM+6IIIe**	55.1	abcde	ABC	−12	27.98	ab	A	6.9	5.42	ab	A	22.9
19	**TCM+7I**	58.5	abcd	ABC	−18.9	29.98	ab	A	0.2	6.22	ab	A	11.5
20	**TCM+7II**	61.1	a	AB	−24.2	35.62	a	A	−18.6	7.53	a	A	−7.1
21	**TCM+7III**	57.2	abcd	ABC	−16.3	29.48	ab	A	1.9	6.06	ab	A	13.8

ABCDE, abcde: no significant difference (P >0.05) between groups have at least one same letter.
* Treated with no compounds.

Table 2
Protective effect of **CSA** analogues to underground parts of **TCM** treated maize seedling

Entry	Compd no	Dry weight of underground parts			
		Five plants (g)	Significance of difference		Inhibition rate (%)
			5%	1%	
1	Control*	3.58	abcd	AB	0
2	**TCM**	3.86	ab	AB	−7.8
3	**TCM+CSA**	4.21	a	A	−17.6
4	**TCM+6Ia**	3.3	abcd	AB	7.8
5	**TCM+6Ib**	2.82	abcd	AB	21.2
6	**TCM+6Ic**	2.97	abcd	AB	17.0
7	**TCM+6Id**	3.06	abcd	AB	14.5
8	**TCM+6Ie**	3.6	abcd	AB	−0.6
9	**TCM+6If**	3.12	abcd	AB	12.8
10	**TCM+6Ig**	2.81	abcd	AB	21.5
11	**TCM+6Ih**	3.72	abc	AB	−3.9
12	**TCM+6Ii**	3.04	abcd	AB	15.1
13	**TCM+6IIa**	2.21	cd	AB	38.3
14	**TCM+6IId**	2.51	bcd	AB	29.9
15	**TCM+6IIe**	2.03	d	B	43.3
16	**TCM+6IIIa**	2.54	bcd	AB	29.1
17	**TCM+6IIId**	2.14	cd	AB	40.2
18	**TCM+6IIIe**	2.95	abcd	AB	17.6
19	**TCM+7I**	2.74	abcd	AB	23.5
20	**TCM+7II**	3.82	ab	AB	−6.7
21	**TCM+7III**	2.41	bcd	AB	32.7

ABCDE, abcde: no significant difference between groups have at least one same letter.
* Treated with no compounds.

compounds isopropyl group (−14%, −6.1%, −12% for **6IIIa**, **6IIId**, **6IIIe**, respectively) on the height of maize seedling, which revealed the cyclopropyl group might be an important pharmacophore. The substitution of methyl groups on **6Ic** and **6Id** with trifluoromethyl groups on **6Ii** and **6If** reduced the inhibition to maize root growth (from −8.1% to −10.8%, and from −22% to −26%, respectively). This result indicated that the introduction of fluorine atom was favorable to the activity improvement of safener.

For the fresh weight and dry weight of aerial parts, similar protective effect could be included from compounds with cyclopropyl, methyl and isopropyl group. Cyclopropyl group was further proved to be an important pharmacophore, because compounds with cyclopropyl group exhibited better protective activity (−11.6%, −11.2%, −8.5% for **6Ia**, **6Id**, **6Ie**, respectively) than compounds with methyl (14.6%, −5.8%, 36.5% for **6IIa** ,**6IId**, **6IIe**, respectively) and compounds with isopropyl group (5.74%, 5.47%, 5.42% for **6IIIa**, **6IIId**, **6IIIe**, respectively). The substitution of methyl groups on **6Ic** and **6Id** with trifluoromethyl groups on **6Ii** and **6If** reduced the inhibition (enhance the protective effect) to maize root growth (4.8–2.3%, −6.4% to −10.1%). This result indicated further that the introduction of fluorine atom would contribute to the increase of the activity.

For the dry weight of underground parts, compounds with cyclopropyl group (**6Ia**, **6Id**, **6Ie**) showed better activity (7.8%, 14.5%, −0.6%) than those with methyl (38.3%, 29.9%, 43.3% for **6IIa**, **6IId**, **6IIe**, respectively) or isopropyl (29.1%, 40.2%, 17.6% for **6IIIa**, **6IIId**, **6IIIe**, respectively). Replace of methyl groups on **6Ic** and **6Id** with trifluoromethyl groups on **6Ii** and **6If** reduced the inhibition to root growth of maize seedling (17–15.1%, 14.5–12.8%). This result revealed again that cyclopropyl group and fluorine atom were very necessary pharmacophores for the design of potential safeners.

For all the compounds, the activity were not be affected significantly by the introduction of an electron-withdrawing or an electron-donating group at ortho position of sulfonamide group on the benzene ring.

Antifungal activity: Phytopathogenic fungi that easily infect many crops are hard to control and risking resistance to the widely used commercial fungicides.[22] Sulfonamides which were extensively employed as effective antimicrobial antifolic agents for the prevention and cure of bacterial infections in human biological systems as early as 70 years ago, have aroused considerable interest in biology and medicine for their diversified pharmacological activities including carbonic anhydrase inhibitors,[23a,b] antifungal,[24] antiviral,[25] antitumor,[26] and anti-inflammatory ones[27] in recent years. The sulfanilamide group in title compounds encouraged us to

Table 3
Antifungal activity of **CSA** analogues

Entry	Compound (50 μg/mL)	Inhibition rate (%)				
		FO	**MA**	**PP**	**AS**	**FG**
1	**CSA**	10.5	13.3	51.9	26.3	27.8
2	**6Ia**	0	13.3	55.6	36.8	44.4
3	**6Ib**	15.8	6.7	51.9	31.6	50
4	**6Ic**	10.5	6.7	70.4	36.8	50
5	**6Id**	10.5	6.7	48.1	31.6	44.4
6	**6Ie**	15.8	26.7	55.6	36.8	38.9
7	**6If**	10.5	26.7	96.3	36.8	55.6
8	**6Ig**	5.3	6.7	77.8	36.8	50
9	**6Ih**	10.5	6.7	92.6	31.6	55.6
10	**6Ii**	0	0	74.1	36.8	61.1
11	**6IIa**	31.6	46.7	96.3	36.8	61.1
12	**6IId**	36.8	26.7	92.6	36.8	66.7
13	**6IIe**	0	20	51.9	52.6	22.2
14	**6IIIa**	15.8	20	92.6	21.1	27.8
15	**6IIId**	5.3	33.3	74.1	42.1	50
16	**6IIIe**	26.3	60	48.1	52.6	38.9
17	**7I**	15.8	13.3	74.1	31.6	44.4
18	**7II**	10.5	20	66.7	26.3	55.6
19	**7III**	15.8	6.7	88.9	26.3	61.1

conjecture whether the title compounds in our work had some antifungal activity or not. It would be meaningful if safeners have antifungal activity as well. Therefore, five typical fungi included *Fusarium oxysporum* (**FO**), *Mycosphaerella arachidicola* (**MA**), *Physollospora piricola* (**PP**), *Alternaria sonali* (**AS**), *Fusarium graminearum* (**FG**) which often occur in Chinese agro-ecosystem were chosen to evaluated the antifungal activity of **CSA** analogues (Table 3).

All the new **CSA** analogues exhibited certain growth inhibition effects against most of the tested fungi. Low inhibition was showed for **FO** (0–36.8%) and **MA** (0–60.0%) with the exception of 60.0% showed by **6IIIe** to **MA**. Moderate inhibition was exhibited for **AS** (21.1–52.6%) and **FG** (22.2–61.1%). While to **PP**, the activities of **6If**, **6Ih**, **6IIa**, **6IId**, **6IIIa** (96.3%, 92.6%, 96.3%, 92.6%, 92.6%, respectively) were excellent. Thus, the findings demonstrated that the new **CSA** analogues might represent a novel chemical skeleton with good activity for inhibiting **PP**.

The inhibition activities against the two fungi **PP** and **FG** were increased when the methoxy group of **6Ic** was replaced by trifluoromethoxy in **6Ii** (70.4–74.1% for **PP**, 50–61.1% for **FG**). Analogously, when the methyl group of **6Id** was replaced by trifluoromethyl in **6If**, the activities especially against **MA** and **PP**, were increased 4-fold (6.7–26.7%) and 2-fold (48.1–96.3%), respectively, which indicated that fluorine atom was possibly favorable to the improvement of the activity as a fungicide especially against **MA** and **PP**.

In summary, in this Letter a new class of acylsulfamoylbenzamide analogues was synthesized and characterized. The activity of the title compounds was evaluated both as safeners and fungicides. The quantitative bioassay could provide information about the reduction in phytotoxicity of **TCM** with title compounds. The preliminary results indicated that fifteen compounds (**6Ia**, **6Ib**, **6Ic**, **6Id**, **6Ie**, **6If**, **6Ig**, **6Ih**, **6Ii**, **6IId**, **6IIIa**, **6IIIe**, **7I**, **7II**, **7III**) showed better protective effect than **CSA** on the fresh weight of aerial parts for maize seedling from the damage of **TCM**, twelve compounds (**6Ia**, **6Ib**, **6Ic**, **6Id**, **6Ie**, **6If**, **6Ig**, **6Ih**, **6Ii**, **6IId**, **7I**, **7II**) exhibited better activity than **CSA** on the dry weight of aerial parts. Most important of all, two compounds (**6Ih**, **7II**) had protective effect on the four aspects of **TCM** treated maize, which could be leading compounds for developing potential safeners. The antifungal activity of title compounds showed five compounds (**6If**, **6Ih**, **6IIa**, **6IId**, **6IIIa**) had excellent antifungal activity against **PP**. The given structure–activity relationship of them was useful for design of more novel potential molecules. Furthermore, the further study of them regarding test on other crops as well as using other herbicides as synergistic composition are ongoing, more extensive details will be reported in future.

Acknowledgment

This work was financially supported by the National High-Tech Program of China (863 Project No. 2012AA020306).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.085.

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