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Studies on Quinazolinones as Dual Inhibitors of Pgp and MRP1 in Multidrug Resistance

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Abstract—The syntheses and SAR studies of various quinazolinone compounds are described for the dual inhibition of Pgp and MRP1 in multidrug resistance.

The appearance of tumor cells resistant to a range of cytotoxic drugs is a serious problem in cancer chemotherapy. This phenomenon is called multidrug resistance (MDR). One form of MDR can be caused by members of the ATP-binding cassette (ABC) family of transport proteins.[1] These are large polytopic membrane proteins that actively transport drugs out of cells, resulting in a decreased intracellular drug concentration. In humans, two ABC transporters have been identified that cause resistance in tumor cells: P-glycoprotein (Pgp) (MDR1)[2] and the multidrug resistance associated protein (MRP1).[3] Pgp transports drugs in an unmodified form, whereas MRP1 transports drugs either conjugated to the anionic ligands glutathione (GSH), glucuronide, or sulfate,[4] or transports them in an unmodified form, probably together with GSH.[5] Among those cytotoxics transported by Pgp are various natural product oncolytics, such as vinca alkaloids, epipodophyllotoxins, anthracyclines and taxanes,[6] most of which are also substrates for MRP1 transport,[7] although taxanes are apparently not subject to MRP1 mediated resistance.[8]

The differential expression and tissue/tumor specificities of Pgp and MRP1 have been reviewed recently,[9,10] although it is also known that Pgp and MRP can be over-expressed at the same time in drug resistant cells.[11]

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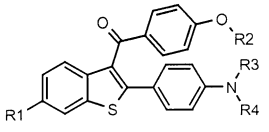
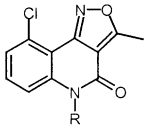
The correlation between drug resistance and expression of the drug efflux pumps, Pgp and MRP1, has spurred considerable efforts in the development of inhibitors of Pgp and MRP1.[12]

Among the specific potent inhibitors of Pgp entered into clinical evaluation are GF120918,[13] LY335979,[14] OC144-093[15] and XR9576.[16] However, the development of specific MRP1 modulators is still in its infancy,[12b] although Eli Lilly has reported the raloxifene analogues (**1**)[17] and isooxazoloquinoline analogues (**2**)[18] to be selective MRP1 inhibitors.



(**1**)



(**2**)

Due to the functional similarity between Pgp and MRP1, many of the reported Pgp modulators have been evaluated for their ability to inhibit the function of MRP1. Several compounds such as cyclosporin and **VX-710** (**3**) have been found to have dual activity and **VX-710** (**3**) has entered clinical trial as a dual inhibitor of Pgp and MRP.[19] The objective of our MDR programme was to identify a potent, selective inhibitor of Pgp and this resulted in XR9576, which displays no activity against MRP1. Nevertheless, we have also identified a quinazolinone molecule (**4**) which possessed dual inhibitory activities against both Pgp and MRP1.

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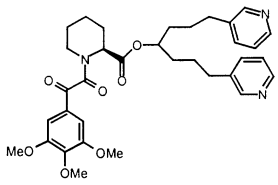
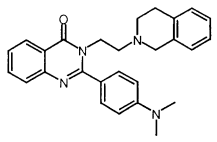


VX-710 (3)

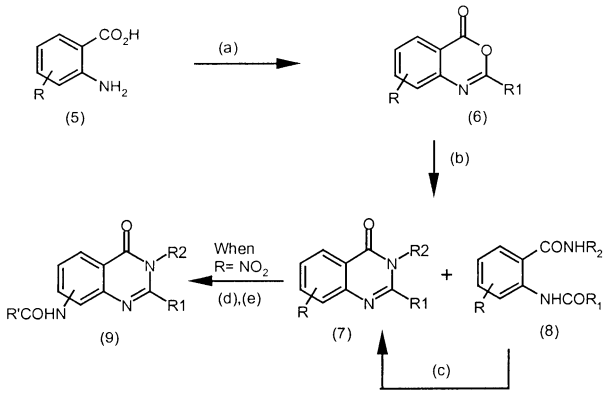


(4)

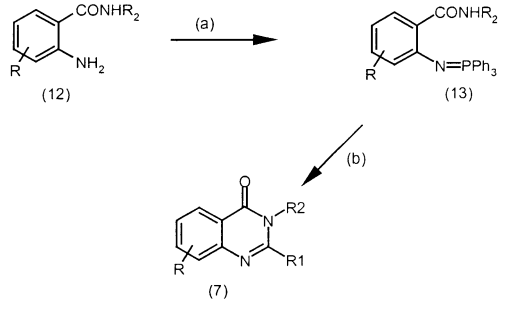
Herein, we describe our syntheses and preliminary SAR studies on the analogues derived from lead molecule (**4**) for their dual inhibitory activity of Pgp and MRP1.

Chemistry

The general quinazolinone analogues were synthesized according to either one of the routes modified from literature methods as shown in Schemes 1[20] and 2.[21]



Scheme 1. Reagents: (a) R_1COCl (**10**), TEA or Pyr, DCM, 0 °C; (b) R_2NH_2 (**11**), Tol., pTSA; (c) $AcOH/H_2SO_4$ (9:1), 100 °C; (d) $SnCl_2{\cdot}2H_2O$, EtOH, reflux; (e) R′COCl, TEA, $CHCl_3$.



Scheme 2. Reagents: (a) PPh_3, Cl_3CCCl_3, benzene, TEA, reflux; (b) R_1COCl (**10**), TEA, tol.

Thus, when anthranilic acids (**5**) were reacted with acid chlorides (**10**) in pyridine at 0 °C, benzooxazinones (**6**) were obtained as crystalline products. The subsequent reflux with amines (**11**) and catalytic amount of pTSA in toluene gave a mixture of quinazolinones (**7**) and uncyclized diamides (**8**), which were converted to products (**7**) by heating in acetic acid and concentrated sulfuric acid (9:1) at 100 °C for 15 min. Further derivatizations were also carried out with the substituents of the benzoquinazolinones (**7**), such as the reduction of the nitro group and subsequent amide couplings, yielding a series of analogues (**9**) ($R = NH_2$, NHCOR′). In Scheme 2, anthranilic amides (**12**) were converted into the corresponding iminophosphorane derivatives (**13**) with triphenyl phosphine/hexachloroethane/triethyl amine.[21] The subsequent aza-Wittig reactions and heterocyclizations with acid chlorides (**10**) in refluxing toluene gave the quinazolinones (**7**) in moderate yields.

Biology Assays

Drug accumulation assay (MRP)

Compounds were assayed for inhibition of MRP1-dependent transport of the radiolabeled cytotoxic agent and MRP substrate, daunomycin. COR.L23/R (MRP expressing non-small cell lung carcinoma MDR subline) cells were seeded 48 h prior to assay into 96-well opaque culture plates. Compounds were serially diluted over a range of concentrations from 100 to 0.015 μM in assay medium containing tritiated daunomycin at 0.3 μCi/mL and incubated with COR.L23/R cells at 37 °C for 2 h before washing and determination of cell associated radioactivity. Results are expressed as an IC_{50} for daunomycin accumulation, where 100% accumulation is that observed in the presence of the known MDR modulator verapamil at a concentration of 100 μM.

Potentiation assay

The ability of modulators to potentiate the cytotoxicity of doxorubicin was evaluated in the COR.L23/R cell line as outlined previously.[22] IC_{50} values for doxorubicin (concentration resulting in 50% inhibition of cell growth) was calculated from plotted results using untreated cells as 100%. EC_{50} values for modulators (concentration required to give 50% of full reversal) were obtained from graphs of potentiation index (ratio of IC_{50} of cytotoxic drug alone/IC_{50} of cytotoxic drug in the presence of modulator) plotted against concentration of modulator.

Similar protocols[22] were applied for Pgp assays with the use of Pgp expressing EMT6/AR1.0 subline.

Results and Discussion

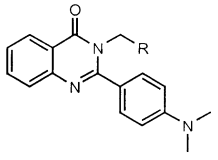
With compound **4** as the lead, SAR was examined at the *N*-3 position of the quinazolinone template with the preparation of a library of compounds at this position, while the rest of the molecule was kept intact. Our initial aim was to quickly screen a variety of *N*-3 substituents bearing different functional and lipophilic groups. The accumulation assay activity of a selection of compounds is shown in Table 1.

Compounds with 3-substitutents bearing no basic centres show reduced or no activities against Pgp or MRP1 (**14**–**16**). Introduction of a pyridyl group in the side chain restores the activities in both resistant cell lines (**17**), compared with the lead compound (**4**). After the first round of screening, the most potent compound (**18**) was found to exhibit dual inhibitory activity against

both Pgp and MRP1. Further modifications of the side chain of this compound (**18**) were carried out by investigating different carbon chain lengths and conformationally restricted and unrestricted ring systems and various lipophilic groups attached to the basic centers. Most of these compounds show dramatically decreased activity against MRP1 while the corresponding Pgp activity is either retained (**19**–**21**) or enhanced (**22**). In compound **22**, the Pgp inhibitory activity is nearly 100-fold greater than that of MRP1.

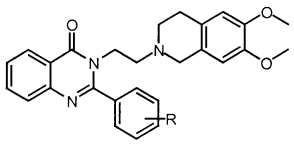
Having achieved one micromolar potency in both Pgp and MRP1 expressing cell lines, we then examined the SAR at the 2-position of the quinazolinone template while maintaining the active 3-substituent of compound **18**. Table 2 illustrates the accumulation assay data for some of the substituted phenyl analogues.

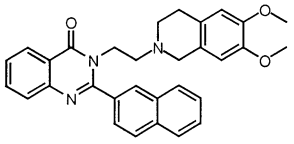
Table 1. Inhibitory activity of 3-substituted compounds in accumulation assays for Pgp (EMT6/AR1.0) and MRP1 (L23/R)



Compd	R	IC_{50} (μM)/Pgp	IC_{50} (μM)/MRP1
4	CH_2-*N*-Tetrahydroisoquinoline	5.0	3.20
14	*n*-Pentyl	40.0	>100
15	Cyclohexyl	14.0	10.0
16	Ph	41.0	>100
17	CH_2-4-pyridyl	10.0	3.33
18	CH_2-*N*-(6,7-dimethoxy)-tetrahydroisoquinoline	1.07	1.05
19	CH_2-*N*-Piperazine-*N*-Bn	2.10	39.0
20	$CH_2N(CH_3)$–$(CH_2)_2$–(3,4-dimethoxy-phenyl)	1.90	37.0
21	CH_2-*N*-Piperazine-*N*-(3,4,5-trimethoxy)-benzoyl	4.20	7.20
22	CH_2-*N*-Piperazine-*N*-2-pyrimidine	0.39	36.0

Table 2. Inhibitory activity of 2-(substituted)phenyl compounds in accumulation assays for Pgp (EMT6/AR1.0) and MRP1 (L23/R)





(**31**)

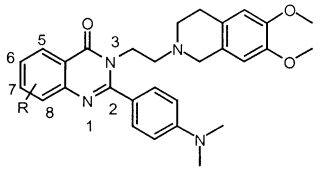
Compd	R	IC_{50} (μM)/Pgp	IC_{50} (μM)/MRP1
18	4-$N(CH_3)_2$	1.07	1.05
23	H	8.0	2.80
24	3-$N(CH_3)_2$	1.60	4.50
25	4-*i*Pr	1.0	0.50
26	4-$N(Et)_2$	1.30	>10.0
27	3-*n*Pr	0.80	1.0
28	3,4-(OCH_2O)	3.70	1.50
29	2-OH	nd[a]	95.0
30	4-Ph	1.03	1.0
31		0.85	0.90

[a]nd, not determined.

As shown in Table 2, removal of 4-*N,N*-dimethylamino group results in decrease of activity in both cell lines (**23**). In contrast, the 3-*N,N*-dimethylamino analogue (**24**) and 3-*n*-propyl and 4-isopropyl analogues (**27**, **25**) show very little variation from compound **18** in Pgp and MRP activities, the activity of **25** is remarkable as the change from *N,N*-dimethyl to isopropyl is a very significant one in electronic terms, whilst the steric aspects are maintained. However, the slightly larger 4-*N,N*-diethlyamino substituted analogue (**26**) shows greatly decreased MRP activity but retains the Pgp activity. A small ring fusion at the 3,4-position (**28**) retains most of the activity, which lends support for the above findings that small non-planar groups at the 3- and 4-positions seem to be tolerated for dual inhibitory activities, but the 2-hydroxy substituted analogue (**29**) results in dramatic loss of MRP activity (only one example). Interestingly, large lipophilic group substituted analogues (**30**, **31**) retain the dual inhibitory activities against both cell lines; this may suggest that planar conformation is tolerated, while concomitant increase of lipophilicity is not detrimental to activity.

After evaluating the active analogues prepared so far in terms of their dual activity and physicochemical properties, we decided to stay with compound **18** for further SAR studies on the fused benzene ring of the quinazolinone template. Table 3 illustrates the activity data from the accumulation assays on some of the selected examples.

Table 3. Inhibitory activity for benzo-substituted quinazolinone compounds in accumulation assays for Pgp (EMT6/AR1.0) and MRP1 (L23/R)



Compd	R	IC_{50} (μM)/Pgp	IC_{50} (μM)/MRP1
18	H	1.07	1.05
32	5-Cl	0.16	3.84
33	6-Cl	1.70	1.56
34	7-Cl	1.35	1.33
35	8-Cl	1.06	2.91
36	8-CH_3	0.63	6.35
37	8-OCH_3	0.05	15.1
38	7-NO_2	1.10	2.02
39	7-NH_2	7.20	18.57
40	6-NO_2	0.60	0.40
41	6-NH_2	2.0	7.60

Table 4. Inhibitory activity of compound **18** and VX-710 in Pgp and MRP potentiation assays

Compd	Pgp EC_{50} (μM)	MRP EC_{50} (μM)
18	3.30	3.01
VX-710[a]	5.0	5.18

[a]Synthesized by a modified procedure[23] developed in-house.

Substitution by a chlorine atom at each position gives dramatically different results; the 5-chloro analogue (**32**) is a potent Pgp selective inhibitor with 24-fold selectivity over MRP, while the 8-chloro analogue (**35**) shows a moderate Pgp selectivity over MRP and good activity in both cell lines. The 6- and 7-chloro analogues (**33**, **34**) display dual activity against Pgp and MRP with retained potency. We then examined the electronic effects at each position, in particular, the 8-position. Thus, the 8-methyl analogue (**36**) gives slightly better Pgp activity but worse MRP activity, while the more electron donating 8-methoxy analogue (**37**) is a Pgp inhibitor with 50 nM potency and is 300-fold selective with respect to MRP. Introduction of electron-withdrawing groups at the 7- and 6-positions retain or improve the dual activity (**38**, **40**), while the more electron-donating groups at these positions give reduced activity, especially in the MRP cell line (**39**, **41**). These results suggest that electron-withdrawing groups are, in general, better tolerated than electron-donating groups for good dual inhibitory activity, particularly at the 6- and 7-positions. Further derivatizations of the 6- and 7-amino analogues (**41**, **39**) to the corresponding amides (**9**), as shown in Scheme 1, failed to give any compounds with better dual activity against Pgp and MRP1.

To further demonstrate the dual inhibitory activities of these quinazolinone compounds, a selection of potent inhibitors were subjected to the potentiation assays.[22] Table 4 illustrates the data (EC_{50} values) of the compound **18** in comparison with those of **VX-710** in our Pgp and MRP potentiation assays.

Compound **18** exhibits equal potentiation activity in both assays and appears to be slightly more active than **VX-710** in reversal of Pgp and MRP1 mediated drug resistance.

In summary, we have identified a series of quinazolinone analogues with potent dual inhibitory activities against both Pgp and MRP1. Although the clinical benefit for developing the dual inhibitors of Pgp and MRP1 remains to be proven, the pursuit of dual inhibitory activity of Pgp and MRP1 inevitably sacrificed the potency levels in comparison with that achieved for selective inhibition of Pgp.[16] Nevertheless, our SAR study in this series of compounds has been useful in our ongoing development of selective MRP1 inhibitors which will be reported in due course.

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