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To be cited as: *ChemMedChem* 10.1002/cmdc.201600580

Link to VoR: http://dx.doi.org/10.1002/cmdc.201600580

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COMMUNICATION

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 has been synthesised and the new compounds have been 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 synthesised as well and the compounds were evaluated for their antiproliferative activity. Among the two series, the amidinourea 3d emerged as a potent anticancer hit compound with IC_{50} = 0.76 μM comparable to tamoxifen.

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 antiproliferative properties[13] probably due to their ability to mimicking the natural nucleobases and thus to interact with DNA. Amidinoureas can be considered as bio-isosters of biguanides and bis-ureas, two classes of organic molecules endowed with inhibitory activity toward a range of cancer cells. As an example, the biguanides metformin and phenformin recently showed potential anticancer effects.[14] Similarly, Woster and coworkers reported that biguanides and bis-urea derivatives possess anticancer activity, including activity against breast cancer, acting as epigenetic modulators.[15] Due to our previous experience in the synthesis of linear amidinoureas[11-12] and their structural correlation with biguanides, we decided to synthesise a narrow library of amidinourea derivatives and evaluate them as potential anticancer agents.

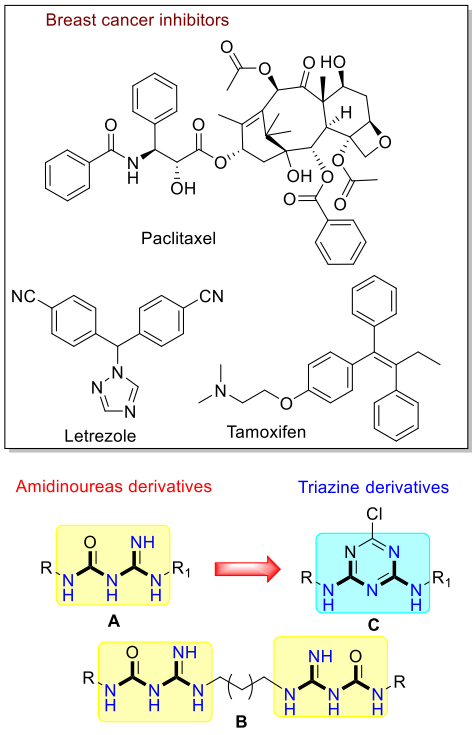
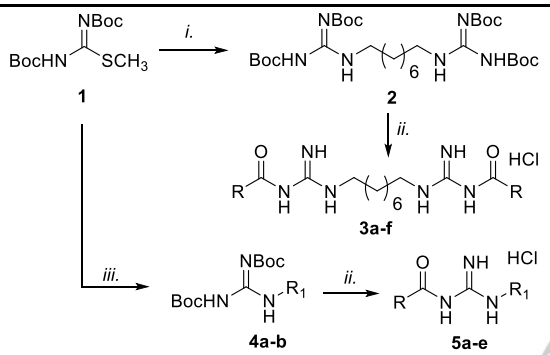
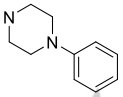
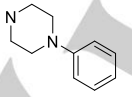
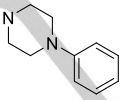
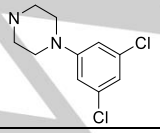


Figure 1. Common drugs active on breast cancer cells. General structures of amidinoureas and triazines.

In particular, as an extension of our previous work, we describe the design and synthesis of two series of mono and bis-amidinourea derivatives (**A** and **B**, Figure 1) and the evaluation of their anti-proliferative activity against MDA-MB-231 human breast cancer cells. In addition, a series of triazine analogues of amidinoureas was designed. In fact, triazines with the general structure **C** represent the rigid congeners of **A** as shown in Figure 1. Chemical rigidification is an established approach in drug discovery which allows to derivatise and improve the activity of a drug by reducing the conformations that a molecule can adopt. Here, we decided to rigidify the amidinourea group into a triazine bioisosteric moiety. Triazines have been shown to possess antitumoral properties,[16] but their activity on breast cancer cells has not yet been fully investigated,[17] thus the synthesis of a narrow library of triazine congeners of amidinoureas was planned.

Table 1. Synthesis of amidinourea derivatives **3** and **5**

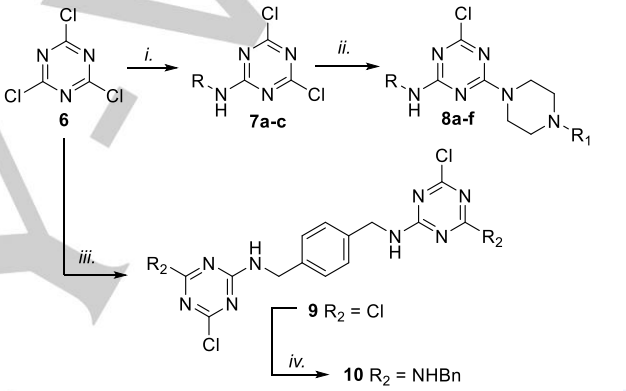


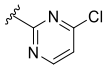
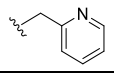
Cmpd	R	R_1
3a	BnNH	-
3b	4-Cl-Ph-NH	-
3c	$PhCH_2CH_2NH$	-
3d		-
3e		-
3f		-
5a		Bn
5b		4-Cl-Ph
5c		Bn
5d		Bn
5e		4-Cl-Ph

[a] Reagents and conditions: *i.* 1,8-diamonoctane, DCM, r.t., 12h. *ii.* Amine, THF, reflux, 12h. *iii.* Amine, DCM, r.t., 12h.

We first focused on the synthesis of amidinoureas with general structures **A** and **B**. Table 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] The triazine analogues were synthesised as described in Table 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**.

Table 2. Synthesis of triazine derivatives **8**, **9** and **10**



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	

[a] Reagents and conditions: *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.

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, 8d** and **8e**, proved to be inactive, whilst most of the bis-amidinoureas showed a good activity profile. In particular, **3b** produced a cell proliferation inhibition of 80% when used at 1-10 μM.

Table 3. Inhibitory efficency of amidinourea and triazine derivatives against the breast cancer cell line MDA-MB-231

	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[18]

Among compounds **3**, the derivative **3b** bearing a *p*-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 group (**3a**), or a 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 longer side 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** 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%.

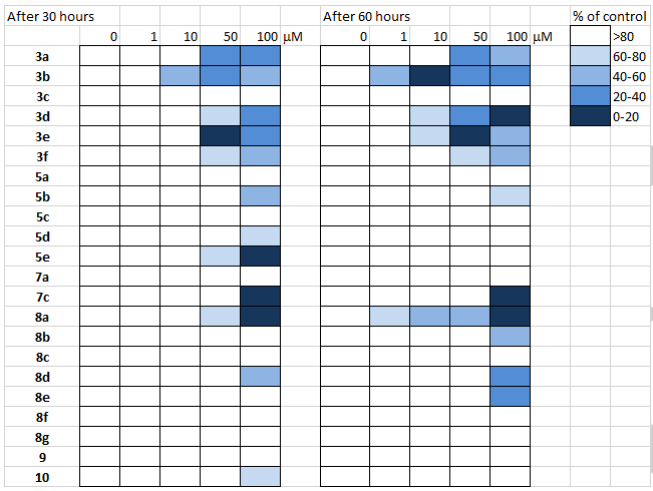


Figure 2. Anti-proliferative activity of amidinourea and triazine derivatives on MDA-MB-231 human breast cancer cells

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 3 and were compared with the data reported for tamoxifen.[18] The three amidinoureas **3d-f** confirmed the data previously observed, thus 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 μM and 1.5 μM 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.

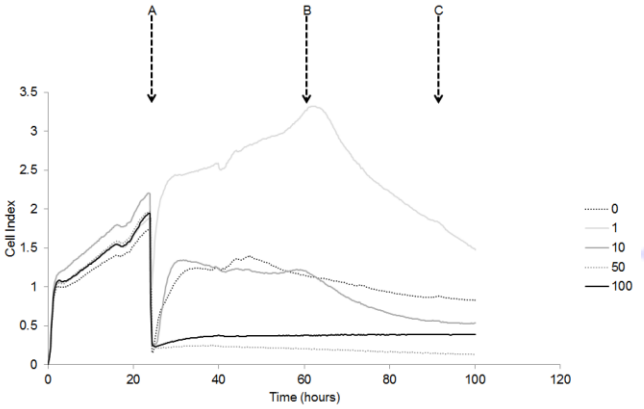


Figure 3. Effect of **3d** on MDA-MB-231 cell growth. The effect of compound **3d** on the proliferation of MDA-MB-231 is shown in the Figure. The cells were incubated for 24 hours prior the addition of **3d** (point A) at 0, 1, 10, 50 100 μM. The cell growth was evaluated after 30h (point B) and 60h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

In conclusion, this work showed the potentiality of amidinourea compounds as potential anticancer agents, leading to the identification of a new promising hit candidate compound **3d** able to inhibit breast cancer cells proliferation at submicromolar concentration. The design and synthesis of additional derivatives are currently under investigation in our lab.

Experimental Section

The procedures for the synthesis of all compounds, the cell proliferation screening and the IC_{50} determination are reported in the Supporting Information.

Acknowledgements

Northumbria University and Royal Society of Chemistry (Research Fund 2015) are acknowledged for financial support.

Keywords: amidinourea • triazine • breast cancer • MDA-MB-231 cells • cell proliferation

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10.1002/cmdc.201600580

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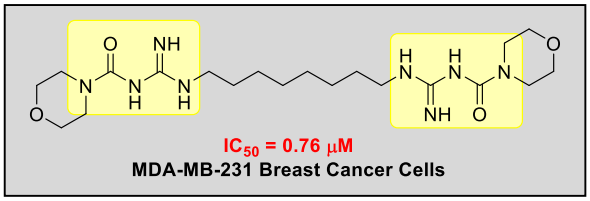

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