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REVERSAL OF P-GLYCOPROTEIN MEDIATED MULTIDRUG RESISTANCE BY NOVEL ANTHRANILAMIDE DERIVATIVES

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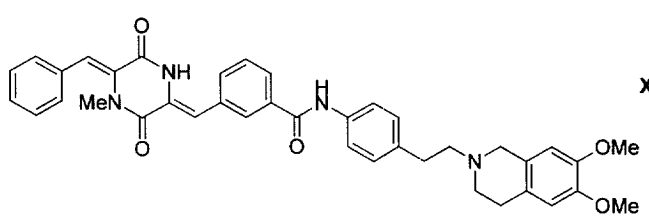
Abstract: We have synthesised and evaluated a series of anthranilamide based modulators of P-glycoprotein. These studies have identified XR9576(**2**), a potent inhibitor of P-glycoprotein *in vitro* and *in vivo*. The general synthesis and the SAR of these compounds are described.

Introduction

In the treatment of cancer the clinical resistance to a range of antineoplastic drugs such as the anthracyclines, vinca alkaloids and taxoids continues to be a major problem.[1] Many types of cancer can display resistance, not only to the original cytotoxic agent used to treat the cancer, but also to agents to which the tumour has not previously been exposed. Furthermore these agents may be unrelated by structure or mechanism of action.[1] This phenomenon is known as multidrug resistance (MDR) and is commonly mediated by overexpression of an ATP dependent 170 kDa plasma membrane protein, P-glycoprotein (P-gp), which functions as a broad specificity efflux pump.[3,10] A large number of structurally unrelated compounds have been identified, such as cyclosporin A and verapamil, which can restore sensitivity to multidrug resistant cells overexpressing P-gp.[2] Unfortunately the use of these first generation compounds as P-gp modulators in the clinic has been precluded by their side-effects and toxicities and thus there is a need for more potent and specific sensitizers.



XR9051 (1)

We have previously reported a novel, potent P-gp modulator, XR9051(**1**) (Accumulation(Acc.) assay, IC_{50} = 220±60 nM), identified from a medicinal chemistry programme based on a naturally occurring diketopiperazine.[3] We believed, however, that it would be possible to further improve both the potency and the

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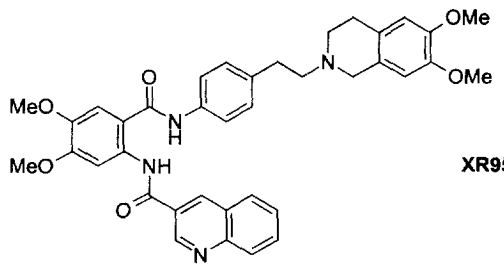
physicochemical properties of XR9051. This letter describes the design and synthesis of a series of novel modulators based on an anthranilamide nucleus and the resulting discovery of XR9576 (**2**), a potent and effective modulator of P-gp *in vitro*[4] and *in vivo*.[5]

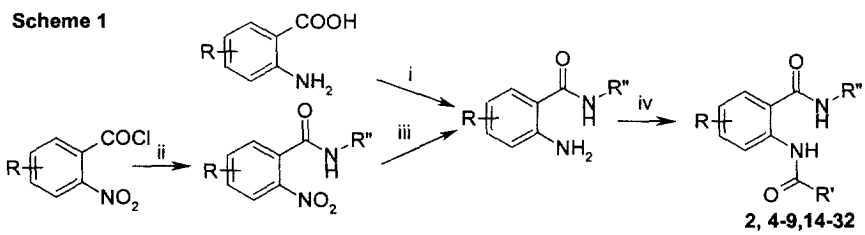


XR9576 (2)

Chemistry

The general strategy employed for preparing the anthranilamides **2, 4-9** and **14-32** is shown in Scheme 1. Two methods were used to couple $R''NH_2$ to the anthranilic acid to yield the intermediate 2-aminobenzamides.[6] The first method used an appropriately substituted anthranilic acid and a carbodiimide-coupling procedure. In the second method the amine $R''NH_2$ was reacted with an *o*-nitrobenzoyl chloride followed by reduction of the nitro group. Reaction of the 2-aminobenzamides with the appropriate carboxylic acid chloride R'COCl or, where the acid chloride was not available, by activation of the carboxylic acid with an appropriate activating reagent, yielded the anthranilamides. These methods allowed a diverse array of substituted anthranilamides to be prepared.[7]

Scheme 1

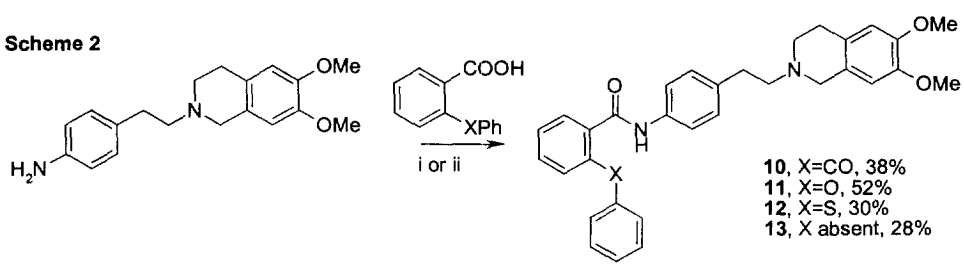


2, 4-9,14-32

i) $R''NH_2$, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulphonate, 1-hydroxybenzotriazole hydrate, Et_3N, CH_2Cl_2 ii) $R''NH_2$, Et_3N, CH_2Cl_2 iii) PtO_2, H_2, ethanol iv) R'COCl or activated acid, CH_2Cl_2.

Compounds **10-13**, were prepared according to Scheme 2 from commercially available carboxylic acids. 2-Phenylsulphanyl-benzoic acid was prepared from thiophenol and 2-chlorobenzoic acid.[8]

Scheme 2



10, X=CO, 38%
11, X=O, 52%
12, X=S, 30%
13, X absent, 28%

i) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulphonate, 1-hydroxybenzotriazole hydrate, Et_3N, CH_2Cl_2 ii) 2-chloro-1-methylpyridinium iodide, Et_3N, CH_2Cl_2.

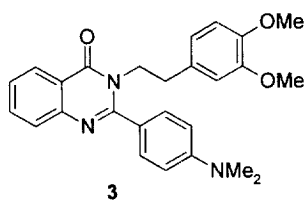
Biology

The effect of the modulators on the accumulation of [^{3}H] daunorubicin in the multidrug resistant mouse mammary carcinoma cell line EMT6/AR 1.0 was assessed. The ability of the compounds to reverse the P-gp dependent accumulation deficit in these cells was performed as reported previously.[3] Results were calculated relative to 100 μM verapamil which restores levels up to that of parental EMT6/P cells. The mean IC_{50} ±SEM from a minimum of three experiments is quoted, unless otherwise stated.

The ability of several of the more potent compounds to potentiate the cytotoxicity of doxorubicin was confirmed in the P-gp over-expressing human small cell lung carcinoma cell line H69/LX4. These potentiation assays were performed as described previously.[3]

Results and Discussion

As a starting point to develop potent, novel modulators of P-gp, we evaluated a series of commercially available compounds which contained features common to XR9051, and frequently found in other known P-gp modulators such as verapamil, S9788[9], GF 120918[10], CP100356[11], VX710[12] and LY335979.[13] These features included a tertiary amine, a dimethoxyphenyl group, and an amide group, and appeared to be important for activity against P-gp mediated drug resistance.



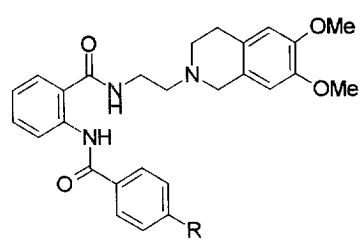
3

From this search we selected the quinazolinone **3** (Acc. IC_{50} = 5000 nM) as a promising initial low molecular weight lead. Although small modifications yielded modest improvements in activity, when cross-screened against a panel of assays, some activity against other targets was observed. However utilising the uncyclised analogues of the quinazolinones, eg **4**, restored specificity for P-gp and yet maintained full activity.

A number of analogues of **4** substituted in the 2-, 3- and 4- positions on the bottom side-chain were prepared and indicated that substitution in the 4- position was beneficial relative to the unsubstituted **7** but these

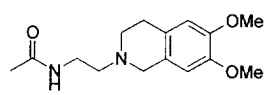
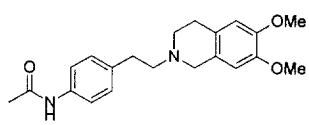
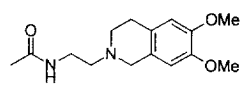
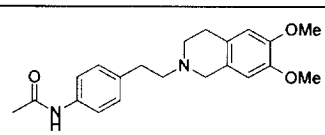
Table 1

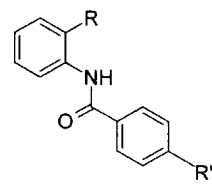
No.	R	IC_{50}, nM Acc.
4	i-Propyl	1100±150
5	Cyclohexyl	1600±300
6	NMe_2	2400±650
7	H	6800±1800



compounds were still of modest potency (Table 1). In an attempt to improve the potency of the anthranilamide series a phenyl ring was introduced into the top side-chain to give a moiety used successfully in XR9051 and shown elsewhere to enhance P-gp modulatory activity.[10] This modification was successful and yielded **8** which showed a considerable increase in potency over **7** (Table 2). Surprisingly though, the analogue of **4** showed only a minimal increase in activity (**9**, Acc. IC_{50} = 850 nM) indicating that changes to R and R' were not additive.

Table 2

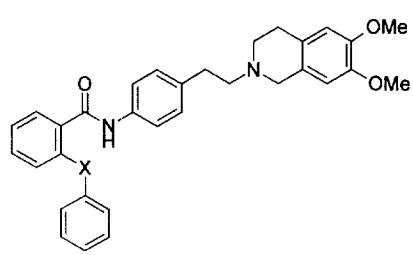
No.	R	R'	IC_{50}, nM Acc.
7		H	6800±1800
8		H	250±25
4		i-Propyl	1100±150
9		i-Propyl	850±370



Further attempts to improve potency through modification of the amide linker to the lower side-chain were unsuccessful (Table 3).

Table 3

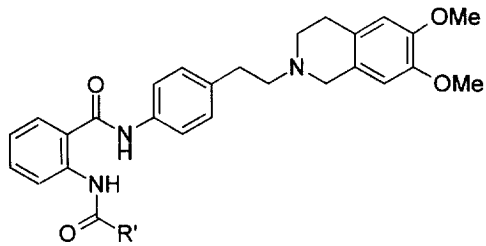
No.	X	IC_{50}, nM Acc.
8	NHCO	250±25
10	CO	1100 (n=1)
11	O	2700±350
12	S	2300 (n=1)
13	Direct bond	1700 (n=1)



An extensive study to optimise the bottom side-chain resulted in a large number of compounds in which substituted aromatics, heteroaroaromatics, and saturated systems were introduced as replacements for the benzamide group in **8** (Table 4). Replacement of the benzene ring in **8** with unsubstituted heteroaromatic rings

Table 4

No	R'	IC_{50}, nM Acc.
8	phenyl	250±25
14	3-thiophenyl	730±270
15	2-furanyl	3300±800
16	3-furanyl	920±520
17	2-pyrazinyl	204±10
18	2-(5-methylpyrazinyl)	67±17
19	2-pyridyl	680±110
20	3-pyridyl	880±380
21	3-(6-methylpyridyl)	67±25
22	2-quinoxalinyl	92±13
23	2-quinolinyl	790±15
24	3-quinolinyl	87±13
25	3-isoquinolinyl	4900 (n=1)

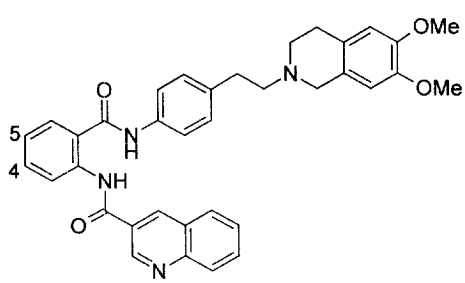


offered no advantage. However the introduction of the 5-methyl-2-pyrazine **18**(Acc. IC_{50} = 67 nM) and the 6-methyl-3-pyridine **21**(Acc. IC_{50} = 67 nM) significantly improved potency, and this potency was maintained with the introduction of the 2-quinoxaline **22**(Acc. IC_{50} = 92 nM) and the 3-quinoline **24**(Acc. IC_{50} = 87 nM). Comparison of **24** with the unsubstituted pyridine **20** demonstrates the importance of the benzo-fused ring. The activities of **24** and the isomeric **23** and **25** illustrate that the position of the nitrogen within the quinoline ring is critical for potency. Importantly, the introduction of the quinoline-3-carboxamide resulted in improved aqueous solubility, and consequently this side-chain was used in all further modifications.

Substitution on the phenyl ring of the anthranilamide moiety was then explored (Table 5). It was found

Table 5

No	4-	5-	IC_{50}, nM Acc.
24	H	H	87±13
26	H	F	60±16
27	F	H	38±13
28	H	Cl	149±26
29	Cl	H	141±19
30	H	Me	220±66
31	H	NMe_2	293±75
32	NO_2	H	45±16
2, XR9576	MeO	MeO	38±18
Cyclosporin A			440±230
Verapamil			5800±2200



that a number of groups were well tolerated in the 4- and 5- positions and some offered modest improvements in activity over the unsubstituted parent compound **24**. Methoxy **2,** fluoro **26** and **27**, and nitro **32** substitutions all showed activity in the 30-60 nM range. The activities of verapamil and cyclosporin A are also shown for comparison.

The activities of a selection of the more active compounds were confirmed in the potentiation assay (Table 6).

Table 6

No.	EC_{50}, nM Potentiation
22	19±2
24	30±12
26	27±11
2, XR9576	16.3±5.3

XR9576 was further profiled against a panel of MDR cell lines using a range of different cytotoxic drugs. These studies confirmed the potency of XR9576 *in vitro*,[4] and more recent work has demonstrated that XR9576 reverses P-gp mediated MDR *in vivo*.[5]

Summary

A range of selective and highly potent P-gp modulators has been designed using a short and versatile synthetic strategy. XR9576 (**2**) is currently undergoing clinical evaluation.

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References and Notes

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7. All final compounds gave satisfactory spectral analyses. For example compound **24**; ^{1}H NMR ($CDCl_3$) 2.73-3.05 (8H, m), 3.66 (2H, s), 3.86 (3H, s), 3.87 (3H, s), 6.53 (1H, s), 6.61 (1H, s), 7.20 (1H, t, J = 7.6 Hz), 7.23-7.37 (2H, m), 7.52-7.74 (5H, m), 7.83 (1H, ddd, J = 1.4, 7.0, 8.4 Hz), 8.01 (1H, d, J = 7.6 Hz), 8.18 (1H, d, J = 8.4 Hz), 8.23 (1H, s, NH), 8.76-8.84 (2H, m), 9.54 (1H, d, J = 2.3 Hz), 12.24 (1H, br s, NH). ESI MS 587 (MH^+, 100). Melting point = 218-220°C. Infra red (solid phase), ν = 3302.0 (m), 1655.2 (m), 1642.11 (s) cm^{-1}. Analysis calculated for $C_{36}H_{34}N_4O_4 \bullet 0.25H_20$; C 73.14%, H 5.88%, N 9.48%. Found C 73.17%, H 5.79%, N 9.44%.
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