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Synthesis and biological activity of 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazoles

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ABSTRACT

Acid hydrazides were coupled with acrylic acid derivatives and cyclodehydration gave 1,3,4-oxadiazoles. Lastly, in-situ nitrile oxide formation from aryl oximes treated with sodium hypochlorite, and subsequent 1,3-dipolar cycloaddition to the exomethylene moiety delivered 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazoles. This library was evaluated in a high-throughput screen at Dow AgroSciences. Several compounds were active against fungal pathogens and pest insects.

1,3,4-Oxadiazoles are an important class of heterocyclic compounds with a variety of biological activities. Substituted 1,3,4-oxadiazoles have shown antibacterial,[1] anti-inflammatory,[1b] antifungal,[1a,2] anticonvulsant and muscle relaxant,[3] insecticide,[4] and anion sensing and fluorescent patterning activities.[5] The widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry establishes this moiety as a member of the privileged structures class.[6] Isoxazolines are also an important heterocycle; molecules containing this moiety have been found to elicit herbicidal,[7] anti-inflammatory,[8] anti-tuberculosis,[9] antifungal,[10] anti-influenza,[11] antibacterial,[12] spermicidal, and anti-HIV properties.[13] As part of 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.[14] These libraries serve to both produce molecules with activity and, simultaneously, provide valuable information to facilitate optimization. This coupled with the broad spectrum of biological activity of 1,3,4-oxadiazole and isoxazoline derivatives prompted us synthesize heterocycle **6** (Figure 1). While the core structure is known in the literature,[15] most examples display diversification only on the isoxazoline moiety. We envisioned that tethering together the two heterocycles with diversification at three points in the molecule could lead to new properties. Due to the importance of each heterocycle in the literature, a library based on this scaffold was pursued. Herein we report the synthesis and biological activity of a library with diversification of three positions in the molecule.

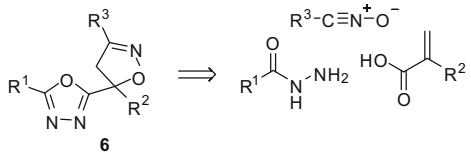
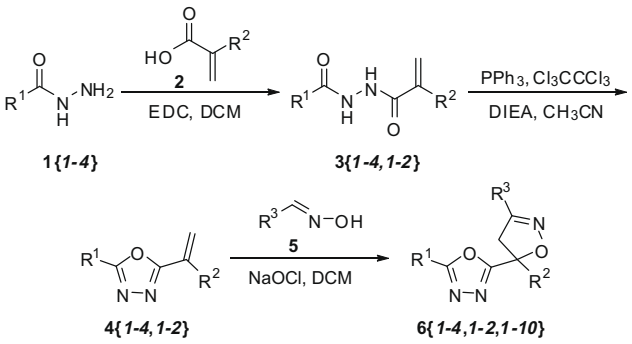


Figure 1. Retrosynthetic analysis of proposed 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazole library.



Scheme 1. Preparation of racemic 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazole library; experimental details and spectral data are provided in Supplementary Data.

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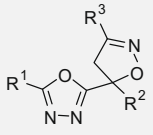
Table 1
Library diversity inputs



6{*1-4,1-2,1-10*}

R^1	R^2	R^3	
{*1*} 4-Cl-C_6H_4	{*1*} CH_3	{*1*} 4-pyridyl	{*6*} 4-NO_2-C_6H_4
{*2*} C_6H_5	{*2*} H	{*2*} 4-F-C_6H_4	{*7*} 2,6-di-Cl-C_6H_3
{*3*} benzyl		{*3*} 4-Cl-C_6H_4	{*8*} C_6H_5
{*4*} 3-Cl-C_6H_4		{*4*} 3-pyridyl	{*9*} 4-OCH_3-C_6H_4
		{*5*} 3-Cl-C_6H_4	{*10*} 4-CH_3-C_6H_4

Table 2
Fungicidal (PYR & SEP) and insecticidal (BAW) activity for subset of 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazole library.

Entry	R^1	R^2	R^3	PYR	SEP	BAW
1	C_6H_5	H	4-Pyridyl	0	0	0
2	C_6H_5	H	4-Cl–C_6H_4	0	0	17
3	C_6H_5	H	4-F–C_6H_4	30	0	17
4	Benzyl	CH_3	4-NO_2–C_6H_4	30	30	17
5	4-Cl–C_6H_4	CH_3	4-Cl–C_6H_4	50	0	17
6	C_6H_5	H	2,6-Di-Cl–C_6H_3	40	30	17
7	4-Cl–C_6H_4	CH_3	4-Pyridyl	30	70	0
8	3-Cl–C_6H_4	CH_3	4-Cl–C_6H_4	40	0	50
9	Benzyl	CH_3	2,6-Di-Cl–C_6H_3	0	0	0
10	3-Cl–C_6H_4	CH_3	4-F–C_6H_4	50	10	0
11	3-Cl–C_6H_4	CH_3	3-Cl–C_6H_4	30	0	33
12	3-Cl–C_6H_4	CH_3	2,6-Di-Cl–C_6H_3	50	0	50
13	3-Cl–C_6H_4	CH_3	C_6H_5	40	10	17
14	C_6H_5	H	3-Cl–C_6H_4	30	0	0
15	4-Cl–C_6H_4	CH_3	4-NO_2–C_6H_4	30	40	0
16	4-Cl–C_6H_4	CH_3	3-Cl–C_6H_4	50	10	50

PYR = *Pyricularia oryzae*; SEP = *Septoria tritici*; data is percent disease control. BAW = *Spodoptera exigua* (beet armyworm); data is percent mortality.

The synthesis of the target scaffold **6**, as seen in Scheme 1, began with EDC-mediated coupling of hydrazide chemset **1** with acrylic acid chemset **2** (Table 1).[22] When methacrylic acid ($R^2 = CH_3$) was used, formation of the product proceeded cleanly in nearly quantitative yields. However, when acrylic acid ($R^2 = H$) was used, significant byproduct, presumably from the polymerization of the acrylic acid, made isolation of the product difficult. Due to the low yielding nature of this reaction and difficulties with purification, only ten derivatives where $R^2 = H$ were synthesized.

There are several known methods for the synthesis of 1,3,4-oxadiazoles,[16] but approaches based on cyclodehydration of diacyl hydrazides are the most commonly used.[1d,6,17] This approach often employs strongly acidic reagents or dehydrating reagents that promote cyclization.[1d] Due to the alkene functionality present in compound **3**, a dehydrating reagent was chosen over strongly acidic conditions. Typical examples of dehydrating reagents include triphenylphosphine, thionyl chloride, triflic anhydride, phosphorus pentoxide, and phosphorus oxychloride.[1d] These reagents are known to result in significant byproduct formation, but due to the sensitive nature of compound **3**, this approach was selected. The method chosen utilizes a mild triphenylphosphine and hexachloroethane mediated cyclodehydration to form the 1,3,4-oxadiazoles.[17] Although byproduct formation was observed, the desired product can be easily isolated from the mixture by purification via flash column chromatography. This method was employed to isolate all 1,3,4-oxadiazole intermediates in moderate to high yields (51–96%).[23]

With the 1,3,4-oxadiazole exomethylene intermediate **4** in hand, the last step to diheterocycle **6** was to introduce the isoxazoline component by way of the nitrile oxide 1,3-dipolar cycloaddition. This was accomplished via biphasic treatment of exomethylenes **4{*1-4,1-2*}** with reagent chemset **5** (aryl oximes, Table 1) and bleach (Huisgen's method[18]) to afford the targeted 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazoles **6{*1-4,1-2,1-10*}** in yields ranging from 40–86%.[24] The lower end yields arise from the chlorine and pyridine diversity elements where solubility was an issue for these compounds. It has been shown that the 5,5-disubstituted isoxazoline is the major regioisomer when reacting a 1,1,-disubstituted alkene with a nitrile oxide.[19] Comparison of the proton NMR isoxazoline methylene protons in **6{*1-4,1-2,1-10*}** to similar systems established that the 5,5-disubstituted regioisomer was the desired product.[20,21]

Sixteen compounds from this collection were screened broadly for herbicidal, fungicidal, and insecticidal activity.[25] This set of compounds was screened against *Helianthus annuus* (sunflower) and *Digitaria sanguinalis* (crabgrass) as a measure of herbicidal activity, and *Pyricularia oryzae* (rice blast) and *Septoria tritici* (wheat leaf blotch) for fungicial activity. While none of the compounds tested displayed any herbicidal activity, several show modest disease control (Table 2), however the observed activity was far below that for fungicide standards such as azoxystrobin. Four of the sixteen compounds in the set passed the HTS insect screens against larvae of *Spodoptera exigua* (beet armyworm), a 25% pass rate. Moreover, some initial trends in the SAR were observed, as seen in entries 8, 11, 12, and 16, which have a 4-Cl or 3-Cl phenyl substituent on the oxadiazole and on the isoxazoline rings, suggesting that chlorine substituents are important for insecticidal activity. Unfortunately, further testing of the four compounds in secondary assays against larvae of *S. exigua* and *Helicoverpa zea* (corn earworm) showed no activity when compared to the insecticide standard spinosad. Future efforts to increase the insect activity will comprise expanding these early SAR trends and surveying bioisosteres of the oxadiazole ring, including screening 5-(thiazol-5-yl)-4,5-dihydro-isoxazoles from a previous library.[20]

In summary, a route to 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazoles has been developed leading to a 50-member library of 1,3,4-oxadiazoloisoxazolines. Key steps included diacyl hydrazide formation, followed by cyclodehydration to give the 1,3,4-oxadiazole, and 1,3-dipolar cycloaddition to give the isoxazoline. These compounds were evaluated in the insecticide HTS and exhibited weak to moderate insecticidal activity. Early SAR trends point to chlorine substituents as important for insecticidal activity. Future efforts to increase the insecticidal activity will include screening other heterocyclic rings isosteres.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.139.

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22. Procedure for Acid Coupling: (3{*1,1*}). 4-Chlorobenzhydrazide (2.50 g, 14.7 mmol) was dissolved in DCM (100 mL) and cooled to 0 °C. EDC (3.09 g, 16.1 mmol) was added and the mixture was stirred for 10 min after which time methacrylic acid (1.24 mL, 14.7 mmol) was added. The reaction mixture was warmed to room temperature, stirred overnight and diluted by the addition of brine. The layers were separated and the aqueous layer was extracted with EtOAc (3×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated under rotary evaporation to afford **3{*1,1*}** in ~100% yield which was used without further purification. A small portion of the product was purified for analytical purposes; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.42 (s, 1H), 10.01 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 9.0 Hz, 2H), 5.81 (s, 1H), 5.49 (s, 1H), 1.92 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 167.0, 164.7, 138.1, 136.6, 131.3, 129.3, 128.6, 120.6, 18.5; ESI-MS m/z 239, 241 $(M+H)^+$. Purity was determined to be 100% by HPLC analysis.
23. Procedure for Oxadiazole Synthesis: (**4{*1,1*}**). Hydrazide **3{*1,1*}** (3.50 g, 14.7 mmol) was dissolved in dry acetonitrile (~300 mL), and triphenylphosphine (6.92 g, 26.4 mmol), and DIEA (14.8 mL, 85.0 mmol) were added and the mixture was stirred for 5 min. Hexachloroethane (4.51 g, 19.1 mmol) was added and the solution was stirred at room temperature for 2 h. The reaction mixture was concentrated and water and EtOAc were added. The aqueous layer was separated and extracted again with EtOAc (2×). The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, and concentrated under rotary evaporation. The crude solid was purified by flash chromatography (EtOAc/Hexane, 1:4) to give **4{*1,1*}** (1.64 g, 51% yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (d, J = 9.0 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 6.07 (s, 1H), 5.58 (s, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.4, 163.7, 138.1, 129.6, 129.5, 129.0, 128.4, 122.5, 18.8; ESI-MS m/z 221 $(M+H)^+$. Purity was determined to be 100% by HPLC analysis.
24. Procedure for Isoxazoline Synthesis: (**6{*1,1,4*}**). Oxadiazole **4{*1,1*}** (0.055 g, 0.25 mmol) and nicotinaldehyde oxime (0.061 g, 0.50 mmol) were dissolved in DCM (2 mL) and cooled in an ice bath. Bleach (laboratory grade, 5.65%, 2.5 mL) was added dropwise and the reaction mixture was stirred overnight. Water (2 mL) and DCM (2 mL) were added and the layers were separated. The organic layer was dried over sodium sulfate, filtered, and concentrated by rotary evaporation. Purification by preparative HPLC gave **6{*1,1,4*}** (0.071 g, 83% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.84 (d, J = 4.8 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.84–7.80 (m, 1H), 7.50 (d, J = 8.4 Hz, 2H), 4.43 (d, J = 17.2 Hz, 1H), 3.58 (d, J = 16.8 Hz, 2H), 2.07 (s, 3H); ESI-MS m/z 341, 343 $(M+H)^+$. Purity was determined to be 99% by HPLC analysis.
25. General Procedure for Bioassays. The insect assays were conducted as described previously by Choung et al.[14d] Fungicidal activity was determined using two fungal pathogens, *Pyricularia oryzae* and *Septoria tritici*. Compounds were tested for inhibition of fungal growth, graded using a nephlometer (Nephelostar Galaxy, BMG Labtechnologies, Offenburg, Germany), in 96 well microtiter plates. The test compounds were diluted to 2.5 mg/mL in dimethylsulfoxide (DMSO) all wells receiving 2.0 μL of the test solution. Plates are inoculated by adding 200 μL of the conidial suspension. Following inoculation, the plates were placed in a shaker incubator (Innova 44R, New Brunswick Scientific Company Inc., Edison, NJ, USA) set at 22 °C for 48 (*P. oryzae*) to 72 (*S. tritici*) hours. The HTS testing media was a synthetic dextrose minimal medium, as described by Adams et al. 1997[26], with exclusion of agar and the addition of phosphate (6% w/v). Herbicidal activity was evaluated using *Helianthus annuus* (sunflower) and *Digitaria sanguinalis* (crabgrass,). Seeds were germinated in a 72-cell plug tray (Dillen Products, Middlefield, OH) filled with Metro-mix 360® soil-less media (Sun Gro, Vancouver, British Columbia) providing a plant density of 1 sunflower and 8 foxtail plants per cell. Five days after planting, the plants in each cell were sprayed (syringe applicator, TeeJet 0.5 nozzle) with the test compound (1 mg dissolved in 100 μL of dimethylsulfoxide (DMSO) and then formulated with 0.9 mL of a spray solution (54.6% v/v (volume/volume) distilled water, 40% v/v acetone, 5% v/v isopropanol, 0.4% v/v crop oil concentrate and 0.01% w/v (weight/volume) Triton X-155). The total application volume was 0.5 mL (ca. 4 kg/ha). After application, plants were held the greenhouse (22 °C, 14 h light/10 h dark cycle) and then visually assessed for growth reduction 5–6 days after treatment.
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