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Synthesis and biological evaluation of novel amidinourea and triazine congeners as inhibitors of MDA-MB-231 human breast cancer cell proliferation

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Abstract

A series of novel amidinourea derivatives was synthesized, and the compounds were evaluated as inhibitors of MDA-MB-231 human breast cancer cell proliferation. In addition, a second series of triazine derivatives designed as rigid congeners of the amidinoureas was synthesized, and the compounds were evaluated for their antiproliferative activity. Among the two series, amidinourea 3d (N-[N-[8-[[N-(morpholine-4-carbonyl)carbamimidoyl] amino]octyl]carbamimidoyl]morpholine-4-carboxamide) emerged as a potent anticancer hit compound with an IC50 value of 0.76 mm, similar to that of tamoxifen

TEXT

Breast cancers are solid tumors which result from a series of non-random molecular alterations, transforming normal cells into cancer cells with invasive and metastatic potential. However, the steps of tumor progression are not yet well elucidated in breast cancer.[1] Breast cancer represents today the most common malignant tumor and the second most lethal cancer among women preceded only by lung cancer.[2-3] Women have a 1 in 8 lifetime risk of developing breast cancer and 1 in 35 risk of breast cancer causing death in the US and Europe. Several studies have established that estrogens are predominantly involved in the initiation and proliferation of breast cancer and much efforts are now being devoted to block estrogen formation and action.[4] Most common breast cancer therapies are based on the use of drugs that stop estrogen and progesterone from helping breast cancer cells grow.[5] These drugs include the natural drug paclitaxel,[6] aromatase inhbitors[7] such as the triazole letrezole and the estrogen receptor modulators tamoxifen and raloxifen.[8] (Figure 1). However, there is constant of need to find novel anticancer molecules with improved activity, selctivity and reduced side effects.

Amidinoureas represent an interesting and underexplored class of compounds.[9-10] We recently discovered both macrocyclic and linear amidinurea derivatives endowed with antifungal[11] and antiviral activity.[12] Some amidinurea derivatives also showed anti-proliferative properties[13] probably due to their ability to mimicking the natural nucleobases and thus to interact with DNA.

Here, as an extension of our previous work, we describe the design and synthesis of two series of mono and bis-amidinourea derivatives and the evaluation of their anti-proliferative activity against MDA-MB-231 human breast cancer cells. In addition, a series of triazine analogues of amidinureas were designed. In fact, triazines with the general structure **A** represent the rigid analogues of **C** as shown in Figure 1. Triazines have been shown to possess antitumoral properties,[14] but their activity on breast cancer cells has not yet been fully investigated.[15]

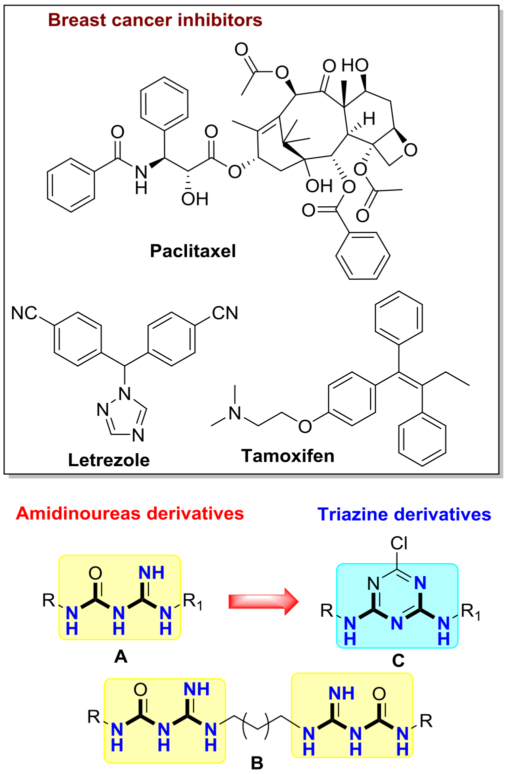
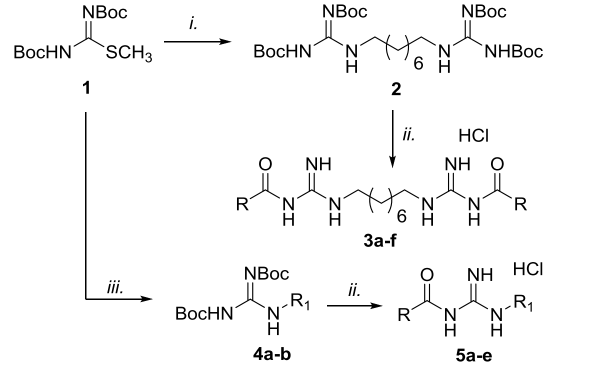
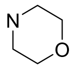
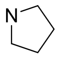
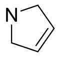
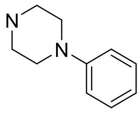
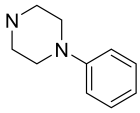
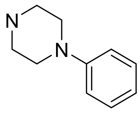
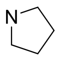
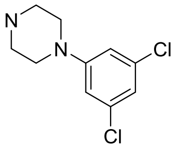


Figure 1. Common drugs active on breast cancer cells. General structures of amidinoureas and triazines

We first focused on the synthesis of amidinureas with general structures **A** and **B**. Scheme 1. The thiopseudourea **1** was reacted with 1,8-diaminooctane affording the biguanide **2**. The latter was then treated with different primary and secondary amines in refluxing THF affording the desired Boc-protected bis-amidinoureas which were in turn converted into the desired products **3a-f** upon treatment with freshly prepared HCl/AcOEt. Similarly, treatment of **1** with benzylamine or p-Cl-aniline led to guanidines **4a-b**, which were reacted with appropriate amines leading, after Boc group cleavage, to the desired amidinureas **5a-e**.[12]

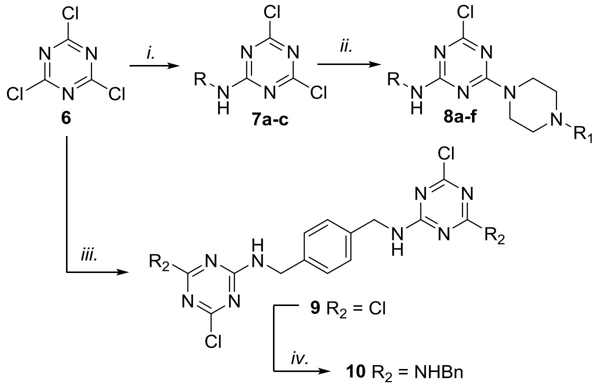


i. 1,8-diamonoctane, DCM, r.t., 12h. *ii.* Amine, THF, reflux, 12h. *iii.* Amine, DCM, r.t., 12h.

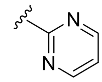
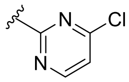
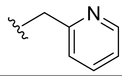
Cmpd	R	R_1
3a	BnNH	-
3b	4-Cl-Ph-NH	-
3c	$PhCH_2CH_2NH$	-
3d		-
3e		-
3f		-
5a		Bn
5b		4-Cl-Ph
5c		Bn
5d		Bn
5e		4-Cl-Ph

Scheme 1. Synthesis of amidinourea substrates

The triazine analogues were synthesised as described in Scheme 2. Cyanuric chloride **6** was first reacted with different amines/anilines affording the derivatives **7a-c**. These latter were then reacted with a series of piperazines leading to the final products **8a-g**. A bis-triazine **9** was also synthesised by reacting **6** with p-xylylenediamine. Compound 9 was further functionalised through reaction with benzylamine leading to derivative **10**.



i. Amine, DCE, -40 °C, 3h. *ii.* Piperazine-R_1, DCE, 80 °C, MW, 20 min. *iii.* p-xylylenediamine, DCE, -40 °C, 3h. *iv.* Benzylamine, DCE, 80 °C, MW, 20 min.

Cmpd	R	R_1
8a	4-Cl-Ph	Ph
8b	Bn	Ph
8c	$PhCH_2CH_2$	Ph
8d	Bn	Boc
8e	Bn	
8f	Bn	
8g	Bn	

All the compounds were then evaluated for their anti-proliferative effects on MDA-MB-231 human breast cancer cells. The inhibition of proliferation was monitored after 30 and 60 hours as shown in Figure 2 and Figure 3 (for compound **3d**). A number of compounds were shown to inhibit cellular proliferation at 50-100 μM. The triazines, with the exception of **8a** and **8e** proved to be inactive, whilst most of the bis-amidinurea showed a good activity profile. In particular **3b** produced a cell proliferation inhibition of 80% when used at 1-10 μM. Among compounds **3**, the derivative **3b** bearing a Cl-phenyl moiety on the amidinurea group proved to be the most promising compound in term of inhibition of cell proliferation. The replacement of the aryl moiety with a benzyl (**3a**), or an heterocycle (morpholine in **3d**, pyrrolidine in **3e**, pyrroline in **3f**) led to derivatives still able to inhibit the cell proliferation but at higher dose than **3b**. Interestingly, compound 3c bearing a longerside chain did not show any activity against MDA-MB-231 cells. The compounds **5a-e** also proved to be not active, thus accounting for the importance of a long aliphatic backbone for the anticancer activity. Similarly, the triazine analogues of compounds **5** also showed poor inhibition of MDA-MB-231 cells cell proliferation. However, at higher concentrations (50-100 μM) the derivatives **7c** and **8a** proved to be able to inhibit the growth of MDA-MB-231 cells at >80%.

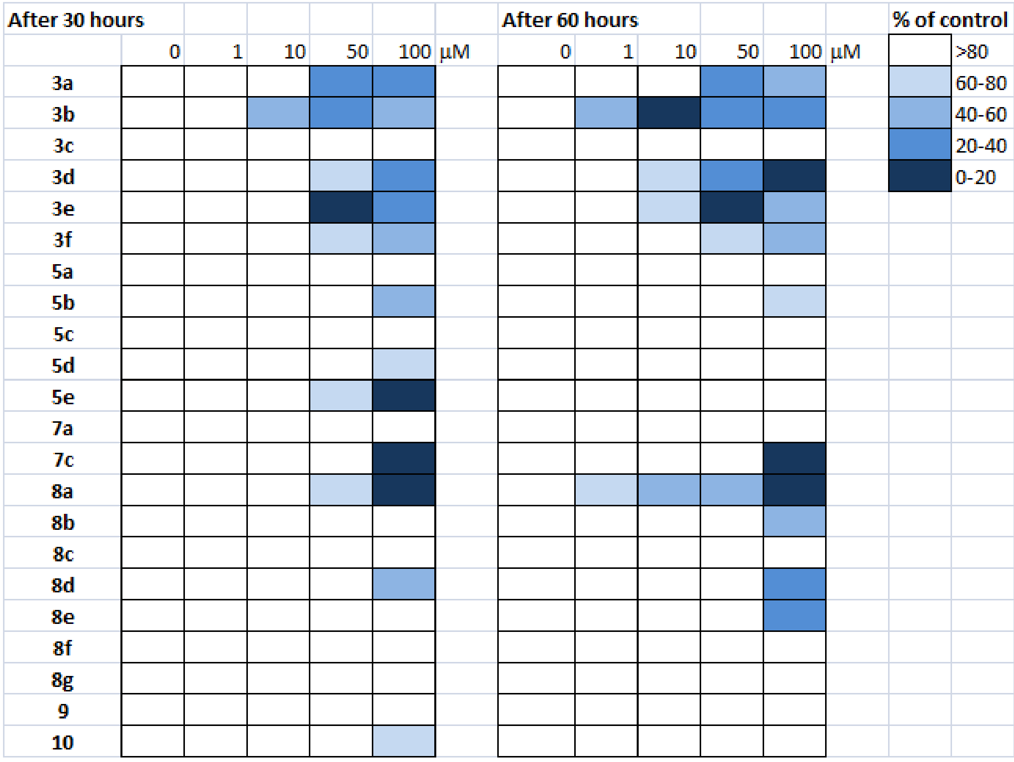


Figure 2. Anti-proliferative activity of amidinourea and triazine derivatives on MDA-MB-231 human breast cancer cells

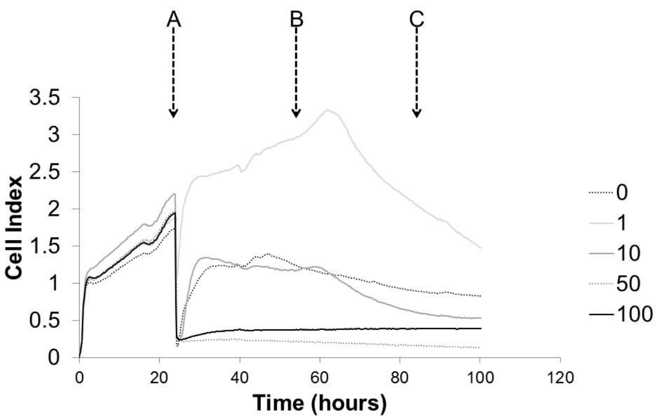


Figure 3. Effect of 3d on MDA-MB-231 cell growth. The effect of compound **3d** on the proliferation of MDA-MB-231 is shown in Figure 3. The cells were incubated for 24 hours prior the addition of **3d** (point A) at 0, 1, 10, 50 100 µM. The cell growth was evaluated after 30h (point B) and 60h (point C) in the presence of **3d**.

The inhibitory efficiency for some of the most active compounds was then evaluated against the breast cancer cell line MDA-MB-231. The IC_{50} values are summarised in Table 1 and were compared with the data reported for tamoxifen.[16] The three amidinureas **3d-f** confirmed the data previously observed emerging as potent breast cancer inhibitors. In particular compound **3d**, bearing a morpholine substituent on the amidinurea moiety showed IC_{50} = 0.76 μM, close to that of tamoxifen, thus proving to be a valuable candidate for further development. Also the derivatives **3e** and **3f** showed a good activity with IC_{50} values of 1.3 and 1.5 respectively, as well as the aryl amidinurea 3b which showed an IC_{50} = 4.9 μM. Again, the triazine derivatives **8a** and **8e** and the amidinurea **5e** showed poor inhibition with IC_{50} values >12 μM.

Table 1.

	Compounds								
	3a	**3b**	**3d**	**3e**	**3f**	**5e**	**8a**	**8e**	**Tamoxifen**
IC_{50} (μM) MDA-MB-231	67.5	4.9	0.76	1.3	1.5	22.1	12	74.7	0.66[16]

In conclusion, this work showed the potentiality of amidinourea compounds as potential anticancer agent, leading to the identification of a new promising hit candidate compounds **3d**. Currently additional derivatives are under investigation in our lab.

References

1. Eccles, S. A.; Paon, L. *Lancet* 2005, **365**: 1006–1007

2. Albrand, G.; Terret, C. *Drugs Aging* **2008**, *25*, 35–45.

3. Ross, C. R.; Temburnikar, K. W.; Wilson, G. M.; Seley-Radtke, K. L. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1715–1717

4. Jorden, V. C.; Gradishar, W. J. *Mol. Aspect Med.* **1997**, *18*, 187.

5. Hassan, M. S.; Ansari, J.; Spooner, D.; Hussain, S. A. *Oncol Rep.* **2010**, *24*, 1121-31.

6. Pivot, X.; Asmar, L.; Hortobagyi, G. N. *Int. J. Oncol.* **1999**, *15*, 381-6.

7. Hong, Y.; Chen, S. *Ann. N. Y. Acad. Sci.* **2006**, *1089*, 237-51.

8. Musa, M. A.; Khan, M.O.; Cooperwood, J. S. *Curr. Med. Chem.* **2007**, *14*,1249-61.

9. Castagnolo, D. *New Strategies in Chemical Synthesis and Catalysis*, Chapter 5, Ed. Bruno Pignataro, Wiley-VCH, **2012**

10. Castagnolo, D.; Schenone, S.; Botta, M. *Chem. Rev.* **2011**, *111*, 5247-5300.

11. a) Sanguinetti, M.; Sanfilippo, S.; Castagnolo, D.; Sanglard, D.; Posteraro, B.; Donzellini, G.; Botta, M. *ACS Med. Chem. Lett.* **2013**, *4*, 852–857. b) Manetti,F.; Castagnolo,D.; Raffi,F.; Zizzari,A. T.; Rajamäki,S.; D'Arezzo,S.; Visca,P.; Cona,A.; Fracasso, M. E.; Doria, D.; Posteraro, B.; Sanguinetti, M.; Fadda, G.; Botta, M. *J. Med. Chem.* **2009**, *52*, 7376-7379.

12. Magri, A.; Reilly, R. Scalacci, N.; Radi, M.; Hunter, M.; Ripoll, M.; Patel, A.; Castagnolo, D. *Bioorg. Med. Chem. Lett.* **2015***, 25,* 5372-5376.

13. Piskala, A.; Hanna, N. B.; Masojidkova, M.; Otmar, M.; Fiedler, P.; Ubik, K. *Collect. Czech. Chem. Commun.* **2003**, *69*, 711-743.

14. Maga, G.; Falchi. F.; Radi, M.; Botta, L.; Casaluce, G.; Bernardini, M.; Irannejad, H.; Manetti, F.; Garbelli, A.; Samuele, A.; Zanoli, S.; Esté, J. A.; Gonzalez, E.; Zucca, E.; Paolucci, S.; Baldanti, F.; De Rijck, J.; Debyser, Z.; Botta, M. *ChemMedChem.* **2011**, *6*, 1371-89.

15. Prinka, S.; Vijay, L.; Kamaldeep, P. *Eur. J. Med. Chem.* **2016**, *117*, 59-69.

16. Nagaiah, K.; Venkatesham, A.; Srinivasa Rao, R.; Saddanapu, V.; Yadav, J. S.; Basha, S. J.; Sarma, A. V. S.; Sridhar, B.; Addlagatta, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3259-3264

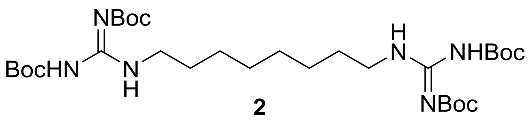
17. Keane, K.M.; Bell, P.G.; Lodge, J.; Constantinou, C.; Jenkinson, S.E.; Bass, R.; Howatson, G. *Eur. J. Nutr.* **2016**, 55(4), 1695-705.

General Methods

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer. ^{1}H and ^{13}C spectra were referenced relative to the solvent residual peaks and chemical shifts (δ) reported in ppm downfield of trimethylsilane ($CDCl_3$ δ H: 7.26 ppm, δ C: 77.0 ppm). Coupling constants (*J*) are reported in Hertz. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or some combination of these. Thin layer chromatography (TLC) was performed using commercially available pre-coated plates and visualized with UV light at 254 nm. Permanganate or nhynidrine were used to reveal the products. Flash column chromatography was carried out using Silica 60 Å.

Compounds **5a-e** were synthesised as previously reported. Magri, A.; Reilly, R. Scalacci, N.; Radi, M.; Hunter, M.; Ripoll, M.; Patel, A.; Castagnolo, D. *Bioorg. Med. Chem. Lett.* **2015***, 25,* 5372-5376.

Synthesis of biguanide 2



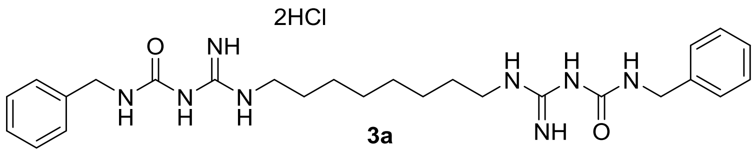
2

Diamine (1 g, 6.93 mmol, 1.1 eq) and 1,3-Bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (3.67 g, 12.65 mmol, 2 eq) were mixed in CH_2CL_2 (10 mL) and the mixture was stirred at r.t. for 12 h. The reaction mixture was then quenched with water and extracted with ethyl acetate (3 x 50 mL). The combined organic phase was concentrated under reduced pressure. Yellow oil was obtained.

^{1}H NMR (400 MHz $CDCl_3$) δ 11.48 (br, 1H), 8.11 (br, 1H), 3.37 (m, 2H), 1.49 (s, 9H), 1.47 (s, 9H), 1.50-1.20 (br, 6H) ppm.

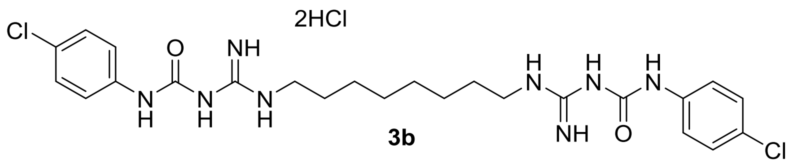
General procedure for the synthesis of compounds 3a-g

Guanidine **2** (400 mg, 0.63 mmol, 1eq) was dissolved in THF (5 mL). The apropriate amine was then added (2eq) and the mixture was stirred overnight at reflux. The reaction mixture was washed with cold water and extracted with ethyl acetate (3x20 mL). The crude product was purified by column chromatography using AcOEt/Hexane 1:4 as eluent affording the Boc-protected compound. This latter was dissolved in AcOEt (1 mL) and then treated with freshly prepared HCl/AcOEt (3 mL). The mixture was stirred for 24h. The solvent was removed affording the desired amidinourea **3**.



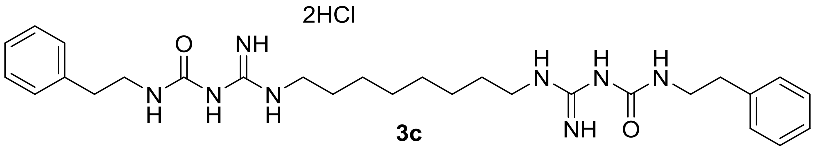
3a

Yield: 61% **^{1}H NMR** (400 MHz MeOD-d6) 7.29-7.28 (m, 5H), 4.34 (s, 2H), 3.25-3.27 (m, 2H), 1.62, (m, 2H), 1.24 (m, 4H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 154.3, 154.0, 138.1, 128.3, 127.2, 127.1, 43.1, 41.2, 28.6, 27.9, 26.18 ppm.



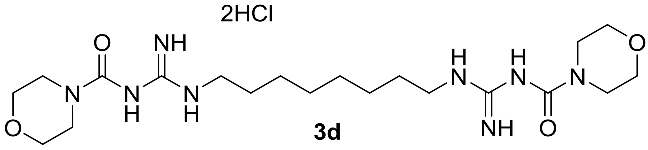
3b

Yield: 42% **^{1}H NMR** (400 MHz MeOD-d6) 7.44 (d, 2H, J = 8 Hz), 7.36 (d, 2H, J = 8 Hz), 3.22 (m, 2H), 1.49, (m, 2H), 1.26 (m, 4H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 153.4, 151.9, 137.1, 129.4, 128.0, 121.6, 41.5, 29.0, 28.3, 26.4 ppm.



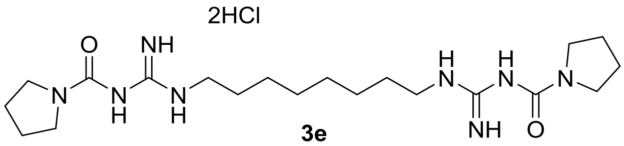
3c

Yield: 53% ^{1}**H NMR** (400 MHz MeOD-d6) 7.27-7.14 (m, 5H), 3.40 (m, 2H), 3.24 (m, 2H), 2.79 (m, 2H), 1.60, (m, 2H), 1.25 (m, 4H) ppm. ^{13}**C NMR** (100 MHz MeOD-d6) δ 154.8, 154.5, 139.4, 129.1, 128.9, 126.8, 41.8, 41.6, 35.8, 29.3, 28.5, 26.8 ppm.



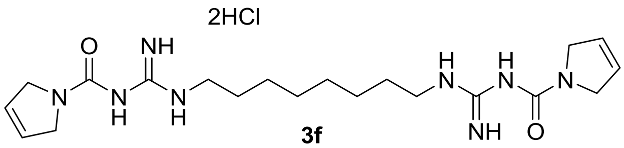
3d

Yield: 40% ^{1}**H NMR** (400 MHz MeOD-d6) 3.66 (m, 4H), 3.52 (m, 4H), 3.28 (m, 2H), 1.63 (m, 2H), 1.28 (m, 4H) ppm. . ^{13}**C NMR** (100 MHz MeOD-d6) δ 155.0, 153.2, 66.1, 44.4, 41.4, 28.8, 28.0, 26.4 ppm.



3e

Yield: 56% ^{1}**H NMR** (400 MHz MeOD-d6) 3.47-3.42 (m, 4H), 3.29-3.26 (m, 2H), 2.01 (m, 2H), 1.89 (m, 2H), 1.64 (m, 2H), 1.39 (m, 4H) ppm. ^{13}**C NMR** (100 MHz MeOD-d6) δ 155.4, 152.9, 46.9, 46.6, 41.8, 29.3, 28.5, 26.8, 26.2, 24.7 ppm.

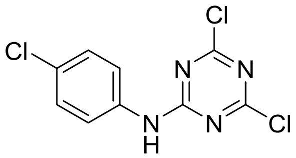


3f

Yield: 55% ^{1}**H NMR** (400 MHz DMSO-d6) 5.89 (s, 2H), 4.26 (m, 2H), 4.09 (m, 2H), 3.21 (m, 2H), 1.48 (m, 2H), 1.26 (m, 4H) ppm. ^{13}**C NMR** (100 MHz DMSO-d6) δ 154.8, 152.2, 125.9, 125.7, 53.9, 53.8, 47.2, 28.9 ,28.3, 26.0 ppm.

General procedure for the synthesis of triazines 7.

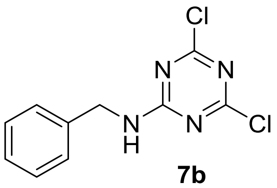
Cyanuric chloride (2.7 mmol) was dissolved in 30 mL of DCE and the mixture was cooled at -40 °C. The appropriate amine/aniline (2.7 mmol) was added and the mixture was stirred at the same temperature for 3h. The mixture was quenched with water. The organic phase was then washed twice with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was used in the next step without any further purification.



7a

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 7.60 (br s, 1H), 7.48 (d, 2H, *J* = 8 Hz), 7.35 (d, 2H, *J* = 8 Hz) ppm.

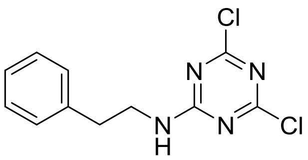
Shahin, Rand; Taha, Mutasem O. Bioorganic & Medicinal Chemistry Volume20 Issue1. Pages377-400



7b

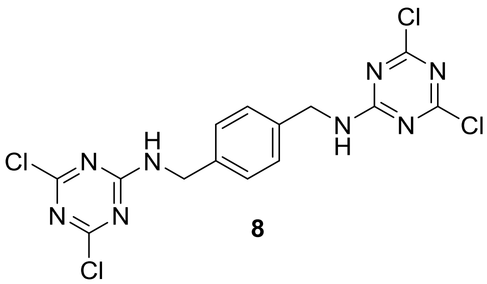
Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 7.36-7.24 (m, 5H), 6.67 (br s, 1H), 4.66 (d, 2H, *J* = 7.8 Hz) ppm.

Maga, Giovanni; Falchi, Federico; Radi, Marco; Botta, Lorenzo; Casaluce, Gianni; Bernardini, Martina; Irannejad, Hamid; Manetti, Fabrizio; Garbelli, Anna; Samuele, Alberta; Zanoli, Samantha; Este, Jose A.; Gonzalez, Emmanuel; Zucca, Elisa; Paolucci, Stefania; Baldanti, Fausto; De Rijck, Jan; Debyser, Zeger; Botta, Maurizio ChemMedChem Volume6 Issue8 Pages1371-1389



7c

Yield: 57% **^{1}H NMR** (400 MHz $CDCl_3$) 7.30-7.16 (m, 5H), 6.63 (br s, 1H), 3.75 (m, 2H), 2.89 (t, 2H, *J* = 7.4 Hz) ppm.

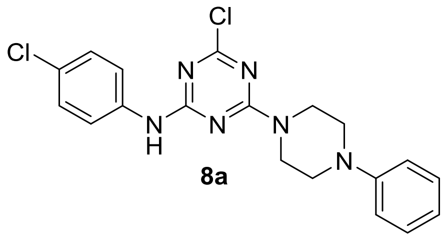


8

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 7.23 (s, 2H), 4.46 (s, 2H) ppm.

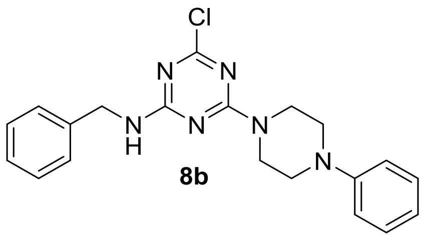
Genaral procedure for the synthesis of triazines 8

The appropriate triazine 7 (1 mmol) was dissolved in 2 mL of DCE in a microwave vial. The appropriate piperazine (1 mmol) was added and the reaction mixture was heated at 80 °C under microwave irradiation for 20 minutes (2 x 10 minutes runs). The mixture was diluted with brine (10 mL) and extracted with AcOEt (5 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel, using hexane/EtOAc (10:1) as eluent.



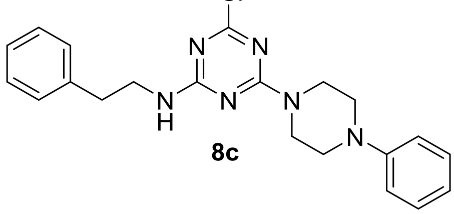
8a

Yield: 82% **^{1}H NMR** (400 MHz $CDCl_3$) 7.47 (d, 2H, *J* = 8 Hz), 7.32-7.27 (m, 4H), 6.96-6.91 (m, 3H), 4.01-3.96 (m, 4H), 3.23-3.22 (m, 4H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 162.1, 161.1, 154.5, 137.2, 136.6, 128.7, 128.6, 127.8, 127.7, 127.6, 127.0, 44.5, 42.1 ppm.



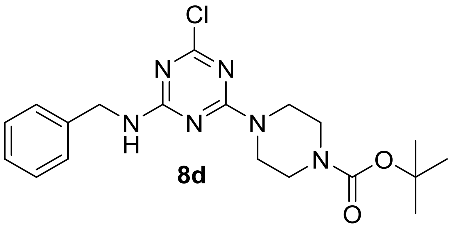
8b

Yield: 76% **^{1}H NMR** (400 MHz $CDCl_3$) 7.33-7.21 (m, 7H), 6.94-6.90 (m, 3H), 5.98 (brs, 1H), 4.61 (d, 2H, *J* = 4Hz), 3.95 (m, 4H), 3.17 (m, 4H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 169.1, 165.6, 164.4, 138.1, 129.3, 128.7, 128.6, 127.8, 127.5, 127.3, 116.8, 49.6, 44.9, 43.3 ppm.



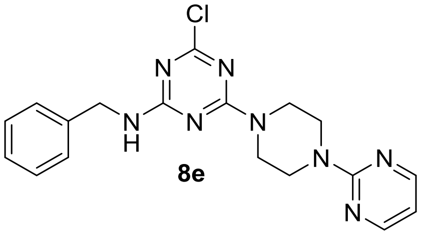
8c

Yield: 67% **^{1}H NMR** (400 MHz $CDCl_3$) 7.30-7.24 (m, 7H), 6.96-6.89 (m, 3H), 5.55 (brs, 1H), 3.97 (m, 4H), 3.67 (M, 2H), 3.21 (m, 4H), 2.88 (m, 2H) ppm.



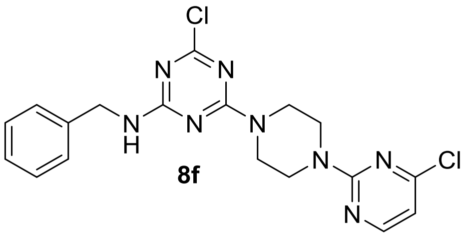
8d

Yield: 87% **^{1}H NMR** (400 MHz $CDCl_3$) 7.31-7.25 (m, 5H), 4.57 (s, 2H), 3.76 (m, 4H), 3.44 (m, 4H), 1.45 (s, 9H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 174.9, 167.5, 161.1, 154.5, 138.8, 128.7, 127.5, 127.4, 80.5, 44.7, 43.8, 43.4, 28.3 ppm.



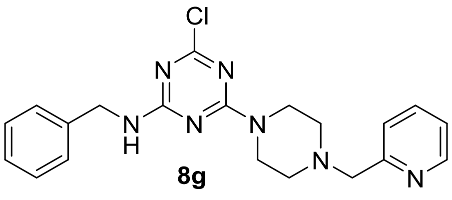
8e

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 8.32 (d, 2H, *J* = 4Hz), 7.32-7.28 (m, 5H), 6.52 (t, 1H, *J* = 4Hz), 5.90 (br s, 1H), 4.60 (s, 2H), 3.84 (br m, 8H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 178.8, 169.1, 165.6, 161.5, 157.8, 138.1, 128.6, 127.5, 127.4, 110.3, 45.0, 43.3, 43.2 ppm.



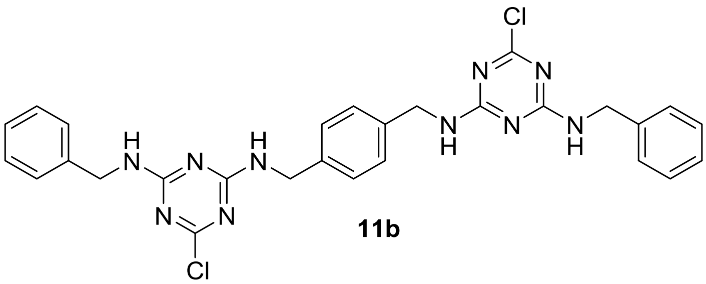
8f

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 8.16 (s, 1H), 7.30-7.23 (m, 5H), 6.51 (s, 1H), 4.57 (s, 2H), 3.81 (br m, 8H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 189.1, 178.9, 169.9, 167.0, 158.9, 158.5, 152.3, 134.3, 128.6, 127.5, 110.6, 44.9, 43.4, 43.2, ppm.



8g

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 8.57 (d, 2H, *J* = 3.4Hz), 7.66 (m, 1H), 7.41 (m, 1H), 7.31-7.26 (m, 5H), 7.20 (m, 1H), 5.71 (br s, 1H), 4.56 (s, 2H), 3.84 (m, 4H), 3.68 (s, 2H), 2.52 (m, 4H) ppm.



11b

Yield: 64% **^{1}H NMR** (400 MHz $CDCl_3$) 7.30-7.14 (m, 7H), 4.48 (s, 2H), 4.26 (s, 2H) ppm. **^{13}C NMR** (100 MHz MeOD-d6) δ 164.4, 163.7, 150.9, 136.3, 129.3, 128.9, 121.7, 120.6, 116.7, 49.2, 43.6 ppm.

Cell proliferation screening and IC50 determination

The MDA-MB-231 cell line was obtained from ATCC and cultured in DMEM, supplemented with 10% (v/v) foetal calf serum (FCS) and penicillin/streptomycin. Cell identity was authenticated by short tandem repeat profiling (DDC, DNA Diagnostics Centre, London, UK). All cell culture reagents were from ThermoFisher Scientific (Paisley, UK).

For all compound testing MDA-MB-231 cells were incubated in media containing only 0.5% FCS for 24 hours prior the start of an experiment to synchronise proliferation. The effect of the synthesised compounds on cell proliferation was determined using an xCELLigence DP real time cell analyser according to the manufacturer's instructions (Acea Biosciences Inc., CA, USA) and as previously reported[17]. Cells were seeded onto an xCELLigence E plate at 20,000 cells/well, in normal growth media for 24 hours prior to the addition of compound or DMSO only as control. Measurements were taken every 15 minutes for up to 100 h to determine cell proliferation.

To determine IC50 MDA-MB-231 were put in a 96-well plate at 5000 cell/well and incubated for 24 hours in the presence of titrated compound. To quantify cell proliferation, WST-1 (Sigma-Aldrich, Dorset, UK) was added to each well and cells were incubated at 37°C for 4 hours to allow colour change to develop. Absorbance at 490nm was measured using a FLUOstar Omega plate reader. IC50 analysis was performed by Origin Software (Silverdale Scientific, Buckinghamshire, UK)