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Synthesis and anti-acetylcholinesterase activity of benzotriazinone-triazole systems

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Abstract. An approach for the construction of benzotriazinone-triazole system is described. The synthesis is based on diazonium chemistry and subsequent intramolecular heteroatom-heteroatom bond formation. The introduction of triazole moiety occurred *via* click reaction catalyzed by nano-sized copper, supported on modified silica mesopore KIT-5 leading to the desired products in excellent yield. Also, *in vitro* acetylcholinesterase (AChE) inhibitory activities of the target compounds were screened by Ellman's method.

Keywords. Triazole; benzotriazinone; diazonium chemistry; nanocatalyst; acetylcholinesterase.

1. Introduction

Nitrogen-containing heterocycles are key structural motifs in natural and synthetic bioactive agents.[1] In the library of six-membered heterocyclic frameworks, 1,2,3-benzotriazines which show exceptional bioactivity are widely used in medicinal chemistry. In the benzotriazine class of compounds, 1,2,3-benzotriazine-4-one[2] is recognized as a core structure in molecules exhibiting various biological effects including antiarthritic,[3] anesthetic,[4] diuretic,[5] sedative,[6] antitumor,[7] and chorismate inhibitory activities.[8] The sustained interest in triazine chemistry was not restricted to the application of these compounds in pharmaceuticals and other bioactive molecules. There are also some reports relying on their application as a substrate, from which a wide variety of new heterocyclic frameworks could be derived.[9]

Considered as a unique class of five-membered N-heterocycles, triazoles are mainly synthesized *via* the rapid and potent pathway, copper-catalyzed azide-alkyne cylcoaddition (CuAAC).[10] Due to the importance of triazoles in diverse applied sciences,[11] this reaction has become one of the well-studied reactions in modern organic chemistry,[12] The pharmacological properties which are depicted in triazole-containing molecules[13] could be attributed to the stereochemical features and the ability of this core in mimicking special bonds, resulting in anti-allergic,[14] antibacterial[15] and anti-HIV[16] activities. Due to the remarkable features of benzotriazinone and triazole ring systems, we decided to synthesize new benzotriazinones-triazoles hybrid heterocycles.

Nanocatalysts have attracted interests in several catalytic organic reactions[17] and in the synthesis of novel heterocyclic cores.[18] Meanwhile, nanocatalysts especially those which are immobilized onto various supports, are considered as a big achievement in the sustainable construction of triazoles.[19] As a result, we decided to introduce nanoparticles copper iodide/APTES-KIT-5 (CuI-AK) in water[20] as a catalyst for the synthesis of 3-(substituted-1,2,3-triazol-4-yl methyl)benzo[*d*][1,2,3]triazin-4(3*H*)-ones, whereby we could meet global concerns about environmental issues along with the high yielding synthesis of new compounds which were not reported before.

Herein, we report our investigation on the synthesis of benzotriazinone-triazole system utilizing nano copper catalyst in water. The reaction proceeded by

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only three-steps and produced 3-(substituted-1*H*-1,2,3-triazol-4-yl methyl)benzo[*d*][1,2,3]triazin-4(3*H*)-ones in overall high yield.

2. Experimental

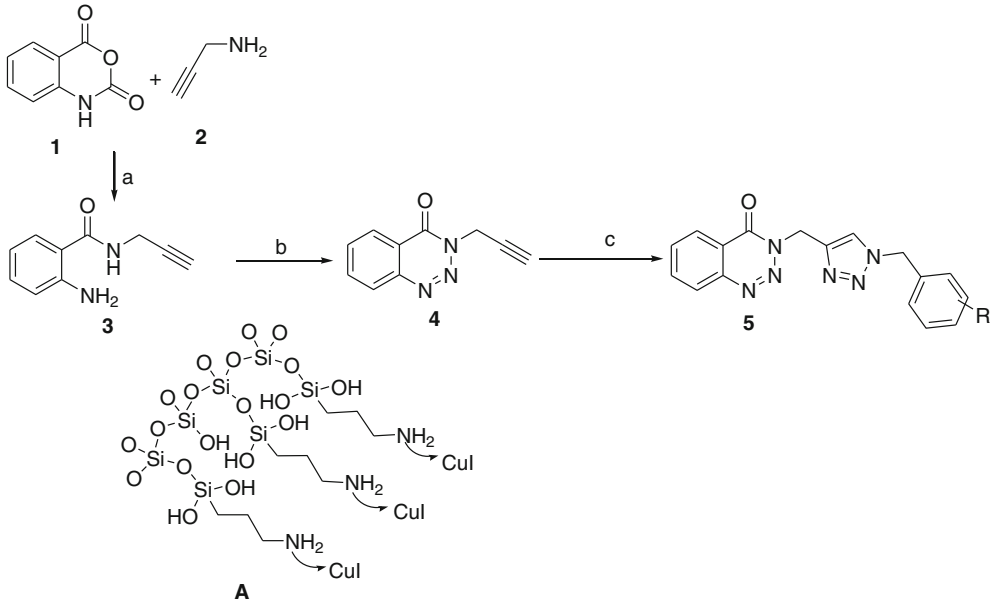
2.1 *Materials and Methods*

Melting points were taken on a Kofler hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-500, using tetramethylsilane (TMS) as an internal standard. The infrared (IR) spectra were obtained on a Nicolet Magna FT-IR 550 spectrophotometer (KBr disks). Mass spectra were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis were carried out with a Perkin-Elmer model 240-C apparatus.

2.1a *Biological activity assay*: Colorimetric Ellman's method[21] was used to evaluate the inhibitory potency of target compounds toward AChE. Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from Electric eel, 1000 unit) was obtained from Sigma–Aldrich. 5, 5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were purchased from Fluka. Donepezil hydrochloride was obtained from Merck, Darmstadt, Germany. In short, to determine IC_{50} values, 50 μL of the five different concentration of the test compounds that produced inhibition in the range of 20–80% was added to the mixture of 3 mL phosphate buffer 0.1 M, pH = 8.0 and 100 μL of DTNB solution (0.1 M) and 50 μL AChE. 10 μL solution of acetylthiocholine iodide (0.15 M) as substrate was added following 10 min incubation at 25°C. The progress curve was plotted by measuring the absorbance at 412 nm for 6 min. The IC_{50} values were determined graphically from inhibition curves (inhibitor concentration vs percent of inhibition). UNICO double beam spectrophotometers 2100 was used for colorimetric measurements.

2.2 *Spectral data of some of the products*

2.2a *3-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)benzo[d][1,2,3]triazin-4(3H)-one (**5a**)*: Pale yellow solid, [0.27 g, 86%]; M.p. 176–178°C; IR (KBr): 1678, 1456, 1331, 1059, 784, 694 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.56 (s, 2H), 5.64 (s, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.32–7.37 (m, 3H), 7.94 (t, J = 7.3 Hz, 1H), 8.09 (t, J = 7.3 Hz, 1H), 8.16–8.18 (m, 1H), 8.19 (s, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 44.6, 52.8, 119.3, 123.9, 124.5, 127.9, 128.0, 128.5, 128.6, 133.0, 135.4, 135.8, 142.2, 143.6, 154.4 ppm; MS: m/z (%) = 318 (51, M$^+$), 227 (29), 146 (100), 91 (22), 77 (39). Anal. Calcd. (%) for $C_{17}H_{14}N_6O$: C, 64.14; H, 4.43; N, 26.40. Found (%): C, 64.21; H, 4.32; N, 26.51.



Scheme 1. Reagents and conditions: (a) H_2O, r.t.; (b) $NaNO_2$, HCl (10%), acetone; (c) NaN_3, benzyl bromides, CuI-AK, H_2O, reflux.

3. Results and discussion

3.1 *Synthesis and characterization*

Within our program for the synthesis of novel heterocycles,[22] herein, we started by stirring isatoic anhydride and propargyl amine in water at room temperature to obtain pure 2-amino-*N*-(prop-2-yn-1-yl)benzamide **3** as a white solid in 85% yield (Scheme 1). In the following step, exposure of **3** to acidic solution of sodium nitrite at 0°C for 1 h, resulted in intramolecular nitrogen-nitrogen bond formation. In the final step, copper nanocatalyst on modified silica-based KIT-5 (CuI-AK), catalyst **A** was used. So, triple bond was subjected to click reaction in the presence of NaN_3, different benzyl bromide derivatives and 0.04 g catalyst **A** in refluxing water. The desired products **5a–j** were obtained after 3 h in good to excellent yields. Water rich solvents like *t*-BuOH/H_2O (1:1.5) and EtOH/H_2O (1:1.5) were also examined at reflux temperature for the synthesis of **5a** which produced the desired product in same yields (86%, 84%, respectively). Nevertheless, we decided to report water as the reaction medium, in response to green chemistry principles.

To test the scope of the reaction, 10 different benzyl bromide derivatives were effectively examined to synthesize **5a–j** in excellent yields (Table 1). In general, the presence of methyl substitution gave higher yields. Electron-withdrawing groups like NO_2, F, Cl, and Br also produced the desired products in good yields.

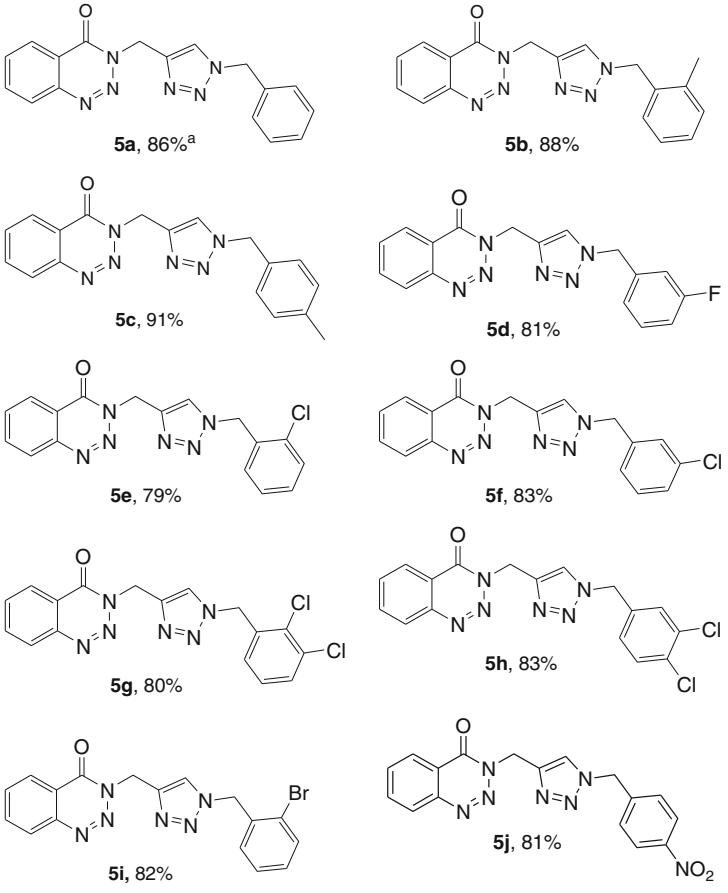
The Cu catalyst could be reused in click reaction, while significant loss of activity was observed in fifth run (Table 2).

Table 2. The recyclability of Cu nanoparticle in the formation of **5a**.[20]

Entry	Run	Yield (%)[a]
1	1	86
2	2	85
3	3	83
4	4	82
5	5	71

[a)] Isolated yields.

Table 1. Substrate scope of 1,2,3-triazol-benzo[*d*][1,2,3]triazin-4(3*H*)-ones.



[a] Isolated yields.

Table 3. Acetylcholinesterase inhibitory activities of compounds **5a**–**5j**.[a]

Entry	Compound	IC_{50} (μ M)
1	**5a**	>25
2	**5b**	1.88 ± 0.091
3	**5c**	>25
4	**5d**	3.97 ± 0.19
5	**5e**	>25
6	**5f**	>25
7	**5g**	1.94 ± 0.077
8	**5h**	5.85 ± 0.27
9	**5i**	>25
10	**5j**	>25
11	**Donepezil**	0.022 ± 0.002

[a]Data are expressed as Mean ± SE (three independent experiments).

3.2 *Biological activity*

Acetylcholinesterase (AChE) is an important enzyme responsible for the hydrolysis of acetylcholine. The reduced amounts of this neurotransmitter is considered as the main reason for Alzheimer's disease. So, finding efficient acetylcholinesterase inhibitors alleviates global concerns and represents a promising treatment approach for this disease. As noted by Sharpless, AChE serves as a selective target for the construction of triazoles *via* click reaction which simultaneously act as an inhibitor for this enzyme.[23] In addition, benzotriazinones are present in molecules with anti-acetylcholinesterase activity.[24] As our synthesized compounds contain both of these heterocyclic moieties, we decided to evaluate the activity of these compounds against acetylcholinesterase. Table 3 summarizes the anti-acetylcholinesterase activities of our products **5a**–**5j**, determined by Ellman's method.

Unfortunately, only four compounds, **5b**, **5d**, **5g** and **5h**, exhibited good inhibitory activities, albeit less than the reference drug, donepezil. It seems that the substituents have a profound effect on the activities in this order 2-Me>2,3-*di*Cl_2>3-F>3,4-*di*Cl_2.

4. Conclusions

In conclusion, we have reported a versatile, mild and three-step strategy for the high yield synthesis of 3-(substituted-1*H*-1,2,3-triazol-4-yl)methyl)benzo[*d*][1,2,3]triazin-4(3*H*)-one derivatives. The click reaction which was the last step was carried out with the recently reported nano copper catalyst in water. Utilizing water as a solvent and reusability of the catalyst are the main advantages of this strategy in compliance with green chemistry principles.

Supplementary Information (SI)

Full experimental details and ^{1}H, ^{13}C NMR spectra are provided in the supplementary information available at www.ias.ac.in/chemsci.

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