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Bioorganic & Medicinal Chemistry Letters 16 (2006) 387–390
Bioorganic & Medicinal Chemistry Letters

Synthesis of *N*-benzylated-2-aminoquinolines as ligands for the Tec SH3 domain

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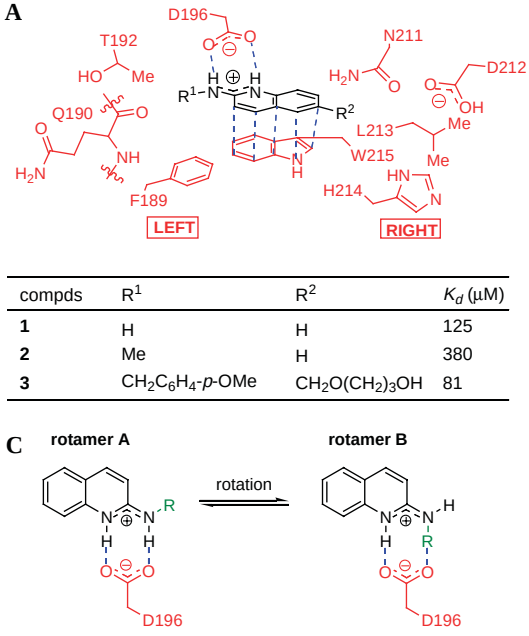
Received 7 April 2005; revised 23 September 2005; accepted 27 September 2005
Available online 2 November 2005

Abstract—In recent work, we have been developing 2-aminoquinolines as ligands for Src Homology 3 (SH3) domains, so far the only reported examples of small-molecule ligands for these domains. In this paper, we report the synthesis of a series of *N*-benzylated-2-aminoquinolines by reductive amination of aryl aldehydes with 2-aminoquinoline. These ligands bound the SH3 domain with ca. one and a half to twofold reduced affinity relative to 2-aminoquinoline; however, some evidence was found to suggest that the benzylic substituents made new contacts with the SH3 domain surface. These results provide useful SAR information that may assist in future ligand design.

Src Homology 3 (SH3) domains are small, non-catalytic protein–protein interaction domains that bind to proline-rich peptides and feature within a range of important cell signaling events and other biological processes (see, Mayer[1] or Zarrinpar[2] for reviews). These domains have long been targets for the development of potential therapeutics, however until recently, no examples of entirely non-peptide ligands for the domain had been reported. Previously, we have reported that 2-aminoquinolines bind the Tec SH3 domain with weak to moderate affinity.[3] The mechanism of the binding of 2-aminoquinoline **1** has been well studied and involves. π–π stacking between the quinoline ring and tryptophan 215 (W215) and a salt bridge between the protonated ligand and aspartate 196 (D196), both highly conserved residues in the proline-rich peptide binding site of the Tec SH3 domain (Fig. 1A).

The highest affinity 2-aminoquinolines identified so far are all 6-substituted ligands with bulky hydrophobic substituents that are thought to make contacts with regions on the 'right' side of the ligand binding site as illustrated in Figure 1A.[3] However, currently little information is known about the types of ligand/protein contacts that may be formed between 'left' surface residues,



compds	R^1	R^2	K_d (μM)
1	H	H	125
2	Me	H	380
3	$CH_2C_6H_4$-*p*-OMe	$CH_2O(CH_2)_3OH$	81

Figure 1. (A) Model for mechanism of binding of 2-aminoquinoline to the Tec SH3 domain, and regions adjacent to both the 'left' and 'right' sides of the binding site, predicted to make contacts with substituents attached to the 2-aminoquinoline nucleus. (B) Summary of some ligand binding results reported previously. [Ligands **1** and **2**, see Ref. 3; ligand **3**; see Ref. 4]. (C) Chemical considerations that assist in the explanation for why *N*-substituted-2-aminoquinolines bind the SH3 domain with reduced affinity.

Keywords: Src Homology 3 domain; 2-Aminoquinoline; Ligand.
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doi:10.1016/j.bmcl.2005.09.073

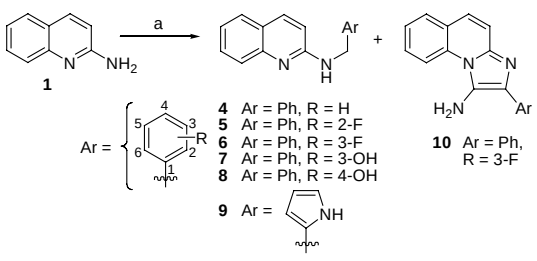
and ligand-substituents placed at other parts of the 2-aminoquinoline platform, for example the amino group as illustrated in Figure 1A. Substitution on the amino nitrogen atom of 2-aminoquinoline with a methyl group (ligand **2**) resulted in a ca. threefold reduction in affinity[3] (Fig. 1B). This may be explained by considering the rotamers that exist around the HRN–C(1) bond, and the formation of the salt bridge involved in the binding of 2-aminoquinolines to the SH3 domain (Fig. 1C). In **1**, (when R = H), both rotamers can be involved in the formation of an 'ideal' salt bridge with D196. But when R = Me (as in **2**), only one rotamer interacts favorably with D196. Thus, there is an entropic cost associated with the binding of **2** to the SH3 domain, leading to lower affinity. However, as part of our synthetic investigations into new methods for the preparation of 2-aminoquinolines, some 6-substituted-2-(4-methoxybenzyl)aminoquinolines were prepared and one of these, compound **3**, was tested for binding to the Tec SH3 domain. [4] The affinity of this ligand (Fig. 1B) was suggestive that the penalty for substitution on the amino nitrogen atom was less severe when the substituent was a 4-methoxybenzyl group, than when the substituent was a methyl group alone. Therefore, this prompted us to further investigate the influence of *N*-benzylation of 2-aminoquinoline on its SH3 binding affinity, and this is the focus of the present study.

A one-pot method for the synthesis of *N*-benzylated-2-aminoquinolines from aryl aldehydes and 2-aminoquinoline was sought. Thus, the method of Mattson[5] involving titanium tetraisopropoxide assisted reductive alkylation of amines was adapted for use with 2-aminoquinoline and aryl aldehydes as illustrated in Scheme 1. By this method, the appropriate aldehyde was stirred with 2-aminoquinoline in titanium tetraisopropoxide for ca. 1 h, prior to the addition of sodium cyanoborohydride in ethanol. In cases where the starting aldehydes were solids, additional titanium tetraisopropoxide and/or THF were added to the mixture to assist in stirring. Using this approach, the *N*-benzylated derivatives **4–9** were prepared in low to moderate yields (3–46%) (Scheme 1). The poor yields were in part attributed to the formation of a by-product each time, the imidazo[1,2-*a*]quinolin-1-ylamine derivatives (see the 3-fluorophenyl derivative **10** in Scheme 1). These by-products were generally not isolated in a pure form, but in the case of the 3-fluorophenyl derivative **10**, pure **10** was isolated in 10% yield and its complete characterization was possible.

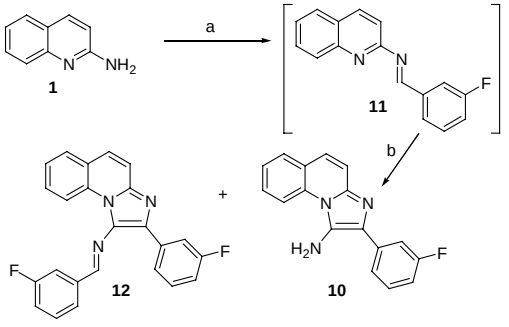
The formation of this structure most likely occurs due to nucleophilic attack by cyanide (from sodium cyanoborohydride) on the imine intermediate **11**, leading to the formation of the new cycle. The formation of the structure was accompanied by a very large downfield change in the ^{1}H NMR chemical shift for H8 of the quinoline ring in 2-aminoquinoline **1** from ca. 7.8 to 9.1 ppm in **10** (H9 in **10**). This is thought to be a result of an anisotropic deshielding effect provided by the partial sp^2 character of the N–C bond at the amino group. The assignment of the signal at 9.1 ppm to H9 was confirmed by the presence of a cross-peak between the 1-amino protons and H9 in the [^{1}H,^{1}H] ROESY NMR spectrum.

Only one example of this heterocyclic skeleton has been previously reported,[6] however, no spectral data were provided to support its structure. Thus, in order to further validate the formation of the structure, **10** was explicitly synthesized by an independent method. Initially, it was attempted to prepare **10** by adapting the one-pot titanium tetraisopropoxide assisted method, but instead using sodium cyanide rather than sodium cyanoborohydride. However, a complex mixture of products was isolated after workup in this case. Instead, as illustrated in Scheme 2, the imine intermediate **11** was prepared first by heating the aldehyde with 2-aminoquinoline in toluene at reflux. This imine was then treated with sodium cyanide in methanol at ca. 50 °C to form **10** in 33% overall yield from 3-fluorobenzaldehyde. A small amount (4%) of a second imine **12** was also isolated following chromatography with silica gel, indicating that some hydrolysis of imine **11** occurred during the second step, and the reformed aldehyde was then condensed with the amine **10** to form **12**.

As has been previously demonstrated in the synthesis of 6-substituted-2-(4-methoxybenzyl)aminoquinolines, [4] *N*-benzylated-2-aminoquinolines could also be synthesized from 2-chloroquinolines by treatment with the appropriate benzylamine. Thus, in order to demonstrate an alternative and much improved method for the synthesis of the desired *N*-benzylated-



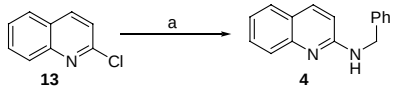
Scheme 1. Reagents and conditions: (a)—(1) ArCHO, Ti(^{i}OPr)$_4$, (THF), rt. (2) $NaBH_3CN$, EtOH. (Experimental details are provided as Supplementary data.)



Scheme 2. Reagents and conditions: (a) 3-Fluorobenzaldehyde/toluene/Δ; (b) NaCN/MeOH/ca. 50 °C. (Experimental details are provided as Supplementary data.)

2-aminoquinolines in the current study, the derivative **4** was also prepared in 97% yield by treatment of 2-chloroquinoline **13** with benzylamine at ca. 140 °C for 30 h as illustrated in Scheme 3.

The *N*-benzylated-2-aminoquinolines **4–9** were all tested for binding to the Tec SH3 domain using NMR chemical shift perturbation, with [^{1}H,^{15}N] HSQC experiments using ^{15}N labeled Tec SH3 protein, to obtain K_d values. The use of this method in the current ligand development studies has been documented.[3] Compounds **4–9** all bound the Tec SH3 domain with approximately one and a half to twofold reduced affinity relative to 2-aminoquinoline **1** (K_ds ca. 180–300 μM for **4–9**, K_d = 125 μM for **1**, Table 1) (see for **4** and **7** in Fig. 2). However, all of these ligands bound with improved affinity relative to the *N*-methylated-2-aminoquinoline derivative **2** (K_d = 380 μM) (Table 1). In the case of ligands **4** and **6**, the improvement in affinity was approximately twofold relative to **2**.



Scheme 3. Reagents and conditions: (a) benzylamine/ca. 140 °C.

Table 1. Equilibrium binding dissociation constants (K_d) for *N*-benzylated-2-aminoquinoline derivatives with the Tec SH3 domain, determined by NMR chemical shift perturbation experiments, and comparison with K_ds from previous studies

Compound	K_d (μM)[a]
4	193(±15)
5	208(±28)
6	177(±34)
7	234(±55)
8	292(±85)
9	285(±46)
2	380(±40)[b]
1	125(±24)[b]

[a] Quoted values are means ± standard deviation over residues whose ^{1}H (H–N) chemical shifts were altered by at least 0.1 ppm at the maximum concentration of ligand.
[b] As reported in Inglis et al.[3]

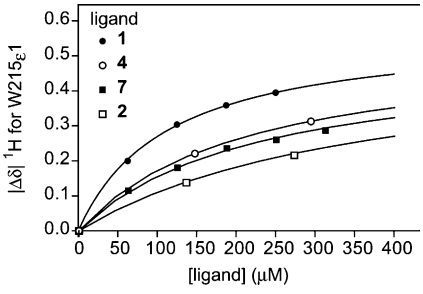


Figure 2. Equilibrium binding of *N*-benzylated-2-aminoquinolines **4** and **7**, and comparison with 2-aminoquinoline **1** and the *N*-methylated derivative **2** (from previous studies)[3] to the Tec SH3 domain as studied by NMR spectroscopy. Overlays of isotherms obtained from independent experiments, represented by the change in ^{1}H (H–N) chemical for the indole H–N proton of tryptophan 215 (W215ε1).

The SAR information provided above confirms that the larger benzyl substituents of ligands **4–9** are better tolerated than the methyl substituent of ligand **2**, suggesting that a new lipophilic contact is formed that mediates the improvement in affinity. This lipophilic contact is able to partially offset the entropic costs that are likely to be associated with the ca. threefold reduction in affinity due to *N*-methylation of 2-aminoquinoline (discussed above and see Fig. 1c). Of the *N*-benzylated ligands, the variation in the affinities may be explained by the different substituents on the phenyl ring. The small inductively withdrawing fluoro substituents of ligands **5** and **6** have little influence on the affinity, as does the 3-hydroxy substituent of **7**, suggesting that substitution at these positions with simple groups is well tolerated. The differences in the electronic character of the phenyl rings of ligands **5**, **6**, and **7** do not appear to significantly alter the affinities. However, substitution with a hydroxyl group at the 4-position as in **8** leads to a small reduction in affinity, suggesting that substitution at this position is less well tolerated, possibly due to either steric or electronic penalties. The reduced affinity of ligand **9** that has a 5-membered electron-rich aryl group may be a result of the smaller size of the ring, and hence a reduced lipophilic contact area.

The above results also suggest that the strongly electronegative fluorine atoms of ligands **5** and **6** do not have a significant impact on the affinity of the ligands. It may also be concluded that the hydroxyl groups on the phenyl rings of ligands **7** and **8** are not involved in 'ideal' hydrogen bonding, as significantly greater improvements in affinity would have been expected if this were the case.

Chemical shift mapping of all residues whose ^{1}H (H–N) resonances were altered by at least 0.1 ppm at the maximum concentration of ligand **4** revealed a similar binding footprint to 2-aminoquinoline **1**, confirming that both ligands bind at the same location of the SH3 domain. Furthermore, additional changes in chemical shift were observed for residues alanine 191 (A191, upfield) and glutamine 190 (Q190, downfield), 'left' of the 2-aminoquinoline binding site, attributed to anisotropic shielding and deshielding effects, respectively, provided by the phenyl ring of **4** (see Supplementary data). This provides additional evidence for the positioning of the phenyl ring and suggests that it indeed interacts with 'left' surface residues. An obvious candidate residue in the region likely to mediate the interaction is the phenyl ring of phenylalanine (F189) (Fig. 1A), which may form a π–π stack with the phenyl ring of the benzyl substituent of the ligand. Implicating F189 in the interaction also assists in the explanation for why the penalty for substituting the amino nitrogen atom with a methyl group (as in ligand **2**) is greater than when bulkier substituents are present (as in ligands **4–9**). The lipophilic contact area of **2** is considerably smaller, resulting in a very small, if any, lipophilic interaction.

In summary, a small series of *N*-benzylated-2-aminoquinolines was synthesized by adapting a reported method[5] for the titanium tetraisopropoxide assisted reductive

alkylation of amines with carbonyl compounds. This method poorly suited the synthesis of the desired amines presented here, because of low yields. However, the low yields were partly accounted for by the formation of the corresponding imidazo[1,2-*a*]quinolin-1-ylamine by-products, an uncommon heterocyclic scaffold, in yields of ca. 10% each time the reaction was performed. *N*-Benzylated-2-aminoquinolines can instead be prepared in much improved yields by synthesis from 2-chloroquinolines by treatment with benzylamines, as was demonstrated by the alternative synthesis of derivative **4**.

Although all the compounds **4–9** bound the Tec SH3 domain with ca. one and a half to twofold reduced affinity relative to 2-aminoquinoline **1**, they all bound with improved affinity relative to the *N*-methylated-2-aminoquinoline compound **2**. This suggests that the bulkier benzyl substituents make new lipophilic contacts, but the overall affinity of the ligand is lower than 2-aminoquinoline **1**, probably as a result of substitution on the amino group. This provides useful SAR information and may assist in future ligand design. Particularly, it would be of interest to prepare 2-aminoquinolines with bulky lipophilic substituents that may target the 'left' surface residues in a similar fashion to the *N*-benzylated ligands, but instead attach the substituent at other points of the 2-aminoquinoline nucleus, for example the 3- or 4-position of the quinoline ring. Thereby, the primary amino group, involved in the important salt bridge with D196, could be retained.

Supplementary material

The experimental details of synthetic procedures presented in this paper together with chemical shift mapping of ligands **1** and **4** onto the SH3 fold are provided as Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.09.073.

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