Novel diversely substituted 1-heteroaryl-2-imidazolines for fragment-based drug discovery

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Abstract

A palladium-catalyzed Buchwald-Hartwig arylation protocol has been applied to achieve high-yielding *N*-heteroarylation of a diverse set of privileged 2-imidazolines. The resulting compounds are of interest as a novel type of molecular tool for fragment-based drug discovery. The potential for combining two 2-imidazoline moieties in a heteroarene-linked dimer via sequential Pd-catalyzed arylation has been demonstrated.

Keywords: fragment-based drug discovery, 2-imidazolines, Rule of Three, Buchwald-Hartwig reaction, 2-haloazines, imidazoline dimers.

Since its invention at Abbott Laboratories in 1996,¹ fragment-based drug discovery² continues to evolve into a remarkably efficient alternative to the traditional high-throughput screening (HTS) technology of lead generation both in terms of compound numbers involved and the existing rational approaches for subsequent follow-up and optimization of the initial hits.³ In particular, X-ray crystallography guided optimization of fragments allows one to achieve significant increase in ligand affinity in strikingly few iterations. Success stories of such streamlined fragment-based drug discovery include nanomolar inhibitors of Aurora kinase⁴ as well as heat shock protein 90 (Hsp90),⁵ which have advanced into clinical trials for cancer.

Typically, fragments display weaker (μM to mM range) affinity to a biological target compared to traditional HTS hits. However, given their much smaller molecular weight, the ligand efficiency of fragment hits (i.e. binding energy per atom)⁶ is quite high. In designing new fragments, one should take into account certain guidelines restricting molecular weight (<300 Da) as well as other calculated properties of the future fragments (the so-called ‘Rule of Three’).⁷ Moreover, fragment compounds should be sufficiently soluble to permit their screening at high concentrations and, ideally, should incorporate privileged⁸ structures. A large number of privileged motifs have been identified to-date⁹ and are suggested as a basis for fragment design.¹⁰ However, many of these cores (in particular, fused aromatic coumarin,

benzothiophene, quinolone, quinoxaline, etc.) are typically not conducive to high solubility of the resulting fragments.

In our program aimed at designing novel fragments for protein affinity screening, attention was devoted to the 2-imidazoline scaffold which has high solubility¹¹ and can be regarded as being privileged. The latter aspect is clearly evidenced by the plethora of biological activities reported for 2-imidazoline-based compounds, such as binding to nicotinic acetylcholine receptors,¹² inhibition of K⁺ channels,¹³ suppression of NF- κ B-mediated inflammatory response,¹⁴ antagonistic modulation of angiotensin II receptors,¹⁵ prominent adrenergic activity,¹⁶ and affinity to specialized imidazoline binding sites (or putative receptors) where discovery of certain subtype (I₁, I₂, I₃) selective modulators can lead to many useful therapeutic applications.¹⁷

2-Substituted 2-imidazolines **1** are very common and can be accessed in a variety of ways from readily available precursors, as recently reviewed by Crouch.¹⁸ We designed a series of 1,2-disubstituted imidazoline fragments **2** where an additional, preferably heteroaromatic moiety would contribute to the protein affinity, while the molecular weight of the resultant compounds would remain low (<300 Da). Electron-deficient nitrogen heterocycles are expected to reduce the basicity (or cationic character¹⁹) of the imidazoline core (thereby reducing chances for non-specific protein binding) and provide opportunities for additional interactions with a protein (Figure 1). Retrosynthetically, **2** can be derived from **1** via heteroarylation of the latter with suitable heteroaromatic halides. Direct nucleophilic substitution of 2-chloroazines with 2-imidazolines has been reported to require the presence of an additional electron-withdrawing (such as nitro²⁰ or carboxamide²¹) substituent in the azine coupling partner, thereby limiting the reaction scope. Transition metal catalyzed *N*-arylation of amidines (2-imidazolines being a special case of a cyclic amidine) has been studied quite extensively, as recently reviewed by Maes et al.²² However, such methodology has not been developed for 2-imidazolines.²³ Herein, we report on the preparation of diversely substituted, fragment-like *N*-heteroaryl 2-imidazolines via Pd-catalyzed Buchwald-Hartwig arylation²⁴ of 2-substituted 2-imidazolines.

A series of 2-substituted 2-imidazolines **1a-k** was synthesized on 10-40 mmol scale from the respective aldehyde precursors via condensation with ethylenediamine (EDA) and treatment with NBS (Scheme 1).²⁵ Compounds **1a-k** were isolated by simple crystallization or chromatographically in good to excellent yields (Table 1).

Having created a range of the starting materials for the target *N*-heteroaryl fragments **2**, we proceeded to identify a suitable *N*-arylation protocol. From the literature review²² on *N*-arylation of amidines, it appeared that methods employing copper-amine complexes to catalyse this reaction are as commonplace as those where palladium(II) complex catalysts are used. However, our attempts to *N*-arylate **1a** with 2-chloropyrazine under such conditions as CuI/DMEDA/K₂CO₃/DMF, 80 °C, 12 h²⁶ or CuI/L-proline/Cs₂CO₃/DMF, 110 °C, 20 h²⁷ led to negligible conversions and the formation of multiple by-products. In selecting an alternative, Pd-catalyzed protocol for the desired *N*-arylation, we were motivated by our prior experience in achieving *N*-arylation of poorly reactive heterocyclic amidines.²⁸ The same

protocol²⁹ was applied to imidazolines **1a-k** and a range of 2-haloazines to prepare a diverse set of the target fragment-like 1,2-disubstituted imidazolines **2a-t** (Scheme 2). The isolated yields after column chromatography were generally good (Table 2).³⁰ Notably, the molecular weights of compounds **2a-t** did not exceed 300 Da thereby making these compounds excellent novel tools for fragment-based drug discovery.³¹

The scope of the Pd-catalyzed³² *N*-(hetero)arylation protocol appears to be limited to highly reactive 2-haloazines. When applied to less reactive 5-bromopyrimidine or 4-cyano-2-fluorophenyl bromide (which we considered sufficiently electron-deficient to participate in the Buchwald-Hartwig coupling, based on literature precedence^{33,34}), the yields of the respective *N*-arylation products **2u-v** were markedly lower (Scheme 3). Simple non-activated aryl halides (iodobenzene, 4-iodobiphenyl) or even 2-haloazines with electron-donating substituents (2-chloro-5-hydroxypyridine, 4-amino-2-chloropyrimidine) failed to undergo the desired reaction.

Finally, we were interested to determine if *N*-heteroaryl imidazolines containing reactive halogen substituents (such as **2c**) could serve as a starting point for introducing further diversity to the novel *N*-heteroaryl 2-imidazoline scaffold. Quite satisfyingly, **2c** could indeed be used to *N*-heteroarylate reactive secondary amines (such as morpholine or hexamethyleneimine) providing high yields of the fragment-like products **3a,b**. Moreover, under the same conditions, **2c** underwent a facile and high-yielding reaction with a second 2-imidazoline providing access to a novel type of heteroarene-linked, non-symmetrical 2-imidazoline dimer, **4** (Scheme 4). Dimeric imidazoline derivatives are of significant interest as potential anti-infective agents.³⁵

In conclusion, we have identified a reliable protocol for *N*-heteroarylation of privileged 2-imidazolines and applied it to the synthesis of a diverse set of novel fragment-like compounds.³⁶ These compounds will be screened for their affinity to therapeutically relevant proteins using Fourier-transform mass spectrometry³⁷ and the results will be reported in due course.

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29. General procedure (0.5 – 3.0 mmol scale) for the preparation of compounds **2a-t**: A thick-glass, screw-capped pressure tube (50 mL) was charged with a suspension of the starting imidazoline (1.0 equiv.), heteroaryl halide (1.1 equiv.) and Cs₂CO₃ (1.0 – 3.0 equiv., an additional equiv. per salt component of the reactants) in toluene (3 mL/mmol). Pd(OAc)₂ (0.02 equiv.) and BINAP (0.4 equiv.) were weighed out into a vial, suspended in toluene (2 mL/mmol) and shaken in a 100 °C oil bath for 2 min. The resulting clear, purple catalyst solution was added at once to the vigorously stirred reaction mixture. The tube was filled with argon, capped, and stirred at 100 °C for 16–20 h. The mixture was cooled, partitioned between EtOAc and water. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography on silica gel using an appropriate gradient of acetone in hexane (or MeOH in CH₂Cl₂) as eluent to provide the target product **2**.
30. Characterization data for representative compounds: **2a** – 85% yield (30% acetone in hexane), clear oil. ¹H NMR (500 MHz, CDCl₃) δ 8.52 (d, *J* = 5.0 Hz, 1H), 7.76–7.81 (m, 2H), 7.45 (m, 1H), 7.16–7.21 (m, 2H), 4.11 (t, *J* = 8.5 Hz, 2H), 3.87 (t, *J* = 8.5 Hz, 2H), 3.00 (d, *J* = 7.0 Hz, 2H), 2.16 (sept, *J* = 7.0 Hz, 1H), 1.01 (d, *J* = 6.5 Hz, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 163.2 (d, *J*_{C-F} = 245.0 Hz), 163.1 (d, *J*_{C-F} = 2.5 Hz), 161.5, 158.5, 158.1, 139.4 (d, *J*_{C-F} = 7.5 Hz), 122.6 (d, *J*_{C-F} = 2.5 Hz), 117.7 (d, *J*_{C-F} = 21.3 Hz), 114.0 (d, *J*_{C-F} = 23.8 Hz), 108.7, 51.6, 48.7, 40.8, 26.1, 22.6; HRMS *m/z* [M+H]⁺ calcd for C₁₇H₂₀FN₄ 299.1667, found 299.1654. **2h** – 91% yield (30% acetone in hexane), waxy solid. ¹H NMR (500 MHz, CDCl₃) δ 8.49 (d, *J* = 4.8 Hz, 2H), 7.94 (s, 2H), 7.60 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.28 (td, *J*_t = 7.7 Hz, *J*_d = 1.5 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.77 (t, *J* = 4.8 Hz, 1H), 4.11 (t, *J* = 9.1 Hz, 2H), 3.98 (t, *J* = 9.1 Hz, 2H), 3.86 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 158.7, 158.2, 157.8, 157.6, 132.2, 129.9, 127.9, 125.6, 120.5, 119.7, 113.2, 111.0, 55.4, 52.0, 48.8; HRMS *m/z* [M+H]⁺ calcd for C₁₆H₁₇N₄O 281.1397, found 281.1384. **2p** – 62% yield (40→50% acetone in hexane), white solid, mp = 123–125 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.06 (d, *J* = 4.6 Hz, 1H), 7.36 (ddd, *J* = 11.6, 8.1, 1.4 Hz, 1H), 7.27 (d, *J* = 1.0 Hz, 1H), 7.04 (ddd, *J* = 8.1, 4.6, 3.4 Hz, 1H), 6.63 (d, *J* = 3.4 Hz, 1H), 6.35 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.08 (m, 2H), 4.01 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 153.1, 151.7 (d, *J*_{C-F} = 257.5 Hz), 145.6, 144.4 (d, *J*_{C-F} = 11.3 Hz), 143.3 (d, *J*_{C-F} = 5.0 Hz), 143.2, 123.9 (d, *J*_{C-F} = 17.5 Hz), 120.8 (d, *J*_{C-F} = 2.5 Hz), 112.4, 111.1, 54.2, 51.6 (d, *J*_{C-F} = 1.3 Hz); HRMS *m/z* [M+Na]⁺ calcd for C₁₂H₁₀FN₃ONa 254.0700, found 254.0692.
31. Although we did not perform quantitative solubility measurements for compounds **2a-t**, all were remarkably soluble in polar organic solvents (CH₂Cl₂, CHCl₃, MeOH, EtOAc).

In order to test for solubility in aqueous medium, we prepared a 100 mM solution of a representative compound **2t** in methanol and added 10 μ L of this solution to a 50x volume of pH 7.2 phosphate buffer solution. There was no observable precipitation.

32. In a control experiment, reaction of **1k** with 2-bromopyrimidine under the general procedure conditions, but in the absence of Pd(OAc)₂, did not produce any of the arylation product **2o**.
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Scheme 1. Preparation of 2-substituted 2-imidazolines.

Scheme 2. *N*-heteroarylation of 2-imidazolines **1a-k**.

Scheme 3. Examples of less reactive haloaromatics.

Scheme 4. Further Buchwald-Hartwig reactions of compound **2c**.

Figure 1. 2-Substituted 2-imidazolines (**1**) and the design of the new 1-heteroaryl 2-imidazoline fragments (**2**).

^a pK_a value was calculated using ACD Labs 6.00 software.

Table 1. 2-Substituted 2-imidazolines **1a-k** prepared in this work.

Compound	Structure	Isolated yield, %
1a		78 ^a
1b		94 ^b
1c		89 ^a
1d		67 ^c
1e		63 ^b
1f		89 ^b
1g		96 ^b
1h		63 ^b
1i		68 ^b
1j		48 ^b
1k		56 ^c

^a Isolated by conversion of the crude product into a hydrochloride salt and crystallization of the latter from MeOH.

^b Isolated by crystallization of the crude product from ethyl acetate-hexanes.

^c Isolated by column chromatography on silica gel using CH₂Cl₂-MeOH-28% aq. NH₄OH (90:9.5:0.5) as eluent.

Table 2. *N*-heteroaryl 2-imidazolines **2a-t** prepared in this work.

Compound	Starting material	Halide	R	HetAr	Isolated yield, %	MW	MS [M+H] ⁺ <i>m/z</i>
2a	1a	Cl			85	298.4	299.3
2b	1i	Br			78	242.3	243.3
2c	1a	Br			93	281.2	282.2
2d	1d	Cl			66	285.4	286.4
2e	1e	Cl			68	263.3	264.3
2f	1f	Cl			54	295.3	296.2
2g	1g	Cl			65	239.3	240.3
2h	1c	Br			91	280.3	281.2
2i	1e	Cl			92	264.3	265.4
2j	1a	Cl			66	280.4	281.4
2k	1a	Cl			78	218.3	219.3
2l	1a	Cl			74	204.3	205.3
2m	1h	Cl			81	239.3	240.4
2n	1h	Cl			76	300.4	301.4
2o	1k	Br			61	268.4	269.4
2p	1b	Cl			62	231.2	232.3
2q	1c	Cl			48	280.3	281.4
2r	1g	Br			85	242.3	243.3
2s	1b	Cl			63	290.3	291.3
2t	1j	Br			93	288.7	289.3