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2,3-Substituted quinoxalin-6-amine analogs as antiproliferatives: A structure–activity relationship study

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ABSTRACT

The quinoxaline core is considered a privileged scaffold as it is found in a variety of biologically relevant molecules. Here we report the synthesis of a quinoxalin-6-amine library, screening against a panel of cancer cell lines and a structure–activity relationship (SAR). This resulted in the identification of a bisfuranylquinoxalineurea analog (**7c**) that has low micromolar potency against the panel of cancer cell lines. We also show that cells treated with quinoxalineurea **7c** results in caspase 3/7 activation, PARP cleavage and Mcl-1 dependent apoptosis.

The definition of privileged scaffold has evolved over the past two decades from ligands for a diverse array of receptors to multiple compounds with similar core structure having biological activity.[1] Quinoxaline is an important class of nitrogen containing heterocycle that is found in drugs (Fig. 1) that aid in smoking cessation (Varenicline), have antiglaucoma activity (Brimonidine) and have antibacterial properties (Quinacillin).[2–4] Not surprisingly, recent high throughput screening (HTS) campaigns against a variety of biological targets have identified quinoxaline analogs as hits. Quinoxaline analogs have been identified as inhibitors of heparin-induced tau fibril formation, cyclophin A, kinases (p110δ of PI3 kinase, JSP-1), phosphatases (cdc25B, MAPK phosphatase-1) and isomerases (peptidyl-prolyl-*cis*–*trans* isomerase).[5–11] Europium and Indium complexes containing substituted quinoxalines show large fluorescence enhancement through ligand to metal transfer, which has been exploited in developing imaging agents.[12] Together these studies indicate that the quinoxaline core is a privileged scaffold.

Our lab is focused on the identification and characterization of small molecule inhibitors of protein–protein interactions.[13,14] The early onset of breast cancer gene 1 (BRCA1) contains multiple functional domains that interact with a plethora of proteins to mediate key signal transduction events that are critical for normal cellular functions. Abnormalities of BRCA1 result in the dysfunction of the corresponding signaling networks and have been implicated in the onset and progression of cancer. A HTS using a fluorescence polarization (FP) assay to identify small molecule inhibitors of the carboxy terminus domains of BRCA1 (BRCT) resulted in the identification of a quinoxalineurea as a validated hit.[10] This prompted us to generate a focused chemical library to explore this scaffold as a BRCT inhibitor. The library was screened using the BRCT FP assay.[15] However, this did not result in the identification of either a compound with improved potency or a discernable structure–activity relationship (SAR).

Recent studies from other labs have shown that quinoxaline analogs have antiproliferative activity with micromolar potency against breast and prostate cancer cell lines.[16,17] Based on these studies and the privileged scaffold status of the quinoxalines we screened our library against a panel of cancer cell lines and estimated their growth inhibitory effects. We identified a compound (**7c**) with anti-tumor activity against a panel of cancer cell lines. We also show that this compound (**7c**) was able to induce activation of caspase 3/7, poly-ADP-ribose polymerase (PARP) cleavage and Mcl-1 dependent apoptosis.

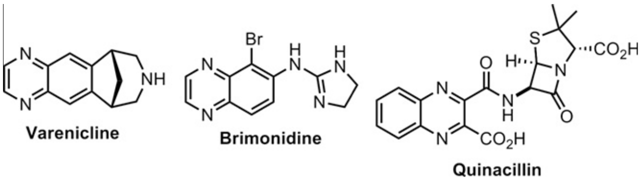

Figure 1. Drugs containing the quinoxaline core.

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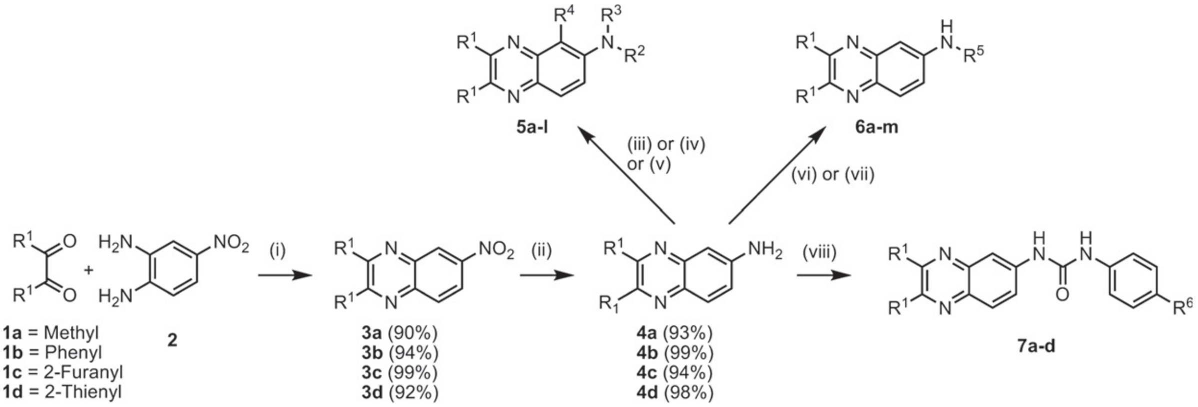

Scheme 1. Synthesis of 2,3-substituted quinoxalin-6-amine analogs. Reagents and conditions: (i) ethanol, reflux, 36–48 h; (ii) Pd/C, H_2, ethanol, room temperature, 6–8 h; (iii) R^2NCO, DIPEA, DCM 24–72 h; (iv) R^2COCl, DCM, 4 h; (v) TsCl, TEA, DCM 6 h; (vi) R^5NCS (2 equiv), DCM, reflux; (vii) triphosgene, DIPEA, DCM, 4 h; amine, DCM, 8–24 h; (viii) $R^6C_6H_4NCO$ (1.5 equiv), DIPEA, DCM, 12–24 h.

A small focused library with substitutions at the 2,3- and 6-positions on the quinoxaline core was envisioned to probe the scaffold for biological activity (Scheme 1). The various analogs and yields for the final step are summarized in Table 1. The variations at the R^1 position were methyl, furan, thiophene and phenyl groups. At the 6-position acetyl, phenylurea, and tolylsulfonamide were explored in the **5** compound series. The compounds (**5a–l**) were generated from 2,3-substituted-6-aminoquinoxaline analogs (**4**, Scheme 1)[11] with the amino group functionalized with acetyl chloride, phenylisocyanate and tosylchloride respectively. The synthesis of acetylated and phenyl urea analogs (**5a–h**) was accomplished using reported methods. However reaction of the quinoxalineamine analogs (**4**) with tosyl chloride yielded rearranged, monosubstituted and disubstituted products depending on the substitution at the 2,3-positions (**5i–l**). The amines (**4a–d**) were also reacted with isothiocyanates to generate the corresponding quinoxalinylthiourea analogs (**6a–f**). In order to generate quinoxaline urea compounds (**6g–m**) with secondary amines, 2,3-substituted quinoxaline isocyanates were generated in situ using triphosgene and reacted with the corresponding secondary amine.

Table 1
Isolated yields of 2,3-substituted quinoxalin-6-amine analogs

Entry	R^1	R^2	R^3	R^4	% Isolated yield
5a	Methyl	$-COCH_3$	–H	–H	64
5b	2-Furanyl	$-COCH_3$	–H	–H	84
5c	2-Thienyl	$-COCH_3$	–H	–H	77
5d	Phenyl	$-COCH_3$	–H	–H	70
5e	Methyl	–C(=O)–NHPh	–H	–H	83
5f	2-Furanyl	–C(=O)–NHPh	–H	–H	77
5g	2-Thienyl	–C(=O)–NHPh	–H	–H	70
5h	Phenyl	–C(=O)–NHPh	–H	–H	80
5i	Methyl	–H	–H	$-SO_2Tol$	65
5j	2-Furanyl	$-SO_2Tol$	$-SO_2Tol$	–H	66
5k	2-Thienyl	$-SO_2Tol$	$-SO_2Tol$	–H	69
5l	Phenyl	$-SO_2Tol$	–H	–H	65
	R^1	R^5			
6a	2-Furanyl	–C(=S)–NHPh			70
6b	2-Thienyl	–C(=S)–NHPh			74
6c	Phenyl	–C(=S)–NHPh			75
6d	2-Furanyl	–C(=S)–NH–(4-nitro)-phenyl			78
6e	2-Thienyl	–C(=S)–NH–(4-nitro)-phenyl			80
6f	Phenyl	–C(=S)–NH–(4-nitro)-phenyl			80
6g	Methyl	–CO-pyrrolidine			55
6h	2-Furanyl	–CO-pyrrolidine			57
6i	Phenyl	–CO-pyrrolidine			40
6j	Methyl	–C(=O)–NH-(4-benzyl)-piperidine			50
6k	2-Furanyl	–C(=O)–NH-(4-benzyl)-piperidine			53
6l	Methyl	–CO-piperdine			54
6m	2-Furanyl	–CO-morpholine			80
	R^1	R^6			
7a	2-Furanyl	–F			80
7b	2-Furanyl	–Cl			81
7c	2-Furanyl	–Br			76
7d	2-Furanyl	–Phenyl			71

The compounds were screened in a growth inhibition assay at 20 μM over a 72 h period in a panel of cancer cell lines (lung-A549; pancreatic-Aspc1; colon-HT29; breast-MDAMB231; prostate-PC3; ovarian-SKOV3 and bone-U2OS) and the results are summarized in Table 2. In the **5** compound series only **5a**, **5b**, and **5f** inhibited growth of the various cancer cell lines. This suggests that the furan substitutions at the 2,3-positions are clearly better than the other three. It also suggests that sulfonamide substitution at the 6-position is not suitable for the growth inhibitory activity. The screening results in the **6** compound series again show that all the furan compounds (**6a**, **6d**, **6h**, **6k**, and **6m**) were active. It also shows that substitution at the 4-position of the phenyl thioureas plays a role in the biological activity (**6b** and **6c** vs **6e** and **6f**). This size effect was also observed with the urea compounds from the secondary amine (**6j** vs **6l** and **6k** vs **6m**).

These results prompted us to synthesize four additional compounds to probe the size effect at the 4-position on a phenyl urea (**7a–d**). Evaluation of these analogs in the growth inhibition assay clearly showed a size effect and compound **7c** with bromo substitution at the 4-position was identified as the best compound. A dose–response study with compound **7c** shows low-μM GI_{50} values against a panel of cancer cell lines (Table 3). In summary this iterative synthesis and screening effort show that the furan substitution at the 2,3-position, a urea at the 6-position and the substituent at the *para*-position of a phenyl urea are important for the biological activity. These studies also resulted in the identification of **7c** with low-μM GI_{50} values against a panel of cancer cell lines.

Caspases are a class of cysteine proteinases that are activated during apoptosis and measuring caspase activity is often used to detect activation of apoptotic signaling. To determine if the growth inhibitory effects observed with **7c** in various cancer cell lines were a result of programmed cell death, we explored the ability of **7c** to induce caspase-3/7. Our results show that **7c** induces caspase 3/7 much more rapidly compared to the positive control (Etoposide) in MDA-MB-231 and PC3 cells (Fig. 2A and B) and the induction is sustained for 72 h in these cell lines.

Bcl-xL, Bcl-2, and Mcl-1 are antiapoptotic proteins that are implicated in the survival of cancer cells.[18,19] Bad3SA is the endogenous inhibitor of Bcl-xL and Bcl-2 but not Mcl-1. Using HeLa cells

Table 2
Screen results of the 2,3-substituted quinoxalin-6-amine analogs

Entry	% Growth inhibition at 20 μM						
	A549	AsPC1	HT29	MDA-MB-231	PC3	SKOV3	U2OS
5a	23.4 ± 3.3	26.2 ± 2.8	31.7 ± 2.8	25.7 ± 5.1	21.8 ± 15.1	4.0 ± 3.6	31.4 ± 3.6
5b	57.8 ± 7.0	Inactive	7.6 ± 5.1	20.3 ± 5.9	57.7 ± 9.4	9.0 ± 3.4	Inactive
5c	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5d	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5e	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5f	54.6 ± 2.6	9.6 ± 6.6	52.1 ± 11.6	31.3 ± 10.7	70.6 ± 1.3	24.7 ± 1.1	59.6 ± 1.6
5g	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5h	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5i	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5j	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5k	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5l	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6a	18.0 ± 0.1	Inactive	26.1 ± 15.0	19.1 ± 12.5	56.8 ± 1.4	Inactive	21.4 ± 8.4
6b	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6c	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6d	19.2 ± 8.9	Inactive	42.0 ± 1.9	9.0 ± 4.7	43.4 ± 9.3	Inactive	32.2 ± 0.1
6e	Inactive	Inactive	23.9 ± 27.8	53.3 ± 7.2	19.5 ± 37.4	Inactive	50.6 ± 3.9
6f	16.6 ± 11.2	55.5 ± 25.4	81.2 ± 0.7	72.6 ± 0.7	81.0 ± 8.5	55.8 ± 30.7	76.1 ± 0.4
6g	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6h	23.3 ± 2.9	11.6 ± 4.2	16.7 ± 3.7	18.2 ± 7.0	53.1 ± 9.9	16.9 ± 4.9	46.5 ± 4.4
6i	11.0 ± 1.3	29.4 ± 9.8	Inactive	16.7 ± 3.9	56.9 ± 2.7	18.5 ± 4.3	54.2 ± 2.2
6j	40.3 ± 1.9	Inactive	16.3 ± 10.1	12.4 ± 5.0	30.6 ± 11.6	8.9 ± 5.1	33.5 ± 0.4
6k	93.1 ± 7.8	>100	55.2 ± 4.1	90.8 ± 4.5	>100	47.6 ± 2.5	80.3 ± 2.4
6l	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
6m	39.7 ± 12.8	Inactive	19.4 ± 3.6	10.9 ± 5.0	48.9 ± 7.5	Inactive	Inactive
7a	>100	>100	74.2 ± 5.2	38.4 ± 2.7	90.7 ± 4.1	Inactive	55.6 ± 6.6
7b	99.2 ± 5.2	81.1 ± 2.0	86.3 ± 7.7	92.5 ± 6.6	86.4 ± 1.1	55.8 ± 24.1	87.4 ± 1.1
7c	>100	>100	88.6 ± 4.6	>100	>100	94.9 ± 7.9	>100
7d	13.8 ± 9.8	Inactive	72.4 ± 7.6	50.2 ± 8.9	88.5 ± 1.9	Inactive	46.9 ± 7.4

Table 3
Dose–response growth inhibition study with **7c**

Cell line	GI_{50} (μM)
A549	6.4 ± 3.0
AsPC1	17.3 ± 0.9
HT29	12.1 ± 7.4
MDA-MB-231	8.4 ± 0.9
PC3	5.9 ± 2.7
SKOV3	16.8 ± 5.2
U2OS	10.8 ± 0.2

that over express Bad3SA, we explored the mechanistic basis for the induction of apoptosis by **7c**. In these cell lines expression of Bad3SA is under the control of Doxycycline (Dox). The apoptosis studies carried out in this cell line are summarized in Figure 3. As with the other cancer cell lines we observe induction of caspase 3/7 and PARP cleavage by **7c** (Fig. 3A and B, respectively). We next carried out a dose–response study with **7c** in untreated cells [(−) Dox] and cells treated with Dox (1 μg/mL for 3 h) to induce Bad3SA [(+) Dox]. The cells were incubated with **7c** and a positive control (DNA damaging agent Camptothecin, CPT) for 12 h. Cell death was measured by counting the number of condensed nuclei.

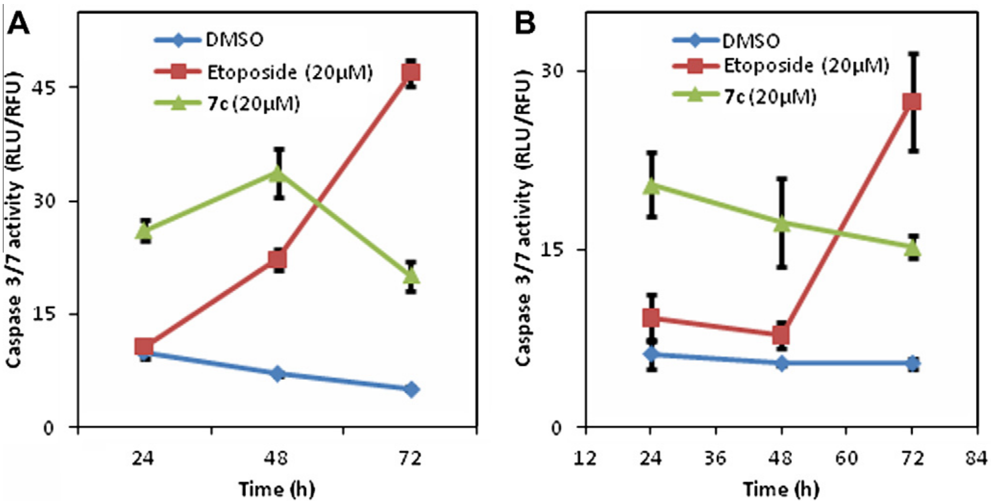

Figure 2. Induction of caspase 3/7 activity by **7c** and Etoposide (a known chemotherapeutic agent) in MDA-MB-231 breast cancer cells (A) and PC3 prostate cancer cells (B).

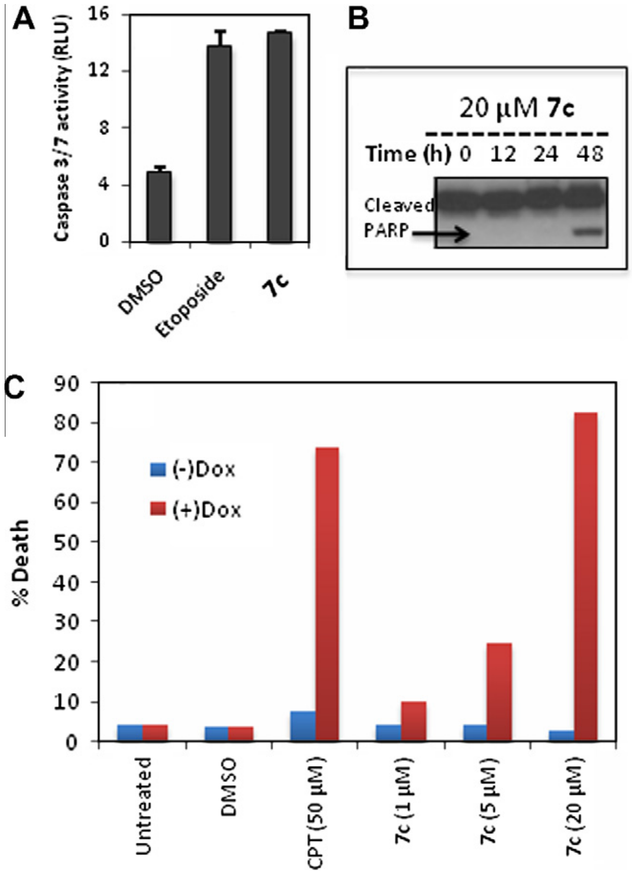

Figure 3. Apoptosis studies in HeLa cells. (A) Induction of caspase 3/7 by **7c** and Etoposide. (B) PARP cleavage induced by **7c** assessed by Western blot. (C) Mcl-1 dependent induction of apoptosis by **7c**.

We observed a dose dependent increase in the induction of apoptosis in the (+) Dox cells, indicating that **7c** induces apoptosis in a Mcl-1 dependent manner (Fig. 3C).

In summary, a focused library of 2,3-substituted quinoxalin-6-amine analogs was synthesized and evaluated in a panel of cancer cell lines for growth inhibition. The preliminary structure–activity relationship (SAR) showed bis-furan substitution at the 2,3-positions was favored. A comparison of a series of linkers between the 2,3-disubstituted quinoxaline and a substituted phenyl ring showed that a urea linker was optimal for the antiproliferative activity. In addition, the size of the substituent at the 4-position of the phenyl ring was important for the activity. This led to the identification of a bisfuranylquinoxalineurea analog (**7c**) with low micromolar potency against the panel of cancer cell lines. The analog **7c** induces caspase 3/7 activation, PARP cleavage and Mcl-1 dependent apoptosis. The molecular target of compound **7c** is currently under investigation and will be reported in due course.

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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.2011.02.055.

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