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ARTICLE

3-(Arylthiomethyl)isoxazole-4,5-dicarboxamides: Chemoselective Nucleophilic Chemistry and Insecticidal Activity

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A collection of 91 3-(arylthiomethyl)isoxazole-4,5-dicarboxamides was prepared starting from dimethyl 3-(chloromethyl)isoxazole-4,5-dicarboxylate. The thioether moieties in these compounds were subsequently oxidized to give the corresponding 3-(arylsulfonylmethyl)isoxazole-4,5-dicarboxamides. By carefully controlling stoichiometry and reaction conditions, the C4 and C5 carbomethoxy groups could be differentially derivatized to carboxamides. A total of 182 trisubstituted isoxazoles are reported and deposited in the National Institutes of Health Molecular Repository; an 80 compound subset was evaluated for insecticidal activity.

KEYWORDS: Isoxazole; insecticidal activity; nucleophilic selectivity

INTRODUCTION

The 5-membered isoxazole provides a valuable scaffold in medicinal chemistry as well as a useful synthon in organic synthesis (*1*). In addition, the aromatic isoxazole displays wide-ranging biological activities, including pharmacological applications such as hypoglycemic, analgesic, antiinflammatory, antibacterial, and HIV-inhibitory activities, as well as agrochemical applications spanning herbicidal, fungicidal, and insecticidal activities (*2*). As part of a collaboration with Dow AgroSciences, lead generation libraries are designed and biologically evaluated for new chemistry as well as product pipeline potential. Indeed, there are several reports of combinatorial libraries as discovery tools for agrochemicals, and these have provided numerous hits and leads (*3*). These libraries serve to both produce molecules with activity and, simultaneously, provide valuable information to facilitate optimization. 3,4,5-Trisubstituted isoxazoles were recently found to have novel PPARδ agonist's properties with good in vivo pharmacokinetics (*4*). The antiepileptic and antiobesity drug zonisamide (*5*) (**Figure 1**) piqued our interest in sulfide/sulfone substituents—functionalities that also have important pharmaceutical applications (*6*)—tethered to the isoxazole heterocycle. We previously reported that the three electrophilic centers of dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate (**1**) are available for diversification and reported the elaboration to a library of 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acids (e.g., **2**) (*7*). Herein, we report the elaboration of **1** to chemsets of 3-(arylthiomethyl)isoxazole-4,5-dicarboxamides (**6** and **9**) and 3-(arylsulfonylmethyl)isoxazole-4,5-dicarboxamides (**7** and **10**).

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MATERIALS AND METHODS

General. All chemicals were purchased from commercial suppliers and used without further purification. Analytical TLC was carried out on precoated plates (silica gel 60, F254) and visualized with UV light. Flash chromatography was performed with silica gel 60 (230−400 mesh). NMR spectra (^{1}H at 300, 400, and 600 MHz; ^{13}C at 75, 100, and 150 MHz) were measured in acetone-d_6 and $CDCl_3$ as solvents indicated individually, and chemical shifts are reported in parts per million (ppm) related to internal deuterated solvents or TMS. The LC-MS analyses were performed on a Waters 2690 with electrospray (+) ionization, mass range 100−1500 Da, 23 V cone voltage, and Xterra MS C_{18} column (2.1 mm × 50 mm × 3.5 μm).

Preparation of Dimethyl 3-Chloromethylisoxazole-4,5-dicarboxylate (1) (*7*). The procedure was adopted from the literature; IR, ^{1}H NMR, and ^{13}C NMR matched literature data.

The intermediate oxime was not purified by distillation which was reported to lead to a violent explosion. In addition, this oxime was reported to irritate the eyes and the mucous membrane of the nose as well as blister the skin. Following the literature routes as well as the protocol outlined in **Scheme 1**, we had no exposure problems.

General Procedure for the Preparation of Sulfide Ether: Dimethyl 3-(Phenylthiomethyl)isoxazole-4,5-dicarboxylate (4{*1*}). To a DMF (18 mL) solution of **1** (1.21 g, 5.19 mmol) under N_2 gas were added thiophenol (**3**{*1*}, 0.629 g, 5.71 mmol) and triethylamine (0.577 g, 5.71 mmol). The resulting solution was stirred at room temperature for 10 min, at which time TLC analysis (hexane/ethyl acetate = 4:1) indicated that the reaction had gone to completion. The reaction mixture was diluted with water (200 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with 5% aqueous NaOH (2 × 50 mL) and brine (1 × 100 mL), dried over anhydrous Na_2SO_4, and filtered; the solvent was removed in vacuo. The resulting yellow oil was purified by flash column chromatography (SiO_2/hexane/ethyl acetate = 4:1) to give **4**{*1*} (1.54 g, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.23 (m, 5H, C_6H_5), 4.26 (s, 2H, CH_2), 3.98 (s, 3H, CH_3), 3.90 (s, 3H, CH_3).

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Following this procedure also delivered **4**{*2*−*7*} (90−95%).

General Procedure for the Preparation of Homobisamides: 3-(Phenylthiomethyl)-*N*^{4},*N*^{5}-dipropylisoxazole-4,5-dicarboxamide 6{*1*,*1*}. Propylamine (**5**{*1*}, 303 mg, 5.12 mmol) was added to a methanol (1 mL) solution of crude **4**{*1*} (157 mg, 0.512 mmol). The resulting solution was stirred at room temperature overnight or 40 °C for 2 h, at which time TLC analysis (hexane/ethyl acetate = 4:1) indicated that the reaction had gone to completion. The reaction mixture was cooled with ice−water for 15 min, and the resulting white precipitate was collected by filtration and washed with cold methanol. Alternatively, methanol can be evaporated from the reaction mixture to give **6**{*1*,*1*} (141 mg, 85%) as a white paste: mp 102−103 °C; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 10.04 (br s, 1H, N−H), 8.62 (br s, 1H, N−H), 7.42−7.19 (m, 5H, phenyl), 4.59(s, 2H, S−CH_2), 3.43 (apparent q, J = 8 Hz, 2H, N−CH_2), 3.31 (apparent q, J = 8 Hz, 2H, N−CH_2), 1.72−1.55 (m, 4H, 2C−CH_2), 1.00−0.94 (m, 6H, 2C−CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 163.6, 159.4, 159.2, 157.6, 136.0, 130.0, 129.1, 126.7, 115.5, 41.6, 41.0, 28.6, 22.6, 22.3, 11.2, 10.9.

Following this procedure, also delivered **6**{*1*−*7*, *1*−*7*}.

General Procedure for the Preparation of Mixed Bisamides: *N*^{5}-Benzyl-3-(phenylthiomethyl)-*N*^{4}-propylisoxazole-4,5-dicarboxamide (9{*1*,*1*}). Benzylamine (**5**{*7*}, 307 mg, 2.87 mmol) was added to a methanol (10 mL) solution of crude **4**{*1*} (881 mg, 2.87 mmol), and the resulting solution was stirred at room temperature for 4 h, at which time a white precipitate had formed. This precipitate was collected by filtration and washed with cold methanol to yield **8**{*1*}, methyl 5-(benzylcarbamoyl)-3-(phenylthiomethyl)isoxazole-4-carboxylate (601 mg, 55%), as a white solid: mp 92−93 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37−7.23 (m, 10H, phenyl C−H), 4.66 (d, J = 5.4 Hz, 2H, N−CH_2), 4.26 (s, 2H, S−CH_2), 3.90 (s, 3H, O−CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0, 163.8, 161.8, 137.8, 134.2, 131.8, 128.7, 128.6, 128.2, 128.1, 128.0, 110.0, 53.8, 44.0, 30.2.

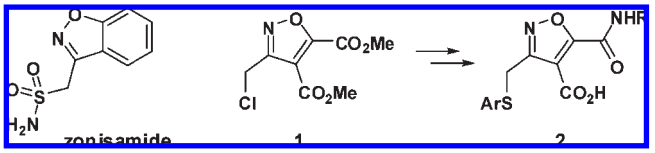


Figure 1. Zonisamide—an antiepileptic and antiobesity drug; 3-chloromethylisoxazole-4,5-dicarboxylate as a discovery platform.

Without further purification, **8**{*1*} (68 mg, 0.178 mmol) was suspended in methanol (1 mL), and *n*-propylamine (**5**{*1*}, 105 mg, 1.78 mmol) was added. The resulting suspension was stirred at room temperature, and the reaction was monitored by TLC analysis (hexane/ethyl acetate = 4:1) After 6 h, TCL analysis indicated that the reaction had gone to completion. The reaction mixture was concentrated (condensed air evaporation) to give **9**{*1*,*1*} (64.8 mg, 89%) as a white powder: mp 93−94 °C; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 9.96 (br s, 1H, N−H), 9.14 (br s, 1H, N−H), 7.42−7.18 (m, 10H, phenyl C−H), 4.67 (d, J = 4.4 Hz, N−CH_2), 4.54 (s, 2H, S−CH_2), 3.31 (apparent q, 2H, J = 8 Hz, N−CH_2), 1.63−1.54 (m, 2H, C−CH_2), 0.98−0.95 (t, 3H, J = 7.4 Hz, C−CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 163.6, 159.2, 157.7, 157.6, 138.1, 136.0, 130.1, 129.9, 129.2, 129.0, 128.8, 128.6, 128.1, 127.9, 127.5, 126.8, 115.8, 43.4, 43.3, 41.1, 22.7, 11.3, 11.2.

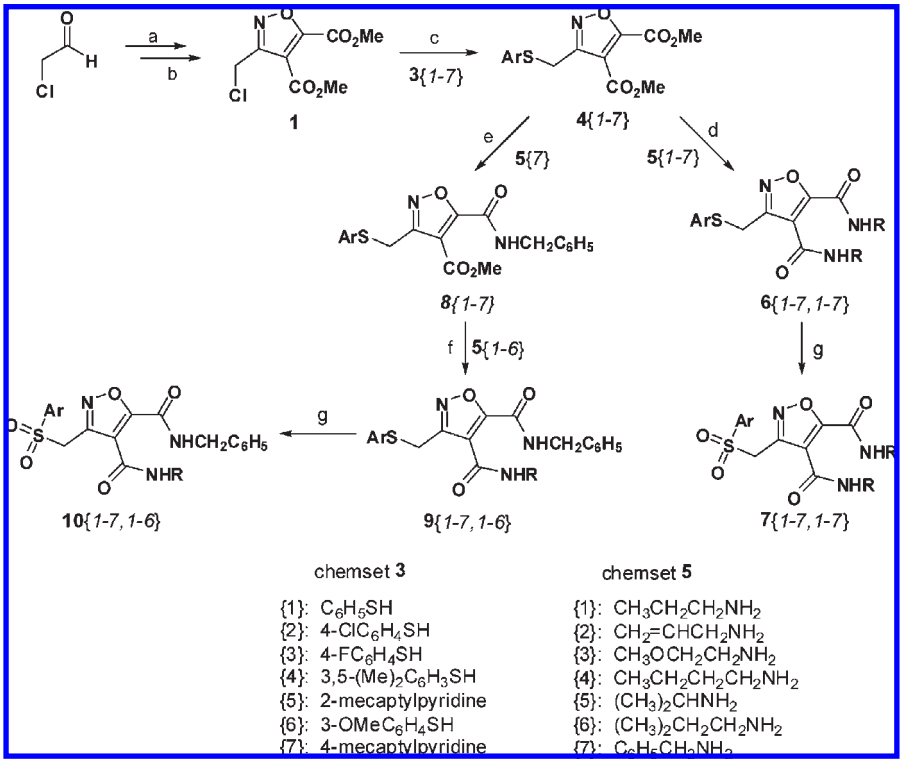
Following this procedure, also chemset **9**{*1*−*7*,*1*−*6*} was delivered.

General Procedure for the Preparation of Homobisamides Sulfones: 3-(Pyridine-2-ylsulfonylmethyl)-*N*^{4},*N*^{5}-dibenzylisoxazole-4,5-dicarboxamide 7{5,7}. Hydrogen peroxide (30%; 39 μL, 0.382 mmol) and catalysts (including Aliquat336, $Na_2WO_4 \cdot 2H_2O$, and $C_6H_5PO_3H$; 0.038 mmol each) were added to an acetic acid (2 mL) and EtOAc (2 mL) solution of **6**{*5*,*7*} (70 mg, 0.153 mmol). The resulting mixture was stirred at room temperature and monitored by TLC analysis (hexane/ethyl acetate = 1:1). After 12 h, TLC analysis indicated the reaction was complete. The reaction mixture was diluted with 5% NaOH (10 mL) and extracted with ethyl acetate (10 mL × 2). The organic layer was collected and dried over anhydrous Na_2SO_4, filtered, and concentrated onto a small amount of silica gel for dry loading flash column. A short column (hexane/ethyl acetate = 1:1) preceded to remove biphasic catalyst to yield **7**{*5*,*7*} (60 mg, 80%) as a white solid: mp 129−130 °C.

Following this procedure, **7**{*1*−*7*, *1*−*7*} as well as the mixed bisamides **10**{*1*−*7*,*1*−*6*} were delivered.

General Procedure for Bioassays. Insecticidal activity was evaluated as described previously (*8*). Briefly, the primary screen used two 96-well-

Scheme 1. Reagents and Conditions: (a) $NH_2OH \cdot HCl$, Water, RT, 30 min, Not Isolated; (b) DMAD, NaOCl, THF, 70%; (c) ArSH **3**{*1*−*7*}, Et_3N, DMF, 10 min, 95%; (d) RNH_2 **5**{*1*−*7*}, 10 equiv, MeOH, 40 °C, 2−4 h, 85−90%; (e) $C_6H_5CH_2NH_2$, **5**{*7*}, 1.1 equiv, MeOH, RT, 4−5 h; (f) RNH_2 **5**{*1*−*6*}, 10 equiv, MeOH, RT, 12 h, 75−80%; (g) H_2O_2, $Q^+HSO_4^-$ (PTC Phase-Transfer-Catalyst), $Na_2WO_4 \cdot 2H_2O$, $C_6H_5PO_3H$, EtOAc, HOAc, RT, 1 h



based high-throughput insect assays, one for beet armyworm (*Spodoptera exigua*: Lepidoptera) larvae and another for yellow fever mosquito (*Aedes aegypti*: Diptera). Artificial diet (100 μL/well, 96-well microtiter plate) that had been pretreated with test compounds (12 μg dissolved in 30 μL of DMSO/acetone/water (2:1:7) mixture, and then dried for several hours) was seeded with eggs of *S. exigua*. Infested plates were then covered and held in the dark (29 °C), and the mortality was recorded 6 days post-treatment. The *A. aegypti* assay used 1-day-old larvae pipetted into each well of pretreated (6 μg/well, dissolved in 15 μL of DMSO/acetone/water (1:1:8) mixture, then allowed to dry) 96-well microtiter plate. The plates were covered with a lid held at room temperature and then graded for mortality 3 days post-treatment. There were six replicates per treatment for each assay. Compounds active in the primary screen (67%) were further evaluated by means of a larger format (3 mL, 128-well diet trays containing 1 mL of diet, and selected dosages of the test compound) diet-based bioassay using second instar of *S. exigua* or *Helicoverpa zea* (corn earworm: Lepidoptera) larvae. The wells (eight per treatment) were covered with clear plastic, and percent mortality (average of the eight wells) was recorded for each treatment 5 days after the assay was initiated. Approximate LC_{50} values (lethal concentration for 50% of the population) were estimated using probit analysis.

RESULTS AND DISCUSSION

In our earlier work, we established that the C5 carbomethoxy of **1** was more reactive than the C4 carbomethoxy and, by exploiting this reactivity difference, we were able to elaborate isoxazole **1** to a 5-alkylcarbamoyl-3-arylsulfanylmethylisoxazole-4-carboxylic acid (**2**) library (*7*). Two additional enabling discoveries are reported here (**Figure 2**). The first is that thiolate nucleophiles chemoselectively engage the C3 chloromethyl electrophile. This observation suggested that isoxazole **1** might lead to homobisamide **6** in two simple transformations: thiolate S_N2 displacement of chloride followed by concomitant C4/C5 ester → amide transformation. In addition, sulfone **7** could potentially be derived from **6** by S-oxidation.

Dimethyl 3-chloromethylisoxazole-4,5-dicarboxylate (**1**), prepared as previously described (*7*), reacts rapidly in the aprotic solvent DMF with aryl thiols **3**{*1*−*7*} in the presence of triethylamine to give the corresponding thioethers in quantitative yield **4**{*1*−*7*}; **Scheme 1**). No evidence for involvement of either ester is detected, and the crude product of this reaction is obtained in such high purity that it can be employed in subsequent reactions without purification.

Treating a methanolic solution of crude bisester **4**{*1*−*7*} with an excess (10 equiv) of various primary amines **5**{*1*−*7*} at 40 °C for 30 min delivered novel homobisamides **6**{*1*−*7*,*1*−*7*} in 85−90% overall yield from the chlorodiester **1**. Under these conditions no mixed ester/amide products were detected upon workup, but TLC monitoring of this reaction did reveal the appearance of the presumed C5-amide/C4-ester intermediate, which, in the presence of excess amine, then proceeds on to bisamide **6**.

The presumed intermediacy of a C5-amide/C4-ester led us to treat a methanol solution of crude bisesters **4**{*1*−*7*} with primary amine **5**{*7*} at near stoichiometric levels and at room temperature (instead of 40 °C). Under these conditions, the reaction required 4−12 h for completion, but cleanly delivered the C5-amide/C4-ester. When TLC indicated complete consumption of bisester **4**{*1*−*7*}, an excess (10 equiv) of primary amine **5**{*1*−*6*} was added, and stirring was continued for an additional 6−12 h. This protocol delivered 42 mixed bisamides of general structure **9**{*1*−*7*,*1*−*6*} in 75−80% overall yield from chlorodiester **1**. Interestingly, the mixed bisamide **9**{*2*,*5*} has very low yield.

Two surprises accompanied this **8** → **9** transformation. First, allowing a methanolic solution of monoamide−monoester **8** and excess (10 equiv) amine to stir at room temperature for multiple days led cleanly to the homobisamide product **6** instead of the mixed bisamide **9**. That is, transamination of the C5 amide occurs under extended reaction times, even at room temperature. This transamination process can be expedited by warming the methanolic reaction mixture to 40 °C (homobisamide obtained in 12 h). Second, and related to the first, concentration of the crude monoamide−monoester **8** plus excess amine reaction mixture must be effected at room temperature; otherwise, a mixture (sometimes only pure bisamide **6**) of homobisamide **6** and mixed bisamide **9** is obtained.

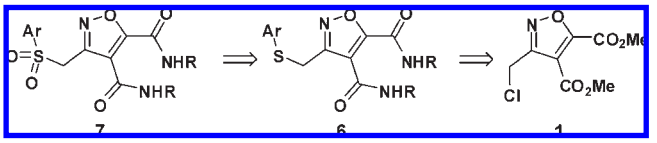


Figure 2. 3-(Arylthiomethyl)isoxazole- and 3-(arylsulfonylmethyl)-isoxazole-4,5-dicarboxamides.

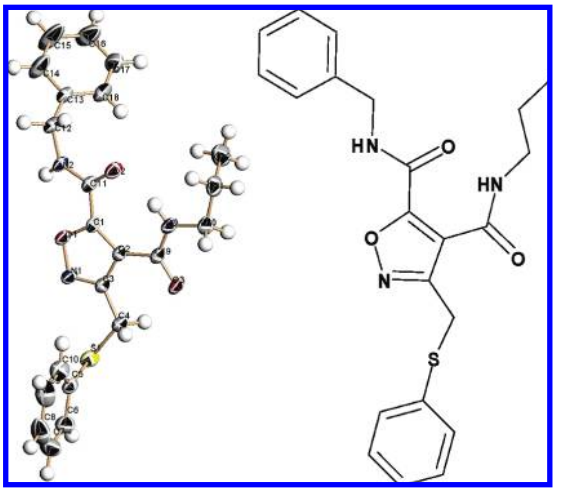


Figure 3. X-ray crystallography-determined structure of **9**{*1*,*1*} [N^5-benzyl-3-(phenylthiomethyl)-N^4-propylisoxazole-4,5-dicarboxamide].

The regioselectivity of these mixed bisamide products **9** was, on the basis of our previous results with **1** (*7*), presumed to derive from the first amine reacting at the C5 ester and the second amine reacting at the C4 ester. To verify this, crystalline mixed bisamide **9**{*1*,*1*}, prepared by treatment first with benzyl amine (1.1 equiv) and subsequently with propyl amine (10 equiv), was evaluated by X-ray crystallographic analysis. As depicted in **Figure 3**, the first amine reacted at the C5 ester, and the second amine reacted at the C4 ester.

The thioether moieties in homobisamides **6**{*1*−*7*,*1*−*7*} and mixed bisamides **9**{*1*−*7*,*1*−*6*} were oxidized to the corresponding sulfones. Although a number of reagents are effective for this transformation, green chemistry consideration led us to select a recently reported tungsten-mediated procedure that employs hydrogen peroxide as the stoichiometric oxidant. This procedure also employs $[CH_3(n\text{-}C_8H_{17})_3N]HSO_4$ as a phase-transfer catalyst and $C_6H_5P(=O)(OH)_2$ as a phosphonic acid promoter (*9*). With this protocol, we were able to use an ethyl acetate/acidic acid solvent system instead of the more typical chlorohydrocarbon solvent. With catalytic Na_2WO_4/ $C_6H_5P(=O)(OH)_2$/$[CH_3(n\text{-}C_8H_{17})_3N]HSO_4$ (physiologically harmless), these oxidation reactions generally went to completion in 12 h at room temperature. TLC analysis indicated the presence of the sulfoxide intermediate and, in cases when sulfone/sulfoxide mixtures were obtained within 12 h, separation of the sulfoxide from the sulfone was accomplished by silica gel chromatography. In those cases when

Table 1. Biological Activity against *Spodoptera exigua* (Beet Armyworm) for Homobisamide Thioether Derivative (Chemset **6**)

entry	Ar	R	% mortality against *S. exigua* in HTS	% mortality against *S. exigua* in secondary assay
1	C_6H_5	$CH_3CH_2CH_2$	50	NT[a]
2	4-Cl-C_6H_4	$CH_3CH_2CH_2$	50	NT
3	4-F-C_6H_4	$CH_3CH_2CH_2$	33	NT
4	3,5-$(Me)_2C_6H_3$	$CH_3CH_2CH_2$	50	NT
5	2-pyridine	$CH_3CH_2CH_2$	50	NT
6	3-$OMeC_6H_4$	$CH_3CH_2CH_2$	50	NT
7	4-pyridine	$CH_3CH_2CH_2$	33	NT
8	C_6H_5	CH_2=$CHCH_2$	33	NT
9	4-Cl-C_6H_4	CH_2=$CHCH_2$	17	NT
10	4-F-C_6H_4	CH_2=$CHCH_2$	67	0
11	3,5-$(Me)_2C_6H_3$	CH_2=$CHCH_2$	17	NT
12	2-pyridine	CH_2=$CHCH_2$	17	NT
13	3-$OMeC_6H_4$	CH_2=$CHCH_2$	67	0
14	4-pyridine	CH_2=$CHCH_2$	17	NT
15	C_6H_5	$CH_3OCH_2CH_2$	83	0
16	4-Cl-C_6H_4	$CH_3OCH_2CH_2$	83	0
17	4-F-C_6H_4	$CH_3OCH_2CH_2$	67	0
18	3,5-$(Me)_2C_6H_3$	$CH_3OCH_2CH_2$	NT	NT
19	2-pyridine	$CH_3OCH_2CH_2$	17	NT
20	3-$OMeC_6H_4$	$CH_3OCH_2CH_2$	67	0
21	4-pyridine	$CH_3OCH_2CH_2$	50	NT
22	C_6H_5	$CH_3CH_2CH_2CH_2$	NT	NT
23	4-Cl-C_6H_4	$CH_3CH_2CH_2CH_2$	33	NT
24	4-F-C_6H_4	$CH_3CH_2CH_2CH_2$	50	NT
25	3,5-$(Me)_2C_6H_3$	$CH_3CH_2CH_2CH_2$	NT	NT
26	2-pyridine	$CH_3CH_2CH_2CH_2$	NT	NT
27	3-$OMeC_6H_4$	$CH_3CH_2CH_2CH_2$	NT	NT
28	4-pyridine	$CH_3CH_2CH_2CH_2$	67	0
29	C_6H_5	$(CH_3)_2CH$	67	0
30	4-Cl-C_6H_4	$(CH_3)_2CH$	83	0
31	4-F-C_6H_4	$(CH_3)_2CH$	50	NT
32	3,5-$(Me)_2C_6H_3$	$(CH_3)_2CH$	NT	NT
33	2-pyridine	$(CH_3)_2CH$	50	NT
34	3-$OMeC_6H_4$	$(CH_3)_2CH$	0	NT
35	4-pyridine	$(CH_3)_2CH$	NT	NT
36	C_6H_5	$(CH_3)_2CH_2CH_2$	33	NT
37	4-Cl-C_6H_4	$(CH_3)_2CH_2CH_2$	100	0
38	4-F-C_6H_4	$(CH_3)_2CH_2CH_2$	100	38
39	3,5-$(Me)_2C_6H_3$	$(CH_3)_2CH_2CH_2$	NT	NT
40	2-pyridine	$(CH_3)_2CH_2CH_2$	NT	NT
41	3-$OMeC_6H_4$	$(CH_3)_2CH_2CH_2$	NT	NT
42	4-pyridine	$(CH_3)_2CH_2CH_2$	83	0
43	C_6H_5	$C_6H_5CH_2$	67	NT
44	4-Cl-C_6H_4	$C_6H_5CH_2$	67	0
45	4-F-C_6H_4	$C_6H_5CH_2$	67	0
46	3,5-$(Me)_2C_6H_3$	$C_6H_5CH_2$	83	0
47	2-pyridine	$C_6H_5CH_2$	67	0
48	3-$OMeC_6H_4$	$C_6H_5CH_2$	50	NT
49	4-pyridine	$C_6H_5CH_2$	NT	NT

[a] NT, not tested.

an allyl amide was present, ^{1}H NMR analysis indicated that the C,C-double bond remained unaffected.

Finally, we attempted a one-pot conversion of 3-(chloromethyl)isoxazole-4,5-dicarboxylate **1** to mixed bisamide **9** by treating **1** sequentially with thiolate **3**{*1*}, benzylamine **5**{*7*} (1.1 equiv), and propan-2-amine **5**{*5*} in methanol. Various procedural modifications (equivalency, reaction temperature, reaction time, microwave vs conventional heating, etc.) generally led to a mixture of homobisamides and mixed bisamides which, due to quite similar R_f values, proved difficult to separate. Consequently, we found it to be more practical to effect **1** → **9** as a three-pot/no intermediate purification procedure.

Table 2. Biological Activity against *Spodoptera exigua* (Beet Armyworm) for Homobisamide Sulfone Derivative (Chemset **7**)

entry	Ar	R	% mortality against *S. exigua* in HTS	% mortality against *S. exigua* in secondary assay
1	C_6H_5	$CH_3CH_2CH_2CH_2$	100	75
2	C_6H_5	$(CH_3)_2CH$	100	0
3	C_6H_5	$CH_3CH_2CH_2$	100	0
4	C_6H_5	CH_2=$CHCH_2$	100	0
5	4-Cl-C_6H_4	$CH_3CH_2CH_2CH_2$	100	0
6	4-Cl-C_6H_4	$CH_3OCH_2CH_2$	100	0
7	4-Cl-C_6H_4	CH_2=$CHCH_2$	100	0
8	C_6H_5	$CH_3OCH_2CH_2$	83	75
9	4-Cl-C_6H_4	$CH_3CH_2CH_2$	83	0
10	4-Cl-C_6H_4	$(CH_3)_2CH$	97	0

Table 3. Biological Activity against *Spodoptera exigua* (Beet Armyworm) for Mixed Bisamide Thioether Derivatives (Chemset **9**)

entry	Ar	R	% mortality against *S. exigua* in HTS	% mortality against *S. exigua* in secondary assay
1	4-Cl-C_6H_4	$(CH_3)_2CH$	100	0
2	4-Cl-C_6H_4	$CH_3CH_2CH_2$	100	0
3	C_6H_5	$CH_3CH_2CH_2CH_2$	100	0
4	4-Cl-C_6H_4	$CH_3CH_2CH_2CH_2$	83	0
5	4-Cl-C_6H_4	CH_2=$CHCH_2$	83	0
6	C_6H_5	$(CH_3)_2CH$	83	0
7	C_6H_5	$CH_3CH_2CH_2$	83	38
8	4-Cl-C_6H_4	$(CH_3)_2CH_2CH_2$	67	0
9	C_6H_5	$(CH_3)_2CH_2CH_2$	67	0
10	C_6H_5	$CH_3OCH_2CH_2$	17	NT[a]
11	4-Cl-C_6H_4	$CH_3OCH_2CH_2$	17	NT
12	C_6H_5	CH_2=$CHCH_2$	0	NT

[a] NT, not tested.

Eighty of the compounds synthesized as part of this study were evaluated for insecticidal activity. The initial assays were high-throughput screening (HTS) assays utilizing larvae of *S. exigua* (beet armyworm) and *A. aegypti* (yellow fever mosquito). None of the compounds prepared via **Scheme 1** were active against *A. aegypti* larvae. In contrast, more than half (68.8%) of the compounds tested exhibited >67% mortality in the *S. exigua* HTS assay (**Tables 1**−**4**), and nearly a fifth (18.8%) of the compounds tested exhibited 100% mortality. Further evaluation of the hits (>67% mortality) from the *S. exigua* HTS assay involved testing in a larger format assay against two lepidopteran species: *S. exigua* and *H. zea* (corn earworm). Although none of the *S. exigua* HTS hits exhibited activity against *H. zea* larvae, 5 of the 44 hits (11.4%) exhibited some level of activity against *S. exigua* larvae in the follow-up assay (**Table 1**, entry 38; **Table 2**, entries 1 and 8; **Table 3**, entry 7; and **Table 4**, entry 5). Although the activity for all five compounds never exceeded 75% mortality at the 50 μg/cm^2 dose, two of the compounds (**Table 2**, entries 1 and 8) exhibited LC_{50} values (lethal concentration for 50% of the population) of approximately 30 and 19 μg/cm^2, respectively. Some structure−activity relationship trends can be gained from these data. In general, it appears that the sulfones are more efficacious than the corresponding thioethers (**Table 1**, chemset **6**, vs **Table 2**, chemset **7**). One potential explanation for this observation could be that the thioether moiety is not oxidized to the corresponding sulfone by the insect larvae and/or that other parts of the molecules (i.e., amide linkages) are metabolized before thio-oxidation occurs. An electron-withdrawing substituent on the S-aryl ring (in both the thioether and the sulfone series) appears to be advantageous for delivering secondary assay

Table 4. Biological Activity against *Spodoptera exigua* (Beet Armyworm) for Mixed Bisamide Sulfone Derivatives (Chemset **10**)

entry	Ar	R	% mortality against *S. exigua* in HTS	% mortality against *S. exigua* in secondary assay
1	C_6H_5	$CH_3OCH_2CH_2$	100	NT[a]
2	C_6H_5	CH_2=$CHCH_2$	100	0
3	C_6H_5	$CH_3CH_2CH_2$	100	0
4	4-Cl-C_6H_4	$CH_3OCH_2CH_2$	100	0
5	4-Cl-C_6H_4	CH_2=$CHCH_2$	100	38
6	4-Cl-C_6H_4	$CH_3CH_2CH_2$	100	0
7	4-Cl-C_6H_4	$CH_3CH_2CH_2CH_2$	83	0
8	C_6H_5	$CH_3CH_2CH_2CH_2$	67	0
9	4-Cl-C_6H_4	$(CH_3)_2CH$	NT	NT

[a] NT, not tested.

insecticidal activity (**Table 1**, entry 38; **Table 4**, entry 5). Although bisamide sulfones exhibited some of the best biological activity, it is interesting that, in general, there seems to be little insecticidal difference between the homobisamide sulfone (**Table 2**, chemset **7**) and the mixed bisamide sulfone (**Table 4**, chemset **10**) series. Thus, there appears to be a degree of chemical flexibility in this particular bioactive scaffold.

Although significantly less active than commercial standards such as spinosad (LC_{50} = 0.06 $\mu g/cm^2$) (*10*), the compounds described herein did exhibit a hit rate that was far higher than typically seen in the random screening of diverse chemsets (~6%, Sparks and Lorsbach, unpublished data). However, the above-normal hit rate in the HTS assay did not translate to high potency in the more demanding secondary assays. One reason for the lack of translation to secondary assays may be related to the non-ag-like physical properties of these compounds. Future efforts around this series of compounds will expand the SAR trends, address potential metabolic handles in the analogues, and include inputs that lead to more ag-like targets with less liphophilicity.

In summary, we have systematically exploited the three electrophilic centers in dimethyl 3-(chloromethyl)isoxazole-4,5-dicarboxylate to prepare 3-(arylthiomethyl)- and 3-(arylsulfonylmethyl)isoxazole-4,5-dicarboxamides. Eighty compounds from this library were evaluated for insecticidal activity, and many of them exhibited HTS activity against *S. exigua*. Despite the fact that insecticidal activity diminished in secondary assays, second-generation libraries will focus on improving the physical properties of the compounds by incorporating ag-like features (*11*). The HTS activity observed confirms that 3,4,5-trisubstituted isoxazoles present a bioactive scaffold that can be utilized to design novel insecticides. Moreover, as a lead generation effort, this library achieved its goal of producing actionable starting points for further exploitation.

Supporting Information Available: ^1H NMR, ^{13}C NMR, and LC-MS spectral data for representative library members and crystallographic data of **9**{*1*,*1*} and CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

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