Bioorganic & Medicinal Chemistry Letters 22 (2012) 1146–1150

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Cu-mediated N-arylation of 1,2,3-triazin-4-ones: Synthesis of fused triazinone derivatives as potential inhibitors of chorismate mutase

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ARTICLE INFO

Article history:
Received 14 September 2011
Revised 4 November 2011
Accepted 24 November 2011
Available online 2 December 2011

Keywords:
1,2,3-Triazin-4-one
Copper
Coupling
Chorismate mutase

ABSTRACT

A rapid and direct access to *N*-aryl substituted fused triazinone derivatives has been accomplished via N-arylation of 1,2,3-triazin-4-one ring involving a Cu-mediated coupling between triazinone derivatives and aryl boronic acids. A combination of $Cu(OAc)_2$–Et_3N in 1,2-dichloroethane was found to be effective and various fused triazinone derivatives have been prepared by using this methodology. Molecular structure of a representative compound was confirmed by single crystal X-ray diffraction study. The scope and limitations of this reaction is discussed. Some of the compounds synthesized were tested for chorismate mutase inhibitory properties in vitro. The in vitro dose response study of an active compound is presented.

1,2,3-Triazin-4-ones being integral part of many bioactive agents represent an important class of nitrogen containing heterocycle and have attracted particular attention in the area of bioorganic and medicinal chemistry.[1] This is exemplified by a range of pharmacological properties reported for this class of compounds that include sedative,[2] diuretic,[3] anesthetic,[4] antiarthritic[5] and antitumor activities.[6] Indeed, the replacement of the pteridyl moiety of folic acid with 1,2,3-benzotriazin-4-ones was achieved without affecting its antitumor activity.[6] Notably, not much known about antitubercular properties of this class of compounds. Despite the availability of a range of effective antibacterial agents, tuberculosis still remains a leading cause of death worldwide due to a number of factors such as (a) long treatment duration (6–9 months), (b) increased incidence of (multi or extensive) drug resistance, (c) co-morbidity with HIV-AIDS and (d) declined effort in anti-infective drug discovery research. Thus, discovery, development and introduction of new treatments for tuberculosis require immediate attention. Shikimate pathway for the biosynthesis of aromatic amino acids such as phenylalanine and tyrosine involve the Claisen rearrangement of chorismate to prephenate in the presence of chorismate mutase or CM (EC 5.4.99.5). Due to the absence of this pathway in animals but not in bacteria CM is considered as an attractive target for the identification of effective antibacterial agents.[7] However, to our knowledge only few small molecules[8] have been reported as inhibitors of CM including the 2,3-diaryl acrylic acid **A** (Fig. 1). Our continuing interest[8d,e] in the identification of novel small molecules as inhibitors of CM prompted us to design the triazinone **C** from **A** via quinolone **B** (Fig. 1). Herein we report for the first time the CM inhibitory properties of 1,2,3-triazin-4-one derivatives synthesis of which has been carried out via a Cu-mediated N-arylation methodology.

Preparation of N-arylated 1,2,3-triazin-4-one derivatives usually involves the construction of the six-membered 1,2,3-triazin-4-one ring[9] with an aryl group at N-3 via a multi-step process or a single-step N-arylation of 1,2,3-triazin-4-one ring at N-3 position under Ullmann-type conditions[10] (Scheme 1). However, all these methods require tedious steps along with harsh reaction conditions whereas Ullmann condensation of 1,2,3-benzotriazin-4-one with iodobenzene provided the desired product only in 14% yield.[10] Moreover, only one example of N-arylation of 1,2,3-benzotriazin-4-one has been studied so far.

Copper promoted C–N bond formation between NH containing heterocycles and organometalloids via cross coupling reactions have emerged as a powerful synthetic tool for the generation of *N*-(hetero)aryl derivatives.[11] This efficient methodology is characterized by mild reaction conditions for example the use of ambient temperature, weaker base and the presence of air. A number of extensions and applications of this methodology have been reported.[12,13] Notably, the use of 1,2,3-triazin-4-ones for successful N-arylation by using this methodology is not common. In continuation of our effort in the synthesis of bioactive molecules via Cu-mediated reactions we became interested in generating a small-molecule library based on 1,2,3-triazin-4-one scaffold where

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doi:10.1016/j.bmcl.2011.11.096

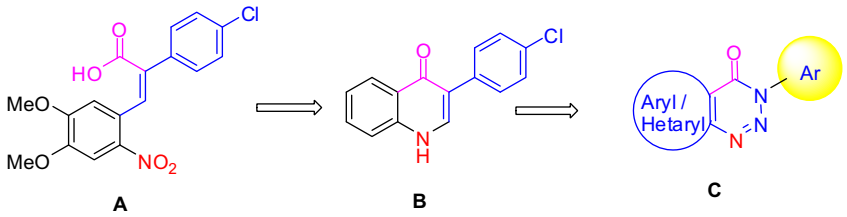
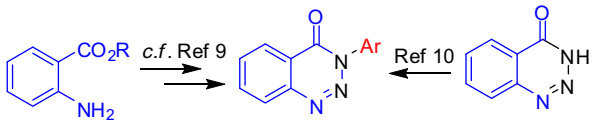


Figure 1. Design of novel inhibitors of chorismate mutase.

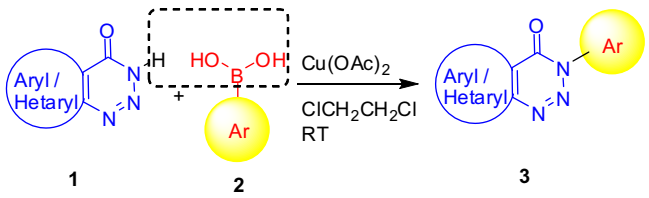


Scheme 1. Reported synthetic approaches to N-arylation of 1,2,3-benzotriazin-4-ones.

Cu-mediated N-arylation was used as a key synthetic step. Thus, aryl boronic acids were coupled with various 1,2,3-triazin-4-ones in the presence of a Cu-salt (Scheme 2) to give the corresponding N-arylated products. The preliminary results of this study are presented.

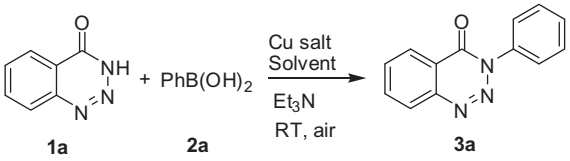
Initially, the coupling of benzo[*d*][1,2,3]triazin-4(3*H*)-one (**1a**) with phenyl boronic acid (**2a**, 1.5 equiv) was used to establish the optimized reaction conditions. Thus **1a** and **2a** was reacted in the presence of a number of Cu-salts in various solvents using Et_3N as a base at room temperature under anhydrous conditions (Table 1). All the reactions were carried out in the presence of air. The desired product that is 3-phenylbenzo[*d*][1,2,3]triazin-4(3*H*)-one[14] (**3a**) was isolated in 80% yield when $Cu(OAc)_2$ (anhydrous) was used in dichloromethane (DCM) (Table 1, entry 1). The reaction was completed within 3 h. The yield was decreased marginally when DMF was used in place of DCM (Table 1, entry 2) but increased to 90% when 1,2-dichloroethane (DCE) was employed (Table 1, entry 3). The reaction was completed within 1 h in DCE. The use of THF decreased the product yield (Table 1, entry 4). We then examined the use of other Cu-salts such as $Cu(OTf)_2$, CuI, and CuBr (Table 1, entries 5–7). While $Cu(OTf)_2$ and CuI was found to be equally effective the other salt was found to be inferior in terms of product yields. Notably, a sharp decrease in yield was observed when $Cu(OAc)_2{\cdot}H_2O$ was used as catalyst indicating moisture sensitive nature of this methodology (Table 1, entry 8). Overall, the combination of anhydrous $Cu(OAc)_2$ and Et_3N in DCE was found to be optimum for the present N-arylation reaction.

Having established the optimum condition, we then decided to examine the reactivity of other 1,2,3-triazin-4-ones with a variety of aryl boronic acids. Accordingly, a number of boronic acids (**2**) were reacted with **1** and the results are summarized in Table 2. The reaction proceeded well irrespective of aryl (**1a**) or heteroaryl (**1b–c**) moiety fused with 1,2,3-triazin-4-one ring. Thus 4,5,6,7-tetrahydrobenzothieno[2,3-*d*][1,2,3]triazin-4(3*H*)-one[15] (**1b**) and 5-methyl-7-propyl-3*H*-pyrazolo[4,3-d][1,2,3]triazin-4(5*H*)-one (**1c**) were employed successfully to afford the desired products **3e–3l** in good yields (Table 2, entries 5–12). The presence of Cl, F and MeO group in aryl boronic acids were well tolerated and the use of naphthalen-2-ylboronic acid (entries 4, 8 and 12) was found to be effective. However, no desired product was isolated when a heteroaryl boronic acid such as pyridin-2-yl boronic acid was reacted with **1b** under the reaction condition employed. Moreover, the use of an alkyl boronic acid such as cyclopropylboronic acid was also found to be ineffective in the present C–N bond forming reaction. All the compounds synthesized were well characterized by spectral data (NMR, MS and IR). Additionally, the molecular structure of a representative compound **3e** was established unambiguously by single crystal X-ray diffraction study (Fig. 2).[16]



Scheme 2. Cu-mediated coupling of 1,2,3-triazin-4-ones (**1**) with arylboronic acids (**2**).

Table 1
Effect of reaction conditions on Cu-mediated coupling of **1a** with **2a**[a]



Entry	Cu salts	Solvent	Time (h)	Yield[b] (%)
1	$Cu(OAc)_2$	CH_2Cl_2	3.0	80
3	$Cu(OAc)_2$	DMF	3.0	70
3	$Cu(OAc)_2$	DCE	1.0	90
4	$Cu(OAc)_2$	THF	4.0	70
5	$Cu(OTf)_2$	DCE	3.0	75
6	CuI	DCE	2.5	75
7	CuBr	DCE	4.0	60
8	$Cu(OAc)_2{\cdot}H_2O$	DCE	6.0	50

[a]All the reactions were carried out using **1a** (1.0 mmol), **2a** (1.5 mmol), a Cu-salt (1.0 mmol) and Et_3N (2.0 mmol) in a solvent (10–15 mL) at room temperature under anhydrous conditions in the presence of air (DCE = 1,2-dichloroethane).
[b] Isolated yield.

Some of the compounds synthesized were tested for CM inhibiting properties in vitro. The assay[17,18] involved determination of activity of enzyme CM which catalyzes the conversion of chorismate to prephenate. Thus determination of activity of CM is based on the direct observation of conversion of chorismic acid to prephenate spectrophotometrically at OD_{274}. This reaction was performed in the presence of test compounds to determine their CM inhibiting activities. A known inhibitor of CM that is 4-(3,5-dimethoxyphenethylamino)-3-nitro-5-sulfamoylbenzoic acid[8a] was prepared and used as a reference compound the IC_{50} value of which was found to be less than 10 μM. Compounds **3d**, **3h**, and **3l** showed significant inhibition of CM in compared to other molecules when tested at 50 μM (Table 3) indicating the importance of naphthyl ring present in these molecules in CM inhibition. Moreover, the maximum inhibition shown by the compound **3l** suggested that the pyrazole ring played a key role in CM inhibition. The compound **3l** showed dose dependent inhibition with an IC_{50} value of 15.13 ± 0.95 μM (Fig. 3).

Table 2
Synthesis of *N*-aryl substituted fused triazinone derivatives (**3**) via $Cu(OAc)_2$ mediated C–N bond forming reaction between **1** and **2** (Scheme 2)[a]

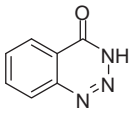
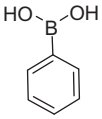
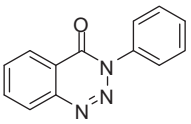
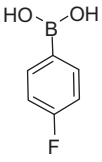
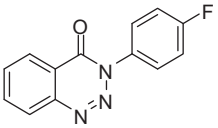
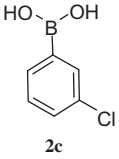
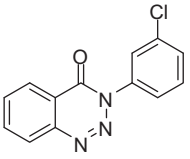
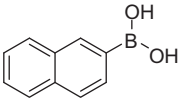
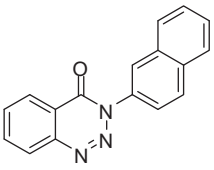
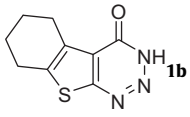
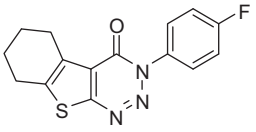
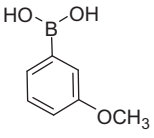
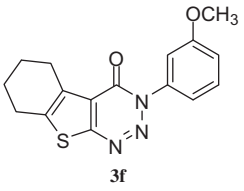
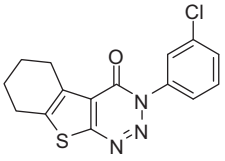
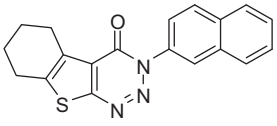
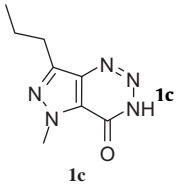
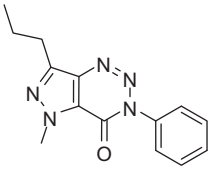
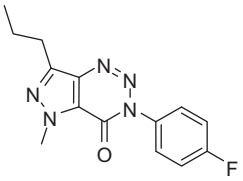
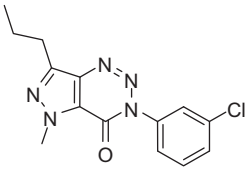
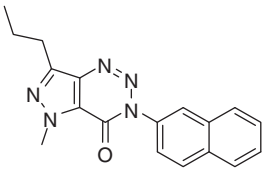
Entry	1,2,3-triazin-4-ones (**1**)	$ArB(OH)_2$ (**2**)	Products (**3**)	Time (h)	Yield[b] (%)
1	 **1a**	 **2a**	 **3a**	1.0	90
2	**1a**	 **2b**	 **3b**	1.0	90
3	**1a**	 **2c**	 **3c**	1.5	90
4	**1a**	 **2d**	 **3d**	1.5	85
5	 **1b**	**2b**	 **3e**	1.0	85
6	**1b**	 **2e**	 **3f**	2.0	81
7	**1b**	**2c**	 **3g**	1.5	90
8	**1b**	**2d**	 **3h**	1.5	80
9	 **1c**	**2a**	 **3i**	1.0	84

Table 2 (*continued*)

Entry	1,2,3-triazin-4-ones (**1**)	$ArB(OH)_2$ (**2**)	Products (**3**)	Time (h)	Yield[b] (%)
10	**1c**	**2b**	 **3j**	1.0	86
11	**1c**	**2c**	 **3k**	1.5	90
12	**1c**	**2d**	 **3l**	1.5	88

[a] All the reactions were carried out using **1** (1.0 mmol), **2** (1.5 mmol), a $Cu(OAc)_2$ (1.0 mmol) and Et_3N (2.0 mmol) in DCE (10–15 mL) at room temperature under anhydrous conditions in the presence of air.
[b] Isolated yield.

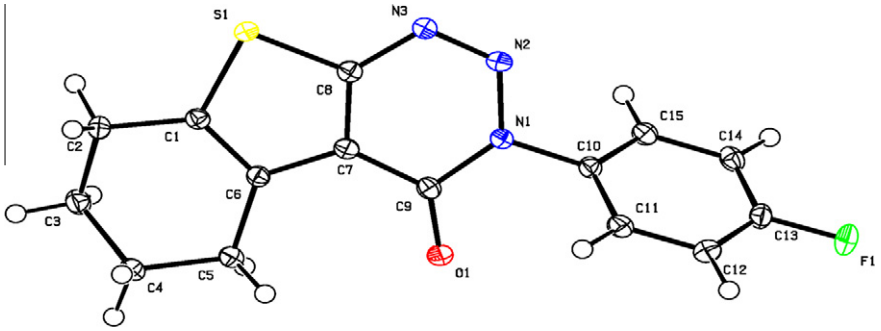


Figure 2. ORTEP representation of the **3e** (Thermal ellipsoids are drawn at 50% probability level).

Table 3
Inhibition of chorismate mutase by triazinones in vitro

Entry	Triazinones (**3**)	% Inhibition[a] @ 50 μM
1	**3a**	12
3	**3d**	40
3	**3e**	25
4	**3h**	37
5	**3i**	10
6	**3j**	24
7	**3k**	30
8	**3l**	61

[a] Average of three experiments.

In conclusion, a mild and efficient method has been developed for direct N-arylation of 1,2,3-triazin-4-one ring which involves a Cu-mediated coupling reaction between triazinone derivatives and aryl boronic acids. Among all the Cu-salts tested $Cu(OAc)_2$ was identified as the best catalyst and a combination of $Cu(OAc)_2$–Et_3N–DCE was found to be optimum. A number of N-aryl substituted fused

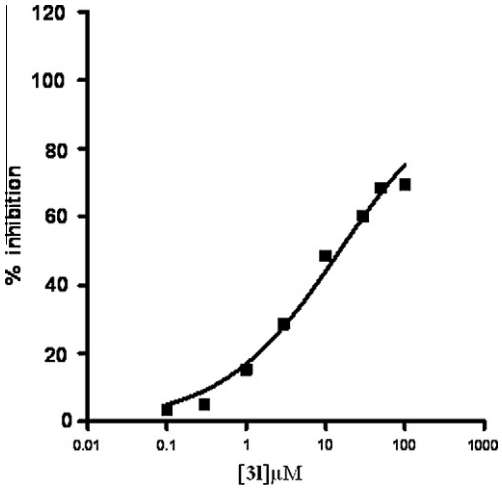


Figure 3. Dose dependent inhibition of CM by compound **3l**

triazinone derivatives have been prepared by using this experimentally simple and ligand-free process within short period of time and molecular structure of a representative compound was confirmed by single crystal X-ray diffraction study. The reaction however, did not work with heteroaryl and alkyl boronic acids. Some of the compounds synthesized were tested for chorismate mutase inhibitory properties in vitro. The in vitro dose response study of an active compound has been presented. Overall, this research has provided a rapid and direct access to a library of compounds based on N-aryl substituted fused triazinone which has been identified as a new scaffold for the development of novel inhibitors of chorismate mutase for the potential treatment of tuberculosis.

Acknowledgments

The authors thank Professor Seyed E. Hasnain and Professor J. Iqbal for encouragement and DBT, New Delhi, India for financial support (Grant NO BT/01/COE/07/02). KS thanks UGC, New Delhi, India for a Dr. D. S. Kothari Post doctoral fellowship [No.F.4-2/2006(BSR)/13-324/2010(BSR)]. RA thanks CSIR, New Delhi, India for a Junior Research Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.096.

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16. *Crystal data of* **3e**: Molecular formula = $C_{15}H_{12}FN_3OS$, Formula weight = 301.35, Crystal system = Monoclinic, space group = $P2_1/c$, a = 9.2519 (15)Å, b = 11.0049(18)Å, c = 13.028(2)Å, V = 1326.1(4)Å^3, T = 298 K, Z = 4, D_c = 1.419 Mg m^{-3}, μ(Mo-$K\alpha$) = 0.71073 mm^{-1}, 16547 reflections measured, 2883 independent reflections, 2672 observed reflections [$I > 2.0\alpha$ (I)], R_1_obs = *0.031*, *Goo*dness of fit = 0.879. Crystallographic data (excluding structure factors) for **3e** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 831731.
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