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Synthesis and SAR Evaluation of Oxadiazolopyrazines as Selective *Haemophilus influenzae* Antibacterial Agents

Xenia Beebe,* Angela M. Nilius, Philip J. Merta, Niru B. Soni, Mai H. Bui, Rolf Wagner and Bruce A. Beutel

Infectious Disease Research, Abbott Laboratories, Abbott Park, IL 60064, USA

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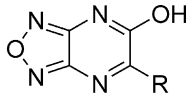
Abstract—The parallel synthesis and antibacterial activity of 5-hydroxy[1,2,5] oxadiazolo[3,4-*b*]pyrazines is reported. The compounds were synthesized by condensing diaminofurazan with α-keto acids to give a variety of aryl-substituted analogues. Halogenated phenyl groups at *C*-6 give rise to the greatest *Haemophilus influenzae* antibacterial activity.

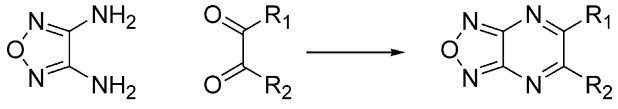
The search for new antibacterial agents is important due to increasing resistance of clinically important pathogens to known classes of antibiotics.[1] In our ongoing research aimed at the identification of novel antibacterial agents, we have discovered that oxadiazolopyrazines **1** selectively inhibit the growth of *Haemophilus influenzae*, a key pathogen involved in community acquired respiratory tract infections. The oxadiazolopyrazine leads were discovered in a high throughput screen designed to identify compounds from chemical libraries that have in vitro antibacterial effects against Gram-positive and Gram-negative bacteria. Herein we describe our efforts to optimize **1** in the context of antibacterial activity. To our knowledge, the synthesis of these compounds has not been described in the literature. However, a search of the literature revealed that the structurally related thiadiazolopyrazines are known to have antifungal and weak antimicrobial activity (Fig. 1).[2]



1

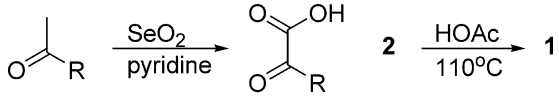
Figure 1. 5-Hydroxy[1,2,5]oxadiazolo[3,4-*b*]pyrazine.

The goal of the project was to optimize the spectrum of antibacterial activity without compromising the eukaryotic cytotoxicity. In order to explore the SAR of the lead series **1**, it was necessary to design a practical synthesis to incorporate the 5-hydroxyl into the oxadiazolopyrazine core and vary the *C*-6 substituent. 5,6-Biaryl oxadiazolopyrazines **4** have been synthesized[3] by condensing diaminofurazan **2** with a diketone **3** to give **4**, as shown in Scheme 1, where R_1, R_2 = aryl. It was discovered that a variety of *C*-6 aryl substituents could be incorporated into the 5-hydroxyloxadiazolopyrazine core by condensing diaminofurazan **2** with different α-keto acids **6** as shown in Scheme 2 where R = aryl. The *C*-6 aryl groups were chosen to vary electronic and steric factors to see what the effect they had on the antibacterial potency, spectrum and cellular selectivity.



2 **3** **4**

Scheme 1. Synthesis of [1,2,5]oxadiazolo[3,4-*b*]pyrazines.



5 **6**

Scheme 2. Synthesis of 5-hydroxy[1,2,5]oxadiazolo[3,4-*b*]pyrazines.

*Corresponding author. Tel.: +1-847-935-8291; fax: +1-847-935-0310; e-mail: xenia.b.searle@abbott.com

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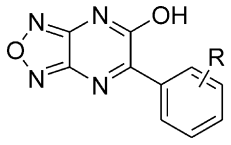
The synthesis of 5-hydroxy-6-aryl oxadiazolopyrazines was accomplished in one step from aryl α-keto acids (Scheme 2). Diaminofurazan **2** was synthesized using the methods of Trudell.[4] α-Keto acids **6** were condensed with the diaminofurazan **2** in acetic acid at 110 °C for 2 h to give the 5-hydroxy oxadiazolopyrazines[5] **1**. Only non-enolizable keto acids, where R = aryl, gave the desired products in this reaction. The structure can also be drawn as the keto/amide tautomer. NMR studies in DMSO indicate that the compound is actually the phenolic structure as shown. A broad signal of the OH is apparent at 13.2–13.5 ppm.

In order to synthesize a variety of 5-hydroxy oxadiazolopyrazines, a source of the α-keto acid starting materials was essential. α-Keto acids can be synthesized by oxidizing acetophenones **5** (R = aryl) with selenium dioxide/pyridine[6] or potassium permanganate.[7] The selenium dioxide oxidations gave near quantitative yields of the α-keto acids and the only side product was the corresponding benzoic acid arising from decarboxylation of the α-keto acid. Since the oxidative conditions were so mild, it was postulated that it might be possible to run the oxidation and condensations in one step. Thus, the desired acetophenone **5** was mixed with diaminofurazan **2** and selenium dioxide in pyridine and heated to 110 °C overnight to afford the corresponding 5-hydroxydiazolopyrazine[8] **1**. About half of the desired 5-hydroxy[1,2,5]oxadiazolo[3,4-*b*]pyrazines were synthesized in this one-pot procedure. Each compound was purified by reverse-phase preparative HPLC[9] and gave satisfactory ^{1}H NMR and MS data. The biological activity of each compound is described in Tables 1 and 2.

The compounds were tested for antibacterial activity against multiple strains of Gram-positive and Gram-negative bacteria using the broth microdilution method (NCCLS-1)[10] and results are reported as the minimum inhibitory concentration (MIC). We also assessed selectivity for bacterial cells versus eukaryotic cells by determining antifungal activity against the yeast *Candida albicans* using the broth microdilution method (NCCLS-2)[11] and eukaryotic cytotoxicity against human Jijoye B cells.[12] The MIC's of the phenyl-substituted analogues are shown in Table 1. The *m*- and *p*-halogenated aryl analogues **7**, **8**, **9**, **10**, **12** and **13** have the most potent antibacterial activity against the Gram-negative species *H. influenzae* and also demonstrate modest activity against the Gram-positive species *Staphylococcus aureus* and *Streptococcus pneumoniae*. The *o*-halogenated analogues **22**, **27**, and **28** are the least active against *H. influenzae* and *S. aureus*. None of the compounds exhibit activity against the other Gram-negative strain *Escherichia coli*. The compounds do not inhibit the growth of the eukaryotic cells, *C. albicans* and Jijoye B cells. With this data in mind, we next explored *meta*- and *para*-substituted phenyl analogues with a variety of electron donating and withdrawing substituents.

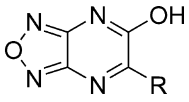
The phenyl substituted compound **14** shows potent *H. influenzae* activity that is comparable to the *p*- and

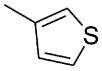
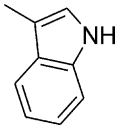
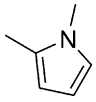
Table 1. Substituted aryl analogues



Compd	R	MIC (μg/mL)					Jijoye B. cell cytotoxicity (μg/mL)
		H. influenzae	*E. coli*	*S. aureus*	*S. pneumoniae*	*C. albicans*	
7	*m*-Br	0.5	>128	32	64	>128	>100
8	*p*-Br	1	>128	16	64	>128	>100
9	*p*-Cl	1	>128	16	128	>128	>100
10	*m*-Cl	1	>128	128	64	>128	>100
11	*m*-NO_2	1	>128	32	64	>128	>100
12	*m*-F	1	128	64	64	>128	>100
13	*p*-F	1	>128	64	64	>128	>100
14	H	1	128	128	128	>128	>100
15	3,4-Dioxolane	2	>128	64	128	>128	>100
16	*m*-CF_3	2	>128	32	128	>128	100
17	*p*-CF_3	4	>128	32	64	>128	>100
18	*p*-NMe_2	4	>128	>128	128	>128	>100
19	*p*-OCH_3	4	>128	64	128	>128	>100
20	*m*-OCH_3	8	>128	128	64	>128	>100
21	*p*-NO_2	8	>128	>128	128	>128	>100
22	*o*-F	8	>128	>128	64	>128	>100
23	*p*-OPh	8	>128	4	64	>128	60
24	*p*-Cyclohexyl	16	>128	64	64	>128	35
25	*p*-*N*-Morpholine	32	>128	>128	64	>128	>100
26	*p*-*N*-Piperidine	64	>128	64	128	>128	>100
27	*o*-Br	128	>128	>128	128	>128	>100
28	*o*-Cl	128	>128	>128	128	>128	>100
29	3,4,5-Trimethoxy	128	>128	>128	128	>128	>100

Table 2. Heterocyclic analogues



Compd	R	MIC (μg/mL)					Jijoye B. cell cytotoxicity (μg/mL)
		H. influenzae	*E. coli*	*S. aureus*	*S. pneumoniae*	*C. albicans*	
30		0.5	128	32	64	>128	>100
31		1	128	64	128	>128	>100
32		4	>128	128	128	128	>100
33		8	>128	>128	64	>128	>100
34		8	>128	>128	128	>128	>100

m-halogenated aryl analogues, but in contrast, has only very weak Gram-positive activity. A few other analogues show some *S. aureus* activity, in conjunction with activity against *H. influenzae*. The most active compound against *S. aureus*, the *p*-phenyl ether analogue **23**, also demonstrates slight eukaryotic cytotoxicity against Jijoye B cells. Electron withdrawing or electron donating groups seem to have no effect on antibacterial activity. The lack of an electronic effect on MIC's implies that the antibacterial activity arises from steric rather than electronic interactions. The compounds with larger aliphatic substituents, like the cyclohexyl **24**, *N*-morpholine **25** and *N*-piperidine **26** analogues, are much less active than the halogenated compounds.

We also synthesized some heterocyclic compounds shown in Table 2. The 2-thiophene analogue **30** has slightly better antibacterial activity than the isosteric phenyl analogue **14** (Table 1) and **30** exhibits Gram-positive activity. The analogues containing pyrrole **32**, indole **33**, dimethyl aniline **18**, piperidine **26**, and morpholine **25** substituents are less active than the thiophene **30** and phenyl **14** substituted analogues against both *H. influenzae* and the Gram-positive strains. It therefore appears that the presence of basic groups decreases the antibacterial activity.

In conclusion, we have discovered a new class of compounds that exhibit antibacterial activity, the 5-hydroxy [1,2,5]oxadiazolo[3,4-*b*]pyrazines. The antibacterial activity is affected by varying substitution in the C-6 position. Small non-basic aryl groups in the C-6 position provide good activity against *H. influenzae* and weak activity against *S. aureus* and *S. pneumoniae*.

Acknowledgements

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5. Typical procedure for the preparation of oxodiazolopyrazines: 500 mg (5.00 mmol) of **2** was combined with 1.114 g (5.00 mmol) of (3-bromo-phenyl)-oxo-acetic acid and 1.5 mL of glacial acetic acid. The reaction was heated to 110 °C for 2 h, and then cooled and 5 mL of water added. The water was removed and the material washed with ether (2×2 mL) and the solid transferred with hexane. The solid was then purified by flash silica gel chromatography using 10–15% EtOAc/hexanes to give 328 mg (22% yield) of **7** as a white crystalline solid: mp 188–190 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 (t, J = 7.91

Hz, 1H) 7.75 (d, $J=8.09$ Hz, 1H) 8.21 (d, $J=8.09$ Hz, 1H) 8.40 (t, $J=1.84$ Hz, 1H); ^{13}C (75 MHz, CD_3OD) δ 122.9, 130.3, 130.9, 134.2, 136.0, 137.5, 146.8, 150.4, 156.2, 163.5; MS (ESI-) m/z 292/294 1:1 ratio isotopes.
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8. Typical procedure for the parallel synthesis of oxodiazolopyrazines: 200 mg (2.00 mmol) of **2** was combined with 259 μL (2.00 mmol) of 3′-chloroacetophenone and 0.5 mL of pyridine. The reaction was heated to 90 °C for 1 h, and then 333 mg (3.00 mmol) of selenium dioxide was added and the reaction heated at 110 °C overnight. The reaction was cooled and taken up in EtOAc and filtered through silica gel. The solid was then purified by reverse-phase HPLC to give 108 mg (22% yield) of **10** as a white crystalline solid: mp 183–184 °C; ^1H NMR (300 MHz, CD_3OD) δ 7.50 (t, $J=7.91$ Hz, 1H) 7.60 (m, 1H) 8.17 (d, $J=8.09$ Hz, 1H) 8.25 (s, 1H); ^{13}C (75 MHz, CD_3OD) δ 129.9, 130.7, 131.3, 133.1, 135.0, 137.2, 146.7, 150.3, 156.1, 163.4; MS (ESI-) m/z 246 (M−H). **15**: 328 mg (2.00 mmol) of 3,4-methylenedioxyacetophenone gave 69 mg (17%) of an orange crystalline solid: mp 207–208 °C; ^1H NMR (300 MHz, CD_3OD) δ 6.07 (s, 1H) 6.94 (d, $J=8.46$ Hz, 1H) 7.80 (d, $J=1.47$ Hz, 1H) 8.05 (dd, $J=8.46$, 1.84 Hz, 1H); ^{13}C (75 MHz, DMSO-d_6) δ 101.5, 107.5, 109.4, 126.3, 127.9, 145.2, 146.9, 148.8, 150.7, 154.5, 161.2; MS (ESI-) m/z 257 (M−H).
9. Samples were purified by preparative HPLC on a Waters Symmetry C8 column (40×100 mm, 7 μm particle size) using a gradient of 10% to 100% acetonitrile/0.1% aqueous TFA over 12 min (15-min run time) at a flow rate of 70 mL/min.
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11. National Committee for Clinical Laboratory Standards. 2002. *Reference Method for Broth Dilution Antifungal Susceptibility Testing. Approved Standard M27-A2*. National Committee for Clinical Laboratory Standards: Wayne, PA.
12. Cytotoxicity was evaluated in Jijoye B cells (ATCC, Rockville, MD, USA) by assessing compound effect on cellular respiration. After plating at a density of 1×10^6 cells/mL, log phase cells were treated with compound and incubated 24 h under normal growth conditions. Alamar Blue reagent (BioSource International, Camarillo, CA, USA) was added, and fluorometrically measured after a 4 h incubation. From a semi log plot, the IC_{50} value of each compound was calculated, and compared to a database of over 10,000 compounds, including commercial antibiotics of every structural class available.