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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1637–1640

Synthesis of N^{τ}-arylhistidine derivatives via direct *N*-arylation

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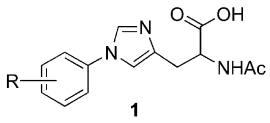
Received 24 December 2003; revised 21 January 2004; accepted 22 January 2004

Abstract—N^{τ}-Aryl-histidine derivatives were synthesized using a modified one-step Cu-catalyzed coupling of aryl halides and *N*-acetylhistidine methyl ester. The latter is much less reactive than imidazole toward aryl halides. *p*-Chloroiodobenzene coupled with iodine displacement only, whereas *m*- and *p*-bromoiodobenzene both gave mixtures of bromo- and iodophenyl products.

1. Introduction

Synthetic *N*-arylimidazole derivatives have shown a number of biological activities of potential therapeutic interest including calcium channel blockade,[1] inhibition of thromboxane synthase,[1,2] phosphodiesterase inhibition,[2,3] inhibition of AMPA receptors,[4] carbonic anhydrase inhibition,[5] and others.[6] *N*-Arylimidazoles also occur naturally in the form of *N*-arylhistidine derivatives. For example, an *N*-arylhistidine moiety has been found to occur at the active site of cytochrome *c* oxidase,[7] and *N*(τ)-(*p*-bromophenyl)-histidine (*desacetyl*-**1a**) can be isolated from hydrolysates of liver proteins from rats treated with the hepatotoxin bromobenzene.[8]

Our laboratory has been interested in characterizing the chemical steps that underlie the metabolic activation, protein covalent binding, and subsequent hepatotoxicity of bromobenzene as a model for small, pharmacologically innocuous but nevertheless hepatotoxic aromatic compounds.[9,10] To facilitate our efforts to identify specific cellular protein targets for cytotoxic arene oxide metabolites of bromobenzene we needed a source of **1a** and several closely related analogues with which to raise anti-**1a** antibodies and characterize their selectivity.



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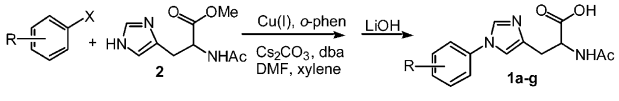
a, R = p-Br
b, R = m-Br
c, R = H
d, R = p-Cl
e, R = p-CH_3
f, R = m-CH_3
g, R = p-OCH_3

The synthesis of *N*-arylimidazoles often involves construction of the imidazole ring onto an aniline-type nitrogen,[6] but this is not practical for the synthesis of **1** and its analogues. We previously reported that *N*-arylation of *N*-Ac-L-His-OMe (**2**) with 4-fluoronitrobenzene led in 4 steps to an 8% yield of **1a**,[11] but this approach is not sufficiently general for many analogues of **1** (e.g., **1b** and **1e–g**). More recently metal-catalyzed procedures for coupling imidazoles directly to arylboronic acids,[12] aryllead derivatives[13] or unactivated aryl halides[14,15] have been described. While the latter copper-catalyzed procedure is directly applicable to simple imidazole derivatives, we found that it fails completely when applied to more complex imidazoles such as **2**. To take advantage of the potential generality of this direct coupling, we modified both the reaction conditions and the workup to obtain the histidine derivatives **1a–g** listed above in useful yields (Scheme 1).

Kiyomori et al.[15] described a modified Ullmann-type coupling of simple imidazoles (3 mmol) with aryl iodides or-bromides (2 mmol) using $Cu(OTf)_2 \cdot PhH$ (0.2 mmol) as a catalyst in the presence of *o*-phenanthroline (*o*-phen, 2 mmol), dibenzylideneacetone (dba, 0.1 mmol) and cesium carbonate (2.2 mmol) in hot xylene; direct flash chromatography afforded clean products in 65–99% yield. Our initial attempts to couple **2** with an excess of iodobenzene using the conditions described gave no apparent product formation, but since it



Scheme 1.

Keywords: *N*-Arylation; Aryl halides; Histidine derivatives; Ullmann-type coupling.
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doi:10.1016/j.bmcl.2004.01.058

appeared that **2** was not soluble in xylene, we changed the solvent to 20 vol% DMF in xylene. Under these conditions, product formation could be detected by TLC and NMR. However, separation of the arylhistidine product from the large excess of *o*-phen ligand by chromatography proved very difficult because the arylhistidine derivatives are much more polar than the simple arylimidazoles described by Kiyomori et al. Thus, after partial purification by silica gel chromatography (hexane/ethyl acetate, 1:1), additional *o*-phen was removed by concentration, crystallization and filtration of solid *o*-phen. Preparative TLC of the mother liquor afforded the methyl ester of **1c** in 12% yield. Hydrolysis of the ester in LiOH solution followed by acidification generated acid **1c**, which was very difficult to extract from aqueous solution at any pH. Hence it was adsorbed from aqueous solution at pH 5 onto a column of C-18 bonded reverse-phase silica gel. The column was washed with water to remove salts, and eluted with methanol to obtain **1c** in 78% yield. The couplings of *m*- and *p*-iodotoluene to **2** gave similar overall yields of **1e** and **1f**.

For the coupling reactions of *p*-chloroiodobenzene and *p*-iodoanisole with **2** we further modified the workup to involve simple partitioning of the reaction mixture between brine and methylene chloride, saponification of the extracted material with a slight excess of LiOH, extraction of *o*-phen with ethyl acetate at high pH, and solid phase extraction of the desired acid using C-18 bonded reverse-phase silica gel. In this way we obtained **1d** and **1g** in a single-step process.

In the synthesis of **1d** from *p*-chloroiodobenzene, the selectivity for displacement of iodine was complete; no sign of the analogous iodine-containing coupling product was detected. Unfortunately, such was not the case with *p*-bromoiodobenzene. Coupling of the latter with **2** afforded **1a** contaminated with ca. 15% of the corresponding *p*-iodo-analogue which could not be separated from **1a** by normal- or reverse-phase chromatography or recrystallization. To obviate the halogen selectivity issue we attempted to couple *para*- and *meta*-dibromobenzene with **2**. No coupled products were formed in either case, although in our hands these halides did undergo coupling with imidazole itself, affording the respective bromophenylimidazole derivatives in 30–50% yields (as assessed by GC/MS). Apparently the imidazole ring of the histidine partner is less reactive than imidazole itself. The reduced reactivity of **2** versus imidazole may result from intramolecular hydrogen bonding or interaction of the sidechain with the catalyst; it is probably not simply steric in origin since 4-methylimidazole and benzimidazole couple to aryl halides as efficiently as imidazole.[15] Thus to obtain **1a** free from contamination, we had to resort to our original method based on *p*-nitrofluorobenzene. Since this route was not amenable to synthesis of meta isomer **1b** we had to resort to coupling **2** with *m*-bromoiodobenzene, which gave a product containing ca. 15% of the *m*-iodo analogue as an impurity (as determined by ^{1}H NMR).

For generation of antibodies against **1a**, two New Zealand white rabbits were immunized with a KLH conjugate of **1a**. After three secondary immmunizations, both rabbits produced an antiserum of high titer ($\geq$1:128,000; data not shown) toward **1a** conjugated to RNase as a coating antigen. To evaluate the selectivity of the antiserum for **1a** versus related structures, an important concern for its intended biological applications, competitive ELISA assays were used. As shown in Figure 1, the original hapten **1a** was most potent in blocking antigen binding sites (IC_{50} $\sim$1 μM). Related compounds **1b–g** are recognized less efficiently as their *N*-aryl moiety becomes less similar to the p-bromophenyl moiety in **1a**. Interestingly, simply moving the bromine atom from the *para* to the *meta* position (i.e., **1b**) caused a drastic decrease in its recognition by antibodies to **1a**. Replacement of the histidine moiety of **1a** with lysine (as in N^{ε}-(*p*-brommophenyl)-N^{α}-Ac-L-lysine, **3**[16]) or cysteine (as in *S*-(*p*-bromophenyl)-*N*-Ac-L-cysteine, **4**[17]) also greatly reduced antibody recognition. These findings are quite significant for the use of the antiserum against **1a** as a probe for adduct structures in proteins from the livers of bromobenzene-treated rats, because metabolically-generated bromobenzene-3,4-oxide also alkylates cysteine and lysine residues of proteins in situ,[8] and at least in principle could generate both *meta* and *para*-bromophenyl derivatives of all three side chains. Additional experiments showed that the following compounds inhibited the ELISA assay only poorly at the highest concentration tested (100 μM): *N*-(*p*-bromophenyl)-imidazole, 48%; *p*-bromoaniline, 12%; *p*-bromophenol, 16%; *N*-acetylhistidine, 15%. Thus, the antiserum raised against KLH-**1a** is both potent and selective for the original hapten, and suitable for use as a selective probe of covalent binding of bromobenzene-3,4-oxide to histidine residues of rat liver proteins.

In summary, whereas copper-catalyzed coupling of aryl iodides and bromides to imidazole and related simple heterocycles gives good to excellent yields of the desired *N*-arylheterocycles,[14,15,18] attempts to apply this method to histidine derivative **2** were somewhat less rewarding. Product isolation was more complicated because the increased polarity of the product made it difficult to separate from the catalyst ligand *o*-phen (which is actually

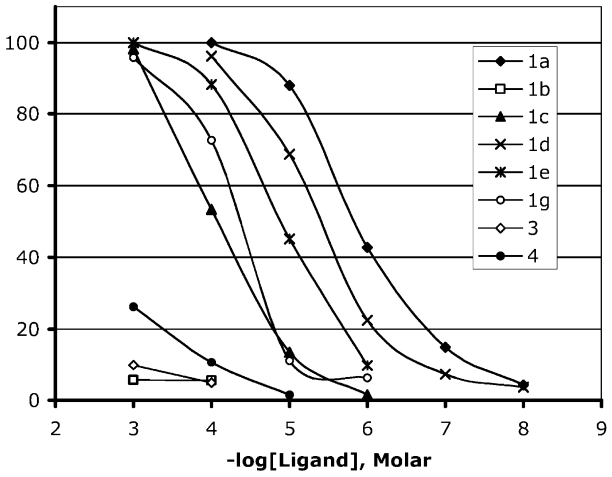

Figure 1. Inhibition of binding of anti-**1a** antibodies to RNase-**1a** conjugate by **1a** and analogues in a competitive ELISA format.

used in stoichiometric excess over the coupling substrates). Other factors, such as the potential for interaction of the protected amino acid side chain with the copper, may also have contributed to the lowered yields. With **2** as the imidazole partner, only aryl iodides were sufficiently reactive as arylating agents to couple successfully, and while *p*-chloroiodobenzene reacted selectively at the aryl-iodine bond, both *p*- and *m*-bromoiodobenzene afforded bromophenyl products contaminated with ca. 15% of the corresponding iodophenyl analogues. In all cases the exclusive regioselectivity of substitution on the imidazole ring was favorable, and the one-step coupling allowed direct access to several compounds (i.e., **1e–g**) that would not have been available using the original *p*-nitrofluorobenzene approach to **1a**. A KLH conjugate of **1a** generated a high-titer immune response in two rabbits and the polyclonal antiserum proved to be highly selective for hapten **1a** versus its cysteine or lysine analogues, its meta isomer **1b**, or other related structures such as **1c–g**.

2. General procedure for synthesis of N^{τ}-aryl-N^{α}-acetylhistidine derivatives

An oven dried 16×100 mm culture tube was charged with $(CuOTf)_2{\cdot}PhH$ (26 mg, 0.05 mmol), 1,10-phenanthroline (135 mg, 0.75 mmol), dibenzylidene acetone (13 mg, 0.055 mmol), and Cs_2CO_3 (270 mg, 0.83 mmol), *N*-Ac-histidine methyl ester (160 mg, 0.75 mmol) and aryl iodode (240 mg, 1.13 mmol). A solvent of 20 vol-% DMF in xylene (0.5 mL) was added, and the tube was purged with nitrogen for 1 min before sealing with a Teflon-lined screw-cap. The mixture was heated at 115 °C for 48 h. After cooling to room temperature, saturated aqueous NH_4Cl solution was added, the mixture was extracted 2–3 times with methylene chloride, and the latter back-washed with brine, water and dried over Na_2SO_4. The solvent was evaporated and the brown residue was subjected to flash chromatography over gel using 0–5% MeOH in $CHCl_3$. The eluent was concentrated to an oil and hydrolyzed with excess 1 M LiOH for 20 h at room temperature, after which residual *o*-phen was removed by extraction with ethyl acetate. The aqueous solution was acidified to pH 5 and passed through a short column of C-18 bonded phase silica gel (ca. 28×65 mm). The column was washed with several column volumes of water and then eluted with methanol. Evaporation of the methanol yielded the *N*-arylhistidine derivatives (12–15% overall) as glassy rather than crystalline solids. However, their excellent purity was ascertained using both ^{1}H- and ^{13}C NMR in combination with electrospray (ESI) MS. For *N*-arylhistidines **1a–g** the collision-induced fragmentation behavior of the respective MH^+ species was consistent for all congeners and included prominent peaks at M-42, M-46, M-88, and M-103.

2.1. N^{α}-Acetyl-N^{τ}-(*m*-bromophenyl)-L-histidine (1b)

Attempted couplings of **2** with *m*-dibromobenzene failed but using *m*-bromoiodobenzene afforded a mixture of **1b** and its *m*-iodo analogue (ca. 85:15). (This small amount of *m*-iodo analogue would not interfere with its use in competitive ELISA assays aimed at demonstrating antibody spcificity). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 8.01–7.20 (m, 6H), 4.54 (m, 1H), 3.13 (m, 2H), 1.91 (s, 3H); ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 178.4, 173.0, 141.2, 139.8, 136.3, 132.9, 131.6, 124.9, 124.4, 120.8, 117.0, 56.4, 32.2, 23.0. ESI-MS: $MH^+ = 352$.

2.1.1. N^{α}-Acetyl-N^{τ}-phenyl-L-histidine (1c). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 8.04 (s, 1H), 7.53 (m, 4H), 7.38 (m, 2H), 4.62 (m, 1H), 3.23 (m, 2H), 1.94 (s, 3H); ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 177.3, 172.9, 140.5, 138.7, 136.1, 131.2, 128.7, 122.2, 117.2, 55.7, 32.1, 22.9. ESI-MS: $M^+H = 274$; EI–MS: $M^+ = 273$.

2.1.2. N^{α}-Acetyl-N^{τ}-(*p*-chlorophenyl)-L-histidine (1d). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 7.98 (s, 1H), 7.49 (m, 4H), 7.30 (s, 1H), 4.55 (m, 1H), 3.12 (m, 2H), 1.91 (s, 3H); ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 178.2, 172.7, 141.2, 137.4, 134.0, 131.2, 130.2, 123.5, 117.0, 56.2, 32.4, 23.0. EI–MS: $(M\text{-}COOH)^+ = 263$.

2.1.3. N^{α}-Acetyl-N^{τ}-(*p*-tolyl)-L-histidine (1e). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 7.90 (s, 1H), 7.28 (m, 5H), 4.56 (m, 1H), 3.15 (m, 2H), 2.35 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 178.3, 172.6, 140.9, 138.7, 136.4, 135.9, 131.6, 122.0, 117.1, 56.3, 32.5, 23.0, 21.7. ESI-MS(+): $MH^+ = 288$; FAB MS: $(M + Li)^+ = 294$; EI–MS: $(M\text{-}COOH)^+ = 242$.

2.1.4. N^{α}-Acetyl-N^{τ}-(*m*-tolyl)-L-histidine (1f). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 7.95 (s, 1H), 7.34–7.15 (m, 4H), 7.14 (s, 1H), 4.57 (s, br, 1H), 3.11 (m, 2H), 2.38 (s, 3H), 1.93 (s, 3H). ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 178.3, 172.7, 141.6, 140.8, 138.7, 135.9, 130.9, 129.3, 122.6, 119.1, 117.1, 56.2, 32.4, 23.0, 21.5. ESI-MS: $MH^+ = 288$.

2.1.5. N^{α}-Acetyl-N^{τ}-(*p*-methoxyphenyl)-L-histidine (1g). ^{1}H NMR (400 MHz, CD_3OD), δ ppm 7.78 (s, 1H), 7.29 (d, 2H), 7.14 (s, 1H), 6.91 (s, 2H), 4.47 (m, 1H), 3.69 (s, 3H), 3.02 (m, 2H), 1.84 (s, 3H); ^{13}C NMR (100.6 MHz, CD_3OD), δ ppm 178.2, 172.7, 160.6, 140.5, 136.1, 132.0, 123.8, 117.6, 116.1, 56.2, 56.1, 32.4, 23.0. EI–MS: $(M\text{-}COOH)^+ = 258$.

2.2. Antibody methods

Using standard methods described previously[19,20] we coupled **1a** to keyhole limpet hemocyanin (KLH) to generate an immunogen, and to ribonuclease A (RNase) to create a target antigen for ELISA assays. Two New Zealand rabbits were immunized with the KLH conjugate followed by three boosts 3–4 weeeks apart. Blood was collected, clotted and centrifuged to obtain serum which was aliquoted and stored at −80 °C. The titer was determined by dilution ELISA. The selectivity of the antiserum from rabbit A was further evaluated by means of competition ELISA assays in which diluted serum (1:128,000) was pre-incubated overnight at 4 °C with various concentrations of **1a** or related ligands.

Acknowledgements

We thank Mr. David Brooks for assistance with antibody preparation. This work was supported in part by NIH grant GM-21784.

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