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Transition metal free hydrolysis/cyclization strategy in a single pot: synthesis of fused furo *N*-heterocycles of pharmacological interest†

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A transition metal free tandem two-step strategy has been developed involving hydrolysis of 2-chloro-3-alkynyl quinoxalines/pyrazines followed by *in situ* cyclization of the corresponding 2-hydroxy-3-alkynyl intermediates in a single pot leading to fused furo *N*-heterocycles as potential inhibitors of sirtuins. A representative compound showed promising pharmacological properties *in vitro* and *in vivo*.

The development of chemical approaches that focus on the use of renewable sources and reduction of waste generation is of prime interest due to the worldwide concern of environmental safety and global warming. Thus the use of aqueous media in organic synthesis has gained high popularity as water is naturally abundant, safe, inexpensive and can be recycled.[1]

Over the years, functionalized fused *N*-heteroaromatics have played key roles in the early stage of drug discovery and many of them have been marketed as successful drugs,[2] *e.g.* blockbuster antibiotic levofloxacin. Fused furo *N*-heterocycles, *e.g.* furoquinoxalines, on the other hand have not been explored extensively as potential bioactive agents[3a] perhaps due to their limited or cumbersome accessibility. However, this class of heterocycles is attractive not only for chemical biology/bioorganic chemistry efforts but also as transient intermediates in organic synthesis.[3b] This and our interest in novel heteroaromatics[4] prompted us to explore a greener route to furo[2,3-*b*]quinoxalines/furo[2,3-*b*]pyrazine (**B**, Fig. 1) as potential inhibitors of sirtuins. Since their over expression has been implicated in several types of cancer, sirtuins (class III NAD-dependent deacetylases) are considered as promising targets for cancer therapeutics.[5] Inhibition of sirtuins allows re-expression of silenced tumor suppressor genes leading to the reduced growth of cancer cells. Our earlier observation on activities of pyrrolo[2,3-*b*]quinoxalines[6] **A** against a panel of cancer cell lines prompted us to design structurally similar **B**, supported by the *in silico* binding studies of a representative compound **C** (Fig. 1) in the catalytic pocket of yeast Sir2 (Fig. 2). The study (docking score −10.2) showed the positioning of the central ring and the side chain of **C** near adenine

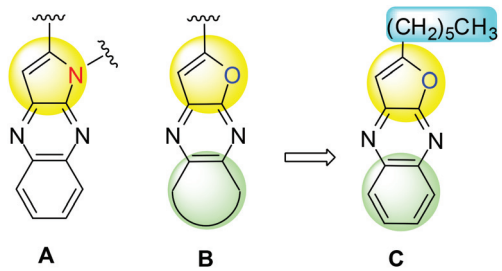

Fig. 1 Design of **B**/**C** as novel inhibitors of sirtuins.

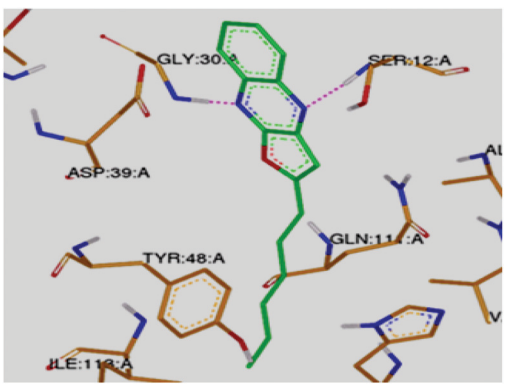

Fig. 2 Binding mode of **C** in yeast Sir2 (PDB ID: 1Q1A).

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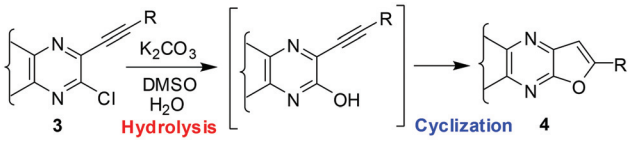
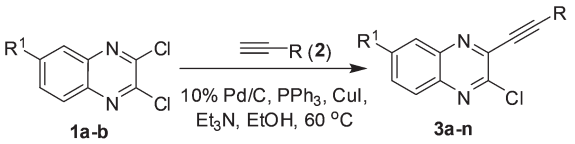


Scheme 1 Hydrolysis–cyclization strategy leading to furo derivatives **4**.

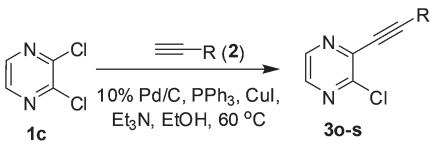
Table 1 Synthesis of 3-alkynyl-2-chloroquinoxalines **3a–n**[a]



Entry	Halide (**1**); R^1 =	Alkyne (**2**); R =	*T*/h	Product (**3**)	Yield[b] (%)
1	**1a**; H	**2a**; Ph	2	**3a**	70
2	**1a**	**2b**; C_6H_4*p*-Me	2	**3b**	62
3	**1a**	**2c**; $(CH_2)_3Me$	2	**3c**	65
4	**1a**	**2d**; $(CH_2)_4Me$	2	**3d**	60
5	**1a**	**2e**; $(CH_2)_5Me$	3	**3e**	66
6	**1a**	**2f**; $(CH_2)_9Me$	2	**3f**	69
7	**1a**	**2g**; CMe_3	3	**3g**	65
8	**1a**	**2h**; CMe_2OH	4	**3h**	60
9	**1a**	**2i**; CH_2OH	4	**3i**	68
10	**1a**	**2j**; $(CH_2)_2OH$	4	**3j**	70
11	**1a**	**2k**; 1-hydroxycyclohexyl	4	**3k**	68
12	**1a**	**2l**; 2-pyridinyl	4	**3l**	60
13	**1b**; Me	**2a**	3	**3m**	63
14	**1b**	**2f**	2	**3n**	67

[a] All reactions were performed by using **1** (1.256 mmol), terminal alkyne **2** (1.256 mmol), 10% Pd/C (0.0125 mmol), PPh_3 (0.0502 mmol), CuI (0.0125 mmol), and Et_3N (1.8844 mmol) in EtOH (4 mL). [b] Isolated yield.

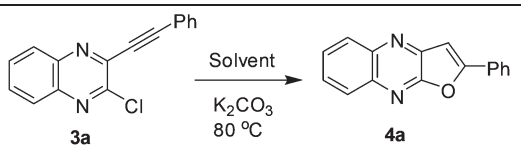
Table 2 Synthesis of 3-alkynyl-2-chloropyrazines **3o–s**[a]



Entry	Alkyne (**2**); R =	*T*/h	Product (**3**)	Yield[b] (%)
1	**2a**	2	**3o**	64
2	**2g**	2	**3p**	70
3	**2h**	4	**3q**	70
4	**2k**	4	**3r**	62
5	**2m**; $SiMe_3$	3	**3s**	60[c]

[a] See footnote "a" of Table 1. [b] Isolated yield. [c] $Pd(PPh_3)_2Cl_2$ (0.0125 mmol) was used in place of Pd/C and PPh_3.

Table 3 Optimization of hydrolysis/cyclization of **3a**[a]



Entry	Solvent	*T*/h	Yield[b] (%)
1	DMSO[c]	4	45
2	DMSO–H_2O (1 : 1)	3	62
3	DMSO–H_2O (1 : 4)	1	78
4	DMSO–H_2O (1 : 8)	4	53
5	H_2O	8	28

[a] Reactions were carried out using **3a** (0.7575 mmol), K_2CO_3 (0.3787 mmol) in solvent (3 mL) at 80 °C. [b] Isolated yield. [c] Commercially available DMSO was used directly without further drying.

and nicotinamide binding site respectively, along with two H-bond interactions of the quinoxaline nitrogens with the backbone –NH of SER 12 and GLY 30 (see ESI†).

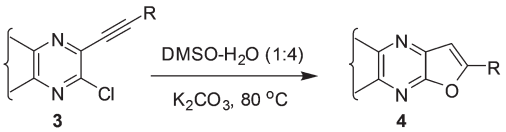
The synthesis of furo[2,3-*b*]quinoxalines/pyrazines is rather uncommon in the literature.[3a,7] While a single compound *i.e.* 2-phenylfuro[2,3-*b*]quinoxaline was prepared by treating 2-chloro-3-phenylethynylquinoxaline with KOH in 1,4-dioxane-H_2O, other "furano-compounds" could not be prepared by using this method.[7b] On the contrary, we have observed that furo[2,3-*b*]quinoxalines/furo[2,3-*b*]pyrazines can be prepared from 2-chloro-3-alkynyl quinoxalines/pyrazines in the presence of K_2CO_3 in DMSO–H_2O. Herein we report our preliminary results on this hydrolysis–cyclization strategy leading to the desired furo derivatives **4** (or **B**, Scheme 1).

The key starting material **3** required was prepared *via* a selective mono alkynylation of 2,3-dichloroquinoxaline/pyrazine (**1**) under Pd–Cu catalysis (Tables 1 and 2).

Initially, the possibility of hydrolysis/cyclization of 2-chloro-3-(phenylethynyl)quinoxaline **3a** leading to **4a** was examined in the presence of K_2CO_3. After assessing a range of solvents an appropriate combination of DMSO–H_2O was found to be effective, perhaps due to the better solubility of **3a** (that was crucial for the success of the reaction) compared to other aqueous media including 1,4-dioxane–H_2O.[7b] It was observed that only DMSO or water alone (entries 1 and 5, Table 3) was less efficient whereas the best yield of **4a** was achieved when 1 : 4 DMSO–H_2O was used (entry 3 *vs.* 2 and 4, Table 3). Moreover, we observed that the reaction was relatively faster in aqueous DMSO rather than in aqueous 1,4-dioxane (*e.g.* 1 *vs.* 3 h). Thus the conditions in entry 3 (Table 3) were found to be optimum and used for our further studies.

We then examined the substrate scope and generality of this method (Table 4). The heteroaryl alkyne **3** containing contrasting groups *e.g.* aryl (**3a–b**, **3m** and **3o**), heteroaryl (**3l**), alkyl (**3c–g**, **3p**, **3g** and **3n**), hydroxyalkyl (**3h–k**, **3q** and **3r**) and trimethylsilyl (**3s**) substituents on the triple bond participated well in the reaction. Both quinoxaline (**3a–n**) and pyrazine (**3o–s**) derivatives showed similar reactivities affording the desired products in acceptable yields. All the nineteen compounds synthesized by this method were well characterized by spectral (NMR, IR and MS) data and the molecular structure of a representative compound **4a** was further confirmed unambiguously by single crystal X-ray diffraction (Fig. 3).[8] The study indicated that the supramolecular interactions between

Table 4 Synthesis of furo[2,3-*b*]quinoxalines/pyrazines **4**[a]



3 4

Entry	Alkyne (3)	Product (4); R =	*T*/h	Yield[b] (%)
1	**3a**	**4a**; Ph	1	78
2	**3b**	**4b**; C_6H_4-*p*-Me	1	73
3	**3c**	**4c**; $(CH_2)_3Me$	6	71
4	**3d**	**4d**; $(CH_2)_4Me$	4	68
5	**3e**	**4e**; $(CH_2)_5Me$	2	71
6	**3f**	**4f**; $(CH_2)_9Me$	6	62
7	**3g**	**4g**; CMe_3	3	76
8	**3h**	**4h**; CMe_2OH	4	66
9	**3i**	**4i**; CH_2OH	3	62
10	**3j**	**4j**; $(CH_2)_2OH$	3	64
11	**3k**	**4k**; 1-hydroxycyclohexyl	3	68
12	**3l**	**4l**; 2-pyridinyl	2	60
13	**3m**	**4m**; Ph	1	73
14	**3n**	**4n**; $(CH_2)_9Me$	5	68
15	**3o**	**4o**; Ph	1	76
16	**3p**	**4p**; CMe_3	2	69[c]
17	**3q**	**4q**; CMe_2OH	3	66
18	**3r**	**4r**; 1-hydroxycyclohexyl	3	62
19	**3s**	**4s**; $SiMe_3$	4	58

[a] Reactions were carried out using **3** (0.7575 mmol), K_2CO_3 (0.3787 mmol) in DMSO–H_2O (1 : 4) (3 mL) at 80 °C. [b] Isolated yield. [c] KOH was used in place of K_2CO_3.

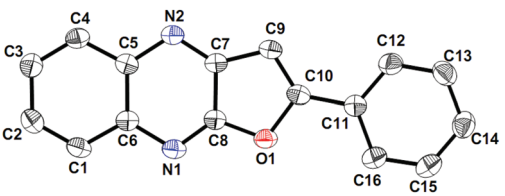


Fig. 3 ORTEP diagram of compound **4a** (30% probability, hydrogen atoms and another asymmetric molecule have been omitted for clarity).

nitrogen and hydrogen are within the range of 2.730–2.770 Å, which resulted two types of interactions *i.e.* (N1···H15 = 2.734 Å, N2···H9 = 2.764 Å). These weak van der Waals interactions resulted in a 1D crystal packing network, as shown in Fig. 4.

It is evident that the present transition metal[9] free hydrolysis–cyclization of alkyne **3** proceeds *via* a 2-hydroxy-3-alkynyl quinoxaline/pyrazine intermediate (Scheme 1).[10] To gain further evidence, 3-(phenylethynyl)quinoxalin-2-ol (**3aa**) prepared from **3a** was isolated and treated under the conditions in entry 3 of Table 3, and **4a** was isolated in good yield. The scope of the present strategy was expanded further by TFAA/H_3PO_4 mediated C-3 benzoylation of **4a** (Scheme 2).

Most of the compounds **4** along with splitomicin[5b] were tested at 50 μM for their ability to inhibit the Sir2 protein [yeast sirtuin family NAD-dependent histone deacetylase (HDAC)] by estimating the inhibition of growth of a yeast strain containing URA3 gene at a telomeric locus, in the presence of 5-fluoroorotic acid (5-FOA) (the yeast cell based

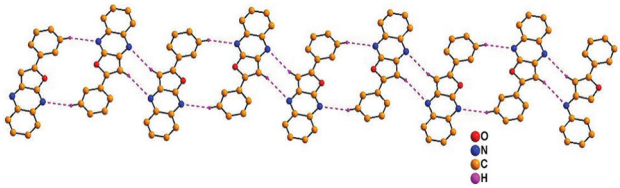
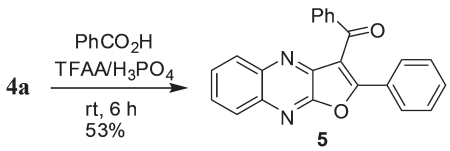


Fig. 4 Supramolecular interactions and crystal packing of compound **4a**.



Scheme 2 TFAA/H_3PO_4 mediated C-3 acylation of **4a**.

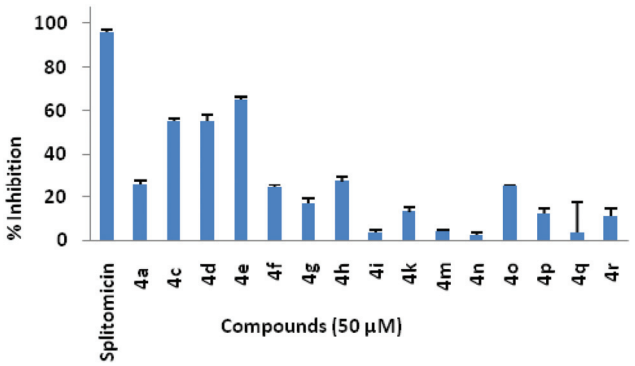


Fig. 5 URA3 silencing assay of known inhibitor splitomicin and **4**.

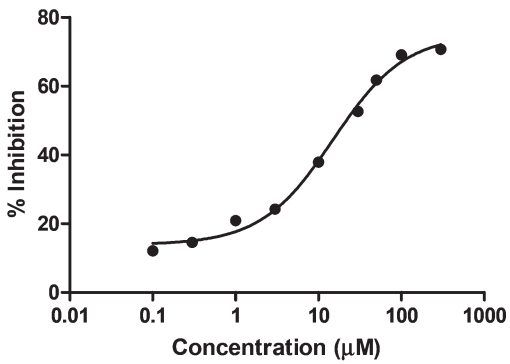


Fig. 6 Dose dependent URA3 silencing assay of **4e**.

reporter silencing assay, see ESI†).[11] As the Sir2 protein is inhibited, the URA3 gene would be de-repressed, resulting in the death of the yeast cell in the presence of 5-FOA. The compounds were also screened in parallel in the absence of 5-FOA to check their cytotoxicity. In general, the furo[2,3-*b*]quinoxalines showed better activities than the furo[2,3-*b*]pyrazines. Among the furo[2,3-*b*]quinoxalines, compounds having a linear alkyl side chain at C-2, *e.g.* **4c**, **4d** and **4e** (or **C** of Fig. 1 and 2), showed significant inhibition (55, 56 and 65%, respectively) in the presence of 5-FOA (Fig. 5) and no

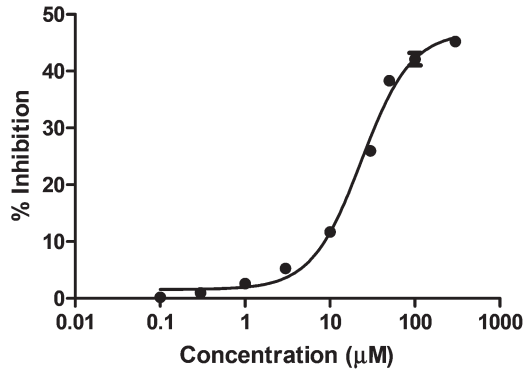


Fig. 7 Dose dependent mammalian SIRT1 assay of **4e**.

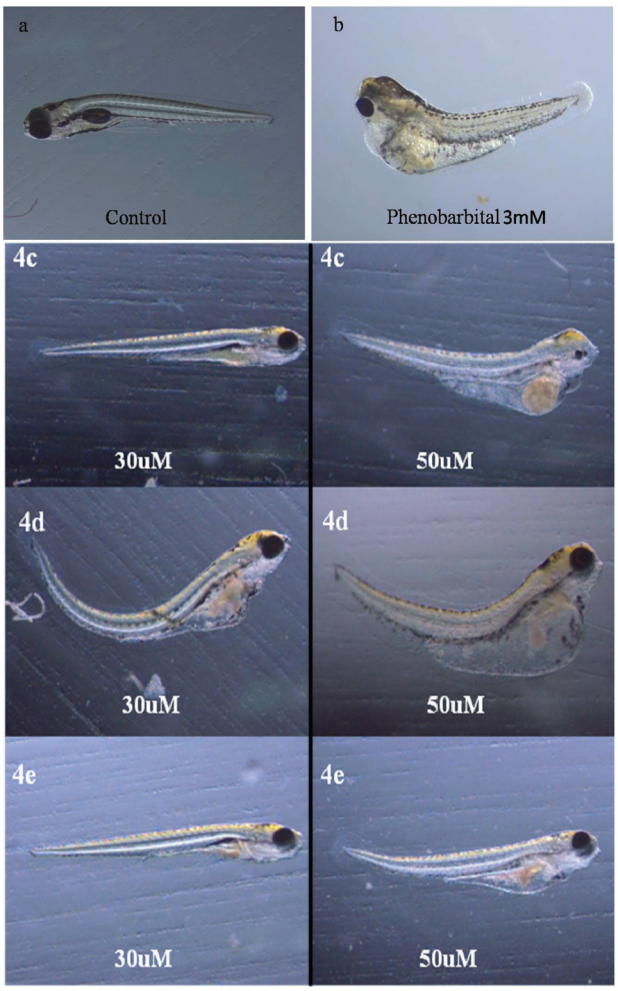


Fig. 8 (a) Control embryo showing normal body; embryo treated with (b) phenobarbital (positive control) showing severe abnormalities, body bent; compound **4c** and **4e** showing slight/no abnormalities at 30 μM and moderate abnormalities at 50 μM; **4d** showing moderate and severe abnormalities at 30 and 50 μM respectively.

significant toxic effect in the absence of 5-FOA. A longer side chain or a hydroxyalkyl or aryl group at C-2 was less effective. In a dose response study **4e** showed an IC_{50} ~ 15.02 μM in

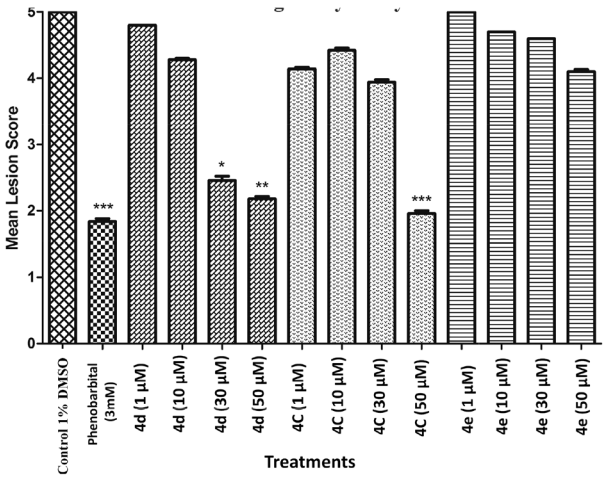


Fig. 9 Teratogenicity assay: mean (±S.D.) lesion score of all parameters different treatment groups. (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). Statistical significance was analyzed as control group *vs.* all groups.

Table 5 Results of zebrafish embryo toxicity studies of **4c**, **4d** and **4e**

	4c	4d	4e
Statistically significant toxic concentration (μM)	50	30, 50	>50
No observed adverse effect level (NOAEL) (μM)	<1	1	30
Minimal toxic concentration (MTC) (μM)	1	10	50

compared to splitomicin's 4.2 μM (Fig. 6). The compound **4e** also showed an IC_{50} ~ 23.5 μM (Fig. 7) against mammalian SIRT1, comparable to splitomicin's 60 μM,[5b] and no adverse effects until 30 μM when tested for toxicity in zebrafish embryos[12] (Fig. 8) at a range 1.0–50 μM [with no observed adverse effect level (NOAEL) ~ 30 μM and minimal toxic concentration (MTC) ~ 50 μM]. The results of this teratogenicity assay is summarized in Fig. 9 and Table 5. Notably, **4c** and **4d** showed low to severe abnormalities in zebrafish embryos (Fig. 8 and 9). In a preliminary MTT assay **4e** inhibited cell growth significantly (>50% inhibition @ ~ 30 μM) when tested against human hepatocellular liver carcinoma (HepG2) and cervical cancer (HeLa) cells.

In conclusion, furo[2,3-*b*]quinoxalines/pyrazines are synthesized as a novel and unique class of fused furo *N*-heterocycles *via* a tandem hydrolysis–cyclization strategy in a single pot. One of the furo[2,3-*b*]quinoxalines showed encouraging pharmacological properties *in vitro*/*in vivo* and is of further interest.

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