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Triazolbenzo[d]thiazoles: efficient synthesis and biological evaluation as neuroprotective agents

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Abstract

A number of (1*H*-1,2,3-triazol-1-yl)benzo[d]thiazoles were synthesized utilizing a versatile Cu-catalyzed azide-alkyne click reaction (CuAAC) on tautomeric benzo[4,5]thiazolo[3,2-*d*]tetrazole (**1**) and 2-azidobenzo[d]thiazole (**2**) starting materials. Moreover, one of the resulting products of this investigation, triazolbenzo[d]thiazole **22**, was found to possess significant neuroprotective activity in human neuroblastoma (SH-SY5Y) cells.

Keywords

Click chemistry; Triazolbenzo[d]thiazoles; Neuroprotective agents

Benzothiazole derivatives exhibit a wide array of biological activities; these include anti-HIV,^{1a} antibacterial,^{1b} antitumor,^{1c} and neuroprotective.^{1d} Of special interest, thiourea derivatives of 2-amino-(6-trifluoromethoxy)benzothiazole, which are analogs of riluzole (a neuroprotective drug in animal models of Huntington's disease), have been shown to significantly decrease neuronal injury.^{1d} 1,2,3-Triazoles have also been shown to exhibit a variety of activities, including antimycobacterial,^{2a} antiprotozoal,^{2b} and anti-inflammatory.^{2c} Given this backdrop, it was envisioned that benzothiazoles derivatized with 1,2,3-triazoles may afford unique biological activities. The compounds reported here are also included in the NIH Molecular Libraries Small Molecule Repository (MLSMR) for high-throughput biological screening.

Previous work reported by one of us^{3a} suggested that benzimidazole and benzothiazole scaffolds often afford neuroprotective compounds. Indeed, a pharmacophore hypothesis for neuroprotection has been developed and suggested to us that triazolbenzo[d]thiazoles would nicely conform to this general scheme. Herein, we report the synthesis of novel

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Supplementary data

Supplementary data associated with (experimental details of synthesis and characterization of the products) this article can be found, in the online version, at XXXXX.

triazolbenzo[*d*]thiazoles as well as the neuroprotective effects of 2-isopropyl-1-((1-(2-morpholinobenzo[*d*]thiazol-6-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indazol-3(2*H*)-one (**22**).

Current methods for the preparation of 2-azidobenzo[*d*]thiazoles include treatment of benzo[*d*]thiazol-2-amine with tert-butyl nitrite followed by treatment with aq. sodium azide,⁴ as well as tosyl azide reaction with heteroarylolithiums (azido transfer).⁵ However, reactions involving 2-azidobenzo[*d*]thiazole could, potentially, be problematic since it is known that an equilibrium exists between benzo[4,5]thiazolo[3,2-*d*]tetrazole (**1a**) and 2-azidobenzo[*d*]thiazole (**2a**) – two tautomeric forms where solvent and substitution patterns govern which tautomer is favored (Figure 1).⁶ A similar tautomeric equilibrium exists with the pyrido- and quinolinotriazoles.^{7a} The structure of **1a** has been confirmed by X-ray crystallographic analysis⁸ and, in our hands, the ¹H-NMR taken in CDCl₃ showed a 1.8:1 ratio of **1a**:**2a** after 15 min, which evolved to a 1:1 ratio after 1 h. Interestingly, it was recently shown that analogous pyridotetrazoles could be employed in click reactions with alkynes to form pyrido-1,2,3-triazole derivatives.^{7a-b} Thus, we envisaged that a variety of substituted triazolbenzo[*d*]thiazoles could be prepared from **1a** $\rightleftharpoons$ **2a** via CuAAC chemistry.⁹

Scheme 1 illustrates that benzothiazolotetrazole **1a** can be obtained from either benzo[*d*]thiazol-2-amine (**3**; method A) or 2-chlorobenzo[*d*]thiazole (**4**; method B). In method A, direct diazotization using 85% phosphoric acid, nitric acid and aq. sodium nitrite followed by addition of aq. sodium azide was employed and led to the rapid formation (>45 min.) of pure **1a**, which precipitated out of solution in the excellent yield of 84%. In method B, **4** was subjected to sodium azide, easily giving **1a** but in only moderate yield (50%) making this a less useful route.

With an effective route to **1a** $\rightleftharpoons$ **2a** in hand, we proceeded by reacting **1a** $\rightleftharpoons$ **2a** with phenyl acetylene and CuSO₄/ascorbate in *t*-BuOH/H₂O. The **1a** $\rightleftharpoons$ **2a** equilibrium mixture proved to be inert to these conditions and triazolobenzothiazole **5** was not obtained.

Fortunately, utilizing stoichiometric CuI in THF at room temperature for 1 h delivered triazolobenzothiazole **5** in excellent yield (84%; Scheme 2). Given this success, we set out to synthesize several triazolobenzothiazoles using **1a** $\rightleftharpoons$ **2a** and a number of diverse alkynes. The yields for these cycloaddition reactions ranged from 64–84% (**5–13**; Table 1) and, as expected, tolerated phenyl, ether, nitrile, ester, and alkene functionality in the alkyne moiety.

We further explored substitution on the benzothiazole ring by employing tetrazole/azide starting materials **1b** $\rightleftharpoons$ **2b** (6-methyl substituted) and **1c** $\rightleftharpoons$ **2c** (6-phenyl substituted) in reaction with a number of alkynes ($\rightarrow$ **10–13**; Table 1). We found that the tetrazole/azide starting materials need not be purified (i.e., could be used crude) without significant loss in yield or complication in product isolation/purification ($\rightarrow$ **12–13**; 64–69% yield; Table 1).

Next, 6-nitrobenzothiazolotetrazole **14** was employed using the same CuI reaction conditions and triazolobenzothiazole **15** was obtained in 50% yield (Scheme 3). It is interesting to note that this reaction proceeded moderately well despite the fact that starting 6-nitrobenzo[4,5]thiazolo[3,2-*d*]tetrazole (**14**) was the near exclusive tautomer as confirmed by ¹H NMR in CDCl₃.

We next set out to prepare triazolobenzothiazoles where the triazole moiety was placed at C6 and an amine placed at C2. The key intermediate for this work, morpholinobenzenzo[*d*]thiazol-6-amine (**19**), was prepared in three steps (50% overall yield) from 6-nitrobenzo[*d*]thiazol-2-amine (**16**; Scheme 4). Diazotization of **16** followed by addition of aq. CuSO₄ and NaCl yielded **17** (75%) and subsequent ipso displacement of

chloride by morpholine yielded aminated product **18** (67%). Zinc-mediated reduction of the nitro group in **18** (Zn powder/AcOH in EtOH) delivered pure **19** in nearly quantitative yield. With **19** in hand, method A conditions (H₂PO₄, HNO₃, aq. NaNO₂, aq. NaN₃; see Scheme 1) were used for the in situ –NH₂ → –N₃ conversion and subsequent one-pot CuI + alkyne cycloaddition in THF gave **20–22** in yields ranging from 51–83%.

6-Triazolo-2-morpholinobenzothiazoles **20–22** were evaluated for their ability to protect the human neuroblastoma cell line SH-SY5Y against serum deprivation. Under these conditions, the cells normally slow their rate of cell division and undergo modest shrinkage. Analog **22** afforded significant neuroprotection as depicted in Figure 2. In addition, **22** caused a small but significant ($p < 0.05$) increase in cell size at both 20 and 40 μ M when compared to control. While these concentrations are higher than desired for a drug candidate, they are typical for hits obtained during screening of compound libraries.^{3b} The positive effects of **22** on the SH-SY5Y cell line could be mediated by several mechanisms, but are most consistent with activation of the serine-threonine kinase Akt. Akt has previously been implicated in neuroprotection, and regulates cell and organismal size.^{3b} Additional studies will be required to confirm a role for this signaling pathway. Compounds **20** and **21** did not protect against serum deprivation; however, they have limited solubility in cell culture medium, which complicates interpretation of these negative results. By contrast, analog **22** was readily soluble beyond 40 μ M. It is noteworthy that **22** resembles a previous neuroprotective hit from screening (Hoechst 33258, LSU-31)^{3a} as judged by automated pharmacophore alignment (Figure 2).

In conclusion, a number of triazolobenzothiazole derivatives were synthesized via the CuAAC reaction of mixed tetrazole $\rightleftharpoons$ azide starting materials. One compound (**22**) was found to be neuroprotective and to also increase cell size similar to previously described screening hits that are structurally related. The novel chemistry and scaffold reported here may provide the basis for deriving highly potent neuroprotective compounds and additional efforts in this regard are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Benzo[4,5]thiazolo[3,2-*d*]tetrazole (**1a**) and 2-azidobenzo [*d*]thiazole (**2a**).

Figure 2.

Neuroprotection by compound **22**. (a) Human neuroblastoma cells were transferred to serum-free medium in the absence (control) or presence of compounds **20–22** at the concentrations indicated. After 3 d, cell viability was measured by conversion of tetrazolium dye (CellTiter 96®) as described previously.³ Cells were assayed in triplicate in 96-well culture plates. Results are expressed as the percentage of control values averaged from 6 experiments. The bars represent the standard deviations, and asterisks indicate significant differences from control, $p < 0.01$. (b) Automated pharmacophore alignment of previous hit, Hoechst 33258 (cyan, middle panel) with **22** (dark blue). This alignment was generated with

MarvinSketch 5.3.2 from ChemAxon. For comparison, the structures of the active compounds are shown in similar orientations.

Scheme 1.

Synthesis of **1a** $\rightleftharpoons$ **2a**. Method A: H_2PO_4 , HNO_3 , aq. NaNO_2 , aq. NaN_3 , 85%. Method B: aq. NaN_3 , DMSO, 100 °C, 50%.

Scheme 2.
Synthesis of 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)benzo[*d*]thiazole.

Scheme 3.
Synthesis of 3-(1-(6-nitrobenzo[d]thiazol-2-yl)-1H-1,2,3-triazol-4-yl)propanenitrile.

Scheme 4.
Synthesis of 6-triazolo-2-morpholinobenzothiazoles **20–22**.

Table 1

Synthesis of 2-(1*H*-1,2,3-triazol-1-yl)benzo[*d*]thiazoles.^{a,b,c} ^aProducts were characterized by ¹H, ¹³C NMR, IR, and ESI MS.^bIsolated yields after purification by chromatography on silica gel.

^cStarting tetrazole/azide ratios (**1:2**) were determined by ¹H NMR in CDCl₃: **1a:2a**::1:1; **1b:2b**::1.5:1.