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Synthesis and cytotoxicity of some biurets against human breast cancer T47D cell line

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ABSTRACT

Design, synthesis and cytotoxicity of several known and novel biurets against human breast cancer T47D cell line in comparison to doxorubicin are described. Biurets incorporating 2-methyl quinoline-4-yl and benzo[d]thiazol-2-ylthio moieties showed higher cytotoxicity and decreased cell viability in a concentration- and time-dependent manner.

Several 4-azolylalkylquinoline derivatives **I**[1,2] and 1-azolylalkyl-4(1H) quinolones **II**[1,3] (Fig. 1), have shown cytotoxicity comparable or superior to adriamycin against various cancer cell lines. While in the preliminary experiments 1-azolylalkyl-4(1H) quinolones **II** inhibited topoisomerases **I**, and **II** and did not bind to DNA,[1] further studies ruled out involvement of these enzymes in their activity.[3] In contrast by using flexible ligand docking technique it has been shown[4] that the most probable mode of action of 4-azolylalkylquinoline derivatives **I** are binding to DNA via intercalation of quinoline moiety between CG base pairs with linker chain and azole moiety binding to minor groove.

Since compounds of general formula **I** and **II** were ineffective in vivo which was attributed to their high lipophilicity,[1] efforts were made to reduce the lipophilicity of compounds by synthesizing water soluble phenol and phenoxy acetic acid derivatives and changing the nature of the chain between azolylalkyl and quinolyl portions of the molecules.[5] While resulting data for effects of reducing lipophilicity on in vitro cytotoxicity were inconsistent, of the synthesised compounds, *N*-(4-quinolyl)azolylalkanamide[5] **III** which can be considered as bioisoster of the 4-azolylalkylquinolines **I** by exchange of the $-CH_2-$ groups of the chain with –NH and –CO groups showed impressive in vitro and moderate in vivo cytotoxic activity.

Additionally, several 1-(2-methylquinolin-4-yl)-3-azolyl urea **IV** (Fig. 1) which could be considered as bioisoster of *N*-(4-quinolinyl)azolylalkanamide **III** by exchange of $-CH_2-$ with –NH– group have shown cytotoxicity comparable or higher than several antitumor agents against human breast cancer T47D cell line.[6] These results prompted us to investigate the effect of further bioisosteric exchange of $-CH_2-$ groups of urea **IV** with –CO and –NH– groups through the synthesis of compounds having biuret functionality

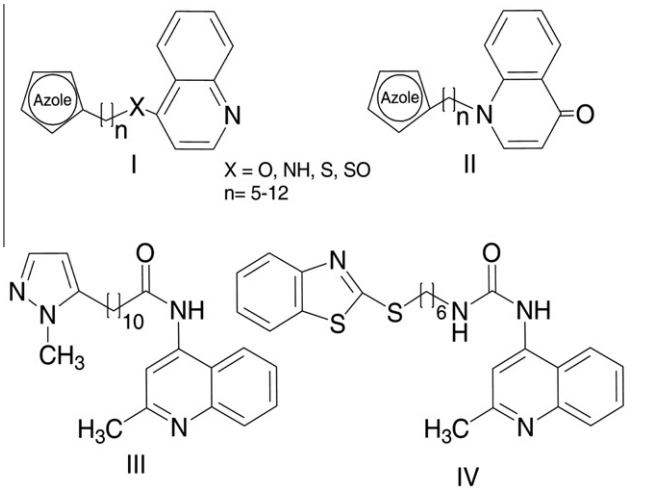

Figure 1. Chemical structures of 4-azolylalkylquinolines **I**, 1-azolylalkyl-4(1H) quinolones **II**, *N*-(4-quinolinyl)azolylalkanamide **III** and 1-(2-methylquinolin-4-yl)-3-azolyl urea **IV**.

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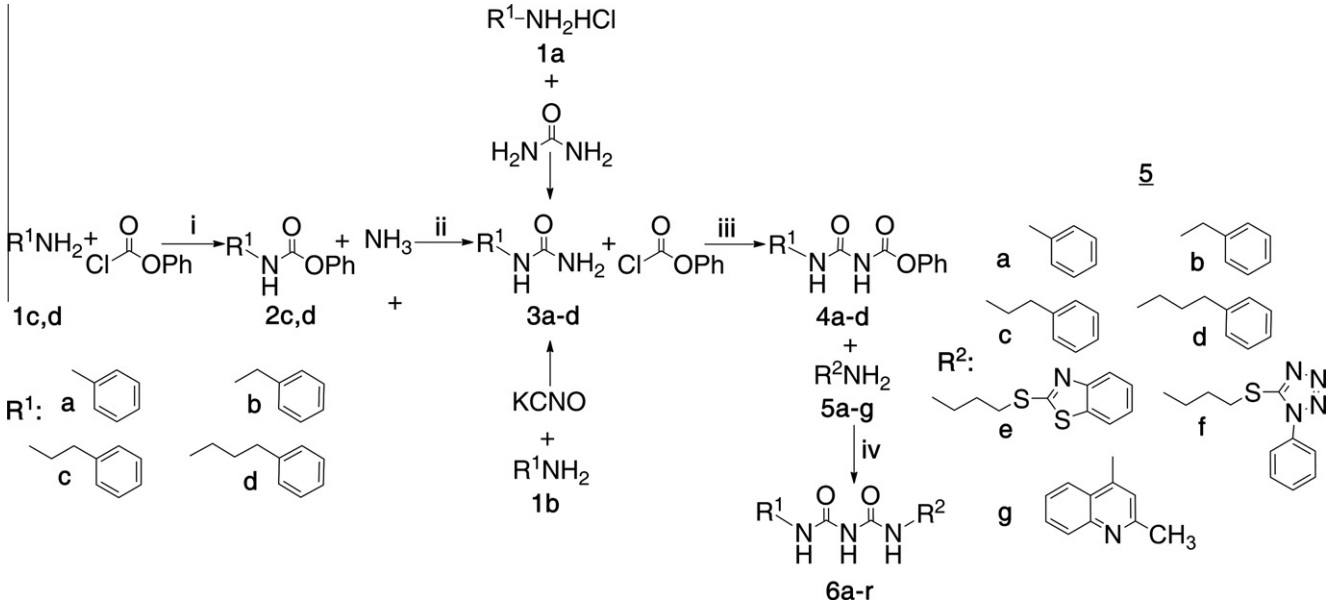

Scheme 1. Synthesis of carbamates **2c**, **d**, ureas **3a–d**, allophanates **4a**, **c**, **d** and biurets **6a–r**. Reagents and conditions: (i) bis(trimethylsilyl)acetamide, DCM; (ii) heat (60–70 °C), 5–6 h; (iii) pyridine,16 h, rt; (iv) K_2CO_3, DCM, reflux, 16 h.

in the chain between two rings. While analgesic,[7] anti-inflammatory,[8,9] hypoglycemic activity[10] and inhibition of the gastric acid secretion[11] for several biurets have been described, the antitumor activity of these compounds have not been reported and this is the first report on their cytotoxicity against a cancer cell line. This paper describes synthesis and cytotoxicity of *N*,*N*′-diphenyl, *N*-phenyl-*N*′-alkylphenyl, and *N*,*N*′-bis alkylphenyl biurets and analogous compounds by replacing one phenyl group with 2-methylquinoline-4-yl, benzo[d]thiazol-2-ylthio and (1-phenyl-1H-tetrazol-5-yl) thio moieties. The rational for selection of the first two heterocyclic moieties were reports on the antitumor activity of 2-methyl-4-aminoquinoline,[12] and benzo[d]thiazole-2-thiol[13] due to DNA intercalation and for 1-phenyl-(1H)-tetrazol was that it is a bioisoster of 1-phenyl triazole[14] which was one of the most effective azole of 4-azolylalkylquinolines **I**. In addition these heterocycles were azole moieties of 1-(2-methylquinolin-4-yl)-3-azolyl urea **IV** which have shown[6] cytotoxicity against human breast cancer T47D cell line. Biurets **6a–r** were prepared (Scheme 1) by the reaction of allophanates **4a–d** with amines **5a–g.**[15] Preparation of biurets **6a**[16] and **6f**[17] under similar conditions, **6e** by the reaction of benzylamine with carbonyl-isocyanate-isothiocyanate[18] and **6o** by the reaction of nitrobiuret with 3-phenylpropylamine[6] have been reported previously. Phenyl allophanates **4a–d** were prepared by the reported method for the preparation of **4a** from the reaction of phenyl chloroformate with ureas **3a–d** in dichloromethane in the presence of pyridine.[19] Of these compounds preparation of **4b** by the reaction of benzylamine and phenoxycarbonylisocyanate has also been described.[20] The known ureas **3a**,**b** were prepared by the reaction of aniline hydrochloride with urea[21] and benzylamine with potassium isocyanate,[22] respectively. Ureas **3c**,**d** were

Table 1
In vitro cytotoxicity of biuret **6a–r** and urea **IV**

Compound name	R^1	R^2	IC_{50}[a] (μM)	Survival[b] (%)
6a	Phenyl	Phenyl	60	82.35
6b	Phenyl	3-Phenylpropyl	50	75.23
6c	Phenyl	2-Methyl-quinoline-4-yl	35	67.25
6d	Phenyl	3-(Benzo[d]thiazol-2-ylthio)propyl	35	64.1
6e	Phenylmethyl	Phenylmethyl	75	91.3
6f	Phenylmethyl	2-Phenylethyl	55	75.97
6g	Phenylmethyl	3-Phenylpropyl	70	88.5
6h	Phenylmethyl	2-(Pyridin-2-yl)ethyl	75	79.22
6i	Phenylmethyl	2-Methyl-quinoline-4-yl	25	49.8
6j	Phenylmethyl	3-(1-Phenyl-1H-tetrazol-5-ylthio)propyl	50	73.62
6k	Phenylmethyl	3-(Benzo[d]thiazol-2-ylthio)propyl	25	54.32
6l	2-Phenylethyl	2-Phenylethyl	50	78.76
6m	2-Phenylethyl	2-Methyl-quinoline-4-yl	10	28.15
6n	2-Phenylethyl	3-(Benzo[d]thiazol-2-ylthio)propyl	45	79.82
6o	3-Phenylpropyl	3-Phenylpropyl	70	84.53
6p	3-Phenylpropyl	2-Methyl-quinoline-4-yl	20	45.72
6q	3-Phenylpropyl	3-(Benzo[d]thiazol-2-ylthio)propyl	60	74.92
6r	3-Phenylpropyl	3-(1-Phenyl-1H-tetrazol-5-ylthio)propyl	55	67.86
IV	—	—	100[6]	—
Doxorubicin	—	—	0.25	50[c]

[a] IC_{50} of compounds was determined after 2 days exposure using MTT assay.
[b] Percent survival of T47D cells following exposure to 25 μM concentration of compounds was determined after 2 days exposure using MTT assay.
[c] Percent survival of T47D cells following exposure to 0.25 μM concentration of doxorubicin was determined after 2 days exposure using MTT assay.

prepared by the reaction of carbamates **2c**,**d** with concentrated ammonia.[23] Preparation of urea **3d**[24] by a similar method for the preparation of **3b** through the reaction 2-phenylethylamine with sodium isocyanate has been previously described. Phenyl carbamates **2c**,**d** were synthesized by the reaction of amines with phenyl chloroformate in dichloromethane in the presence of bis(trimethylsilyl)acetamide.[25] Amines **5a–d**,**g** are commercial and 3-(benzo[d]thiazol-2-ylthio)propan-1-amine **5e**[26] was prepared by the reaction of the corresponding benzo[d]thiazole-2-thiol with bromopropylammonium bromide salt and **5f** was prepared by a similar method. Mp's of the known compounds which were in agreement with the literature values and physicochemical data for the novel compounds which were consistent with their structures are presented in the Supplementary data.

Initially the cytotoxicity of biurets **6a–r** against human breast cancer T47D cells by MTT assay[27] after 2 days of exposure was evaluated at concentration of 250 nM which doxorubicin inhibited 50% cell viability.[27] However, the synthesized compounds at this concentration and even up to 1 μM showed no significant cytotoxicity and as a result concentration of 25 μM was used for evaluation and comparison of cytotoxicity of these compounds with doxorubicin at concentration of 250 nM.

Results for each compound as the percentage of growth of the treated cells in comparison to untreated cells are shown in Table 1. The most active compounds were biurets **6m**, **6p** and **6i** bearing 2-methylquinoline-4-yl moiety which exhibited 28.15%, 45.72% and 49.8% of survival, respectively. Next to these compounds, biuret **6k** bearing benzo[d]thiazol-2-ylthio showed higher activity similar to **6i**. Replacement of the benzo[d]thiazol-2-ylthio with (1-phenyl-1H-tetrazol-5-yl)thio (compounds **6r** and **6j**) reduced the activity. Among biurets without heterocyclic rings, the most active compounds were biuret **6b** and **6o** and in general, percentage of the growth of cells treated with symmetrical biurets **6a**, **6e**, **6l** and **6p** were higher than those of other compounds of this study which were unsymmetrical. The calculated IC_{50} values of all tested compounds after two days exposure showed that the order of the cytotoxicity from highest to lowest were **6m** > **6p** > **6i** = **6k** > **6c** = **6d** > **6n** > **6b** = **6j** = **6l** > **6f** = **6r** > **6a** = **6q** > **6g** = **6o** > **6e** = **6h**.

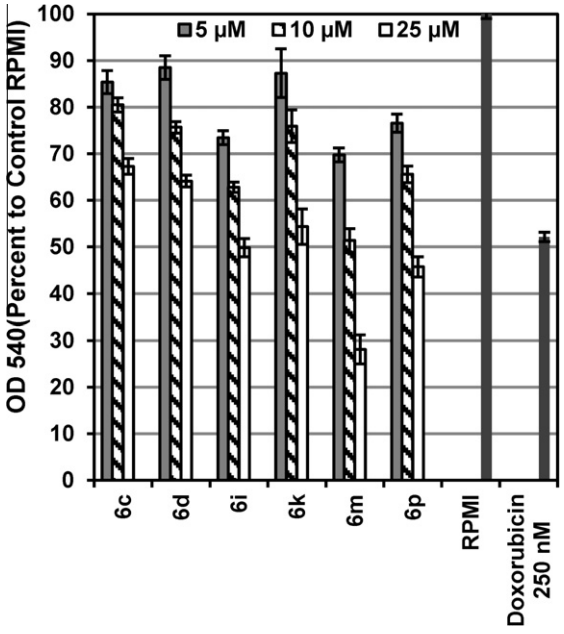

Figure 2. Concentration-dependency of cytotoxic activity of selected compounds **6c**, **d**, **i**, **k**, **m**, **p**. Data are mean ± SE of three separate experiments performed as triplicate using MTT assay after 2 days exposure.

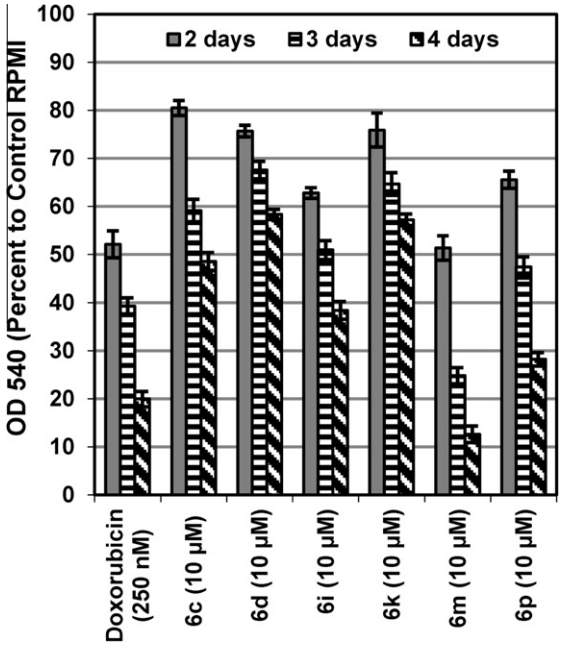

Figure 3. Time-dependency of cytotoxic activity of selected compounds **6c**, **d**, **i**, **k**, **m**, **p**. Data are mean ± SE of three separate experiments performed as triplicate using MTT assay.

On the basis of the preliminary results, compounds **6m**, **6p**, **6i**, **6k**, **6c** and **6d** which showed highest cytotoxicity were selected for further studies to determine the concentration-dependency at three concentrations of 5, 10 and 25 μM and also time-dependency after 2, 3 and 4 days exposure to T47D cells. These compounds decreased cell viability at different concentrations in a concentration- and time-dependent manner (Figs. 2 and 3). The highest cytotoxicity after 2 days exposure was exhibited by compound **6m** at concentration of 10 μM followed by **6p** at concentration of 20 μM and **6i** and **6k** at concentration of 25 μM that were significantly (p <0.001) different from control RPMI. The cytotoxicity of **6m** after 4 days exposure was even greater than growth inhibitory effect of doxorubicin at concentration of 250 nM (Fig. 3).

Preliminary results show that **6m** and **6p** the most active compound of this study are also cytotoxic on human colorectal HT-29 cells and were not significantly cytotoxic on NIH 3T3 cell line where at concentration of 10 μM inhibited only 10% of cell viability (unpublished data).

From the results of this study it appears that further exploration of biurets **6** by incorporation of azoles of the most active 4-azolylalkylquinolines and/or 4-oxoquinoline instead of 2-methylquinoline might lead to compounds with both in vivo and in vitro activity.

This approach is currently underway and results will be reported in the course of time.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.137.

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