Preparation of a Spiroisoxazolinopiperidinylbenzamide-Based Scaffold

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Abstract: A route to spiroisoxazolinopiperidinylbenzamides has been developed. *N*-Boc-4-piperidone underwent a Wittig olefination and Boc-deprotection followed by a nucleophilic substitution reaction with 4-fluoro-3-nitrobenzoic acid to yield the starting scaffold **3** in excellent yields. Diversification of the acid with primary amines, followed nitrile oxide formation in situ (aryl oximes treated with bleach) and subsequent 1,3-dipolar cycloaddition to the exomethylene moiety delivered the spiroisoxazolinopiperdines. Reduction of the arylnitro group followed by acylation with acid chlorides or reductive amination with aldehydes yielded the spiroisoxazolinopiperidinylbenzamide library.

Key words: nitrile oxide, isoxazolines, nucleophilic substitution, cycloaddition, piperidines

Small-molecule heterocyclic compounds are attractive due to their ability to interact with biological systems. Isoxazoline-containing compounds have been shown to display a wide range of biological activities, including herbicidal,[1] anti-inflammatory,[2] anti-tuberculosis,[3] antifungal,[4] anti-influenza,[5] and antibacterial activities.[4] This moiety, when spiro-connected, is also found in bioactive natural products, including 11-deoxyfistularin-3 and purealidin Q, which both have anticancer properties, and in aerothionin, zamamistatin, and agelorin B, which exhibit antibiotic, antifungal, or antimycobacterial activities.[6] Natural products containing this substructure, araplysillins-I and –II as well as purealidin B, have shown antimicrobial activities.[7]

Another biologically relevant heterocycle is the piperidine moiety. Compounds containing this heterocycle have shown antioxidant and anti-inflammatory activity[8] as well as anticoagulant activity.[9] It is interesting to note that when this heterocycle is spiro-connected, various other activities arise, including antiviral activity.[10] In fact, the spiropiperidine substructure is so abundant in biologically pertinent molecules, it has earned 'privileged structure' status.[11]

Due to the strong biological activity of each individual heterocycle and the additional activities when connected in a spiro fashion, we set out to prepare a library containing the spiroisoxazolinopiperidine substructure. Although this substructure is known in the literature,[12] here it was tethered to a diversifiable molecule in order to synthesize a library containing this moiety. Library synthesis increases the possibility of finding a highly active compound by creating a target scaffold with diversity elements that probe chemical space. Often, biological evaluation produces a hit that can be further refined and improved to a valuable lead compound.[13] We report that the targeted library (**A**) can be synthesized from trifunctionalized scaffold **3** (Figure 1).

The synthesis of target scaffold **3** began with commercially available *N*-Boc-4-piperidone (**1**; Scheme 1). Subjecting **1** to Wittig olefination conditions delivered the exomethylene moiety in high yield. Exomethylenepiperidine **2** was Boc-deprotected and then treated with 4-fluoro-3-nitrobenzoic acid in the presence of DIPEA to promote the nucleophilic substitution. It should be noted that attempts to isolate the neutral amine failed as the compound proved to be highly water-soluble. Instead, the TFA salt was neutralized to pH 8 with triethylamine and then used in the nucleophilic substitution. The carboxylic acid was treated with base prior to the addition of the exomethylenepiperidine and the nucleophilic substitution then proceeded in high yield (94%) to deliver the targeted scaffold **3**.

Diversification of this trifunctional scaffold (Scheme 2) began with an EDC-mediated coupling of amine chemset

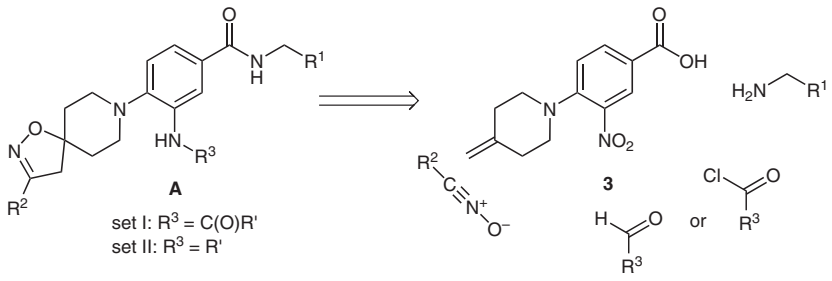

Figure 1 Spiroisoxazolinopiperidinylbenzamide (**A**) reagents

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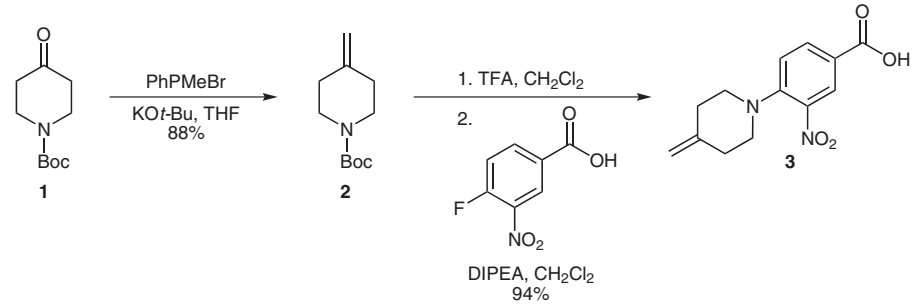
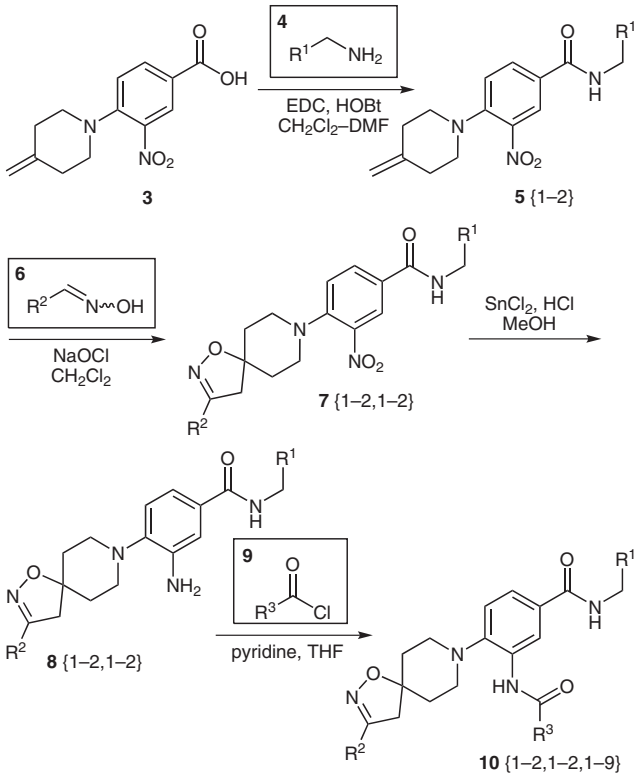


Scheme 1 Synthesis of the trifunctional scaffold

4 (Figure 2) to carboxylic acid **3**, which proceeded in good to excellent yields (78–98%). The route to benzamides **5** could also be achieved by amidation of 4-fluoro-3-nitrobenzoic acid followed by the nucleophilic substitution with the exomethylenepiperidine, but synthesis of the trifunctional scaffold proved to be more efficient.

With benzamide **5** in hand, introduction of the spiroisoxazoline group proceeded via a nitrile oxide 1,3-dipolar cycloaddition. Initial attempts involved the use of hydroximoyl chlorides and triethylamine to form the reactive nitrile oxide intermediate. Although product formation was observed, yields were not consistent. However, the use of oximes and bleach, which is the most common source for sodium hypochlorite, proved to be more reliable and consistently produced higher yields.

Most commonly, the biphasic nitrile oxide 1,3-dipolar cycloaddition reaction is performed by addition of bleach to a solution of the alkene and oxime[14] with the nitrile oxide,



Scheme 2 Preparation of the spiroisoxazolinopiperidinylbenzamide library

which is generated in situ, reacting with the alkene preferentially rather than dimerizing. However, changing the order of addition by adding a solution of the oxime to the alkene and bleach increases dipole reaction with the alkene and thus minimizes dimerization. This modified addition procedure using aryl oxime chemset **6** (Scheme 2) delivered the sprioisoxazolinopiperdine **7** in moderate to good yields (50–89%). It has been demonstrated that the 5,5-disubstituted isoxazoline is the major regioisomer when reacting a nitrile oxide with a 1,1-disubstituted alkene.[6,12a,15,16] Comparison of the proton NMR isoxazoline methylene AB quartet in **7** to similar systems established that, indeed, the 5,5-disubstituted regioisomer was obtained.[12a,16]

Installation of the final diversity element began with tin(II) chloride mediated reduction of the arylnitro functional group. Although literature protocols have shown that treatment of an isoxazoline with metal-based reducing agents can cleave the N–O bond,[17] we have shown the isoxazoline moiety remains intact under tin(II) chloride reducing conditions.[15] Thus, the reduction of the arylnitro group was performed in the presence of concentrated hydrochloric acid under refluxing conditions to yield the arylamine in good yields (74–93%).[18] The reduction was sluggish until the addition of hydrochloric acid and, although these conditions appear harsh, NMR revealed that the isoxazoline and amide moieties remained unaffected.

Finally, HOBt-mediated coupling of the aryl amine in **8** with carboxylic acids was attempted, but yields were poor as this amine is relatively non-nucleophilic and too hindered to attack the activated ester.

Fortunately, coupling with acyl chloride chemset **9** (Figure 2) provided amide **10{1–2,1–2,1–9}** in good yields (47–94%). Alternatively, a reductive amination (Scheme 3) with aldehyde chemset **11** (Figure 3) provided amine **12{2,1–2,1–4}** in moderate yields (48–62%). Both of these diversification methods led to the targeted spiroisoxazolinopiperidinylbenzamide library.

In summary, a route to novel spiroisoxazolinopiperidinylbenzamides has been developed leading to a 44-member library.[19] Key steps included EDC-mediated coupling of amines, nitrile oxide 1,3-dipolar cycloaddition, arylnitro reduction, and either acylation with acid chlorides or reductive amination with aldehydes to give the trifunctionalized heterocycle. The work presented here is in

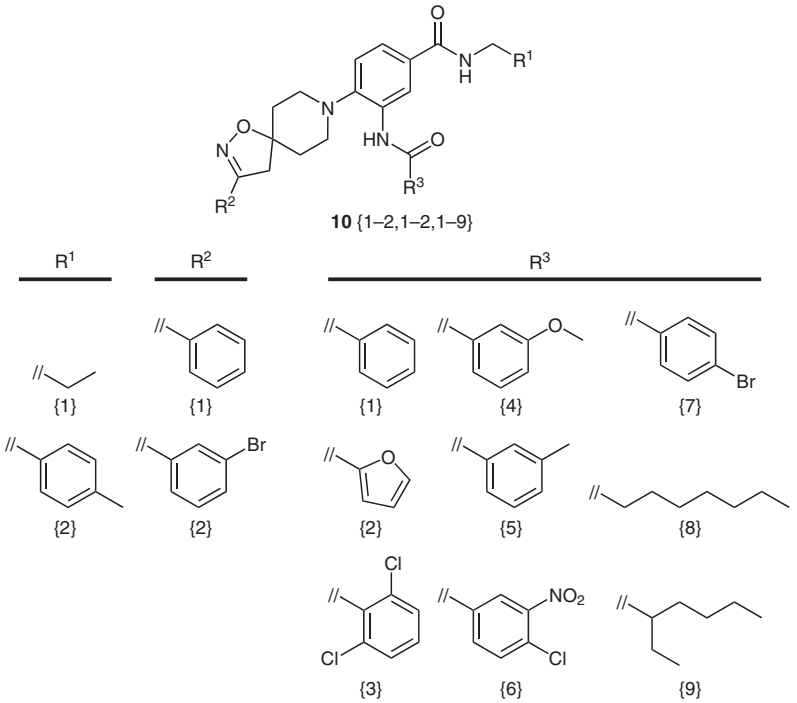
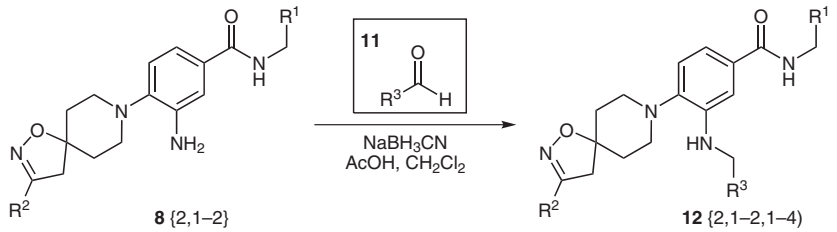


Figure 2 Diversity inputs for the spiroisoxazolinopiperidinylbenzamide library



Scheme 3 Preparation of Set II arylamines

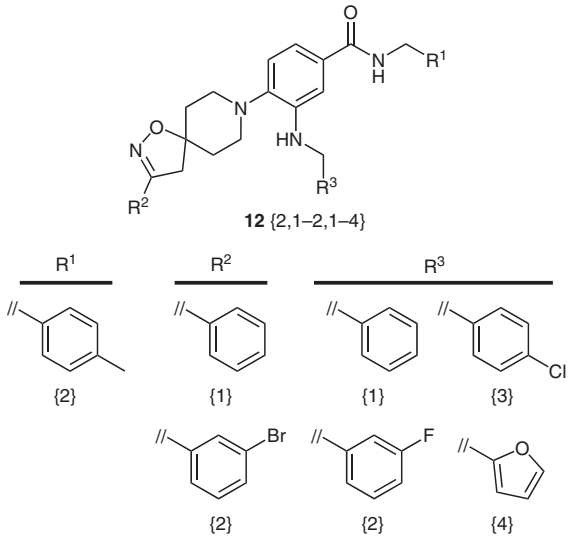


Figure 3 Additional diversity inputs for the spiroisoxazolinopiperidinylbenzamide library

collaboration with the National Institute of General Medical Sciences (NIGMS) to create pilot-scale diversity libraries.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(19) **Procedure for Olefin Synthesis: *tert*-Butyl 4-Methylenepiperidine-1-carboxylate (2).**
Methyltriphenylphosphonium bromide (35.86 g, 110.4 mmol) was dissolved in anhydrous THF (100 mL) and cooled in an ice bath. Potassium *tert*-butoxide (1.0 M in THF, 105.4 mL, 105.4 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min, then warmed to room temperature for 30 min, and warmed to reflux for 1 h. The reaction mixture was cooled in an ice bath and a solution of *N*-Boc-4-piperidone (10.00 g, 50.19 mmol) in anhydrous THF (50 mL) was added. The mixture was removed from the ice bath and warmed to reflux until TLC showed the reaction was complete (~4 h). The reaction mixture was diluted with water, concentrated by rotary evaporation, and extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (EtOAc–hexane, 1:9) gave **2** as a colorless oil (8.76 g, 88% yield). IR (neat): 2977, 2940, 2907, 2865, 1692, 1652, 1416, 1365, 1235, 1165, 1114 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.74 (s, 2 H), 3.42 (t, *J* = 5.7 Hz, 4 H), 2.18 (t, *J* = 5.7 Hz, 4 H), 1.47 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.9, 145.5, 109.2, 79.6, 45.5, 34.7, 28.6; MS (ESI): *m/z* = 198 [$C_{11}H_{20}NO_2^+$]. Purity was determined to be 89% by HPLC analysis.

Procedure for Scaffold Synthesis: 4-(4-Methylene-piperidin-1-yl)-3-nitrobenzoic Acid (3). *tert*-Butyl 4-methylenepiperidine-1-carboxylate (**2**; 4.71 g, 23.9 mmol) was dissolved in CH_2Cl_2 (50 mL) and trifluoroacetic acid (50 mL) was added. The reaction mixture was stirred at room temperature for 2 h after which it was concentrated by rotary evaporation. The residue was dissolved in CH_2Cl_2 (20 mL) and triethylamine was added until the solution reached pH 8. 4-Fluoro-3-nitrobenzoic acid (2.21 g, 12.0 mmol) was dissolved in a separate flask in CH_2Cl_2 (20 mL) and DIPEA (8.33 mL, 47.8 mmol) was added at 0 °C. The mixture was stirred for 30 min at which time the pH 8 solution of deprotected amine was added dropwise and stirred overnight while warming to room temperature. The reaction mixture was concentrated by rotary evaporation, the crude oil was dissolved in ethyl acetate and water, and the pH was adjusted to pH ~3 with 1 M HCl. The layers were separated and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (MeOH–$CHCl_3$, 1:9) gave **3** as a bright orange solid (2.95 g, 94% yield). A small portion of the product was further purified for analytical purposes; mp 137–138 °C; IR (neat): 2949, 2905, 2854, 2168, 1676, 1600, 1526, 1491, 1428, 1388, 1348, 1293, 1264, 1231, 1206, 1160, 1127, 1065 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ = 13.08 (s, 1 H), 8.28 (d, *J* = 1.8 Hz, 1 H), 8.00 (dd, *J* = 9.0, 1.8 Hz, 1 H), 7.34 (d, *J* = 9.0 Hz, 1 H), 4.81 (s, 2 H), 3.18 (t, *J* = 5.4 Hz, 4 H), 2.32 (t, *J* = 5.4 Hz, 4 H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 165.7, 148.1, 144.1, 139.0, 134.2, 127.8, 121.1, 120.5, 109.6, 51.7, 33.6; MS (ESI): *m/z* = 263 [$C_{13}H_{15}N_2O_4^+$]. Purity was determined to be ~100% by HPLC analysis.

General Procedure for Amine Coupling to 5{1–2}. Compound **3** (1 equiv), HOBt (1.4 equiv), and EDC (1.4 equiv) were dissolved in a mixture of CH_2Cl_2–DMF (4:1, 50 mL) at 0 °C and stirred for 30 min. The requisite amine (1.8 equiv) was added dropwise and the solution was stirred overnight while warming to room temperature. The resulting solution was concentrated by rotary evaporation and taken up in EtOAc (50 mL). The solution was then extracted with saturated aq. $NaHCO_3$ (50 mL), 1 M HCl (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (EtOAc–hexane) gave **5{1–2}** in 78–98% yield.

General Procedure for Isoxazoline Synthesis to give 7{1–2,1–2}. Compound **5{1–2}** (1 equiv) was dissolved in CH_2Cl_2 (5 mL) and bleach (laboratory grade, 5.65%, 4 equiv) was added at 0 °C. A solution of the requisite oxime **6{1–2}** (2 equiv) in CH_2Cl_2 (5 mL) was added via addition funnel to the reaction mixture. The resulting solution was stirred overnight. Water (20 mL) and CH_2Cl_2 (20 mL) were added and the layers were separated. The organic layer was extracted with water (2×) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (EtOAc–hexane) gave **7{1–2,1–2}** in 50–89% yield.

General Procedure for Aryl Nitro Reduction to give 8{1–2,1–2}. Compound **7{1–2,1–2}** (1 equiv) and tin(II) chloride dihydrate (3 equiv) were dissolved in MeOH (10 mL) and stirred for 10 min. Concentrated HCl (6 equiv) was added dropwise and the mixture was warmed to reflux until TLC showed the reaction was complete. The solution was cooled to room temperature and concentrated by rotary evaporation. The resulting oil was dissolved in CH_2Cl_2 (10 mL) and water (10 mL) and the mixture was adjusted to pH ~7 with 1 M NaOH. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (EtOAc–hexane) gave **8{1–2,1–2}** in 74–93% yield.

General Procedure for Acid Chloride Coupling to give 10{1–2,1–2,1–9}. Compound **8{1–2,1–2}** (1 equiv) was dissolved in anhydrous THF (5 mL) and pyridine (1.5 equiv) was added. The solution was cooled to 0 °C and the requisite acid chloride (1.2 equiv) was added dropwise. The mixture was stirred overnight, after which time TLC showed the reaction was complete. Water (5 mL) and diethyl ether (5 mL) were added and the solution was adjusted to pH ~7 with 1 M NaOH. The layers were separated and the aqueous layer was extracted with diethyl ether (2×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by flash chromatography (EtOAc–hexane) gave **10{1–2,1–2,1–9}** in 47–94% yield.

General Procedure for Reductive Amination to give 12{2,1–2,1–4}. Compound **8{2,1–2}** (1 equiv) was dissolved in CH_2Cl_2 (5 mL) and the requisite aldehyde (1.5 equiv) and acetic acid (4 equiv) were added. The solution was stirred for 6 h after which time sodium cyanoborohydride (5 equiv) was added. The mixture was stirred overnight, concentrated by rotary evaporation, and taken up in ethyl acetate (10 mL). The organic layer was washed with saturated aq. $NaHCO_3$, 1M HCl, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification by MPLC gave **12{2,1–2,1–4}** in 48–62% yield.