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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticancer activity of some new s-triazine derivatives

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Abstract New s-triazine derivatives **13a–h** were synthesized for the structure–activity relationship studies as potent anticancer agents. The prepared analogues were evaluated for their in vitro inhibitory activity against the growth of PA-1 (Ovarian cancer), A549 (Lung cancer), MCF-7 (Breast cancer), and HT-29 (Colon cancer). Tri-substituted s-triazine derivatives (**13e–h**) with morpholino group on s-triazine scaffold exhibited potent anticancer activities compared to di-substituted s-triazine derivatives. Compounds **13e–h** also showed relatively selective PA-1 and HT-29 cancer cell inhibition over other cancer cell lines. Structure–activity relationships provided useful insights in these classes of compounds and paved the way to design novel analogues with more potency.

Keywords S-triazine derivatives · Anticancer activity · Structure–activity relationships · Hit identification

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Introduction

s-Triazine is a six-membered heterocyclic ring, with three nitrogens situated at 1st, 3rd and 5th positions. Its analogues, melamine, cyanuric acid and cyanuric chloride are important starting compounds for various materials with wide range of applications in textile, plastic, pharmaceuticals and rubber industries. These compounds are also used as pesticides, dyestuffs, optical bleaches, explosives and surface active agents (Bartholomew, 1996; Comins and O'Connor, 1988; Quirke, 1984; Smolin and Rapport, 1959). s-Triazine compounds have been studied extensively and are the subject of many reviews (Blotny, 2006; Giacomelli *et al*., 2004; Sathiakumar *et al*., 2011; Therrien, 2010). This s-triazine scaffold attracted many researchers, as its symmetrical structure facilitates to synthesize diverse set of analogues (ease of synthesis) such as 2, 4, 6-mono, di- or tri-substituted, symmetrical and nonsymmetrical compounds bearing different substituents and in particular, cyanuric chloride is the most important one in this aspect (Blotny, 2006). The s-triazine scaffold also provides the basis for the design of biologically relevant molecules with widespread application as therapeutics. For example, these compounds possess potent antiprotozoal (Klenke *et al*., 2001), antimalarial (Manohar *et al*., 2010; Melato *et al*., 2008), antiviral (Mahajan *et al*., 2009; Maarouf *et al*., 2012; Xiong *et al*., 2008), anticancer (Baindur *et al*., 2005; Leftheris *et al*., 2004; Manohar *et al*., 2010; Moon *et al*., 2002; Sun *et al*., 2010; Zheng *et al*., 2007), antimicrobial (Patel *et al*., 2012a, b, c; Raval *et al*., 2011; Singh *et al*., 2011; Srinivas *et al*., 2005, 2006; Desai *et al*., 2008), antituberculosis (Patel *et al*., 2012a; Sunduru *et al*., 2010) etc. Recently, the s-triazine derivatives were extensively investigated for anticancer activity with a particular target to mTOR/PI3K pathway (Menear *et al*., 2009; Poulsen *et al*., 2012; Tanneeru *et al*., 2012; Venkatesan *et al*., 2010;

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Verheijen *et al*., 2010; Zask *et al*., 2010; Zhang *et al*., 2011). Some of the compounds possessing s-triazine scaffold reported in the literature are presented in Fig. 1. Our continuous interest in the field of bioactive heterocyclic compounds (Kumar *et al*., 2010, 2011; Ravinder *et al*., 2012; Rao *et al*., 2011; Srinivas *et al*., 2009), we have got interest in new s-triazine derivatives as cancer therapeutics. In the context of identification of new chemical entities (NCEs) for cancer therapy, we have selected s-triazine as core scaffold as many reports indicate its significance (Fig. 1) and structural modifications were made at three positions (1st, 3rd and 5th positions) with various pharmacophores. In this article, synthesis of new s-triazine derivatives is presented. All the synthesized compounds were screened against four cancer cell lines and discussed to develop the structure–activity relationships (SAR) of this series. Among all, compounds **13e–h** exhibited potent inhibitory activity against ovarian (PA-1) and colon (HT-29) cancer cell lines

Chemistry

4-(Benzo[d]oxazol-2-yl) aniline (**3**) was prepared from the simple condensation of *o*-amino phenol (**1**) with *p*-amino benzoic acid in the presence of polyphosphoric acid at 200 °C (Scheme 1) (Chua *et al*., 1999). Nucleophilic substitution of one of the chlorines of the cyanuric acid (**4**) with substituted anilines (**5–7** & **11**) in the presence of diisopropylehtylamine (DIPEA) yielded mono-substituted triazine derivatives **8–10** & **12,** respectively, in good yields (mono substitution w.r.t. cyanuric chloride) and the synthetic scheme is presented in Scheme 2 (Zheng *et al*., 2007). Compounds **8–10** and **12** were reported in the literature (Hunter *et al*., 1994; McKay *et al*., 2006; Maga *et al*., 2011). Synthesis of compounds **13a–d** involves a coupling reaction between mono-substituted s-triazine derivatives (**8–10** & **12**) and one equivalent of compound **3** in the presence of K_2CO_3 (Scheme 3). Compounds **13e–h** were synthesized by the replacement of third chlorine atom of di-substituted s-triazine derivatives (**13a–d**) in the presence of K_2CO_3 (Scheme 4). All the intermediates and final compounds were purified by column chromatography and characterized by spectroscopic techniques.

Biology

The anticancer activities of all the synthesized compounds were evaluated by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] method using PA-1 (Ovarian cancer), A549 (Lung cancer), MCF-7 (Breast cancer) and HT-29 (Colon cancer) cell lines. The effect of compounds on the cell viability for each cell line after exposure to different concentrations were depicted in the Fig. 2 and their respective IC_{50} values are presented in Table 1. Synthesized compounds can be classified into two categories based on the presence of the chlorine atom and morpholino group *i.e.* di-substituted s-triazine derivatives (**13a–d**) and tri-substituted s-triazine derivatives (**13e–h**), respectively. Among the two categories, tri-substituted s-triazine derivatives were more active against in all tested human cancer cell lines than di-substituted s-triazine derivatives which suggest the key role of morpholino group for activities. In case of PA-1 cell lines, among **13e–h**, compounds **13e–g** are more potent and are equally potent as Doxorubucin. This indicates the importance of substituted aniline groups on s-triazine compared to benzylamine group. The activity difference in compounds **13e** and **13h** is more than one magnitude (**13e**: 0.61 ± 0.16 μM and **13h:** 10.5 ± 0.51 μM) and this attributes to bridge between

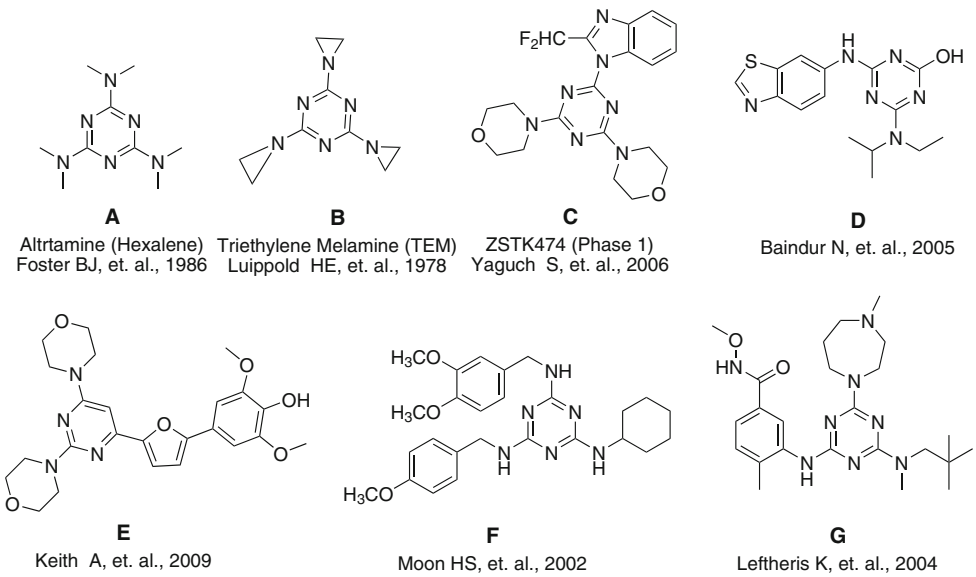
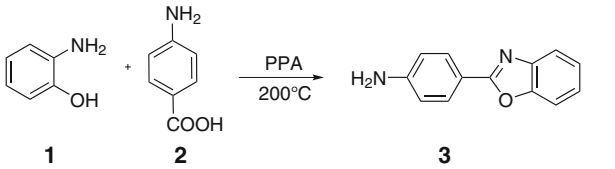
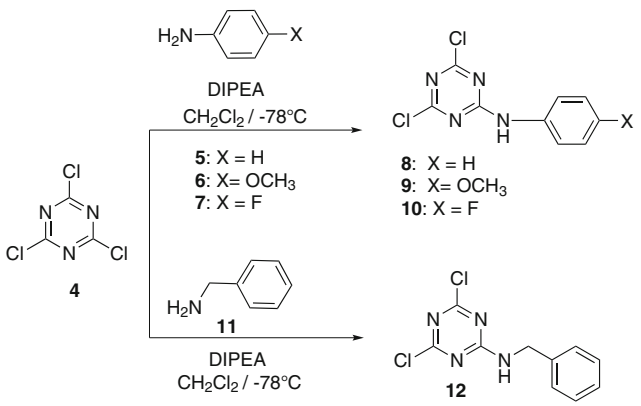


Fig. 1 List of potent anticancer compounds possessing s-triazine scaffold

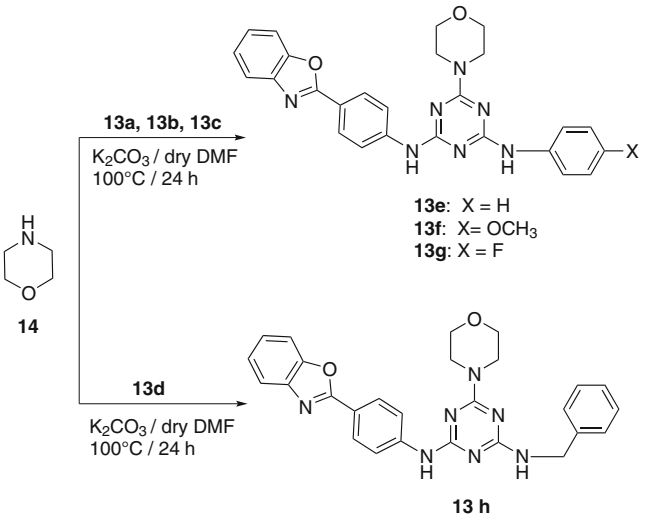
s-triazine and substituted group i.e. –NH– and –NH–CH2– groups, respectively. The di-substituted s-triazine derivatives (**13a–d**) were inactive against A549 (lung cancer) cell lines, whereas tri-substituted derivatives have exhibited relatively better inhibitory activity. Among the compounds **13e–h**, compound **13f** is relatively potent with IC_{50} at 12.9 ± 2.43 μM and other compounds (**13e**, **13g–h**) exhibited twofolds less inhibitory activity. In case of MCF-7 cell lines, compounds **13f** and **13h** are more potent than reference compound, doxorubicin. Among the all compounds in the series, **13h** is most potent. Compounds **13e** and **13g** exhibited moderate anticancer activity against MCF-7 cell lines. Compounds **13e–h** exhibited potent anticancer activity against HT-29 (Colon cancer) cell lines (Fig. 1) and these are more potent than reference compound, doxorubicin. Among the **13e–h**, compound **13e–g** are more potent with IC_{50} of 0.27 ± 0.02, 0.14 ± 0.04 and 0.31 ± 0.15 μM, respectively and compound **13h** is relatively less potent with IC_{50} at 0.92 ± 0.51 μM (Table 1). The disparity in magnitude of activity within **13e–h** is due to the structural dissimilarity i.e. the presence of anilino and substituted anilino groups in case of **13e–g** and the presence of benzylamino group in case of **13h**. In summary, compounds **13e–h** exhibited selective cytotoxicity in HT-29 & PA-1 when compared to other cell line (A549 and MCF-7) and compound **13f** is found to be most potent in respective cell lines. Structure–activity relationships (SAR) reveals that the presence of morpholino group, substituted aniline groups along with benzoxazole moiety on s-triazine ring are essential to exhibit potent anticancer activity. Structural modifications on aniline group and benzoxazole group can provide more insights and give better hits/leads and focus of our future work is on structural modifications on these classes of compounds to



Scheme 1 Synthesis of 4-(benzo[d]oxazol-2-yl) aniline



Scheme 2 Synthesis of compounds **8–10** & **12**



Scheme 4 Synthesis of compounds **13e–h**

Scheme 3 Synthesis of compounds **13a–d**

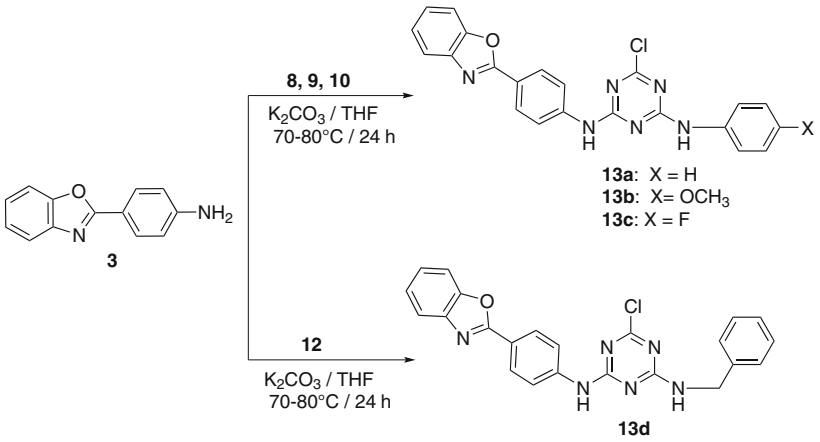
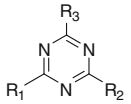
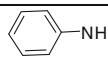
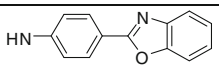
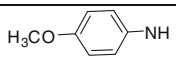
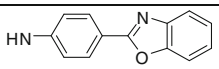
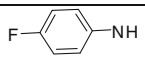
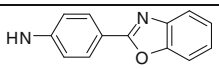
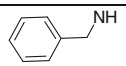
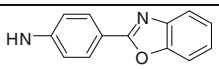
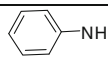
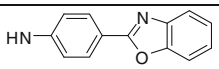
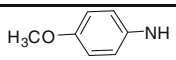
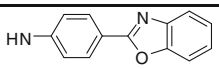
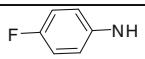
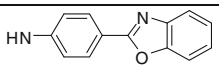
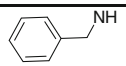
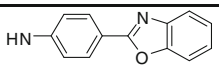


Table 1 Chemical structures of compounds **13a–h** and their inhibitory effects on the growth of tumour cell lines



Compound	R_1	R_2	R_3	IC_{50} (μM)			
				PA-1[a]	A549[b]	MCF-7[c]	HT-29[d]
13a			Cl	169±2.78	225±5.64	55.2±3.21	32.6±2.65
13b			Cl	172±6.21	278±3.98	269.7±6.98	139±5.47
13c			Cl	186±3.21	254±6.21	68.4±3.41	47±2.54
13d			Cl	195±5.43	296±2.45	66.1±2.48	44±3.59
13e				0.61±0.16	22.0±1.11	10.56±1.56	0.27±0.02
13f				0.25±0.19	12.9±2.43	6.48±0.21	0.14±0.04
13g				0.45±0.21	26.7±0.67	12.39±1.38	0.31±0.15
13h				10.5±0.51	23.4± 1.70	2.46±0.15	0.92±0.51
Doxorubicin ((Reference compound)				0.64±0.13	1.88±0.56	10.9±1.76	1.76±0.23

[a] Ovarian cancer cells; [b] Lung cancer cells; [c] Breast cancer cells, [d] Colon cancer cells

improve the anticancer activity and on understanding of this activity through mechanistic studies.

Conclusions

In conclusion, two series of s-triazine derivatives *i.e.* di-substituted (**13a–d**) and tri-substituted derivatives (**13e–h**) were synthesized as anticancer agents. All the synthesized compounds were screened against four cancer cell lines viz. PA-1 (Ovarian cancer), A549 (Lung cancer), MCF-7 (Breast cancer) and HT-29 (Colon cancer). This preliminary study demonstrated that tri-substituted derivatives **13e–h** exhibited potent anticancer activity over the di-substituted derivatives. Among these derivatives, compound **13f** is the most potent in respective cell lines. Structure–activity relationships in these classes of compounds laid foundations to develop more potent compounds as leads for cancer chemotherapy.

Experimental

Chemistry

*Synthesis of 4-(benzo[d]oxazol-2-yl) aniline (**3**) (Chua et al., 1999; Shi et al., 1996)*

To a stirring solution of polyphosphoric acid (PPA; 85 g), 2-Aminothiophenol **1** (5.78 g, 0.053 mol), para amino benzoic acid **2** (7.26 g, 0.053 mol) were added, heated at 220 °C for 4 h. After confirming the reaction completion by TLC (Thin Layer Chromatography), it was cooled, and poured into ice-cold 10 % aqueous sodium carbonate. The solid product was collected, washed with water and recrystallized with methanol water. Yield: 65 %; mp 180–183 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.07–8.03 (d, 2H, *J* 8.3 Hz), 7.73–7.67 (m, 1H), 7.55–7.49 (m, 1H), 7.31–7.27 (m, 2H), 6.78–6.74 (d, 2H, *J* 9.0 Hz) and 4.05 (brs, 2H); ESI–MS: *m/z* 211 (M + H).

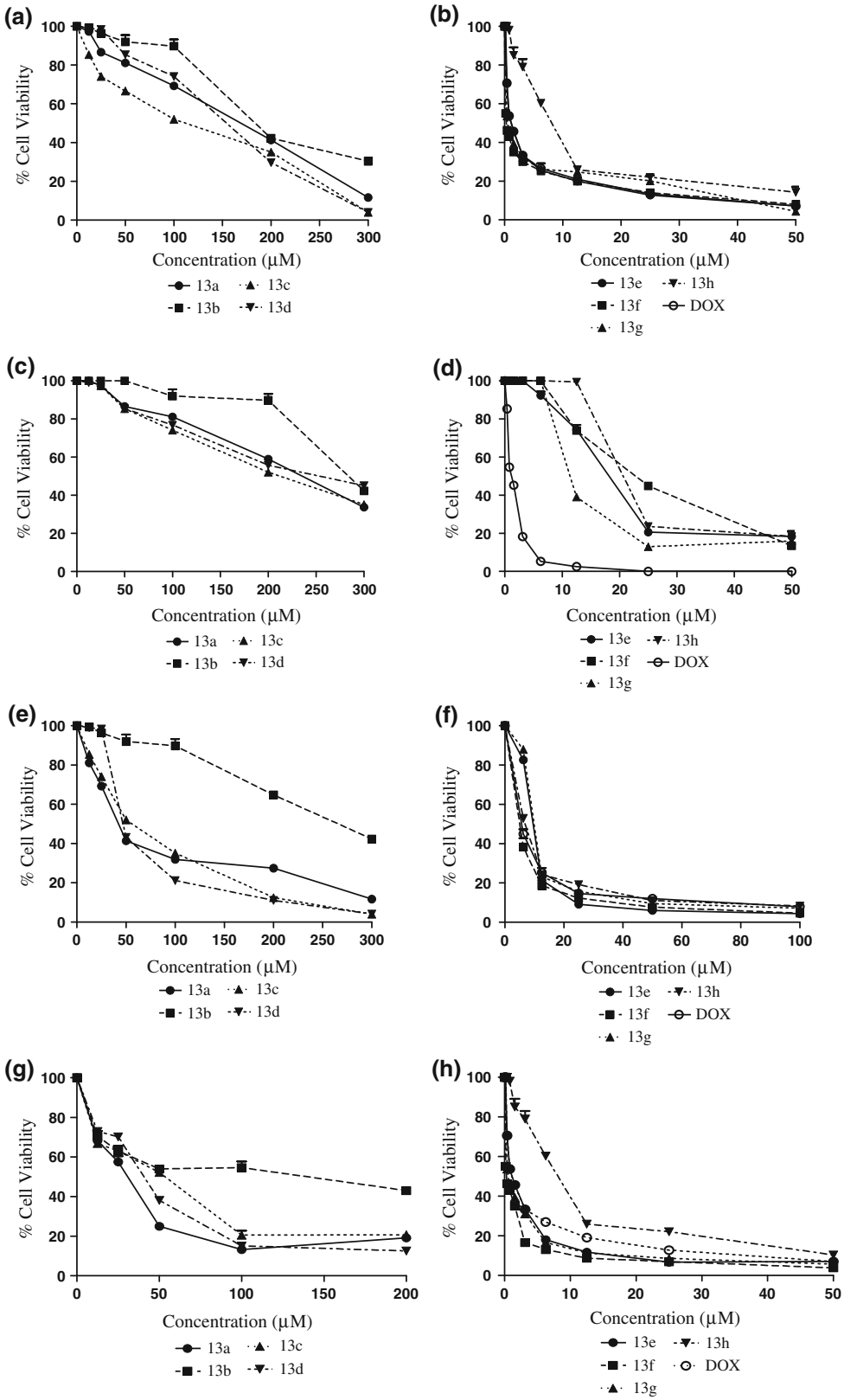


Fig. 2 Dose response of compounds (**13a–13h**) against **a**, **b** PA-1, **c**, **d** A549, **e**, **f** MCF-7, **g**, **h** HT-29 cancer cell lines

General procedure for synthesis of mono-substituted cyanuric chloride derivatives (Zheng et al., 2007)

Compounds **8**, **9**, **10** and **12** were synthesized using cyanuric chloride **4** (10 g, 0.054 mol) in dichloromethane (DCM), *N*,*N*- Di- isopropyl ethylamine (DIPEA). DIPEA (9.26 ml, 0.054 mol) was added slowly drop wise to the reaction mixture at −78 °C (maintained by using acetone and dry ice) for 10 min then it was allowed to stir for 10 min. Aniline **5** (4.94 ml, 0.054 mol) or 4-methoxyaniline **6** (6.66 g, 0.054 mol) or 4-fluoroaniline **7** (5.19 ml, 0.054 mol) or benzyl amine **11** (5.9 ml, 0.054 mol) were added respectively and allowed to stir for 15 min. The reaction was monitored by TLC. After completing the reaction, the reaction mixture was filtered, washed with water, extracted with chloroform, the organic layer was dried over Na_2SO_4 and then concentrated to get the desired mono-substituted s-triazines in excellent yield. Compounds **8–10** and **12** were reported in the literature (Hunter *et al*., 1994; McKay *et al*., 2006; Maga *et al*., 2011).

General procedure for synthesis of di-substituted s-triazines

Di-substituted cyanuric chloride derivatives **(13a–d)**, were synthesized using mono-substituted cyanuric chloride **8** (1 eq), **9** (1 eq), **10** (1 eq) and **12** (1 eq) in dry THF (30 ml), K_2CO_3 (2 eq). The reaction mixture was allowed to stir for 5 min then the benzoxazolephenylamine **3** (1 eq) was added and refluxed for 24 h at 70–80 °C. After confirming the reaction completion by TLC, K_2CO_3 was decanted and the THF was removed, water was added and extracted with EtOAc. The organic layer was dried over Na_2SO_4, concentrated and purified to give the desired di-substituted s-triazines.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-6-chloro-N^4-phenyl-1,3,5-triazine-2,4-diamine (**13a**):* Yield: 80 %; mp: 263–265 °C; IR (KBr): 3,273, 3,109, 1,610, 1,585, 1,243 and 989 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.32 (brs, 1H), 10.03 (brs, 1H) 8.11 (m, 2H), 7.93 (m, 2H), 7.74–7.68 (m, 2H), 7.57 (m, 1H) and 7.32 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.1, 150.1, 150.0, 142.0, 141.5, 128.8, 128.5, 127.8, 125.0, 124.7, 120.6, 120.4, 119.4, 110.8, 110.7; ESI–MS: *m/z* 415 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{22}H_{16}ClN_6O$ $[M + H]^+$ 415.8471, found 415.8342.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-6-chloro-N^4-(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (**13b**):* Yield: 82 %; mp: 275–276 °C; IR (KBr): 3,278, 3,185, 2,835, 1,232 and 992 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.36 (brs, 1H), 10.00 (brs, 1H), 8.13–8.07 (m, 3H,), 7.90 (s, 1H), 7.70–7.55 (m, 4H), 7.5 (s, 1H), 7.35 (m, 2H), 6.90 (m, 2H) and 3.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 165.1, 164.3, 164.3, 155.0, 151.9, 142.3, 142.2, 133.3, 133.2, 127.1, 123.4, 122.2, 121.9, 119.8, 119.7, 113.8, 79.6, 79.3, 79.1, 78.7; ESI–MS: *m/z* 445 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{23}H_{18}ClN_6O_2$ $[M + H]^+$ 445.1102, found 445.1112.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-6-chloro-N^4-(4-fluorophenyl)-1,3,5-triazine-2,4-diamine (**13c**):* Yield: 77 %; mp: 268–269 °C; IR (KBr): 3,384, 3,111, 1,508, 1,242 and 995 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 9.42 (brs, 1H), 9.16 (brs, 1H), 8.18–8.12 (m, 4H), 7.69–7.67 (m, 2H), 7.59–7.57 (m, 1H), 7.32–7.29 (m, 3H), 7.05–7.01 (t, 2H, *J* 8.5, 17.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 162.1, 160.5, 148.5, 140.1, 132.8, 126.0, 122.9, 122.6, 121.5, 119.2, 118.5, 117.6, 113.3, 108.6, 93.9; ESI–MS: *m/z* 433 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{22}H_{15}ClFN_6O$ $[M + H]^+$ 433.8376, found 433.8362.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-N^4-benzyl-6-chloro-1,3,5-triazine-2,4-diamine (**13d**):* Yield: 76 %; mp: 278-279 °C; IR (KBr): 3,268, 3,111, 2,993, 1,240 and 987 cm^{-1}; ^1H NMR (75 MHz, $CDCl_3$): δ 8.30–8.26 (m, 1H), 8.22–8.17 (m, 2H), 7.78–7.74 (m, 3H), 7.58–7.56 (m, 1H), 7.40–7.39 (m, 2H), 7.37–7.31 (m, 4H), 6.05 (brs, 1H), 5.77 (brs, 1H) and 4.72–4.66 (t, 2H, *J* 5.2, 10.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 148.9, 140.9, 140.6, 126.9, 126.8, 126.4, 125.6, 125.5, 118.4, 118.3, 118.0, 117.9, 117.8, 109.1, 109.0; ESI–MS: *m/z* 429 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{23}H_{18}ClN_6O$ $[M + H]^+$ 429.8737, found 429.8725.

General procedure for synthesis of tri-substituted s-triazines

Tri-substituted s-triazine derivatives **(13e–h)** were synthesized from di-substituted cyanuric chloride derivatives **13a–d** (1 eq) in dry DMF (20 ml), K_2CO_3 (2 eq). The reaction mixture was allowed to stir for 5 min then morpholine **14** (1 eq) was added and stirred for 24 h at 100 °C. The reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice-cold water, extracted with ehtylacetate and dried over Na_2SO_4. Solvent was evaporated and solid was purified to get desired product in good yield.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-6-morpholino-N^4-phenyl-1,3,5-triazine-2,4-diamine (**13e**):* Yield: 82 % mp: 259–260 °C; IR (KBr): 3,402, 3,299, 2,925–2,855 and 1,242 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 9.08 (brs,

1H), 8.67 (brs, 1H), 8.14–8.11 (d, 2H, *J* 8.6 Hz), 7.98–7.96 (d, 2H, *J* 8.4 Hz), 7.72–7.70 (t, 3H, *J* 7.9, 5.3 Hz), 7.61–7.58 (t, 1H, *J* 7.1, 14.3 Hz), 7.35–7.27 (m, 4H), 7.03–6.98 (m, 1H), 3.84 (d, 4H, *J* 3.2 Hz), 3.75 (d, 4H, *J* 3.3 Hz);); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 164.5, 163.9, 163.9, 162.4, 150.0, 143.7, 141.6, 139.8, 128.3, 127.8, 124.8, 124.6, 122.1, 122.0, 119.5, 119.3, 118.9, 10.6, 65.9, 43.4; ESI–MS: *m/z* 466 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{26}H_{24}N_7O_2$ $[M + H]^+$ 466.5065, found 466.5023.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-N^4-(4-methoxyphenyl)-6-morpholino-1,3,5-triazine-2,4-diamine (**13f**):* Yield: 80 %; mp: 252–253 °C; IR (KBr): 3,400, 3,307, 2,963, 2,922, 2,855 and 1,227 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.52 (brs, 1H), 9.08 (brs, 1H), 8.04–7.99 (d, 2H, *J* 8.3 Hz), 7.97–7.92 (m, 2H), 7.72–7.66 (m, 2H), 7.53–7.50 (d, 2H, *J* 8.3 Hz), 7.35–7.26 (m, 2H), 3.8 (m, 7H) and 3.7 (d, 4H, *J* 4.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 164.6, 163.8, 154.4, 151.4, 141.6, 132.6, 126.6, 122.8, 121.6, 121.3, 119.2, 113.3, 79.0, 78.8, 78.6, 78.1, 65.9, 54.9, 43.3; ESI–MS: *m/z* 496 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{27}H_{26}N_7O_3$ $[M + H]^+$ 496.2325, found 466.2091.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-N^4-(4-fluorophenyl)-6-morpholino-1,3,5-triazine-2,4-diamine (**13g**):* Yield: 84 %; mp: 246–247 °C; IR (KBr): 3,424, 3,297, 2,924–2,856, 1,508 and 1,243 cm^{-1}; ^{1}H NMR (75 MHz, DMSO-d_6): δ 8.22–8.19 (d, 2H, *J* 8.3 Hz), 7.75–7.72 (m, 3H), 7.58–55 (m, 1H), 7.53–7.48 (m, 2H), 7.37–7.31 (m, 2H), 7.08–7.02 (m, 3H), 6.99 (brs, 1H) 6.79 (brs, 1H) 3.84 (d, 4H, *J* 4.5 Hz) and 3.76 (d, 4H, *J* 4.5 Hz)**;);** ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 164.6, 164.0, 163.9, 151.4, 143.8, 141.7, 139.8, 134.8, 128.3, 126.7, 123.2, 122.0, 121.9, 121.4, 120.1, 119.5. 118.3, 110.9, 6539, 43.3; ESI–MS: *m/z* 484 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{26}H_{23}FN_7O_2$ $[M + H]^+$ 484.1970, found 484.1891.

*N^2-(4-(benzo[d]oxazol-2-yl)phenyl)-N^4-benzyl-6-morpholino-1,3,5-triazine-2,4-diamine (**13h**):* Yield: 78 %; mp: 240–242 °C; IR (KBr): 3,403, 3,296, 2,924, 2,853 and 1,241 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.33–9.29 (brs, 2H), 8.19–8.16 (d, 2H, *J* 8.3 Hz), 7.71–7.68 (d, 2H, *J* 8.3 Hz), 7.52–7.49 (d, 2H), 7.29–7.27 (m, 2H), 7.1 (m, 3H), 7.09–7.06 (m, 1H), 6.86 (s, 1H), 6.66–6.62 (d, 2H, *J* 7.9 Hz), 3.78–3.76 (d, 4H, *J* 4.5 Hz), 3.72–3.70 (d, 4H, *J*4.9 Hz) and 3.55 (s, 2H);); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 136.3, 163.1, 150.0, 138.6, 126.3, 125.6, 125.2, 125.0, 121.1, 119.9, 117.6, 117.5, 64.4, 42.0, 41.6; ESI–MS: *m/z* 480 $[M + H]^+$; HRMS (ESI) m/z Calcd. for $C_{27}H_{26}N_7O_2$ $[M + H]^+$ 480.5331, found 480.2103.

Biological evaluation

Materials

PA-1 (Ovarian cancer), A549 (Lung cancer) cell lines MCF-7 (Breast cancer) and HT-29 (Colon cancer) cell line were obtained from the National center for Cell science (NCCS), Pune, India. MEM, DMEM, RPMI, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], Trypsin–EDTA were purchased from Sigma Chemicals Co (st. Louis, MO), Fetal bovine serum were purchased from Gibco, USA, 96-well flat bottom tissue culture plates were purchased from Tarson.

Maintenance of cell lines

PA-1 (Ovarian cancer) A549 (Lung cancer) cell line were grown as adherent in MEM medium, MCF-7 (Breast cancer) cell line was grown as adherent in DMEM medium and HT-29 (Colon cancer) cell line was grown as adherent in RPMI medium supplemented with 10 % fetal bovine serum, 100 μg/ml penicillin, 200 μg/ml streptomycin, 2 mM L-glutamine, and culture was maintained in a humidified atmosphere with 5 % CO_2.

Preparation of samples for cytotoxicity

Stock solution for compounds **13a–13d** of 30 mM and compounds **13e–13f** of 10 mM stock solution in DMSO were prepared, from the above stock various dilutions were made with sterile PBS to get required concentration.

Cytotoxicity screening using MTT assay

MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay is a standard colourimetric assay for measuring cellular proliferation. MTT is a tetrazolium salt, which is yellow in colour and is photosensitive. MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] is taken by the living cells and reduced by a mitochondrial dehydrogenase enzyme to a purple formazan product that is impermeable to the cell membrane. Solubilisation with solvents like DMSO leads to liberation of product and amount of purple formazan product is directly related to the cell viability. 1×10^4 Cells (counted by Trypan blue exclusion dye method)) in 96-well plates were incubated with compounds and standard Cisplatin with series of concentrations for 24 h at 37 °C in MEM with 10 % FBS medium. Then, the above media was replaced with 90 μl of fresh serum free media and 10 μl of MTT reagent (5 mg/ml) and plates were incubated at 37 °C for 4 h, there after the above media was replaced with 200 μl of DMSO and incubated at 37 °C for 10 min. The

absorbance at 570 nm was measured on a spectrophotometer (spectra max, Molecular devices) IC_{50} values were determined from plot: % cell viability (from control) versus concentration.

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References

Baindur N, Chadha N, Brandt BM, Asgari D, Patch RJ, Schalk-HiHi C, Carver TE, Petrounia IP, Baumann CA, Ott H, Manthey C, Springer BA, Player MR (2005) 2-Hydroxy-4,6-diamino-[1,3,5]triazines: a novel class of VEGF-R2 (KDR) tyrosine kinase inhibitors. J Med Chem 48:1717–1720

Bartholomew D (1996) 1,3,5-Traizenes. In: Boulton AJ (ed) Comprehensive heterocyclic chemistry II. Pergamon, Oxford, pp 6–575

Blotny G (2006) Recent Applications of 2,4,6-trichloro-1,3,5-triazine and its derivatives in organic synthesis. Tetrahedron 62: 9507–9522 and references cited therein

Chua MS, Shi DF, Wrigley S, Bradshaw TD, Hutchinson I, Shaw N, Barrett DA, Stanley LA, Stevens MFG (1999) Antitumor benzothiazoles. 7. Synthesis of 2-(4-acylaminophenyl) benzothiazoles and investigations into the role of acetylation in the antitumor activities of the parent amines. J Med Chem 42:381

Comins DL, O'Connor S (1988) In: Katrizsky AR (ed) Advances in heterocyclic chemistry, vol 44. Academic, New York, p 243

Desai RM, Dodiya DK, Trivedi AR, Shah VH (2008) Simple and efficient synthetic routes to bioactive s-triazinyl dithiocarbamate derivatives. Med Chem Res 17:495–506

Foster BJ, Harding BJ, Leyland-Jones B, Hoth D (1986) Hexamethylmelamine: a critical review of an active drug. Cancer Treat Rev 38:197–217

Giacomelli G, Porcheddu A, De Luca L (2004) [1,3,5]-Triazine: a versatile heterocycle in current applications of organic chemistry. Curr Org Chem 8:1497–1519

Hunter A, Renfrew M, Taylor JA, Whitmore JMJ, Williams A (1994) Nucleophilic aromatic substitution in heterocycles: alcoholysis and hydrolysis of 2-anilino-4,6-dichloro-l,3,5-triazines. J Perk Trans 2:2389–2393

Keith A, Menear SG, Karine M, Christine B, Kristel B, Xiao-Ling C, Sally E, Alexandra F, Armelle LG, Gesine H, Luisa S, Mihiro S, Thomas P, Eleanor T, Ian H, Niall MB, Martin GCM, Smith KG, Pike KG (2009) Identification and optimisation of novel and selective small molecular weight kinase inhibitors of mTOR. Bioorg Med Chem Lett 19:5898–5901

Klenke B, Stewart M, Barrett MP, Brun R, Gilbert IH (2001) Synthesis and biological evaluation of *s*-triazine substituted polyamines as potential new anti-trypanosomal drugs. J Med Chem 44:3440–3452

Kumar PA, Raman D, Murthy USN, Rao VJ (2010) Stereoselective synthesis of (+)-neophrosteranic acid by ring–closing metathesis approach and its biological evaluation. Synth Commun 40:686

Kumar ChNSSP, Parida DK, Santhoshi A, Kota AK, Sridhar B, Rao VJ (2011) Synthesis and biological evaluation of tetrazole containing compounds as possible anticancer agnets. Med Chem Commun 2:486–492

Leftheris K, Ahmed G, Chan R, Dyckman AJ, Hussain Z, Ho K, Hynes J, Letourneau J, Li W, Lin S, Metzger A, Moriarty KJ, Riviello C, Shimshock Y, Wen J, Wityak J, Wrobleski ST, Wu H, Wu J, Desai M, Gillooly KM, Lin TH, Loo D, McIntyre KW, Pitt S, Shen DR, Shuster DJ, Zhang R, Diller D, Doweyko A, Sack J, Baldwin J, Barrish J, Dodd J, Henderson I, Kanner S, Schieven GL, Webb M (2004) The discovery of orally active triaminotriazine aniline amides as inhibitors of p38 MAP kinase. J Med Chem 47:6283–6291

Luippold HE, Gooch PC, Brewen JG (1978) The production of chromosome aberrations in various mammalian cells by triethylenemelamine. Genetics 88:26–317

Maarouf AR, Farahat AA, Selim KB, Eisa HM (2012) Synthesis and antiviral activity of benzimidazolyl and triazolyl-1,3,5-triazines. Med Chem Res 21:703–710

Maga G, Falchi F, Radi M, Botta L, Casaluce G, Bernardini M, Irannejad H, Manetti F, Garbelli A, Samuele A, Zanoli S, Este JA, Gonzalez E, Zucca E, Paolucci S, Baldanti F, Rijck JD, Debyser Z, Botta M (2011) Toward the discovery of novel anti-HIV drugs. second-generation inhibitors of the cellular ATPase DDX3 with improved anti-HIV activity: synthesis, structure–activity relationship analysis, cytotoxicity studies, and target validation. Chem Med Chem 6:1371–1389

Mahajan DH, Pannecouque C, De Clercq E, Chikhalia KH (2009) Synthesis and studies of new 2-(Coumarin-4-yloxy)-4,6-(substituted)-s-triazine derivatives as potential anti-HIV agents. Arch Pharm 342:281–290

Manohar S, Khan SI, Rawat DS (2010) Synthesis, antimalarial activity and cytotoxicity of 4-aminoquinoline–triazine conjugates. Bioorg Med Chem Lett 20:322–325

McKay GA, Reddy R, Arhin F, Belley A, Lehoux D, Moeck G, Sarmiento I, Parr T, Gros P, Pelletier J, Fara AR (2006) Triaminotriazine DNA helicase inhibitors with antibacterial activity. Bioorg Med Chem Lett 16:1286–1290

Melato S, Prosperi D, Coghi P, Monti D (2008) A combinatorial approach to 2,4,6-trisubstituted triazines with potent antimalarial activity: combining conventional synthesis and microwave-assistance. Chem Med Chem 3:873–876

Menear KA, Gomez S, Malagu K, Bailey C, Blackburn K, Cockcroft XL, Ewen S, Fundo A, Le Gall A, Hermann G, Sebastian L, Sunose M, Presnot T, Torode E, Hickson I, Martin NMB, Smith GCM, Pike KG (2009) Identification and optimisation of novel and selective small molecular weight kinase inhibitors of mTOR. Bioorg Med Chem Lett 19:5898–5901

Moon HS, Jacobson EM, Khersonsky SM, Luzung MR, Walsh DP, Xiong WN, Lee JW, Parikh PB, Lam JC, Kang TW, Rosania GR, Schier AF, Chang YT (2002) A novel microtubule destabilizing entity from orthogonal synthesis of triazine library and zebrafish embryo screening. J Am Chem Soc 124:11608–11609

Patel AB, Patel RV, Kumari P, Rajani DP, Chikhalia KH (2012) Synthesis of potential antitubercular and antimicrobial s-triazine-based scaffolds via Suzuki cross-coupling reaction. Med Chem Res doi:10.1007/s00044-012-0041-y

Patel D, Patel R, Kumari P, Patel N (2012b) In vitro antimicrobial assessment of coumarin-based s-triazinyl piperazines. Med Chem Res 21:1611–1624

Patel RV, Kumari P, Rajani DP, Chikhalia KH (2012) Discovery of 2-(4-cyano-3-trifluoromethylphenyl amino)-4-(4-quinazolinyloxy)-6-piperazinyl (piperidinyl)-s-triazines as potential antibacterial agents. Med Chem Res doi:10.1007/s00044-011-9950-4

Poulsen A, Williams M, Nagaraj HM, William AD, Wang H, Soh CK, Xiong ZC, Dymock B (2012) Structure-based optimization of morpholino-triazines as PI3K and mTOR inhibitors. Bioorg Med Chem Lett 22:1009–1013

Quirke ME (1984) 1,3,5-Triaziines. In: Katrizsky AR, Rees CW (eds) Comprehensive heterocyclilc chemistry, vol 3. Pergamon, New York, pp 457–530

Rao BA, Naidu VGM, Rajpooth RS, Srinivas K, Ramakrishna S, Rao VJ (2011) Synthesis and in vitro anticancer activity of novel rhodanine derivatives. Int J Pharm Bio Sci 2:191

Raval JP, Patel NH, Patel HV, Patel PS (2011) In vitro antimycobacterial activity of novel N0-(4-(substituted phenyl amino)-6-(pyridin-2-ylamino)-1,3,5-triazin-2-yl) isonicotinohydrazide. Med Chem Res 20:274–279

Ravinder M, Mahender B, Mattapally S, Hamsini KV, Reddy TN, Rohit C, Srinivas K, Banerjee SK, Rao VJ (2012) Synthesis and evaluation of novel 2-pyridone derivatives as inhibitors of phosphodiesterasse3 (PDE3): a target for heart failure and platelet aggregation. Bioorg Med Chem Lett 22:6010–6015

Sathiakumar N, MacLennan PA, Mandel J, Delzell E (2011) A review of epidemiologic studies of triazine herbicides and cancer. Cirt Rev Toxicol 41:1–34

Shi DF, Bradshaw TD, Wrigley S, McCall CJ, Lelieveld P, Fichtner I, Stevens MFG (1996) Antitumor benzothiazoles. Synthesis of 2-(4 aminophenyl) benzothiazoles and evaluation of their activities against breast cancer cell lines in vitro and in vivo. J Med Chem 39:3375–3384

Singh UP, Singh RK, Bhat HR, Subhashchandra YP, Kumar V, Kumawat MK, Gahtori P (2011) Synthesis and antibacterial evaluation of series of novel tri-substituted-s-triazine derivatives. Med Chem Res 20:1603–1610

Smolin EM, Rapport L (1959) s-Triazine and derivatives in the chemistry of heterocyclic compounds inter science. Wiley, New York

Srinivas K, Srinivas U, Rao VJ, Bhanuprakash K, Kishore HK, Murthy USN (2005) Synthesis and antibacterial activity of 2,4,6-tri substituted s-triazine. Bioorg Med Chem Lett 15:1121–1123

Srinivas K, Srinivas U, Rao VJ, Bhanuprakash K, Kishore HK, Murthy USN (2006) Synthesis and antibacterial activity of various substituted s-triazines. Eur J Med Chem 41:1240–1246

Srinivas Ch, Kumar ChNSSP, Raju BC, Rao VJ, Naidu VGM, Ramakrishna S, Diwan PV (2009) First stereoselective total synthesis and anticancer activity of new amide alkaloids of roots of pepper. Bioorg Med Chem Lett 19:5915

Sun D, Melman G, Le Torneau NJ, Hays AM, Melman A (2010) Synthesis and antiproliferating activity of iron chelators of hydroxyamino-1,3,5-triazine family. Bioorg Med Chem Lett 20:458–460

Sunduru N, Gupta L, Chaturvedi V, Dwivedi R, Sinha S, Chauhan PMS (2010) Discovery of new 1,3,5-triazine scaffolds with potent activity against Mycobacterium tuberculosis H37Rv. Eur J Med Chem 45:3335–3345

Tanneeru K, Reddy BM, Guruprasad L (2012) Three-dimensional quantitative structure–activity relationship (3D-QSAR) analysis and molecular docking of ATP-competitive triazine analogs of human mTOR inhibitors. Med Chem Res 21:1207–1217

Therrien B (2010) Coordination chemistry of 2,4,6-tri (pyridyl)-1,3,5-triazine ligands. J Organomet Chem 696:637–651

Venkatesan AM, Dehnhardt C, Santos ED, Chen Z, Santos OD, Ayral-Kaloustian S, Khafizova G, Brooijmans N, Mallon R, Hollander I, Feldberg L, Lucas J, Yu K, Gibbons J, Abraham RT, Chaudhary I, Mansour TS (2010) Bis (morpholino-1,3,5-triazine) derivatives: potent adenosine 50-triphosphate competitive phosphatidylinositol-3-kinase/mammalian target of rapamycin inhibitors: discovery of compound 26 (PKI-587), a highly efficacious dual inhibitor. J Med Chem 53:2636–2645

Verheijen JC, Richard DJ, Curran K, Kaplan J, Yu K, Zask A (2010) 2-Arylureidophenyl-4-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl) triazines ashighly potent and selective ATP competitive mTOR inhibitors: optimization of human microsomal stability. Bioorg Med Chem Lett 20:2648–2653

Xiong YZ, Chen FE, Balzarini J, De clercq E, Pannecouque C (2008) Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: structural modulations of diaryltriazines with potent anti-HIV activity. Eur J Med Chem, 43:1230–1236 and references cited therein

Yaguch S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T (2006) Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. J Natl Cancer Inst 98:545–556

Zask A, Verheijen JC, Richard DJ, Kaplan J, Curran K, Toral-Barza L, Lucas J, Hollander I, Yu K (2010) Discovery of 2-ureidophenyltriazines bearing bridged morpholinesas potent and selective ATP-competitive mTOR inhibitors. Bioorg Med Chem Lett 20:2644–2647

Zhang YJ, Duan Y, Zheng XFS (2011) Targeting the mTOR kinase domain: the second generation of mTOR inhibitors. Drug Discov Today 16:325–331

Zheng M, Xu C, Ma J, Sun Y, Du F, Liu H, Lin L, Li C, Dian J (2007) Synthesis and antitumor evaluation of a novel series of triaminotriazine derivatives. Bioorg Med Chem Lett 15:1815–1827