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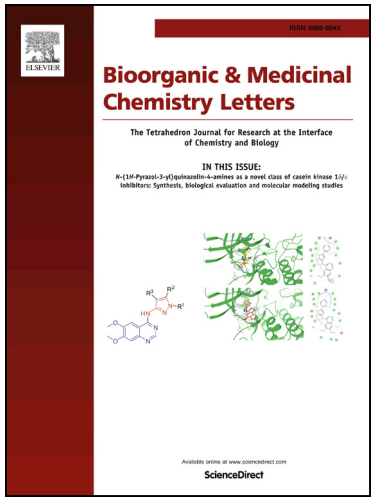

Design, synthesis and biological evaluation of benzamide and phenyltetrazole derivatives with amide and urea linkers as BCRP inhibitors

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Design, synthesis and biological evaluation of benzamide and phenyltetrazole derivatives with amide and urea linkers as BCRP inhibitors

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ABSTRACT

Breast cancer resistant protein (BCRP/ABCG2), a 72 kDa plasma membrane transporter protein is a member of ABC transporter superfamily. Increased expression of BCRP causes increased efflux and therefore, reduced intracellular accumulation of many unrelated chemotherapeutic agents leading to multidrug resistance (MDR). A series of 31 benzamide and phenyltetrazole derivatives with amide and urea linkers has been synthesized to serve as potential BCRP inhibitors in order to overcome BCRP-mediated MDR. The target derivatives were tested for their cytotoxicity and reversal effects in human non-small cell lung cancer cell line H460 and mitoxantrone resistant cell line H460/MX20 using the MTT assay. In the benzamide series, compounds **6** and **7** exhibited a fold resistance of 1.51 and 1.62, respectively at 10 μM concentration which is similar to that of FTC, a known BCRP inhibitor. Compounds **27** and **31** were the most potent analogues in the phenyltetrazole series with amide linker with a fold resistance of 1.39 and 1.32, respectively at 10 μM concentration. For the phenyltetrazole series with urea linker, **38** exhibited a fold resistance of 1.51 which is similar than that of FTC and is the most potent compound in this series. The target compounds did not exhibit reversal effect in P-gp overexpressing resistant cell line SW620/Ad300 suggesting that they are selective BCRP inhibitors.

Cancer is the second leading cause of death in the United States.[1] Although a wide variety of drugs are available to fight cancer, Multidrug resistance (MDR) is a major contributor towards the failure of cancer chemotherapy.[2] Cancer cells can be primarily resistant to chemotherapy or acquire MDR during treatment, making chemotherapy ineffective.[3] ATP-binding cassette (ABC) proteins belong to a superfamily of proteins that consists of 49 members classified from A through G subfamily. ABC transporters use ATP hydrolysis to transport substrates across cell membrane against their electrochemical gradient and many of the human ABC proteins including P-glycoprotein (P-gp/ABCB1) and Breast Cancer Resistance Protein (BCRP/ABCG2) are efflux transporters.[4] The decreased accumulation of chemotherapeutic agents in the tumor cells is one of the most common causes of MDR. BCRP (ABCG2) is the second member of the subfamily G, consisting of 655 amino acids, first identified in a multidrug resistant human breast cancer cell line MCF-7/AdrVp.[5, 6] BCRP transports a wide variety of molecules and plays a key role in protecting the body against xenobiotics.[7]

BCRP actively transports many structurally unrelated chemotherapeutic agents such as mitoxantrone, methotrexate, topotecan, SN38 and flavopiridol out of the cells leading to the development of multidrug resistance (MDR).[8] Overexpression of BCRP in cancer cells is a significant mechanism behind MDR. Efforts have been made to design potential modulators in order to overcome BCRP-mediated MDR.[9] Fumitremorgin C (FTC) which was isolated from *Aspergillus fumigatus*, is a highly potent and specific inhibitor of BCRP. However, it is not suitable for therapeutic use due to its neurotoxicity.[10, 11]

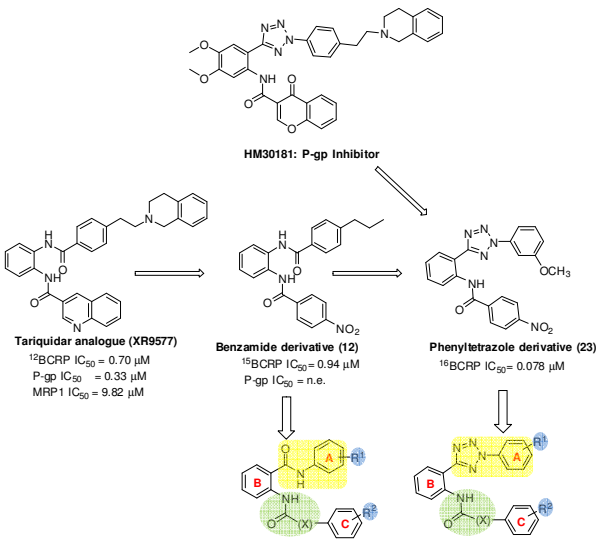
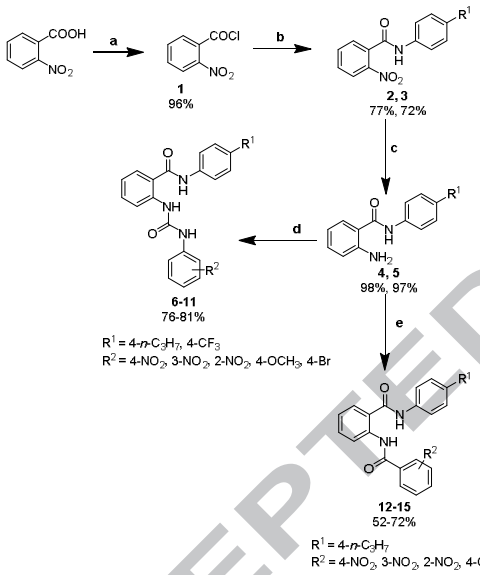


Figure 1. SAR development of benzamide and phenyltetrazole series.

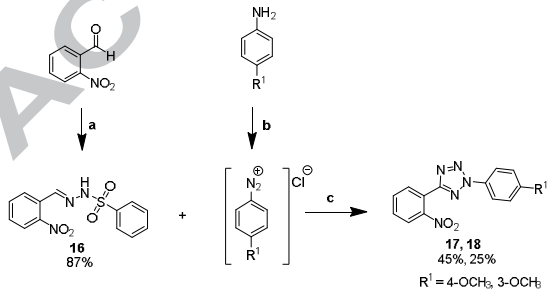
Tariquidar, a third generation P-gp inhibitor is two times more selective in inhibiting P-gp than BCRP.[12] Removal of the

tetrahydroisoquinoline ring of tariquidar has been found to impart selectivity towards BCRP inhibition.[13] Various modifications related to tarquidar and HM30181 have led to the identification of the benzamide derivative **12** (IC_{50} = 0.94 μM in Hoechst assay using MCF-7 MX cells) and the phenyltetrazole derivative **23** (IC_{50} = 0.078 μM in Hoechst assay using MDCKII BCRP cells) which have been used as the lead compounds in the present study.[13, 14, 15, 16] A series of benzamide and phenyltetrazole derivatives with amide or urea linkers between rings B and C, and various substitutions were introduced at R^1 and R^2 on rings A and C, respectively to impart a range of steric, electronic and solubility characteristics to further explore the SAR.

The target compounds **6-15** and **21-41** were synthesized as illustrated in Schemes 1-4. 2-Nitrobenzoyl chloride **1** was obtained by refluxing 2-nitrobenzoic acid and thionyl chloride in DCM (Scheme 1).[17] Reacting 2-nitrobenzoyl chloride **1** with 4-*n*-propyl aniline or 4-(trifluoromethyl)aniline in presence of triethylamine led to amide analogues **2** and **3**, respectively. Hydrogenation using 10% Pd/C resulted in reduction of the nitro analogues **2** and **3** to the amines **4** and **5** in quantitative yields. The amines were then reacted with various substituted aryl isocyanates to obtain the urea analogues **6-11** and with various substituted benzoyl chlorides to obtain the amide analgoues **12-15**.

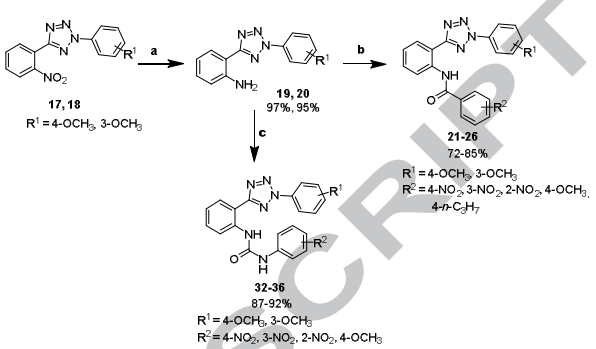


Scheme 1. Synthesis of benzamide analogues **6-15**. (**a**) $SOCl_2$, DCM, reflux, 3 h; (**b**) Substituted anilines, Et_3N, DCM, 0 °C to rt, 16 h; (**c**) H_2, Pd/C, 50 psi, reagent alcohol, 7 h; (**d**) Substituted phenylisocyanate, THF, rt, 3 h; (**e**) Substituted benzoyl chloride, Et_3N, DCM, 0 °C to rt, overnight.

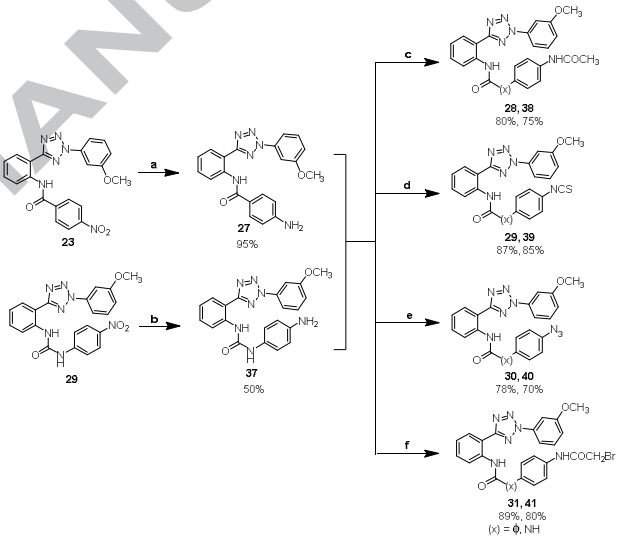


Scheme 2. Synthesis of compounds **17** and **18**. (**a**) Benzenesulfonylhydrazine, EtOH , rt; (**b**) $NaNO_2$, conc. HCl, EtOH/ H_2O, < 0 °C; (**c**) Pyridine, −10 to −15 °C.

Sulfonyl hydrazide **16** was prepared from commercially available 2-nitrobenzaldehyde and benzenesulfonylhydrazine (Scheme 2). The intermediate aryl diazonium salts were prepared from substituted anilines using sodium nitrite and hydrochloric acid in water and ethanol mixture.[18] 1,5-Dipolar cyclization of **16** with aryl diazonium salts yielded the tetrazole analogues **17** and **18**. The amines **19** and **20** were obtained by Pd/C catalyzed hydrogenation of **17** and **18** (Scheme 3) and were reacted with various substituted benzoyl chlorides to obtain the amide analgoues **21-26** and with various substituted aryl isocyanates to obtain the urea analogues **32-36**.



Scheme 3. Synthesis of phenyltetrazole analogues **21**-**26** and **32**-**36**. (**a**) H_2, 10% Pd/C, 50 psi, reagent alcohol, 7 h; (**b**) Substituted benzoyl chloride, Et_3N, DCM, 0 °C to rt, overnight; (**c**) Substituted phenylisocyanate, DCM, rt, 3 h.



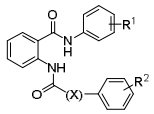
Scheme 4. Synthesis of phenyltetrazole analogues. (**a**) H_2, Pd/C, 50 psi, reagent alcohol, 7 h; (**b**) H_2, Pd/C, 60 psi, DMF, reagent alcohol, overnight; (**c**) Acetic anhydride, Et_3N, DCM, rt; (**d**) Di-2-pyridyl thionocarbonate, DCM, rt; (**e**) $NaNO_2$, NaN_3, HCl/H_2O, 0 °C to rt; (**f**) Bromoacetylbromide, DCM, 0 °C to rt.

Pd/C catalyzed reduction of nitro derivatives **23** and **29** led to the amines **27** and **37**, respectively which were acetylated using acetic anhydride to obtain acetamide analogues **28** and **38**.[19] Isothiocyanate analogues **29** and **39** were synthesized from the amino analogues **27** and **37** by reacting them with di-2-pyridyl thionocarbonate.[20] The azide analogues **30** and **40** were obtained from amines **27** and **37** by diazotization and subsequent treatment with sodium azide.[21] Acylation of the amino analogues **27** and **37** using bromoacetylbromide yielded analogues **31** and **41**.[22]

The human cancer cell lines, H460 (non-small cell lung cancer) and its MX-selected derivative ABCG2-overexpressing cell line H460/MX20 were used to determine the cytotoxicity as well as reversal effects using the MTT assay. FTC was chosen as a positive control and the reversal of mitoxantrone resistance was calculated.[23]

In the benzamide series, all the target compounds with amide or urea linkers **6**-**15** exhibited IC_{50} values greater than 100 µM in both H460 and H460/MX20 cell lines (Table 1). In the phenyltetrazole series, the target compounds with the amide linker **21**-**31**, the IC_{50} values of analogues **21**, **23**, **27**, **28**, and **31** were greater than 100 µM and the remaining analogues exhibited IC_{50} values in the range of 20 and 60 µM in the two cell lines.

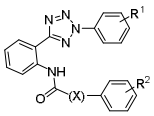
Table 1. Cytotoxicity of benzamide derivatives using MTT assay



Cmpd #	X	R^1	R^2	H460 (µM)	H460/MX20 (µM)	QPlogP
6	NH	4-*n*-C_3H_7	4-NO_2	> 100	> 100	3.83
7	NH	4-*n*-C_3H_7	3-NO_2	> 100	> 100	3.94
8	NH	4-*n*-C_3H_7	2-NO_2	> 100	> 100	3.84
9	NH	4-*n*-C_3H_7	4-Br	> 100	> 100	4.85
10	NH	4-*n*-C_3H_7	4-OCH_3	> 100	> 100	4.53
11	NH	4-CF_3	4-NO_2	> 100	> 100	3.78
12	-	4-*n*-C_3H_7	4-NO_2	> 100	> 100	4.67
13	-	4-*n*-C_3H_7	3-NO_2	> 100	> 100	4.70
14	-	4-*n*-C_3H_7	2-NO_2	> 100	> 100	4.91
15	-	4-*n*-C_3H_7	4-OCH_3	> 100	> 100	5.49

[a]IC_{50} values are represented as mean ± SD of three independent experiments. QPlogP is the log $P_{o/w}$ value calculated using QikProp, Schrödinger.

Table 2. Cytotoxicity of phenyltetrazole derivatives using MTT assay

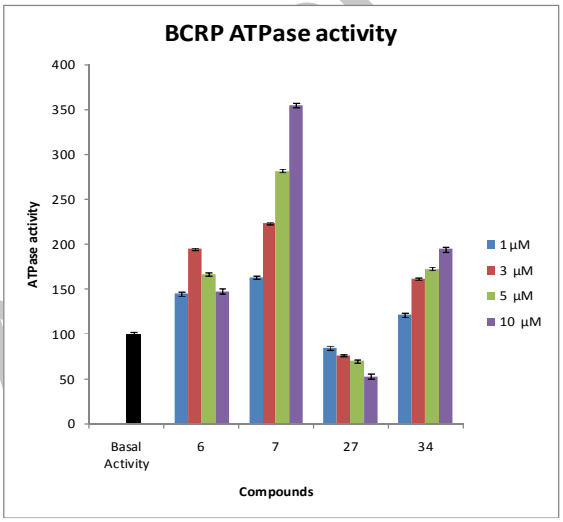


Cmpd #	X	R^1	R^2	H460 (µM)	H460/ MX20 (µM)	QPlogP
21	-	4-OCH_3	4-OCH_3	> 100	> 100	3.51
22	-	4-OCH_3	4-NO_2	24.73 ± 2.62	27.44 ± 3.14	2.90
23	-	3-OCH_3	4-NO_2	> 100	> 100	2.92
24	-	3-OCH_3	3-NO_2	19.85 ± 1.55	25.41 ± 2.95	3.02
25	-	3-OCH_3	2-NO_2	38.90 ± 3.68	44.71 ± 4.21	3.18
26	-	3-OCH_3	4-*n*-C_3H_7	26.97 ± 3.25	40.60 ± 2.55	4.52
27	-	3-OCH_3	4-NH_2	> 100	> 100	2.80
28	-	3-OCH_3	4-$NHCOCH_3$	> 100	> 100	3.08
29	-	3-OCH_3	4-NCS	44.70 ± 6.67	59.01 ± 6.19	3.37
30	-	3-OCH_3	4-N_3	20.30 ± 1.97	21.51 ± 1.75	2.35
31	-	3-OCH_3	4-$NHCOCH_2Br$	> 100	> 100	3.71
32	NH	4-OCH_3	4-OCH_3	52.54 ± 7.51	57.41 ± 6.78	3.63
33	NH	4-OCH_3	4-NO_2	21.69 ± 5.53	28.53 ± 3.19	2.30
34	NH	3-OCH_3	4-NO_2	> 100	> 100	2.16
35	NH	3-OCH_3	3-NO_2	> 100	> 100	2.95
36	NH	3-OCH_3	2-NO_2	> 100	> 100	2.65
37	NH	3-OCH_3	4-NH_2	> 100	> 100	2.50
38	NH	3-OCH_3	4-$NHCOCH_3$	> 100	> 100	2.25
39	NH	3-OCH_3	4-NCS	> 100	> 100	3.01
40	NH	3-OCH_3	4-N_3	> 100	> 100	2.09
41	NH	3-OCH_3	4-$NHCOCH_2Br$	> 100	> 100	2.87

[a]IC_{50} values are represented as mean ± SD of three independent experiments. QPlogP is the log $P_{o/w}$ value calculated using QikProp, Schrödinger.

In the phenyltetrazole series, among the analogues containing the urea linker **32**-**41**, compound **32** with 4-OCH_3 substitution at both R^1 and R^2 positions exhibited IC_{50} values of 52.54 ± 7.51 µM and 57.41 ± 6.78 µM in H460 cells and H460/MX20 cells, respectively (Table 2). Phenyltetrazole derivative **33** with 4-OCH_3 substitution at R^1 and 4-NO_2 substitution at R^2 exhibited IC_{50} values of 21.67 ± 5.53 µM and 28.53 ± 3.19 µM in H460 cells and H460/MX20 cells, respectively. All the other analogues in the phenyltetrazole series containing the urea linker exhibited an IC_{50} value greater than 100 µM.

For the reversal assay, all the target compounds were tested at 10, 3, and 1 µM concentrations (Tables 3-5). The fold resistance of mitoxantrone was found to be 28.66-fold against H460/MX20 cell line which was reduced to 1.49-fold in presence of 10 µM FTC, a known BCRP inhibitor.



[a]ATPase activity is represented as mean ± SD of two independent experiments

Figure 2. Effect of selected target compounds on ATPase activity of ABCG2.

In the benzamide series, the lead compound **12** with amide linker, 4-*n*-C_3H_7 at R^1 and 4-NO_2 at R^2 exhibited fold resistance of 2.76 at 10 µM concentration (Table 3). Compounds **6**, **7**, **11**, and **13** exhibited a fold resistance lower than that of the lead compound **12**. Among benzamide compounds possessing a urea linker, compound **6** with 4-*n*-C_3H_7 at R^1 and 4-NO_2 at R^2 and compound **7** with 4-*n*-C_3H_7 at R^1 and 3-NO_2 at R^2 exhibited a fold resistance of 1.51 and 1.62 at 10 µM concentration which is similar to that of FTC.

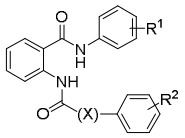
In the phenyltetrazole series with amide linker, compound **22** with 4-OCH_3 at R^1 and 4-NO_2 at R^2 and compound **23** with 3-OCH_3 at R^1 and 4-NO_2 at R^2 were the lead molecules with a fold resistance of 1.76 and 1.43, respectively (Table 4).[16] Compounds **26**, **27** and **31** exhibited a fold resistance lower than the lead compounds **22** and **23** as well as FTC at all three concentrations. Compound **26** with 3-OCH_3 at R^1 and 4-*n*-C_3H_7 at R^2 exhibited the lowest fold resistance, however, as previously described, in the cytotoxicity assay it exhibited IC_{50} values of 26.97 and 59.01 µM, respectively in H460 and H460/MX20 cell lines (Table 2). Compounds with lower IC_{50} values can have intrinsic cytotoxic effect, which in turn can result in ostensibly lower fold resistance. Compounds **27** with 3-OCH_3 at R^1 and 4-NH_2 at R^2 and **31** with 3-OCH_3 at R^1 and 4-$NHCOCH_2Br$ at R^2 were the

most potent analogues in this series with a fold resistance of 1.39 and 1.32, respectively at 10 μM concentration.

In the phenyltetrazole series with the urea linker, compounds **32**, **33**, **34**, **37**, and **38** exhibited fold resistance lower or similar to FTC (Table 5). However, as discussed previously compounds **32** with 4-OCH_3 at R^1 and 4-OCH_3 at R^2 and **33** with 4-OCH_3 at R^1 and 4-NO_2 at R^2 had a lower IC_{50} value in the cytotoxicity assay as compared to the other analogues (Table 2). Compound **38** with 3-OCH_3 at R^1 and 4-$NHCOCH_3$ at R^2 exhibited a fold resistance of 1.51 which was found to be similar to that of FTC and was the most potent member of this series followed by compound **37** with 3-OCH_3 at R^1 and 4-NH_2 at R^2 and **34** with 3-OCH_3 at R^1 and 4-NO_2 at R^2. A similar trend in the fold resistance was observed at 3 μM and 1 μM concentrations for all three series of analogues.

All the target compounds (**6**-**15** and **21**-**41**) were tested at three different concentrations (10, 3 and 1 μM) for the reversal assay in parental human colon cancer SW620 cells and paclitaxel resistant SW620/Ad300 cells. Verapamil was chosen as the positive control and the fold resistance of paclitaxel was calculated. Verapamil was found to have a fold resistance of 2.31, 2.65 and 3.54 at 10, 3, and 1 μM concentrations, respectively. All the target compounds exhibited a fold resistance greater than 45 which is comparable to the fold resistance of paclitaxel. They did not affect the reversal of paclitaxel in P-gp overexpressing resistant cell line SW620/Ad300 suggesting that these analogues are selective towards ABCG2-mediated MDR.

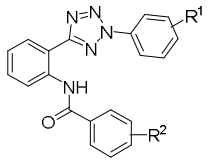
Table 3. Reversal effect of benzamide on mitoxantrone resistant ABCG2 overexpressing H460/MX20 cells



Cmpd #	X	R^1	R^2	Conc. (μM)	H460 IC_{50} ± SD (μM)	H460/MX20 IC_{50} ± SD (μM)	Fold Resistance
	Mitoxantrone				0.215±0.011	6.161±0.172	28.66
				10	0.199±0.023	0.325±0.024	1.51
6	NH	4-*n*-C_3H_7	4-NO_2	3	0.211±0.017	0.482±0.053	2.24
				1	0.209±0.014	0.622±0.031	2.89
				10	0.201±0.054	0.348±0.042	1.62
7	NH	4-*n*-C_3H_7	3-NO_2	3	0.205±0.021	0.501±0.072	2.33
				1	0.206±0.011	0.651±0.022	3.03
				10	0.212±0.062	1.120±0.077	5.21
8	NH	4-*n*-C_3H_7	2-NO_2	3	0.225±0.016	2.563±0.137	11.92
				1	0.221±0.017	3.662±0.214	17.03
				10	0.214±0.025	0.952±0.142	4.43
9	NH	4-*n*-C_3H_7	4-Br	3	0.217±0.015	2.121±0.161	9.87
				1	0.217±0.021	2.655±0.117	12.35
				10	0.211±0.026	0.985±0.072	4.58
10	NH	4-*n*-C_3H_7	4-OCH_3	3	0.218±0.014	2.173±0.112	10.12
				1	0.221±0.016	3.371±0.356	15.68
				10	0.222±0.013	0.452±0.058	2.12
11	NH	4-CF_3	4-NO_2	3	0.221±0.023	0.605±0.045	2.81
				1	0.217±0.011	0.711±0.055	3.31
				10	0.213±0.051	0.594+0.057	2.76
12	-	4-*n*-C_3H_7	4-NO_2	3	0.218±0.015	0.783+0.075	3.64
				1	0.212±0.015	0.833+0.043	3.87
				10	0.205±0.021	0.512+0.064	2.38
13	-	4-*n*-C_3H_7	3-NO_2	3	0.209±0.012	0.727+0.022	3.38
				1	0.211±0.025	0.785+0.072	3.65
				10	0.221±0.012	1.713+0.044	7.70
14	-	4-*n*-C_3H_7	2-NO_2	3	0.206±0.011	2.151+0.132	9.36
				1	0.215±0.012	2.814+0.252	11.75
				10	0.211±0.062	1.656+0.11	7.97
15	-	4-*n*-C_3H_7	4-OCH_3	3	0.224±0.016	2.012+0.11	10.00
				1	0.209±0.016	2.526+0.173	13.10
				10	0.148±0.058	0.321±0.052	1.49
	FTC			3	0.168±0.013	0.451±0.031	2.10
				1	0.186±0.007	0.612±0.034	2.85

[a]IC_{50} values are represented as mean ± SD of three independent experiments. Fold resistance was calculated as by dividing the IC_{50} values of the substrate (MX) in presence or absence of inhibitor by the IC_{50} of parental cells without inhibitors.

Table 4. Reversal effect of phenyltetrazole derivatives with amide linker on mitoxantrone resistant ABCG2 overexpressing H460/MX20 cells

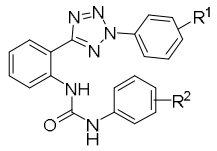


Cmpd #	R^1	R^2	Conc. (μM)	H460 IC_{50} ± SD (μM)	H460/MX20 IC_{50} ± SD (μM)	Fold Resistance
Mitoxantrone				0.215±0.011	6.161±0.172	28.66
			10	0.149±0.061	0.492±0.011	2.29
21	4-OCH_3	4-OCH_3	3	0.156±0.013	0.505±0.035	2.35
			1	0.305±0.054	0.746±0.025	3.47
			10	0.121±0.012	0.378±0.024	1.76
22	4-OCH_3	4-NO_2	3	0.143±0.121	0.535±0.035	2.49
			1	0.244±0.021	0.587±0.064	2.73
			10	0.230±0.043	0.307±0.032	1.43
23	3-OCH_3	4-NO_2	3	0.239±0.032	0.387±0.022	1.80
			1	0.240±0.052	0.585±0.055	2.72
			10	0.345±0.044	0.866±0.056	4.03
24	3-OCH_3	3-NO_2	3	0.561±0.013	1.39±0.15	6.47
			1	0.715±0.016	1.801±0.72	8.38
			10	0.333±0.022	1.10±0.32	5.12
25	3-OCH_3	2-NO_2	3	0.414±0.045	1.32±0.27	6.14
			1	0.501±0.019	1.88±0.152	8.74
			10	0.121±0.051	0.131±0.022	0.61
26	3-OCH_3	4-n-C_3H_7	3	0.173±0.012	0.207±0.031	0.96
			1	0.207±0.112	0.354±0.051	1.65
			10	0.211±0.031	0.299±0.057	1.39
27	3-OCH_3	4-NH_2	3	0.212±0.061	0.491±0.072	2.28
			1	0.217±0.041	0.549±0.072	2.55
			10	0.52±0.022	1.202±0.012	5.58
28	3-OCH_3	4-$NHCOCH_3$	3	0.554±0.086	1.325±0.037	6.16
			1	0.725±0.084	1.363±0.025	6.34
			10	0.352±0.015	0.952±0.053	4.43
29	3-OCH_3	4-NCS	3	0.379±0.052	1.05±0.051	4.88
			1	0.457±0.065	1.221±0.17	5.68
			10	0.415±0.026	1.267±0.23	5.89
30	3-OCH_3	4-N_3	3	0.455±0.056	1.501±0.33	6.98
			1	0.558±0.014	1.615±0.63	7.51
			10	0.250±0.061	0.283±0.056	1.32
31	3-OCH_3	4-$NHCOCH_2Br$	3	0.175±0.073	0.446±0.025	2.07
			1	0.241±0.033	0.605±0.054	2.81
			10	0.148±0.058	0.321±0.052	1.49
FTC			3	0.168±0.013	0.451±0.031	2.10
			1	0.186±0.007	0.612±0.034	2.85

[a]IC_{50} values are represented as mean ± SD of three independent experiments. Fold resistance was calculated as by dividing the IC_{50} values of the substrate (MX) in presence or absence of inhibitor by the IC_{50} of parental cells without inhibitors.

ABCG2 utilizes energy derived from the hydrolysis of ATP to efflux their substrates across the membrane against a concentration gradient, and thus ATP consumption reflects its ATPase activity.[24] To assess the effect of the target compounds on the ATPase activity of ABCB2, ABCB2-mediated ATP hydrolysis was measured at various concentrations. ATPase assay was carried out for selected potent analogues **6**, **7**, **27**, and **34** (Figure 2). Among these analogues **6**, **7**, and **34** were found to stimulate the basal BCRP ATPase activity by 1.47, 3.55 and 1.94 folds, respectively at 10 μM concentration. Compound **27** was found to inhibit the basal BCRP ATPase activity by 0.52-fold. Increased ATPase activity suggests that the analogues **6**, **7**, and **34** may be competitively inhibiting ABCG2 while compound **27** may be serving as a non-competitive inhibitor of ABCG2. Analogues **7**, **27** and **34** showed dose dependent effect on ATPase activity; however compound **6** showed an increase in ATPase activity up to 3 μM concentration followed by decrease in ATPase activity at higher concentrations.

Table 5. Reversal effect of phenyltetrazole derivatives with urea linker on mitoxantrone resistant ABCG2 overexpressing H460/MX20 cells



Cmpd #	R^1	R^2	Conc. (μM)	H460 IC_{50} ± SD (μM)	H460/MX20 IC_{50} ± SD (μM)	Fold Resistance
Mitoxantrone				0.215±0.011	6.161±0.172	28.66
			10	0.211±0.019	0.355±0.041	1.56
32	4-OCH_3	4-OCH_3	3	0.229±0.021	0.382±0.035	1.78
			1	0.255±0.025	0.442±0.062	2.06
			10	0.122±0.025	0.331±0.051	1.54
33	4-OCH_3	4-NO_2	3	0.174±0.021	0.541±0.027	2.52
			1	0.194±0.011	0.718±0.032	3.34
			10	0.113±0.042	0.403±0.054	1.87
34	3-OCH_3	4-NO_2	3	0.190±0.016	0.544±0.013	2.53
			1	0.179±0.035	0.832±0.124	3.87
			10	0.144±0.024	0.750±0.024	3.49
35	3-OCH_3	3-NO_2	3	0.148±0.115	0.807±0.161	3.75
			1	0.254±0.071	0.967±0.145	4.50
			10	0.222±0.058	0.882±0.104	4.10
36	3-OCH_3	2-NO_2	3	0.235±0.014	0.978±0.056	4.55
			1	0.246±0.011	1.172±0.112	5.45
			10	0.176±0.028	0.379±0.044	1.76
37	3-OCH_3	4-NH_2	3	0.216±0.045	0.571±0.065	2.66
			1	0.228±0.064	0.838±0.075	3.90
			10	0.128±0.053	0.325±0.094	1.51
38	3-OCH_3	4-$NHCOCH_3$	3	0.275±0.062	0.454±0.052	2.11
			1	0.341±0.058	0.627±0.022	2.92
			10	0.217±0.044	0.550±0.047	2.56
39	3-OCH_3	4-NCS	3	0.233±0.086	0.667±0.032	3.10
			1	0.294±0.052	1.515±0.21	7.05
			10	0.252±0.054	0.685±0.151	3.19
40	3-OCH_3	4-N_3	3	0.281±0.071	0.691±0.012	3.21
			1	0.279±0.026	0.913±0.32	4.25
			10	0.185±0.025	0.472±0.06	2.20
41	3-OCH_3	4-$NHCOCH_2Br$	3	0.211±0.055	0.583±0.075	2.71
			1	0.254±0.054	0.798±0.132	3.71
			10	0.148±0.058	0.321±0.052	1.49
FTC			3	0.168±0.013	0.451±0.031	2.10
			1	0.186±0.071	0.612±0.034	2.85

[a]IC_{50} values are represented as mean ± SD of three independent experiments. Fold resistance was calculated as by dividing the IC_{50} values of the substrate (MX) in presence or absence of inhibitor by the IC_{50} of parental cells without inhibitors.

In conclusion, a series of novel benzamide (**6-15**) and phenyltetrazole derivatives (**21-41**) consisting of urea and amide linkers were designed and synthesized as potential and BCRP/ABCG2 selective inhibitors. The compounds were evaluated for their cytotoxicity and reversal effects on mitoxantrone resistance in two cell lines, parental cell line H460 and BCRP overexpressing cell line H460/MX20, using the MTT assay. In the benzamide series, compounds **6** and **7** which exhibited QPlogP values of 3.83 and 3.94, respectively were found to have reversal effects on the cytotoxicity of mitoxantrone in H460/MX20 cells comparable to the known BCRP inhibitor, FTC at three different concentrations. In the tetrazole series, compounds **27**, **31**, **34**, **37**, and **38** which exhibited QPlogP values in the range of 2.16 - 3.71 were found to have reversal activity comparable to that of FTC. Compounds **6**, **7**, **27,** and **34** were further evaluated using ATPase assay. Compounds **6**, **7**, and **27** were found to be stimulators of basal ATPase, while compound **34** was found to be an inhibitor of ATPase. Target compounds did not exhibit any reversal effect on paclitaxel resistant P-gp overexpressing SW620/Ad300 cells, suggesting that these analogues are selective BCRP (ABCG2) inhibitors. The target compounds can serve as lead molecules for developing

inhibitors to overcome multi-drug resistance caused by overexpression of BCRP in cancer cells.

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The authors thank Dr. Tanaji Talele and Udaykiran Velagapudi for assistance with the QPlogP calucaltions. Support for this research was provided by Saint John's University.

A. Supplementary data

All experimental procedures (for both synthesis and biological assays) and full characterization of compounds. Supplementary data associated with this article can be found, in the online version, at

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Graphical Abstract

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Design, synthesis and biological evaluation of benzamide and phenyltetrazole derivatives with amide and urea linkers as BCRP inhibitors

Nehaben A. Gujarati, Leli Zeng, Pranav Gupta, Zhe-Sheng Chen and Vijaya L. Korlipara*

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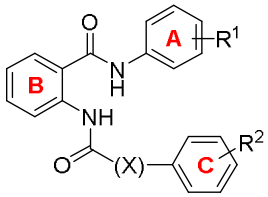
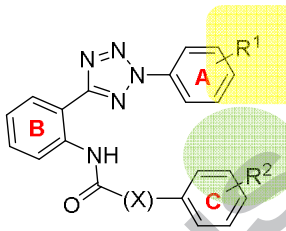
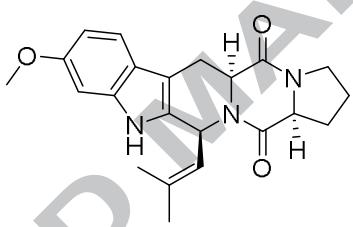


6: X = NH, R^1 = 4-*n*-C_3H_7, R^2 = 4-NO_2,*FR = 1.51
7: X = NH, R^1 = 4-*n*-C_3H_7, R^2 = 3-NO_2, *FR = 1.62



27: R^1 = 3-OCH_3, R^2 = 4-NH_2, *FR = 1.39
31: R^1 = 3-OCH_3, R^2 = 4-$NHCOCH_2Br$, *FR = 1.32
38: X = NH, R^1 = 3-OCH_3, R^2 = 4-$NHCOCH_3$, *FR = 1.51



Fumitremorgin C, *FR = 1.49
*Fold resistance at 10 μM

27.