Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 6800

Received 18th June 2014,
Accepted 10th July 2014

DOI: 10.1039/c4ob01268b

www.rsc.org/obc

Ligand-free MCR for linking quinoxaline framework with a benzimidazole nucleus: a new strategy for the identification of novel hybrid molecules as potential inducers of apoptosis†

Rajnikanth Sunke,[a] P. Vijaya Babu,[a,b] Swapna Yellanki,[a,c] Raghavender Medishetti,[a,c] Pushkar Kulkarni[c] and Manojit Pal*[a]

We report a true MCR involving the reaction of *N*-(prop-2-ynyl)-quinoxalin-2-amine derivatives with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and Et_3N in DMSO to afford the pre-designed hybrid molecules containing quinoxaline framework linked with a benzimidazole nucleus. The MCR proceeds in the absence of any ligand and/or lateral addition of the catalyst/base affording products within 30 min in good yields, some of which showed encouraging apoptosis inducing properties in zebrafish.

Apoptosis, also termed as programmed cell death, is a series of genetically controlled events that result in the elimination of damaged or abnormal cells. Being an important method of cellular control, any disruption of apoptosis leads to abnormal growth of cells *e.g.* cancer. Thus, the induction of apoptosis in tumor cells is considered as an effective approach in the management and therapy of cancer as well as its prevention.[1] Indeed, many of the known anticancer agents and drugs work by inducing apoptosis in cancer cells. While various natural products and small molecules have been explored as inducers of apoptosis earlier, there is still a continued need for a new framework or scaffold for the design and discovery of potential novel apoptotic agents.

The hybrid molecules are generally defined as agents with two (or more) structural frameworks having different biological functions and dual activity (*e.g.*, **A**, Fig. 1), and can act as two distinct pharmacophores.[2a] However, the strategy of a hybrid molecule is also used to enhance the pharmacological activities of the individual pharmacophore (*e.g.* **B**, Fig. 1). For example, the weak cytotoxicity of distamycin A (Fig. 1) has been enhanced by tethering it with the known antitumor compounds or simple active moieties of known antitumor agents.[2b,c] Prompted by this idea we adopted a similar strategy to design our target hybrid molecules as potential apoptotic agents.

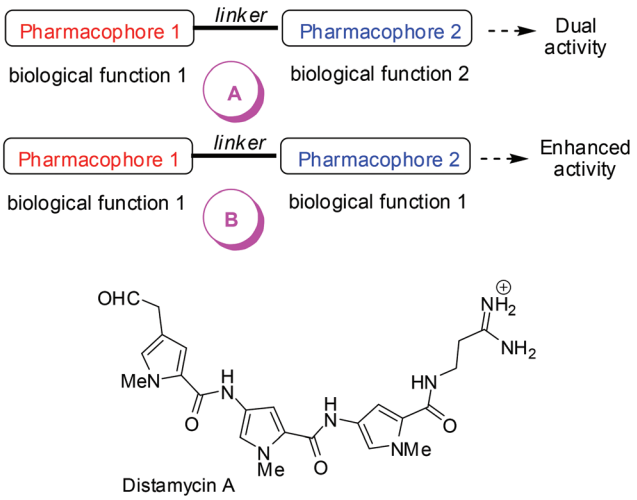


Fig. 1 The strategy of hybrid molecule **A** and **B** in the design and discovery of potential new drugs.

The quinoxaline framework has been reported to be an integral part of several anticancer agents.[3] The benzimidazole nucleus on the other hand has also found to be present in various antitumor/anticancer agents.[4] Thus we anticipated

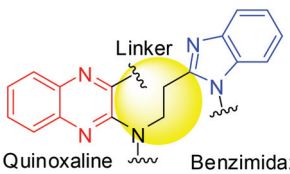
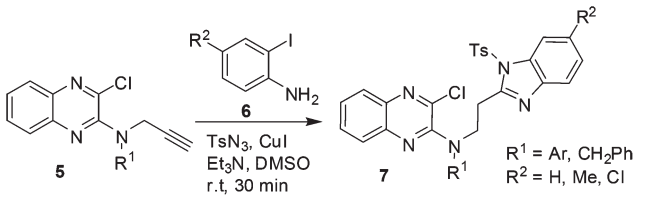


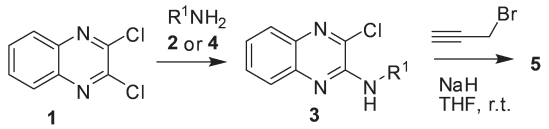
Fig. 2 New hybrid framework (**C**) for the design and identification of novel inducers of apoptosis.

[a]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India. E-mail: manojitpal@rediffmail.com; Tel: +91 40 6657 1500*
[b]*Chemistry Division, Institute of Science and Technology, JNT University, Kukatpally, Hyderabad 500072, India*
[c]*Zephase Therapeutics (an incubated company at the DRILS), University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India*

†Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds. See DOI: 10.1039/c4ob01268b

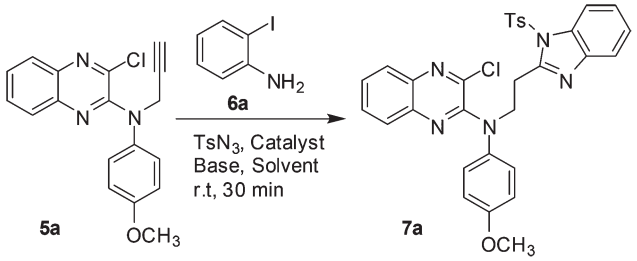


Scheme 1 Synthesis of hybrid molecules **7** based on **C** *via* a ligand-free MCR.



Scheme 2 Synthesis of compound **5**.

Table 1 Optimization of reaction conditions[a]



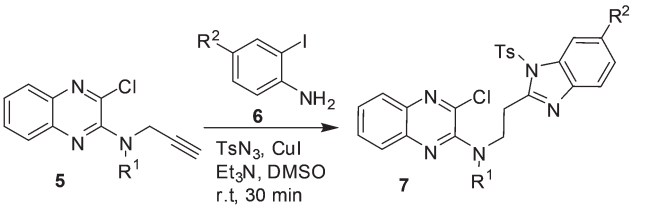
Entry	Catalyst	Base	Solvent	Yield[b] (%)
1	CuI	Et_3N	THF	32
2	CuI	Et_3N	CH_3CN	57
3	CuI	Et_3N	DCM	65
4	CuI	Et_3N	DCE	66
5	CuI	Et_3N	Toluene	48
6	CuI	Et_3N	DMSO	94
7	CuI	Et_3N	DMF	88
8	CuI	Et_3N	DMSO	94[c]
9	$Cu(OTf)_2$	Et_3N	DMSO	10
10	CuI	K_2CO_3	DMSO	70

[a] All the reactions are carried out using compound **5a** (0.30 mmol), **6a** (0.34 mmol), TsN_3 (0.36 mmol), in the presence of a Cu catalyst (0.03 mmol) and base (0.36 mmol) in a solvent (2 mL) at room temp for 30 min under nitrogen. [b] Isolated yield. [c] The reaction was carried using 30 mol% CuI and completed within 10 min.

that the combination of these two moieties connected through an appropriate linker in a single molecule may provide a new framework (**C**, Fig. 2) suitable for the design and identification of novel inducers of apoptosis. Indeed, the strategy was found to be operative in our case. Herein, we report our preliminary findings on the synthesis and pharmacological evaluation of compounds based on **C** as potential inducers of apoptosis. To the best of our knowledge, pharmacological evaluation of this class of compounds, especially as apoptotic agents, has not been explored earlier.

To achieve our project goal, we required a direct and straightforward synthetic method for the rapid supply of our

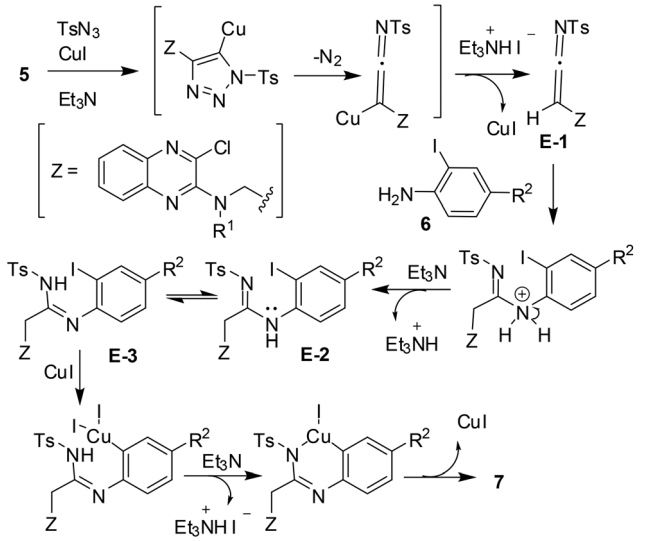
Table 2 Synthesis of *N*-substituted 3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]-imidazol-2-yl)ethyl)quinoxalin-2-amines (**7**)[a]



Entry	Alkyne (**5**); R^1 =	Aniline (**6**); R^2 =	Product (**7**); R^1, R^2 =	Yield[b] (%)
1	**5a**-C_6H_4OMe-*p*	**6a** H	**7a**-C_6H_4OMe-*p*, H	94
2	**5a**	**6b** Me	**7b**-C_6H_4OMe-*p*, Me	89
3	**5a**	**6c** Cl	**7c**-C_6H_4OMe-*p*, Cl	90
4	**5b**-C_6H_4Me-*p*	**6a**	**7d**-C_6H_4Me-*p*, H	92
5	**5b**	**6b**	**7e**-C_6H_4Me-*p*, Me	86
6	**5b**	**6c**	**7f**-C_6H_4Me-*p*, Cl	90
7	**5c**-C_6H_4Cl-*p*	**6b**	**7g**-C_6H_4Cl-*p*, Me	89
8	**5d**-C_6H_4Br-*p*	**6a**	**7h**-C_6H_4Br-*p*, H	95
9	**5d**	**6b**	**7i**-C_6H_4Br-*p*, Me	91
10	**5d**	**6c**	**7j**-C_6H_4Br-*p*, Cl	89
11	**5e**-C_6H_4F-*p*	**6a**	**7k**-C_6H_4F-*p*, H	93
12	**5e**	**6b**	**7l**-C_6H_4F-*p*, Me	86
13	**5f**-CH_2Ph	**6a**	**7m**-CH_2Ph, H	73
14	**5f**-CH_2Ph	**6b**	**7n**-CH_2Ph, Me	81

[a] All the reactions are carried out using compound **5** (0.30 mmol), **6** (0.34 mmol), TsN_3 (0.36 mmol), TEA (0.36 mmol) and CuI (0.03 mmol) in DMSO (2 mL), rt, N_2, 30 min. [b] Isolated yield.

target molecules based on **C**. A literature search revealed that the 2-substituted benzimidazole synthesized *via* the reaction of 1,2-phenylenediamines with the corresponding carboxylic acids or with aldehydes followed by oxidation[5] can be functionalized further, for example, by sulfonylation using an alkyl or aryl sulfonyl chloride.[6] However, this method appeared to be less attractive for the synthesis of molecules based on **C** as the introduction of quinoxaline moiety seemed to be difficult.



Scheme 3 Proposed reaction mechanism.

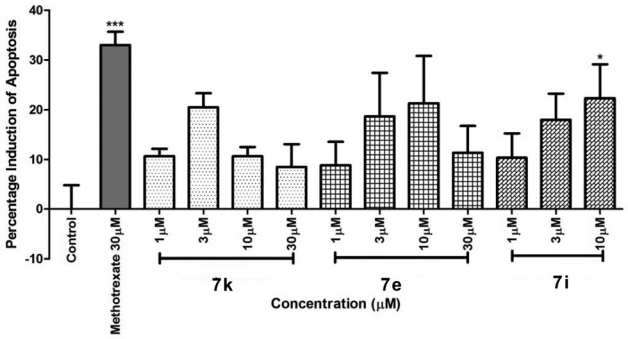


Fig. 3 Results of apoptosis assay: the percentage induction of apoptosis caused by compounds **7k**, **7e** and **7i** at different concentrations along with methotrexate (*p < 0.05, ***p < 0.001). All the statistical analysis was performed using GraphPad Prism® software.

This problem was found to be common with the other methods of constructing 1,2-disubstituted benzimidazole[7] moieties possessing the required quinoxaline[8] substituent.[9] Nevertheless, during the literature search a recent report on Cu-catalyzed 3-component cascade reaction of sulfonyl azides, alkynes and 2-bromoaniline leading to the functionalized benzimidazoles[10] attracted our attention. The substitution pattern on the benzimidazole ring was very similar to that we were looking for. Indeed, we envisioned that the incorporation of quinoxaline moiety into the alkyne reactant may afford our desired product in a single step. However, a closer look at the reported reaction[10] revealed that the methodology required the use of a ligand and lateral addition of an extra quantity of Cu catalyst as well as a base to facilitate the second step of the cascade sequence making it not truly an MCR (multi-component reaction[11]). Moreover, the methodology involved the overall use of 20 mol% of Cu catalyst and required a total reaction time of 6 h to complete the reaction. The yields of the products obtained were also not particularly high being in the range of 43%–78%. In contrast, we have observed that the reaction of *N*-(prop-2-ynyl)quinoxalin-2-amine derivative (**5**) (prepared *via* selective amination[12] of 2,3-dichloroquinoxaline **1** followed by propargylation, Scheme 2) with 2-iodoanilines (**6**) and tosyl azide in the presence of 10 mol% of CuI and Et_3N in DMSO afforded the desired target compound **7** (Scheme 1) within 30 min in the absence of any ligand and/or lateral addition of the catalyst/base. The methodology was used to prepare a range of target compounds in good to excellent yields, the details of which are presented here.

The reaction of 3-chloro-*N*-(4-methoxyphenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (**5a**) with 2-iodoaniline (**6a**) and tosyl azide was used to establish the optimized reaction conditions. The reaction was initially performed in the presence of CuI (10 mol%) and Et_3N at room temperature in the absence of any ligand in a number of solvents, such as THF, MeCN, DCM, toluene, DMSO and DMF (entries 1–7, Table 1). While the reaction proceeded well in all these solvents affording the desired product **7a** in variable yields, the best result was obtained when the reaction was performed in DMSO (entry 6, Table 1) affording **7a** in 94% yield. We were delighted with this observation as the reaction proceeded in the absence of any ligand and was completed within 30 min. Moreover, the use of 10 mol% of CuI was found to be enough to catalyze this MCR though the use of higher quantity of catalyst *e.g.*, 30 mol% of CuI completed the reaction within 10 min (entry 8, Table 1). Since the yield of **7a** was not improved further in this case, the conditions in entry 6 were identified as the best reaction

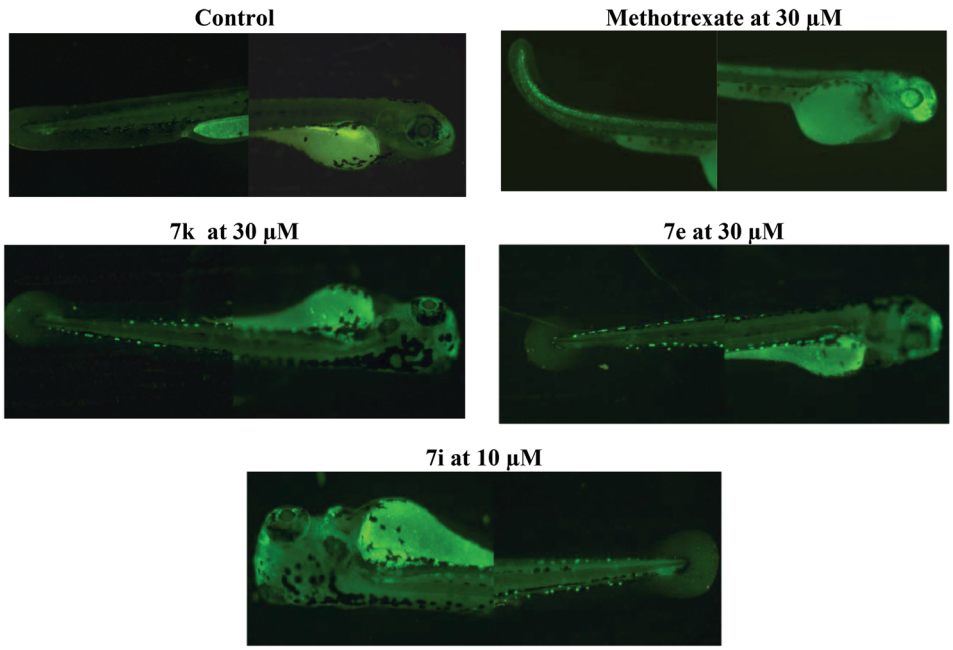


Fig. 4 Representative images of the embryos treated with compounds assayed for apoptosis.

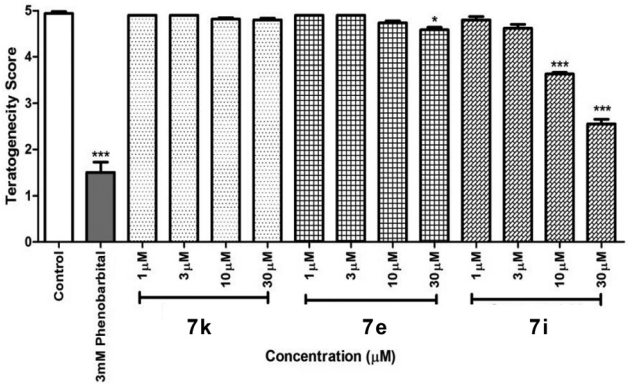


Fig. 5 Results of teratogenicity assay: each embryo was scored based on their level of toxicity from 5 being non toxic and 0.5 being highly toxic (*$p < 0.05$, ***$p < 0.001$). Statistical analysis for scoring was done using GraphPad Prism® software using two-way ANOVA. The graph represents the teratogenic scoring given compared to the positive control Phenobarbital.

conditions for further studies. We also examined the use of another catalyst $Cu(OTf)_2$ (entry 9, Table 1) and base K_2CO_3 (entry 10, Table 1), but these were found to be less efficient in terms of product yield. Nevertheless, being truly an MCR, the present method seemed to have advantages over the earlier method that was more of a cascade reaction.

The ligand-free MCR was further explored to expand its scope and generality. Thus a range of alkynes (**5**) along with a number of 2-iodoaniline derivatives were employed in the present MCR, and the results are summarized in Table 2 (see also Table S1 in ESI†). The reaction proceeded well in all these cases affording a variety of *N*-substituted 3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amines (**7**) in good to excellent yield (73%–95%).

From the view point of the reaction mechanism, the present MCR seems to proceed *via* a number of steps, including the formation of (i) ketenimine, (ii) tosylamide and finally (iii) intramolecular C–N bond as shown in Scheme 3. The terminal alkyne **5** reacts with the tosyl azide in the presence of CuI and Et_3N to form the ketenimine species **E-1** *via* a copper-catalyzed azide–alkyne cycloaddition (CuAAC) process[13] followed by the release of nitrogen gas from the resulting triazolo-Cu intermediate. **E-1** then undergoes nucleophilic attack at the sp-carbon by the 2-iodoaniline derivative (**6**), which triggers several changes including the tautomerism of *N*-tosylamidine intermediate **E-2** to the tosylamide **E-3**. The Cu-catalyzed intramolecular C–N bond formation of **E-3** then shifts the tautomerism equilibrium from towards **E-3** and affords product **7**. Thus, the organo-copper(III) species generated from **E-3** and CuI undergoes intramolecular cyclization, involving the initial formation of N–Cu(III) bond followed by the reductive elimination of CuI to give **7**.

While the reason for the (i) rapid reaction, (ii) low catalyst loading, (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products in the present case in compared to the earlier protocol[10] is not clear if the use of the 2-iodoaniline derivative in place of 2-bromo analog could be a possible reason. It is well known

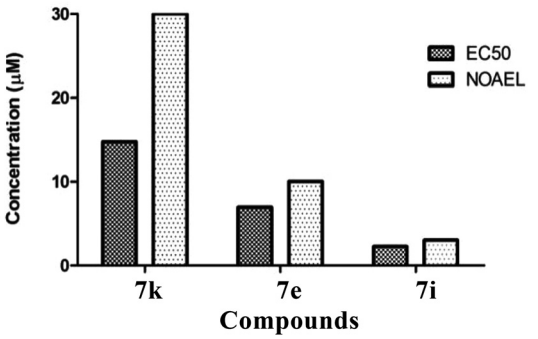


Fig. 7 The EC_{50} (apoptosis) and NOAEL of test compound **7k** (EC_{50} = 14.76 μM & NOAEL = 30 μM), **7e** (EC_{50} = 6.94 μM & NOAEL = 10 μM), and **7i** (EC_{50} = 2.23 μM & NOAEL = 3 μM) (*$p < 0.05$). The overall therapeutic index (ratio of NOAEL/EC_{50}) of **7k** is 2.032, **7e** is 1.44, and **7i** is 1.34.

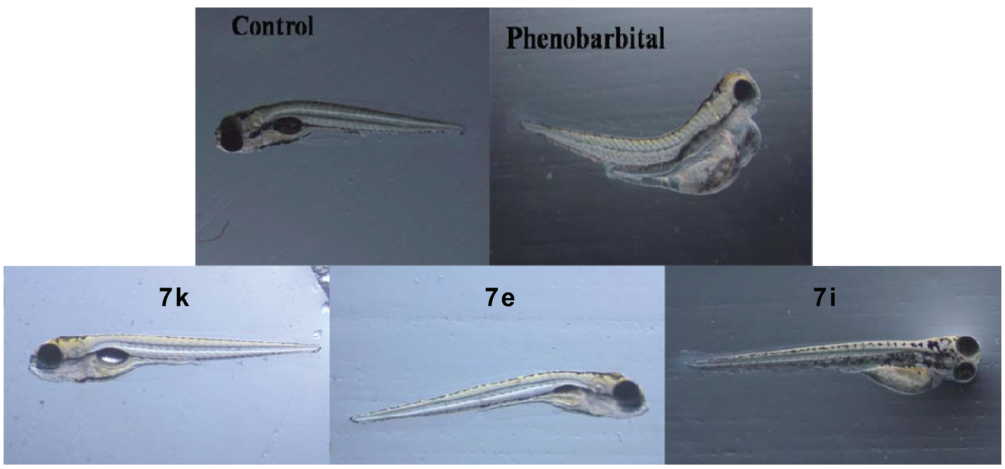


Fig. 6 Representative zebrafish images from the teratogenicity assay of compounds tested at 30 μM.

Table 3 Summary of the pharmacological evaluations of compounds **7k**, **7e**, and **7i**

Pharmacological evaluations				Test compounds data		
Tests	Endpoint	Positive control	Parameters	**7k**	**7e**	**7i**
Apoptosis	Acridine Orange staining of apoptotic cells	Methotrexate	EC_{50}	14.76	6.94	2.23
Teratogenicity	Morphological assessment of phenotypic changes	Phenobarbital	NOAEL	30	10	3
Overall therapeutic index	Ratio of NOAEL/EC_{50}	—	Therapeutic index	2.032	1.44	1.34

that the reactivity order of the halogen substituent towards the transition metal catalyst is I > Br > Cl and therefore 10 mol% Cu catalyst alone in the presence of Et_3N was found to be enough to facilitate the overall transformation efficiently. The other reason could be the nature of terminal alkynes used. The alkynes (**5**) used in the present MCR contain a tertiary amino group, which because of its bulkiness, could force the orientation of the tosylamide moiety of **E-3** to a position favorable for intramolecular cyclization thereby accelerating the Cu-catalyzed C–N bond forming step.

In order to assess their potential to induce apoptosis, the synthesized compounds were tested in zebrafish embryos[14] along with a known drug methotrexate[15] at 30 μM. Based on their considerable effects in the present assay of apoptosis compounds **7k**, **7e** and **7i** were further tested at 1, 3, 10 and 30 μM along with methotrexate (Fig. 3 and 4). While the compound **7k** showed an increase in its apoptotic activities up to 3 μM, a decrease in activity was observed at 10 and 30 μM. The embryos were found to be safe at all concentrations. In the case of compound **7e**, the increase in apoptotic activities was observed with the increase of concentration from 1 to 10 μM, but the activity was decreased at 30 μM. Compound **7i** showed significant apoptotic activity at 10 μM; however, the embryos were dead when the concentration was increased to 30 μM.

These compounds were also evaluated for their potential toxicities[16] *e.g.* teratogenicity in the zebrafish embryo at a range of 1.0–30 μM. The toxicological evaluation was carried out in a blind fashion. All the embryos in the control group were found to be normal. Phenobarbital (3 mM) was used as a positive control in this assay (Fig. 5 and 6). The compound **7k** was found to be non-toxic in all the tested concentrations. While the compound **7e** showed mild toxicity at 30 μM, it was found to be safe at lower concentrations *e.g.*, 1, 3 and 10 μM. Compound **7i** was found to be safe at 1 and 3 μM but showed toxicity at 10 and 30 μM.

Based on the summary of EC_{50} values (apoptosis), NOAEL (no observed adverse effect level) and the overall therapeutic index (Fig. 7 and Table 3), the safety order of the tested compounds appears to be **7k** > **7e** > **7i**. Overall, the compound **7k** was found to be safest whereas **7i** was identified as the most potent inducer of apoptosis in zebrafish,[17] indicating the present class of compounds are of further interest. To assess their anti-proliferative properties *in vitro*, compound **7i** and **7k** were tested against cancer cell line derived from tongue tissue *e.g.* CAL 27 at 10 μM using the sulphorhodamine B (SRB) assay.[18] Two compounds *e.g.*, 3-chloro-*N*-(4-fluorophenyl)quinoxalin-2-amine[12] (**8**) and 1-tosyl-1*H*-benzo[*d*]imidazole[19] (**9**) in addition to the reference compound gemcitabine[20] were also included in this assay to test the concept presented in Fig. 1 (*e.g.* **B**). While **7i** and **7k** showed 61% and 46% growth inhibition of CAL 27, respectively, the compound **8** and **9** showed only 20%–25% inhibition, indicating the usefulness of this concept.

In conclusion, an efficient MCR has been developed involving the reactions of *N*-(prop-2-ynyl)quinoxalin-2-amine derivatives with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and Et_3N in DMSO to afford the pre-designed target compounds containing the quinoxaline framework linked with a benzimidazole nucleus. In contrast to the previously reported cascade reaction for the synthesis of a similar class of compounds, the present MCR seemed to have the following favorable features *e.g.*, (i) rapid reaction (30 min), (ii) low catalyst loading (10 mol%), (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products (73%–95%). A range of novel hybrid molecules originally designed as potential inducers of apoptosis were prepared using this methodology and tested for apoptosis, and teratogenicity in zebrafish embryos. Furthermore, some of these compounds showed encouraging apoptosis inducing properties and therefore seem to have potential medicinal value. MCR presented here could be useful in building a library of hybrid molecules that are helpful for medicinal/pharmaceutical chemistry and drug discovery efforts.

RS thank CSIR for a research fellowship. The authors thank the management of DRILS and CSIR [Grant 02(0127)/13/EMR-II] for support.

Notes and references

1 P. Workman, in *Apoptosis and Cell Cycle Control in Cancer*, ed. N. S. B. Thomas, BIOS Scientific Publishers Ltd, Oxford, 1996, pp. 205–232.

2 (*a*) For an account, see: B. Meunier, *Acc. Chem. Res.*, 2008, **41**, 69; (*b*) L. F. Tietze, H. P. Bell and S. Chandrasekhar, *Angew. Chem., Int. Ed.*, 2003, **42**, 3996; (*c*) P. G. Baraldi, A. N. Zaid, D. Preti, F. Fruttarolo, M. A. Tabrizi, A. Iaconinoto, M. G. Pavani, M. D. Carrion, C. L. Cara and R. Romagnoli, *ARKIVOC*, 2006, **vii**, 20.

3 (*a*) E. Sprague, S. D. Undevia, F. Innocenti, J. Ramirez, L. K. House, H. L. Kindler, D. A. Singh, A. A. Desai, R. L. Schilsky and M. J. Ratain, *J. Clin. Oncol*, (Meeting Abstracts) July 2004, **22**(14_suppl), 2021; (*b*) N. C. Kakodkar, R. Peddinti, M. Kletzel, Y. Tian,

L. J. Guerrero, S. D. Undevia, D. Geary, A. Chlenski, Q. Yang, H. R. Salwen and S. L. Cohn, *Pediatr. Blood Cancer*, 2011, **56**, 164, DOI: 10.1002/pbc.22639; (*c*) R. R. Becklin, C. L. Chepanoske, J. M. Peltier, L. Qi, P. B. Robbins, S. R. Sahasrabudhe, R. Selliah, K. Simmon, B. R. Stockwell, R. G. Venkat, M. von Rechenberg and E. Y. Zhen, *US Patent application number* US20090214465 A1, Aug 27, 2009.

4 (*a*) P. Xiang, T. Zhou, L. Wang, C.-Y. Sun, J. Hu, Y.-L. Zhao and L. Yang, *Molecules*, 2012, **17**, 873; (*b*) M. M. Ramla, M. A. Omar, A.-M. M. El-Khamry and H. I. El-Diwani, *Bioorg. Med. Chem. Lett.*, 2006, **14**, 7324.

5 (*a*) M. Raban, H. Chang, L. Craine and E. Hortelano, *J. Org. Chem.*, 1985, **50**, 2205; (*b*) L. Garuti, M. Roberti and C. Cermelli, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2525; (*c*) L. M. Dudd, E. Venardou, E. Garcia-Verdugo, P. Licence, A. J. Blake, C. Wilson and M. Poliakoff, *Green Chem.*, 2003, **5**, 187; (*d*) J. Charton, S. Girault-Mizzi and C. Sergheraert, *Chem. Pharm. Bull.*, 2005, **53**, 492; (*e*) S. Y. Lin, Y. Isome, E. Stewart, J. F. Liu, D. Yohannes and L. Yu, *Tetrahedron Lett.*, 2006, **47**, 2883; (*f*) B. Das, H. Holla and Y. Srinivas, *Tetrahedron Lett.*, 2007, **48**, 61.

6 (*a*) A. Puratchikody, V. Sivajothi, A. Jaswanth, K. Ruckmani and M. Nallu, *Indian J. Heterocycl. Chem.*, 2002, **11**, 241; (*b*) L. Garuti, M. Roberti and E. D. Clercq, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2707; (*c*) H. Foks, D. Pancechowska-Ksepko, W. Kuzmierkiewicz, Z. Zwolska, E. Augustynowicz-Kopec and M. Janowiec, *Chem. Heterocycl. Compd.*, 2006, **42**, 611.

7 For a recent reviews on benzimidazole synthesis, see: S. S. Panda, R. Malik and S. C. Jain, *Curr. Org. Chem.*, 2012, **16**, 1905.

8 For a recent review on quinoxaline synthesis, see: Y. V. D. Nageswar, K. H. V. Reddy, K. Ramesh and S. N. Murthy, *Org. Prep. Proced. Int.*, 2013, **45**, 1.

9 For the synthesis of benzimidazole linked quinoxaline derivatives, see: L.-H. Chen, C.-M. Chang, D. B. Salunke and C.-M. Sun, *ACS Comb. Sci.*, 2011, **13**, 391.

10 H. Jin, X. Xu, J. Gao, J. Zhong and Y. Wang, *Adv. Synth. Catal.*, 2010, **352**, 347.

11 (*a*) R. C. Cioc, E. Ruijter and R. V. A. Orru, *Green Chem.*, 2014, **16**, 2958; (*b*) A. Dömling, W. Wang and K. Wang, *Chem. Rev.*, 2012, **112**, 3083.

12 B. Prasad, K. S. Kumar, P. V. Babu, K. Anusha, D. Rambabu, A. Kandale, G. R. Vanaja, A. M. Kalle and M. Pal, *Tetrahedron Lett.*, 2012, **53**, 6059.

13 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; (*b*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057; (*c*) E. J. Yoo, M. Ahlquist, I. Bae, K. B. Sharpless, V. V. Fokin and S. Chang, *J. Org. Chem.*, 2008, **73**, 5520.

14 (*a*) M. Westerfield, *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)*, University of Oregon Press, Eugene, OR, 4th edn, 2000; (*b*) A. Nakhi, S. Archana, G. P. Seerapu, K. S. Chennubhotla, K. L. Kumar, P. Kulkarni, D. Haldar and M. Pal, *Chem. Commun.*, 2013, **49**, 6268; (*c*) J. M. Panzica-Kelly, C. X. Zhang, T. L. Danberry, A. Flood, J. W. DeLan, K. C. Brannen and K. A. Augustine-Rauch, *Birth Defects Res., Part B*, 2010, **89**, 382, DOI: 10.1002/bdrb.20260.

15 Y.-X. Chen, W.-G. Lv, H.-Z. Chen, F. Ye and X. Xie, *Eur J. Obstet. Gynecol. Reprod. Biol.*, 2009, **142**, 107.

16 For results of zebrafish embryo toxicity study with toxicological indices and major organs/systems affected in positive control and at MTC (maximum tolerable concentration) of test compounds, see Table S2 in the ESI.†

17 (*a*) While, the observed order of EC_{50} (apoptosis) of compounds *e.g.* **7i** > **7e**> **7k** (low to high) is not clear at this stage the possibility of different rate of metabolism under *in vivo* conditions employed could play an important role. For example, a possible metabolic site *i.e.* C-5 position of the benzimidazole ring was blocked by the Me group in case of **7i** and **7e** but not in case of **7k**. Secondly, a medium sized group like Br or Me at C-4 of the N–Ph ring (*e.g.* **7i** and **7e**) seemed to be beneficial rather than a smaller group like F (*e.g.* **7k**); (*b*) Although the compound **7k** showed slightly better "therapeutic index", the compound **7i** however appeared to be a better candidate in terms of drug like properties. We thank one of the reviewers for pointing out this.

18 (*a*) L. V. Rubinstein, R. H. Shoemaker, K. D. Paull, R. M. Simon, S. Tosini, P. Skehan, D. A. Scudiero, A. Monks and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1113; (*b*) P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.

19 Z. Peng, J. W. Wong, E. C. Hansen, A. L. A. Puchlopek-Dermenci and H. J. Clarke, *Org. Lett.*, 2014, **16**, 860.

20 E. Chu and V. T. DeVita, *Physicians' Cancer Chemotherapy Drug Manual, 2007*, Jones & Bartlett, 2007.