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Synthesis of highly substituted dibenzo[*b*,*f*]azocines and their evaluation as protein kinase inhibitors

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Abstract—Synthetic routes towards highly substituted eight membered ring heterocycles fused to aryl rings such as the dibenzo[*b*,*f*]azocine system are still lacking. Herein, we present a convenient convergent synthetic route towards this heterocyclic class of compounds with possible variations at positions 4, 7, and 11. One member of a library of dibenzo[*b*,*f*]azocines with different substituents at position 11 was identified to inhibit protein kinase A activity (IC_{50} = 122 μM) but not protein kinase C.

Medium-size heterocycles fused to aryl rings are found in many drugs and preclinical leads. An important scaffold is the dibenzo[*b*,*f*]azepine present in psychotropic drugs like Imipramine, Desipramine and Bonnecore.[1] While the mechanism of action of this class of compounds has been associated with protein kinase activity, especially cAMP-dependent kinase (PKA) and phospholipid-dependent protein kinase C (PKC),[2] little is known about their mechanism of influence on the signal transduction mediated by these kinases.[3,4] Recently, we described a novel strategy to synthesize these heterocycles along with the synthesis of dibenzo[*b*,*f*]azocines.[5] In order to explore their potential activity on PKA and PKC, we produced and tested a focused library utilizing this route. We used isoquinolinesulfonyl based protein kinase inhibitors H-89[6] and H-7[7] as controls for the activity studies as they have been exhaustively studied and are known to inhibit the ATP binding site of the PKs (Fig. 1).[8] Closely related heterocycles have also been applied as inhibitors of neuronal Na^+ channels,[9] ligands for human EP_1 prostanoid receptor[10] and arginine vasopressin antagonists.[11]

The dibenzo[*b*,*f*]azocine scaffold was synthesized using precursor **4** and **8** (Schemes 1 and 2).

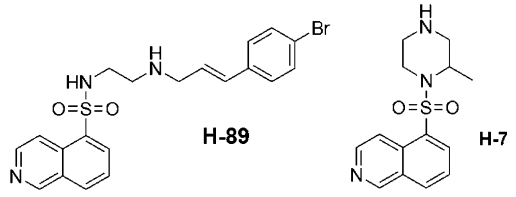
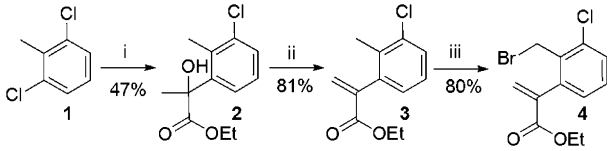


Figure 1. Protein kinase inhibitors H-89 and H-7.



Scheme 1. Synthesis of **4**. Reagents and conditions: (i) a—Mg (2 equiv), 1,2-dibromoethane (cat.), THF, 60 °C, 12 h; b—ethyl pyruvate (1 equiv), THF, 0–25 °C, 3 h; (ii) P_2O_5 (2 equiv), benzene, 80 °C, 8 h; (iii) *N*-bromosuccinimide (1.5 equiv), benzoyl peroxide (cat.), CCl_4, 80 °C, 18 h.

Commercially available 2,6-dichlorotoluene was converted into the corresponding mono Grignard reagent in the presence of magnesium metal and 1,2-dibromoethane as an activating agent. Addition of ethyl pyruvate at 0 °C gave the tertiary alcohol **2** in 60% yield. The dehydration of **2** was carried out using P_2O_5 in benzene giving analytically pure **3** in good yield. Subsequent benzylic bromination of **3** with NBS gave **4** in 80% yield.

The synthesis of **8** was realized in three steps with an overall yield of 41% (Scheme 2). This synthesis includes

Keywords: Dibenzo[*b*,*f*]azocine; Kinase inhibitor.
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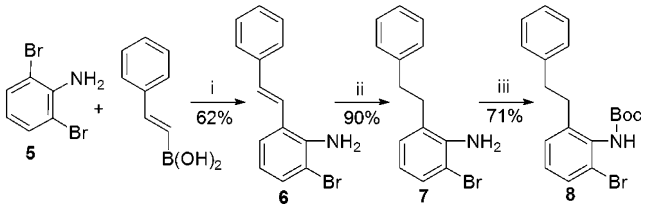
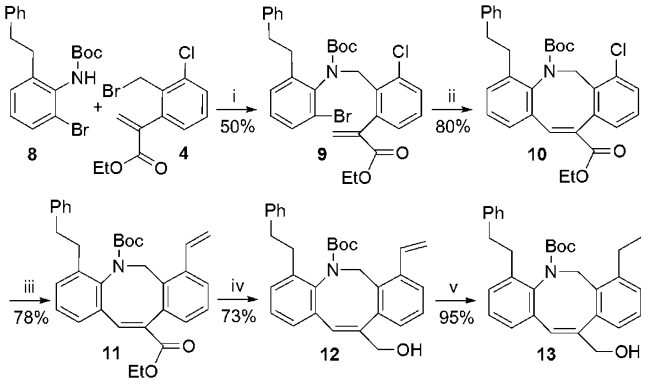


Scheme 2. Synthesis of **8**. Reagents and conditions: (i) Na_2CO_3 (5 equiv), Bu_4NBr (1 equiv), $Pd(PPh_3)_4$ (0.015 equiv), water/toluene (1:2), 100 °C, 18 h; (ii) 10% Pt/C (0.02 equiv of Pt), H_2 (36 psi), ethyl acetate, rt, 14 h; (iii) a—sodium hexamethyldisilazide (2.2 equiv), THF, rt, 15 min; b—Boc-anhydride (1.1 equiv), rt, 3 h.

the introduction of a 2-phenylvinyl substituent under Suzuki coupling reaction conditions. Because of the great variety of commercially available boronic acid derivatives this reaction is well suited for the introduction of various substituents. Unprotected 2,6-dibromoaniline and *trans*-2-phenylvinylboronic acid reacted in a two-phase system (water/toluene) using the phase-transfer catalyst Bu_4NBr. In the presence of 1.5 mol% tetrakis-(triphenylphosphine)palladium compound **6** could be isolated in 62% yield after 18 h at 100 °C. The major side products were the dialkylated aniline (15%) and unreacted starting material (15%). Subsequent hydrogenation of **6** in the presence of a Pt/C catalyst gave **7** in very good yield. Protection of the aniline with di-*tert*-butyldicarbonate was realized by using two equivalents of NaHMDS, a sterically hindered strong base.[12] Compound **8** was isolated in 71% yield.

The synthesis of dibenzo[*b*,*f*]azocine **13** was carried out in five steps using compounds **4** and **8** as starting materials (Scheme 3). Deprotonation of the aniline **8** in the presence of sodium hydride followed by the slow addition of **4** at −50 °C gave **9** in 50% yield. One side reaction is the debromination of **4** which became more dominant at higher temperature. The intramolecular Heck reaction of **9** in the presence of palladium acetate, tetraethylammonium chloride and dicyclohexylmethyl-

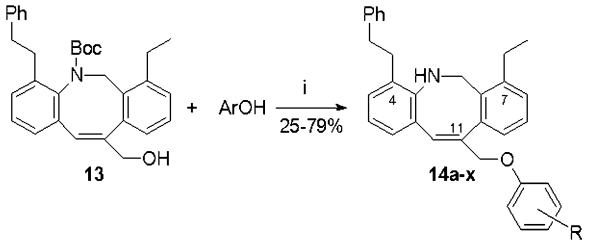


Scheme 3. Synthesis of **13**. Reagents and conditions: (i) a—**8** (1 equiv), NaH (1.1 equiv), dimethylformamide, rt, 1 h; b—**4** (1.1 equiv), −50 °C to rt, 18 h; (ii) $Pd(OAc)_2$ (0.03 equiv), Et_4NCl (1 equiv), Cy_2NMe (1.5 equiv), *N*,*N*-dimethylacetamide, 90 °C, 6 h; (iii) tributyl(vinyl)tin (1 equiv), $Pd(t\text{-}Bu_3)_2$ (0.045 equiv), CsF (2 equiv), dioxane, 100 °C, 16 h; (iv) DIBAL-H (2 equiv), toluene, −78 °C to rt, 3 h; (v) 10% Pt/C (0.07 equiv of Pt), H_2 (36 psi), ethyl acetate, rt, 14 h.

amine proceeded smoothly at 90 °C.[13] The ring closure product **10** could be isolated in 80% yield after 6 h. The introduction of a vinyl substituent at position 7 was realized using a Stille coupling employing a new catalytic system reported by Fu et al.[14] Under these conditions compound **10** reacted with tributyl(vinyl)tin giving compound **11** in 73% yield after 16 h at 100 °C. Reduction with diisobutylaluminium hydride followed by an acid work-up resulted in the formation of allylic alcohol **12**. Selective hydrogenation using Pt/C at 35 psi (H_2) gave compound **13** in 95% yield.

Compound **13** was used as starting material to synthesize a library of dibenzo[*b*,*f*]azocines. The allylic alcohol underwent a Mitsunobu reaction with different phenols in the presence of di-*tert*-butylazodicarboxylate (Scheme 4).[15] The results are summarized in Table 1.

The phenols used were mono-substituted bearing functional groups like nitro, ester, chloro, fluoro, methoxy,



Scheme 4. Synthesis of dibenzo[*b*,*f*]azocine library. Reagents and conditions: (i) a—di-*tert*-butylazodicarboxylate (1.5 equiv), PPh_3 (1.5 equiv), THF, rt, 3 h; b—trifluoroacetic acid (5 equiv), DCM, rt, 12 h.

Table 1. Summary of dibenzo[*b*,*f*]azocine library

Entry	Phenol (ArOH)	Product	Overall yield[a] (%)
1	2-Nitrophenol	**14a**	59 (90)
2	3-Nitrophenol	**14b**	41 (76)
3	4-Nitrophenol	**14c**	51 (68)
4	2-Hydroxymethylbenzoate	**14d**	46 (74)
5	3-Hydroxymethylbenzoate	**14e**	25 (67)
6	4-Hydroxymethylbenzoate	**14f**	35 (80)
7	2-Chlorophenol	**14g**	43 (91)
8	3-Chlorophenol	**14h**	63 (70)
9	4-Chlorophenol	**14i**	48 (78)
10	2-Fluorophenol	**14j**	66 (88)
11	3-Fluorophenol	**14k**	51 (81)
12	4-Fluorophenol	**14l**	56 (85)
13	2-Methoxyphenol	**14m**	38 (70)
14	3-Methoxyphenol	**14n**	39 (70)
15	4-Methoxyphenol	**14o**	55 (87)
16	Trifluoro *o*-cresol	**14p**	76 (91)
17	Trifluoro *m*-cresol	**14q**	49 (83)
18	Phenol	**14r**	79 (84)
19	2-Acetamidophenol	**14s**	39 (64)
20	3-Acetamidophenol	**14t**	—[b]
21	4-Acetamidophenol	**14u**	—[b]
22	2-Hydroxypyridine	**14v**	–(<10)[c]
23	3-Hydroxypyridine	**14w**	–(<10)[c]
24	4-Hydroxypyridine	**14x**	–(<10)[c]

[a] Isolated yield and yield of Mitsunobu reaction in parentheses.
[b] Complex reaction mixture.
[c] Less than 10% conversion.

trifluoromethyl and acetamido substituents in ortho, meta and para positions to the hydroxy functionality. Most of the phenols could be coupled successfully except hydroxypyridines and *m*-and *p*-acetamidophenols (Table 1). The hydroxypyridines gave less than 10% conversion after 12 h and the 3- and 4-acetamidophenols gave complex reaction mixtures (Table 1, entries 20–24). The products **14a–s** were separated from di-*tert*-butyl hydrazinedicarboxylate, the reduction product of di-*tert*-butylazodicarboxylate, and triphenylphosphine oxide by column chromatography and isolated in 64–91% yield. Subsequent deprotection in the presence of TFA gave the corresponding compounds **14a–s** in 25–79% yield over two steps.

We investigated the ability of these compounds to inhibit the phosphorylation of kemptide in the presence of PKA. The screen was carried out using a commercially available luminescence kinase assay (Promega). This assay quantifies the remains of adenosine triphosphate (ATP) by converting luciferin to oxyluciferin in the presence of luciferase. The results are depicted in Figure 2.

Among compounds **14a–s** of the dibenzo[*b*,*f*]azocine library only compound **14s** inhibited partially the phosphorylation of kemptide in the presence of PKA at 100 μM. The other derivatives showed no inhibition. Control experiments were carried out using protein kinase inhibitors H-7 and H-89. H-89 fully inhibited PKA activity, whereas H-7 was only partially active. Furthermore, we included DMSO as negative control and the experiment in the absence of PKA as a positive control.

To quantify the inhibition of PKA by small molecules we performed dose response studies of **14s**, H-89 and H-7 (Fig. 3). The small molecules were diluted and incubated with kemptide and PKA in the presence of ATP. The unconsumed ATP was quantified. Under these assay conditions we calculated the following IC_{50} values: 1.2 μM (H-89) and 122 μM (**14s**), respectively. The inhibition constant of H-7 could not be calculated because of the lack of saturation at the measured concentrations. Thus, **14s** is a fairly good inhibitor of PKA activity in comparison with the control compounds tested.

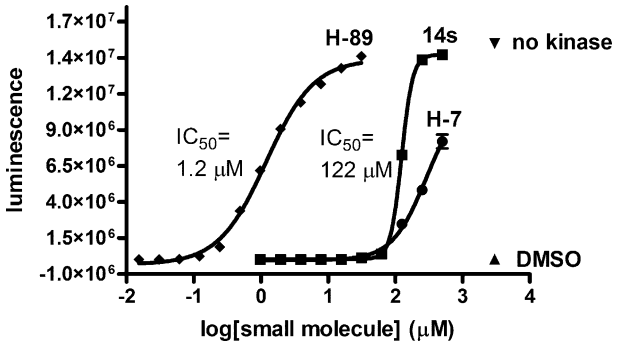


Figure 3. Dose–response analysis of **14s**, H-89 and H-7 using a luminescence based PKA activity assay. The conditions are identical to Figure 2. Compounds were serial diluted in DMSO. IC_{50} values were obtained by fitting data to the following equation (y = min + (max − min)/1 + (x/IC_{50}) Hill slope). ■ (**14s**), ▼ (no kinase), ♦ (H-89), ● (H-7), ▲ (DMSO).

To define the biological mechanism of action of the small molecules, we examined the activity of PKA in the presence of **14s** and H-89 at increasing concentrations of ATP (Fig. 4).

Three concentrations of ATP (25, 5, and 1 μM) were examined in the presence of 41 μg/ml PKA and 10 μM kemptide. The analysis showed that the IC_{50} values of **14s** and H-89 increased with increasing concentration ATP. In case of APT-competitive inhibitor H-89, this behaviour has been reported.[6]

Finally, we investigated the influence of the compounds **14a–s** on the protein kinase C. In this case, we added phosphatidylserine, diacylglycol and $CaCl_2$ to the buffer solution to activate PKC. The results are depicted in Figure 5.

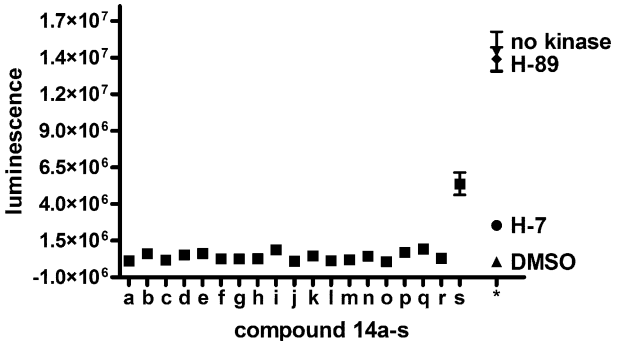


Figure 2. Luminescence based PKA kinase screen. A solution of PKA (41 μg/ml), kemptide (10 μM) and compound (100 μM) in 9 μl reaction buffer (40 mM Tris, pH 7.5, 20 mM $MgCl_2$ and 0.1 mg/ml BSA) was equilibrated for 1 h. Ten minutes after the addition of 1 μl ATP (1 μM), 10 μl Kinase-Glo® plus was added and luminescence was measured. Values represents means of two replicates. ▼ (no kinase), ♦ (H-89), ● (H-7), ▲ (DMSO), ■ compound.

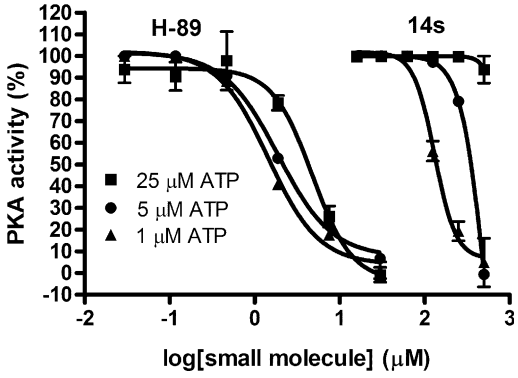


Figure 4. Kinetic analysis of **14s** and H-89. The conditions are identical to Figure 2. H-89 and **14s** were serial diluted in DMSO. Kinase activity was determined by normalizing data to luminescence signal measured for full activity (DMSO) and no activity (exclusion of kinase). ■ (25 μM), ● (5 μM) and ▲ (1 μM).

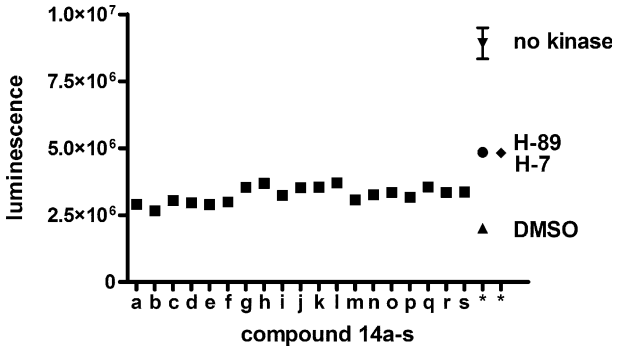


Figure 5. Luminescence based PKC kinase screen. A solution of PKC (0.33 μg/ml), neurogranin (20 μM) and compound (100 μM) in 4.5 μl reaction buffer (20 mM Tris, pH 7.5, 10 mM $MgCl_2$, and 0.1 mg/ml BSA, 250 μM EGTA and 400 μM $CaCl_2$) and 4.5 μl PKC lipid activator solution (Upstate; 0.5 mg/ml phosphatidylserine, 0.05 mg/ml diacylglycol, 20 mM MOPS, pH 7.2, 25 mM β-glycerol phosphate, 1 mM dithiothreitol and 1 mM $CaCl_2$) was equilibrated for 1 h. Thirty minutes after the addition of 1 μl ATP (1 μM), 10 μl Kinase-Glo® Plus was added and luminescence was measured. Values represents means of two replicates. ▼ (no kinase), ♦ (H-89), ● (H-7), ▲ (DMSO), ■ compound.

The compounds **14a**–**s** could not inhibit the phosphorylation of neurogranin in the presence of PKC and ATP. We used the kinase inhibitors H-7 and H-89 as control compounds that showed only partial inhibition of PKC at a concentration of 100 μM. The negative control (DMSO) exhibits still a substantial amount of ATP (2,500,000 counts) due to the low concentration of PKC. However, this concentration was sufficient to give clear controls (DMSO and no kinase). In contrast to H-7 and H-89 compound **14s** showed no activity towards PKC although it was identified to inhibit PKA.

We present herein a convenient synthesis for highly substituted dibenzo[*b*,*f*]azocines. The starting materials to synthesize dibenzo[*b*,*f*]azocine scaffolds are readily available in three steps. The presented synthetic route leaves three independent positions available for the introduction of different substituents at positions 4, 7 and 11 (Scheme 4). Compound **5** can be coupled with different boronic acid derivatives under the developed Suzuki reaction conditions. Furthermore, scaffold **10** can be reacted with commercially available organotin compounds under Stille reaction conditions. The final position of diversity was successfully employed in the synthesis of a dibenzo[*b*,*f*]azocine library (compounds **14a**–**s**).

We showed that one member of this library (compound **14s**) could successfully inhibit the phosphorylation of kemptide in the presence of PKA. Additionally, this compound exhibits selectivity among the serine/threonine kinases PKA and PKC. No inhibition of PKC was detected in the presence of **14s**. The activity of the acetamido functionality regarding the other functionalities employed in this library is remarkable. This is the first example of a protein kinase inhibitor based on a dibenzo[*b*,*f*]azocine scaffold.

We plan to synthesize a second library addressing the suggested two positions of diversity. Furthermore, we will explore the influence of these compounds on the activity of different protein kinases.

Acknowledgments

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