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Cu-Catalyzed ligand-free synthesis of rosuvastatin based novel indole derivatives as potential anticancer agents†

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Rosuvastatin based novel indole derivatives designed as potential anticancer agents were synthesized *via* a newly developed ligand-free, simple, straightforward and inexpensive one-pot method. The methodology involved a Cu-catalyzed coupling-cyclization of a rosuvastatin based alkyne with *o*-iodoanilides in the presence of CuI and K_2CO_3 in PEG-400. Three of the synthesized compounds showed promising anti-proliferative activities against cancer cell lines and an increase of *p21* mRNA expression and apoptotic effects in zebrafish embryos/larvae.

The inhibitors of 3-hydroxy-3-methylglutaryl coenzyme **A** (HMG-CoA) reductase *e.g.* statins are a well known therapy for the treatment of cardiovascular diseases especially hypercholesterolemia.[1] They have also attracted enormous interest as potential anticancer agents[2] because of preclinical evidence on their antiproliferative, proapoptotic, anti-invasive and radiosensitizing properties. Indeed, their inhibition of HMG-CoA reduces mevalonate synthesis thereby decreasing the downstream products of the mevalonate pathway, which in turn reduces protein farnesylation and geranylgeranylation. This causes decrease in (i) the expression of matrix metalloproteinase-9 and *E*-selectin (implicated in tumor cell metastasis), (ii) angiogenesis *via* inhibition of TNF-α thereby limiting tumor growth and (iii) translocation of Ras and Rho proteins to the cell membrane thereby decreasing tumor cell proliferation and migration. Studies have shown that statin's use is associated with a decreased risk of cancer-specific mortality in breast, colorectal and prostate cancer[3] and has an excellent long term safety.[4]

Rosuvastatin (**A**, Fig. 1), a member of the statin family, has been reported to show anti-proliferative as well as apoptotic effects when tested against papillary thyroid[5] and breast cancer cell lines.[6] All these reports prompted us to explore rosuvastatin as a starting point for the identification of new anti-cancer agents. While the side chain of rosuvastatin is considered as the pharmacophore for its lipid lowering activities the heteroaryl part appeared to be an interesting scaffold for the design of new anticancer agents. Indeed, 4-aryl substituted pyrimidin-2-amine derivatives have been explored as anticancer agents (selective adenosine A1 receptor antagonists) earlier.[7] In view of anticancer properties of compounds[8] containing 2-substituted indole framework we replaced the side chain of **A** by this moiety to design a new template **B** for the generation of small molecules as potential anticancer agents (Fig. 1). We therefore required a direct, efficient and inexpensive synthetic method for accessing a focused library of molecules based on **B** for pharmacological screen.

Among the various methods reported for the synthesis of indoles,[9] intramolecular cyclization of 2-alkynylanilid(n)es catalyzed by a range of transition metal catalysts[10] including copper, molybdenum, iridium, mercury, gold, platinum, and rhodium has been explored extensively. Indeed, because of their economic advantages and potential uses in large-scale reactions

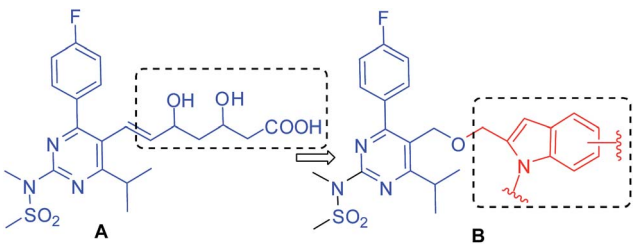

Fig. 1 Design of rosuvastatin-indole based new template for the identification of potential anticancer agents.

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† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, and copies of spectra. See DOI: 10.1039/c6ra20148b

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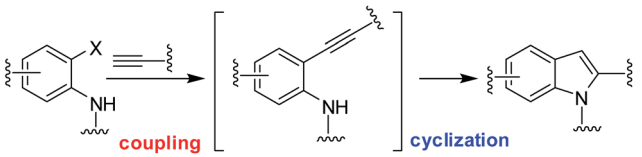
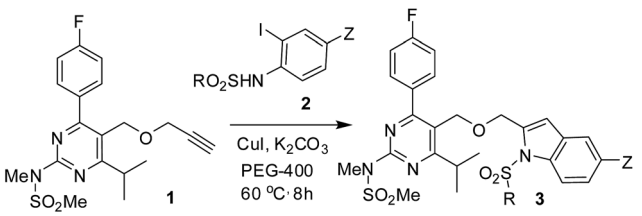


Scheme 1 Synthesis of indoles *via* the one-pot coupling-cyclization strategy.

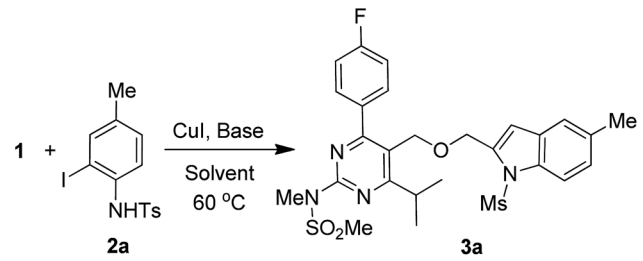
the copper catalysts have attracted particular attention for this purpose.[11] Apart from their uses in the cyclization of 2-alkynylanilid(n)es copper catalysts have also been explored in the one-pot coupling-cyclization process [*i.e. in situ* generation of 2-alkynylanilid(n)es followed by cyclization in the same pot] (Scheme 1) leading to indole derivatives.[12] However, these methods involved the use of expensive or complex ligands such as PPh_3 or $[Cu(phen)(PPh_3)_2]NO_3$ or L-proline *etc.* and a hazardous organic solvent such as toluene or DMF. In our effort we have reported the synthesis of indole derivatives *via* the coupling-cyclization strategy (Scheme 1) using Pd/C–CuI–PPh_3 as a catalyst system.[13] Though appeared to be effective and useful these methods however involve the use of a bimetallic catalyst system and an expensive phosphine ligand. Recently, we have demonstrated that the coupling-cyclization strategy can also be performed using a Cu-salt as the single and only catalyst in PEG-400.[14] The methodology is free from the use of expensive and toxic palladium catalysts, ligands and harmful organic solvents and has been explored for the synthesis of isocoumarins and isoquinolin-1(2*H*)-ones earlier. In further continuation of this work we now report a straightforward and direct synthesis of rosuvastatin based novel indole analogues (**3**) *via* the coupling-cyclization of terminal alkynes (**1**) with various *o*-iodoanilides (**2**) in the presence of CuI and K_2CO_3 in PEG-400 (Scheme 2). The synthesized indoles were evaluated for their cytotoxic/pro-apoptotic effects *in vitro* and *in vivo* the preliminary results of which are presented.

The key starting alkyne **1** was prepared *via* the reaction of a rosuvastatin intermediate[15a] with propargyl bromide (see ESI†). Subsequently, the reaction of **1** with *N*-(2-iodo-4-methylphenyl)methanesulfonamide (**2a**) was carried out under various conditions. Initially, the reaction was performed in PEG-400 in the presence of 15 mol% CuI and K_2CO_3 (entry 1, Table 1) when the desired indole **3a** was obtained in 50% yield. The increase of catalyst loading from 15 to 20 mol% improved the product yield to 82% (entry 2, Table 1) though a further increase



Scheme 2 Cu-Catalyzed Pd/ligand-free synthesis of rosuvastatin based indole derivatives in PEG-400.

Table 1 The optimization of coupling of terminal alkyne **1** with **2a**[a]



Entry	CuI (mol%)	Base	Solvent	Time (h)	Yield[b] (%)
1	15	K_2CO_3	PEG-400	8	50
2	20	K_2CO_3	PEG-400	8	82
3	30	K_2CO_3	PEG-400	8	60
4	20	K_2CO_3	PEG-400	12	80
5	30	K_2CO_3	PEG-400	6	72[c]
6	20	K_2CO_3	H_2O	8	30
7	20	K_3PO_4	PEG-400	12	65
8	20	$CsCO_3$	PEG-400	12	72
9	20	K_2CO_3	PEG-400	8	55[d]

[a] Reactions were carried out using alkyne **1** (1 equiv.), **2a** (1 equiv.), base (2.0 mmol), and CuI in a solvent (5.0 mL) under nitrogen. [b] Isolated yield. [c] The reaction was performed at 80 °C. [d] The reaction was performed at 25 °C.

(*i.e.* to 30 mol%) did not improve the yield (entry 3, Table 1). Performing the reaction for a longer time or at elevated temperature (entry 4 and 5, Table 1) also did not improve the yield. The use of water in place of PEG-400 (entry 6, Table 1) or other bases *e.g.* Cs_2CO_3 and K_3PO_4 (entry 7 and 8, Table 1) was examined but found to be less effective. The lowering of reaction temperature was also found to be less effective (entry 9, Table 1). Thus, the combination of CuI–K_2CO_3 in PEG-400 was identified as the best reaction condition (entry 2, Table 1) for the synthesis of **3a** and therefore was used for the preparation of other analogues. We then prepared a range of desired indoles (**3**) (Table 2) *via* reacting the alkyne (**1**) with various *o*-iodosulphanilides (**2**) under the optimized reaction conditions.[15b] The reaction proceeded well in all these cases irrespective of presence of groups such as Me, F, Cl, Br and CF_3 on the anilide ring affording the desired indoles **3** in good to acceptable yields. All the products were characterized by spectral data. The presence of indole ring in compound **3** was confirmed by the appearance of a singlet in the range ~6.6–6.8 δ due to the C-3 indole proton in the corresponding ^{1}H NMR spectra. Further, the appearance of two singlets near ~4.9 and 4.5 δ indicated the presence of $-CH_2-O-CH_2-$ moiety in compound **3**. To expand the scope of this Cu-catalyzed methodology further, a selective desulfonylation of compound **3d** and **3e** were performed using Cs_2CO_3 in THF–MeOH (2 : 1) to afford the indole derivative **4a** and **4b**, respectively (Scheme 3).

A working mechanism is proposed (Scheme 4) for the present Pd/ligand-free Cu-catalyzed coupling-cyclization[16a,b] method leading to indoles. Since the solvent PEG-400 can play the role of reaction medium as well as a ligand hence a Cu(I) complex (**A**) is formed *via* the interaction of CuI with PEG.[16b,c]

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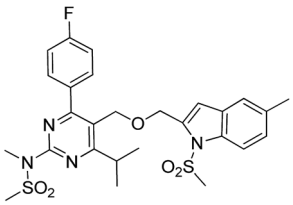
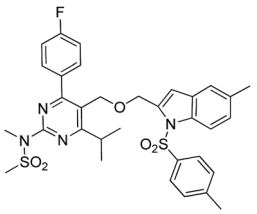
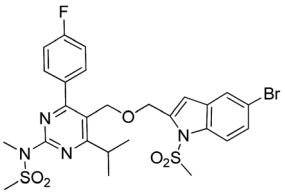
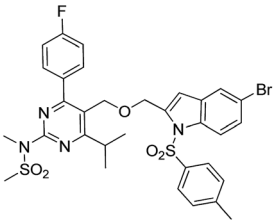
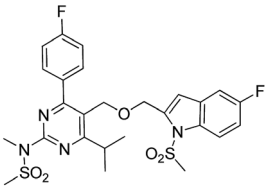
Table 2 Synthesis of rosuvastatin based novel indole derivatives *via* Cu-catalyzed coupling-cyclization in PEG-400 (Scheme 2)[a,b]

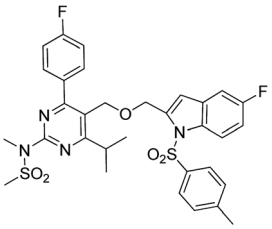

3a; 82%

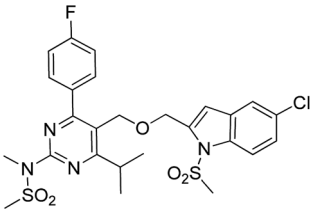

3b; 80%

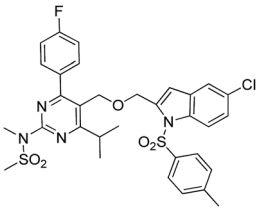

3c; 80%

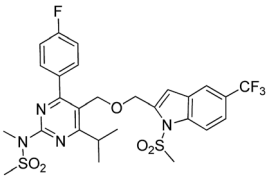

3d; 80%

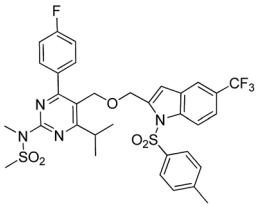

3e; 78%

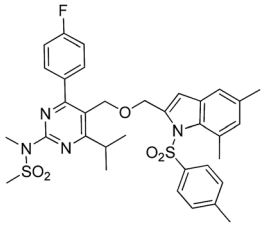

3f; 80%


3g; 78%


3h; 75%


3i; 79%


3j; 81%


3k; 80%

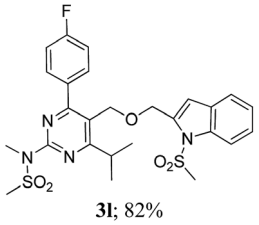
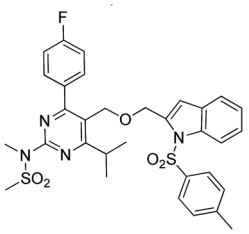

3l; 82%

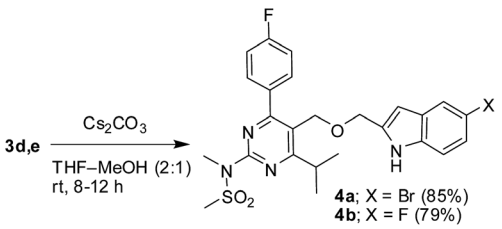
Table 2 (*Contd.*)


3m; 82%

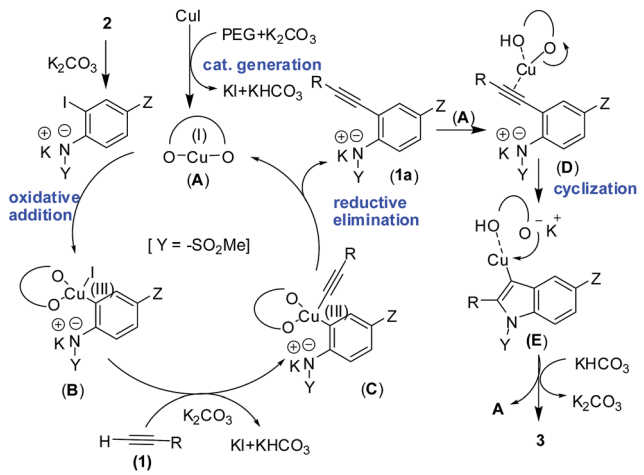
[a] All the reactions were carried out by using **1** (1.0 mmol), an appropriate *o*-iodoanilide (**2**, 1.0 mmol), K_2CO_3 (2.0 mmol), and CuI (20 mol%) in PEG-400 (5.0 mL) at 60 °C under nitrogen. [b] Yields reported are isolated yield.

The complex **A** then on oxidative addition with *o*-iodoanilide forms the arene–Cu(III) species **B.** The interaction of alkyne **1** with **B** in the presence of K_2CO_3 affords the arene–Cu(III)–alkyne species C, which on reductive elimination furnished the *o*-alkynyl anilide intermediate **1a.**[16d] The intermolecular cyclization of **1a** in the presence of **A** affords the desired indole **3** with the regeneration of active Cu(I) catalyst **A.**

To assess their anticancer properties some of the compounds synthesized were tested *in vitro* at 10 μM against A549 (human lung carcinoma cells) and TZM-BL (human cervical carcinoma cells) cell lines using a sulphorhodamine **B**



Scheme 3 Selective desulfonylation of compound **3d** and **3e**.



Scheme 4 The proposed reaction mechanism.

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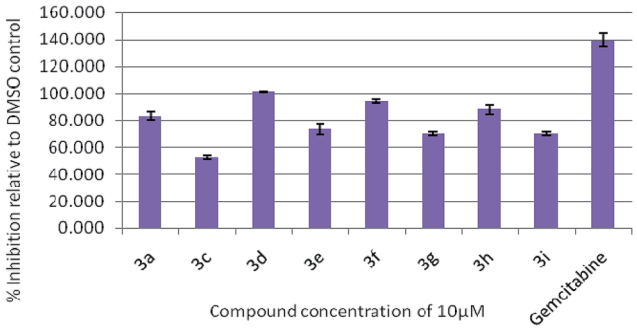

Fig. 2 Effect of compounds on the growth of A549 cell line at 10 μM.

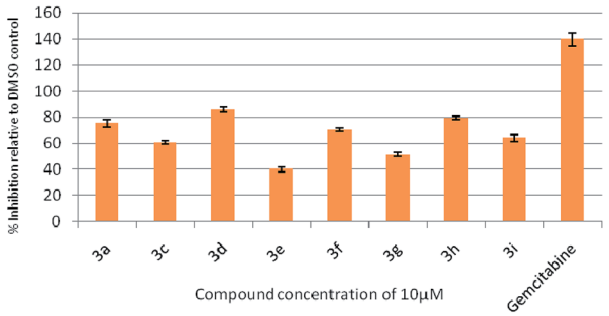

Fig. 3 Effect of compounds on the growth of TZM-BL cell line at 10 μM.

(SRB) assay with gemcitabine as a reference compound. In general, compound **3a**, **3d**, **3f**, **3h** and **3i** showed marginally better activities than **3c**, **3e**, and **3g** against both the cell lines (Fig. 2 and 3). Having found them active in cell based assay we then tested the best active compounds *i.e.* **3i**, **3f** and **3d** for their cytotoxic effect in the zebrafish embryos and larvae at different doses (Tables 3 and 4).[17] It was observed that the embryos treated with 24 μM of **3i** and **3f** ($n = 20$ each) showed phenotypic changes (with short eye, pericardial and yolk sac edema, defective upper and lower jaw) whereas embryos treated with compound **3d** (and the control embryos treated with 0.1% DMSO) did not show any phenotypic changes at this dose (Table 3 and Fig. 4). However, an increase of the concentration of **3d** to 36 μM led to mild phenotypic changes (Table 3 and Fig. 4). Notably, the observed phenotypic changes in embryos were due to the possible cytotoxic effects of test compounds. Nevertheless, the survival rate of embryos at 24 μM was found to be 20% for compound **3i**, 60% for **3f** and 90% for **3d**. Thus the NOAEL (No Observed Adverse Effect Level) of these compounds appeared to be <12 μM for **3i**, <12 μM for **3f** and <24 μM for **3d** ($n = 20$ each). To assess their effect at the stage when the zebrafish larvae become active and start swimming, the same concentrations of **3i**, **3f** and **3d** were used to treat larvae from 4 till 7 dpf (Table 4). Notably, no severe phenotypic changes were observed (Fig. 5). The mild phenotypic changes detected included pericardial and yolk sac edema at 24 μM for **3i** and **3f** ($n = 20$ each) and 36 μM for **3d** (Table 4). Even though liver toxicity was observed in all cases the survival rate was ~90% for all compounds. The NOAEL of these compounds appeared to be <12 μM for **3i**, and **3f** and <24 μM for **3d** ($n = 20$ each).

In order to gain further insight regarding apoptotic effects[18] of these compounds we examined their possible role in the increase of *p21* mRNA expression level in zebrafish. *P21* is a direct target of p53.[19] Indeed, p53 loss is responsible for the development of majority of cancers in human and in zebrafish, p53 similarly acts as a tumor suppressor and key mediator of apoptosis.[20] Thus, we evaluated[21] the *p21* mRNA level in zebrafish larvae treated with the test compounds both at NOEAL and MTC for 48 h (from 4 dpf to 6 dpf). Interestingly, a significant 4–5 fold increase in *p21* expression level (*** $p < 0.0001$) was observed at NOEAL that was doubled at MTC in all cases (Fig. 6). These observations indicated that all these compounds considerably activated the apoptotic pathway. Notably, compounds at the lower concentration also activated the apoptotic pathway (where no obvious phenotypes were observed) and initiated cell death that is beneficial particularly for the protection from cancer. Nevertheless, the MTC appeared

Table 3 Results of zebrafish embryo study (from 1-dpf embryos to 5-dpf larvae). The major organs/systems affected in embryos treated with 0.1% DMSO (control) and the test compounds[a]

		Concentrations (μM)												
	DMSO	**3i**				**3f**				**3d**				
Phenotypical changes	0.1%	3	6	12	24	3	6	12	24	3	6	12	24	36
Body shape	—	—	—	—	x	—	—	—	x	—	—	—	—	—
Head	—	—	—	xx	xxx	—	—	—	x	—	—	—	—	—
Eye	—	—	—	xx	xxx	—	—	—	x	—	—	—	—	—
Intestine	—	—	—	xx	xxx	—	—	—	x	—	—	—	—	x
Liver	—	—	—	xx	xxx	—	—	—	x	—	—	—	x	xx
Heart	—	—	—	x	xx	—	—	—	x	—	—	—	—	x
Jaw	—	—	—	xx	xx	—	—	—	—	—	—	—	—	x
NOAEL (μM)	—	<12				<12				<24				
MTC (μM)		24				24				36				

[a] The graded levels of phenotypical changes: (—) nil; (x) mildly toxic; (xx) medium toxic; (xxx) highly toxic. dpf: days post fertilization. NOAEL: No Observed Adverse Effect Level. MTC: maximum tolerated concentration.

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Table 4 Results of zebrafish larvae study (from 4-dpf larvae to 7-dpf larvae). The major organs/systems affected in embryos treated with 0.1% DMSO (control) and the test compounds[a]

		Concentrations (μM)												
	DMSO	**3i**				**3f**				**3d**				
Phenotypical changes	0.1%	3	6	12	24	3	6	12	24	3	6	12	24	36
Body shape	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Head	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eye	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Intestine	—	—	—	—	x	—	—	—	—	—	—	—	—	—
Liver	x	—	—	xx	xx	—	—	—	xx	—	—	—	x	xxx
Heart	x	—	—	—	xx	—	—	—	xx	—	—	—	—	x
Jaw	—	—	—	—	xx	—	—	—	xx	—	—	—	—	x
NOAEL (μM)	—	<12				<12				<24				
MTC (μM)		24				24				36				

[a] The graded levels of phenotypical changes: (—) nil; (x) mildly toxic; (xx) medium toxic; (xxx) highly toxic. dpf: days post fertilization. NOAEL: No Observed Adverse Effect Level. MTC: maximum tolerated concentration.

to be the cytotoxic dose for these compounds. Overall, the present class of compounds that showed growth inhibition of two cancer cell lines, and apoptotic effects in zebrafish embryos and larvae as visualized by an increase of *p21* mRNA expression deserves further study as potential anticancer agents.

In conclusion, a library of indole based novel small molecules derived from rosuvastatin was designed as potential anticancer agents. These compounds were synthesized using a ligand-free Cu-catalyzed coupling-cyclization method that involved the reaction of a rosuvastatin based alkyne with *o*-iodoanilides in the presence of K_2CO_3 in PEG-400. The present method afforded a range of desired products in good to acceptable yields. Three of the synthesized compounds *e.g.* **3i**, **3f** and **3d** showed promising anti-proliferative properties when tested against human lung carcinoma and human cervical carcinoma cell lines. They also showed proapoptotic effects in zebrafish embryos/larvae *via* activation of p53 pathway as indicated by an increase in the expression of *p21*, a direct target of p53. Overall, our study not only highlights the development of an operationally simple, straightforward and inexpensive one-pot method leading to indoles but also suggests that the described rosuvastatin-indole based framework could be

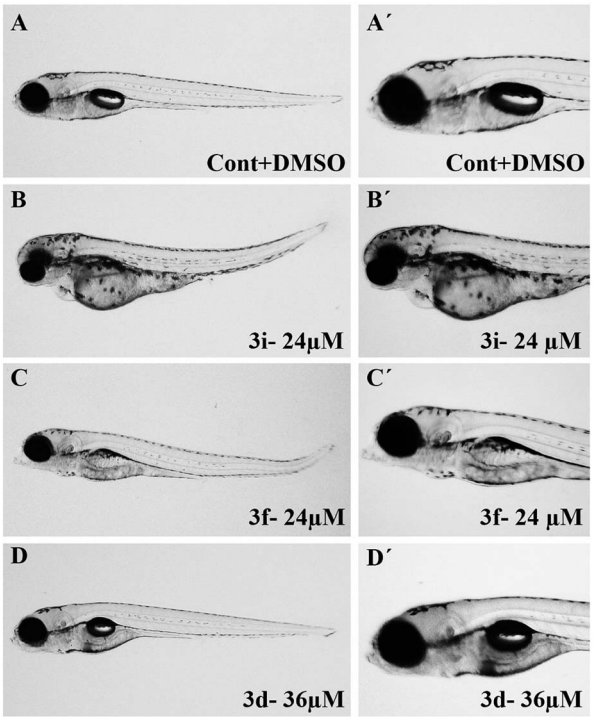

Fig. 4 Representative images of zebrafish larvae (5-dpf) treated with test compounds at MTC.

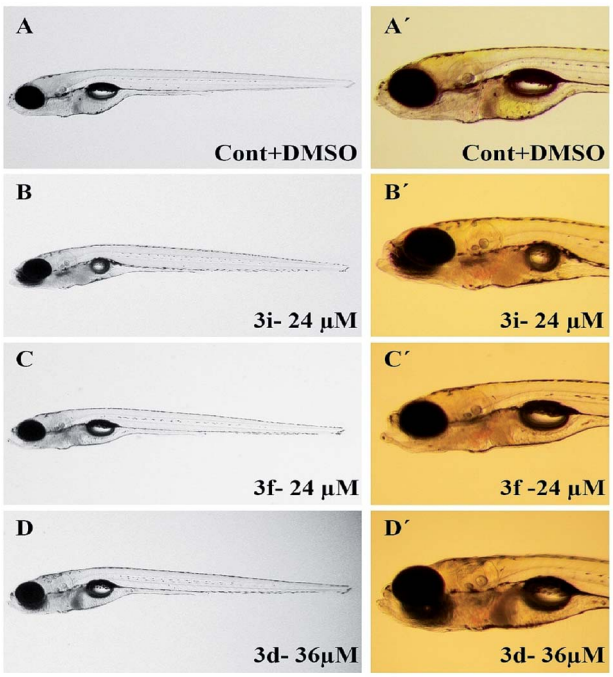

Fig. 5 Representative images of zebrafish larvae (7 dpf) treated between 4 and 7 dpf with test compounds at MTC.

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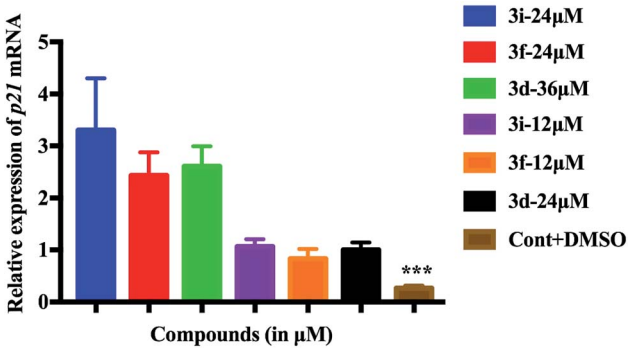

Fig. 6 Activation of the apoptotic pathway in zebrafish larvae as visualized by significantly increased *p21* expression after exposure to NOAEL and MTC of compounds.

a useful template for the design and discovery of potential anticancer agents.

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Notes and references

1 C. A. Aguilar-Salinas, H. Barrett and G. Schonfeld, *Atherosclerosis*, 1993, **141**, 203.
2 H. K. Takahashi, G. Weitz-Schmidt, H. Iwagaki, T. Yoshino, N. Tanaka and M. Nishibori, *J. Leukocyte Biol.*, 2006, **80**, 215.
3 (*a*) C. R. Cardwell, B. M. Hicks, C. Hughes and L. J. Murray, *J. Clin. Oncol.*, 2014, **32**, 3177; (*b*) S. F. Nielsen, B. G. Nordestgaard and S. E. Bojesen, *N. Engl. J. Med.*, 2012, **367**, 1792; O. Yu, M. Eberg, S. Benayoun, A. Aprikian, G. Batist, S. Suissa and L. Azoulay, *J. Clin. Oncol.*, 2014, **32**, 5.
4 M. F. Demierre, *Curr. Opin. Oncol.*, 2006, **18**, 180.
5 N. D. Zeybek, N. E. Gulcelik, F. F. Kaymaz, C. Sarisozen, I. Vural, E. Bodur, H. Canpinar, A. Usman and E. Asan, *J. Endocrinol.*, 2011, **210**, 105.
6 H. Erbaş, O. Bal and E. Cakır, *Balk. Med. J.*, 2015, **32**, 89.
7 L. C. W. Chang, R. F. Spanjersberg, J. K. V. F. D. Künzel, T. M. Krieger, G. V. D. Hout, M. W. Beukers, J. Brussee and A. P. IJzerman, *J. Med. Chem.*, 2004, **47**, 6529.
8 U. Jacquemard, N. Dias, A. Lansiaux, C. Bailly, C. Loge, J. M. Robert, O. Lozach, L. Meijer, J. Y. Merour and S. Routier, *Bioorg. Med. Chem.*, 2008, **16**, 4932.
9 For selected reviews, see: (*a*) S. Cacchi and G. Fabrizi, *Chem. Rev.*, 2005, **105**, 2873; (*b*) G. Leni and R. C. Larock, *Chem. Rev.*, 2004, **104**, 2285; (*c*) D. A. Horton, G. T. Bourne and M. L. Smythe, *Chem. Rev.*, 2003, **103**, 893; (*d*) H.-J. Knolker and K. R. Reddy, *Chem. Rev.*, 2002, **102**, 4303.
10 (*a*) K. Hiroya, S. Itoh and T. Sakamoto, *Tetrahedron*, 2005, **61**, 10958; (*b*) K. Hiroya, S. Itoh and T. Sakamoto, *J. Org. Chem.*, 2004, **69**, 1126; (*c*) S. Cacchi, G. Fabrizi and L. M. Parisi, *Org. Lett.*, 2003, **5**, 3843; (*d*) K. Hiroya, S. Itoh, M. Ozawa, Y. Kanamori and T. Sakamoto, *Tetrahedron Lett.*, 2002, **43**, 1277; (*e*) J. Ezquerra, C. Pedregal, C. Lamas, J. Barluenga, M. Pérez, M. A. García-Martín and J. M. González, *J. Org. Chem.*, 1996, **61**, 5804; (*f*) I. Ambrogio, A. Arcadi, S. Cacchi, G. Fabrizi and F. Marinelli, *Synlett*, 2007, 1775; (*g*) Y. Zhang, J. P. Donahue and C. J. Li, *Org. Lett.*, 2007, **9**, 627; (*h*) X. Li, A. R. Chianese, T. Vogel and R. H. Crabtree, *Org. Lett.*, 2005, **7**, 5437; (*i*) T. Kurisaki, T. Naniwa, H. Yamamoto, H. Imagawa and M. Nishizawa, *Tetrahedron Lett.*, 2007, **48**, 1871; (*j*) F. E. McDonald and A. K. Chatterjee, *Tetrahedron Lett.*, 1997, **38**, 7687; (*k*) T. Shimada, I. Nakamura and Y. Yamamoto, *J. Am. Chem. Soc.*, 2004, **126**, 10546; (*l*) B. M. Trost and A. McClory, *Angew. Chem., Int. Ed.*, 2007, **46**, 2074.
11 For an excellent review, see: S. Cacchi, G. Fabrizi and A. Goggiamani, *Org. Biomol. Chem.*, 2011, **9**, 641.
12 (*a*) F. Liu and D. Ma, *J. Org. Chem.*, 2007, **72**, 4844; (*b*) S. Cacchi, G. Fabrizi and L. M. Parisi, *Org. Lett.*, 2003, **5**, 3843; (*c*) S. Cacchi, G. Fabrizi, L. M. Parisi and R. Bernini, *Synlett*, 2004, 287; (*d*) G. A. Slough, V. Krchnak, P. Helquist and S. M. Canham, *Org. Lett.*, 2004, **6**, 2909.
13 (*a*) M. Pal, V. Subramanian, V. R. Batchu and I. Dager, *Synlett*, 2004, 1965; (*b*) M. Layek, U. Lakshmi, D. Kalita, D. K. Barange, A. Islam, K. Mukkanti and M. Pal, *Beilstein J. Org. Chem.*, 2009, 5, DOI: 10.3762/bjoc.5.46; (*c*) A. Nakhi, B. Prasad, U. Reddy, R. M. Rao, S. Sandra, R. Kapavarapu, D. Rambabu, G. R. Krishna, C. M. Reddy, K. Ravada, P. Misra, J. Iqbal and M. Pal, *Med. Chem. Commun.*, 2011, **2**, 1006; (*d*) R. M. Rao, U. Reddy, A. Nakhi, N. Mulakayala, M. Alvala, M. K. Arunasree, R. R. Poondra, J. Iqbal and M. Pal, *Org. Biomol. Chem.*, 2011, **9**, 3808; (*e*) B. Prasad, R. Adepu, S. Sandra, D. Rambabu, G. R. Krishna, C. M. Reddy, G. S. Deora, P. Misra and M. Pal, *Chem. Commun.*, 2012, **48**, 10434.
14 (*a*) R. G. Chary, G. R. Reddy, Y. S. S. Ganesh, K. V. Prasad, S. K. P. Chandra, S. Mukherjee and M. Pal, *RSC Adv.*, 2013, **3**, 9641; (*b*) R. G. Chary, G. Dhananjaya, K. V. Prasad, S. Vaishaly, Y. S. S. Ganesh, B. Dulla, K. S. Kumar and M. Pal, *Chem. Commun.*, 2014, **50**, 6797.
15 (*a*) N. Joshi, A. S. Khile, Y. B. Kajale and H. H. Kamble, World patent Application No. WO 2008/059519 A2, 2008; (*b*) While all the reactions were performed using *o*-iodosulphanilides (**2**) the use of an *o*-bromosulphanilide *e.g.* *N*-(2-bromo-4-methylphenyl)-4-methylbenzenesulfonamide was also examined. When reacted with the alkyne **1** under the optimized reaction conditions (*i.e.* entry 2 of Table 1) it afforded the desired compound **3a** in low yield (12%) indicating higher reactivity of iodo derivative over bromo analogue under the condition employed.
16 (*a*) V. Declerck, J. Martinez and F. Lamaty, *Synlett*, 2006, **18**, 3029; (*b*) J. Mao, J. Guo, F. Fang and S. J. Ji, *Tetrahedron*, 2008, **64**, 3905; (*c*) N. Yoshikai and E. Nakamura, *Chem. Rev.*, 2012, **112**, 2339; (*d*) Though Pd-free Cu-catalyzed

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Sonogashira reaction have been reported earlier (see for example, H. Jiang, H. Fu, R. Qiao, Y. Jiang and Y. Zhao, *Synthesis*, 2008, 2417), studies have indicated that these reactions can proceed even with ppb levels of Pd, see: Z. Gonda, G. L. Tolnai and Z. Novák, *Chem.–Eur. J.*, 2010, **16**, 11822.

17 (*a*) Zebrafish is an excellent model for screening of potential drugs due to the transparency and conserved gene expression applicable for high throughput analysis, see: Y. Gibert, M. C. Trengove and A. C. Ward, *Curr. Med. Chem.*, 2013, **20**, 2458; (*b*) Zebrafish lines wild-type Turku were maintained and raised at the zebrafish core facility Biomedicum, Helsinki, Finalnd. All animal experiments were performed in compliance with the national ethical guidelines, following the regulations set by the European Union, and were approved by the National Animal Experiment Board. Additionally, informed consent was obtained for any experimentation with human subjects.

18 J. F. Kerr, A. H. Wyllie and A. R. Currie, *Br. J. Cancer*, 1972, **26**, 239.

19 U. Langheinrich, E. Hennen, G. Stott and G. Vacun, *Curr. Biol.*, 2002, **12**, 2023.

20 N. Y. Storer and L. I. Zon, *Cold Spring Harbor Perspect. Biol.*, 2010, a001123, DOI: 10.1101/cshperspect.a001123.

21 For our earlier study, see: S. N. Dash, E. Lehtonen, A. A. Wasik, A. Schepis, J. Paavola, P. Panula, W. J. Nelson and S. Lehtonen, *J. Cell Sci.*, 2014, **127**, 1476.