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Structure–Activity Relationships for Analogues of the Phenazine-Based Dual Topoisomerase I/II Inhibitor XR11576

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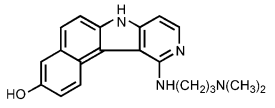
Abstract—As part of a programme to identify further analogues of the dual topo I/II inhibitor XR11576, we describe here the syntheses and SAR studies of various 'minimal' and 3,4-benzofused phenazine chromophores of the phenazine template of XR11576.

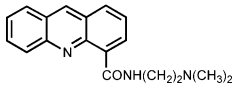
Considerable efforts have been devoted to the development of dual inhibitors of the enzymes topoisomerase I and topoisomerase II that play vital roles in DNA replication and transcription, maintaining the desired topology of DNA by catalyzing the breakage and rejoining of strands.[1] Topo I catalyzes the passage of DNA strands through a transient single strand break, while topo II catalyzes the passage of DNA double strands through a transient double strand break.[2,3] During the process, a covalent linkage is formed between topoisomerase and DNA, termed as 'cleavable complexes'.[4] The majority of topoisomerase inhibitors act as poisons by stabilizing the 'cleavable complexes' and forming ternary complexes[5] and hinder a specific step in the catalytic cycle, that is the reclosure of DNA breaks. In contrast, direct inhibition of topoisomerase catalytic activity without DNA breakage is also known.[6] Topoisomerase II was initially recognized as an important therapeutic target with the development of a number of topo II inhibitors, such as doxorubicin and etoposide,[7] while camptothecin analogues were shown to inhibit topo I.[8]

Topo I and topo II are expressed at different absolute levels in different cell types; the expression of topo I is marked in colon cancer cell lines,[9] while that of topo II is high in many breast and ovarian cell lines.[10] Furthermore, topo I expression shows no major changes during the cell cycles, whereas topo II expression increases in response to DNA replication, reaches a maximum at the G2-M phase and decreases rapidly after that.[11] The expression of either enzyme appears to be sufficient to support cell division and the development of resistance to topo I inhibitors is often accompanied by a concomitant rise in the level of topo II and vice versa.[12–14]

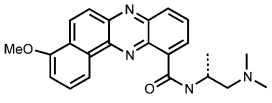
Dual inhibitors of topo I and II may thus have advantages and there has been much interest in such compounds in clinical evaluation, including intoplicine[15] (**1**) and XR 5000[16] (**2**). More recently, we have reported a new phenazine-based dual inhibitor, XR11576[17] (**3**), a development candidate which circumvents multi-drug resistance (MDR) and atypical MDR due to the down-regulation of topo II.[18]



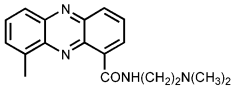
Intoplicine (1)



XR 5000 (2)



XR 11576 (3)



Phenazine (4)

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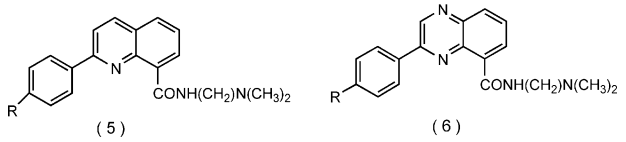
Herein we report a series of synthetic and structure–activity studies around this new phenazine-based lead compound.

'Minimal Chromophore' 3-Aryl Quinoxaline-5-carboxamides (6)

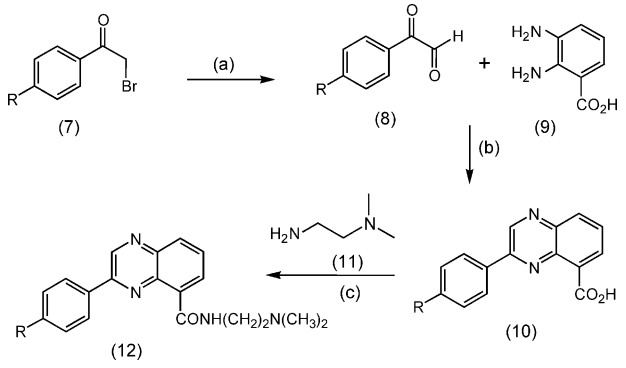
Previous work has demonstrated that suitably substituted 2-aryl quinoline-8-carboxamides (**5**) can act as the 'minimal chromophore' for DNA intercalation.[19] A broad spectrum of anti-tumor activity (in vivo) has been shown for these compounds due to their superior distributive properties. This prompted us to assess the corresponding phenazine derived chromophore (**6**) as a potential series with desired physicochemical properties for good distribution.



The preparation of 3-aryl quinoxaline-5-carboxamides (**6**) is outlined in Scheme 1.

Aryl glyoxals (**8**) were prepared by heating α-bromoacetoketones (**7**) and 48% HBr in DMSO at 50 °C for 24 h.[20] The subsequent regioselective condensation with 2,3-diaminobenzoic acid (**9**)[17] in ethanol gave the quinoxaline acids (**10**) as the major products. The regioselectivity observed in this reaction was confirmed by NOESY experiment on **10** (R = H). The acids (**10**) were then coupled with diamine (**11**) in the presence of CDI to afford the amide products (**12**) in good yields. Initially, the cytotoxic activity of these compounds was measured using the H69 parental (H69/P) human small cell lung carcinoma cell line and the multi-drug resistant human small cell lung carcinoma cell line (H69/LX4), which over-expresses P-glycoprotein (P-gp).[17] These H69 cell lines represent typical examples of solid tumor cell lines.

The activity of selected examples of the quinoxaline analogues is shown in Table 1. These data illustrate that inhibitory activity is observed in both cell lines with nearly equal potency for all the compounds examined.



Scheme 1. Reagents and conditions: (a) DMSO, 48% HBr, 50 °C, 46%; (b) EtOH, reflux, 93%; (c) CDI, DMF, **11**, rt, 56%.

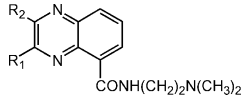
The R_f value observed indicates that the active compounds are not substrates for P-gp. The 4′-substituted analogue (**12b**) appears to offer some scope for improving the activity over the parent analogue (**12a**), as observed by Denny[19] in 2-phenyl quinoline-8-carboxamide series; compound **12c** is twofold more potent than analogue **12a** and this can be attributed to the presence of a larger chromophore. By analogy, these data clearly suggest that the tricyclic carboxamides not possessing a complete fused chromophore can still act as the 'minimal' DNA intercalating agents for cytotoxic activity. Indeed, severe steric crowding, as in compound **13**, would prevent chromophore planarity, leading to complete loss of activity. Predictably, the 2,3-naphthyl fused analogue (**14**) with a large planar chromophore shows a 10-fold increase in potency.

It is clear that the levels of activity in the 'minimal chromophore' series are significantly less than those in the 8,9-benzofused phenazine series (XR11576, $IC_{50} = 20$ nM in both H69/P and H69/LX4).[17] Without further enlargement of the chromophore (as in the pentacyclic **14**), the potential to achieve the same level of potency appears to be limited, and therefore, considering the potential metabolic liability of pentacyclic compounds such as **14**, we quickly moved on to another series with a tetracyclic chromophore.

3,4-Benzofused Phenazine Chromophore: Benzo[*a*]-phenazine-6-carboxamides (15)

Our recent development candidate, XR11576, was developed through the chromophore addition by fusing the benzene ring to the 8,9 positions of the DNA intercalating phenazine template (**4**).[21] The angular 8,9-benzofused phenazine *tetracyclic* chromophore overlays well with that of intoplicine (**1**). Meanwhile, we were also interested in exploring further another angular *tetracyclic* chromophore (**15**), by fusing the benzene ring to the 3,4 positions of the phenazine template (**4**), as the naked 3,4-benzofused phenazine-6-carboxamide was shown to be a potent cytotoxin against P388 and Lewis lung carcinoma cell lines.[21]

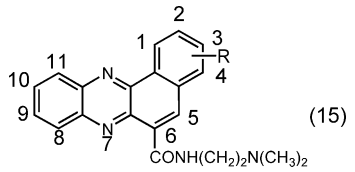
Table 1. Cytotoxic activity of quinoxaline carboxamides (**12**)



Compd	R_1	R_2	H69/P IC_{50} μM	H69/LX4 IC_{50} μM	R_f LX4/P[a]
12a	Ph	H	3.93	3.59	0.9
12b	4-(NO_2)Ph	H	2.40	3.38	1.41
12c	2-Naphthyl	H	1.82	1.67	0.92
13[b]	Ph	Ph	> 100	> 100	
14[b]			0.35	0.33	0.93

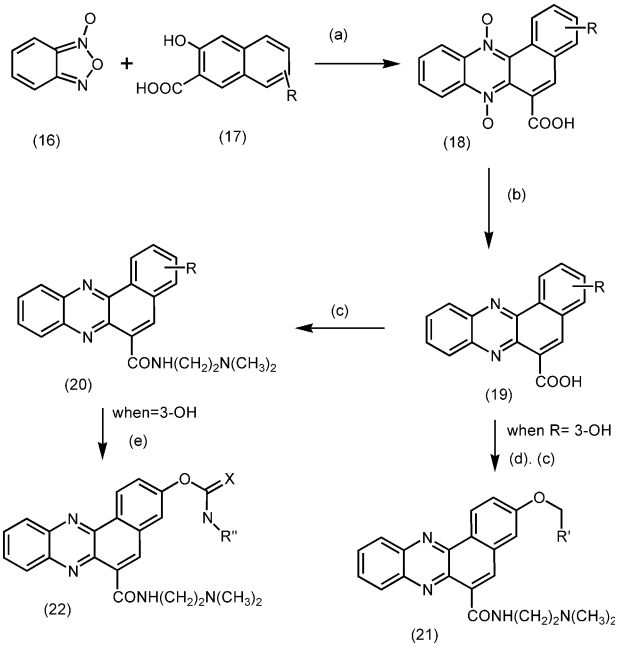
[a] R_f LX4/P: the ratio of the IC_{50} value on the H69/LX4 cell line over the IC_{50} value on the H69 parental cell line.
[b] Compounds **13** and **14** were prepared via the condensation of the respective diketone with 2,3-diaminobenzoic acid (**9**).

The general synthesis of (substituted) benzo[*a*]-phenazine-6-carboxamides is outlined in Schemes 2 and 3.[22]

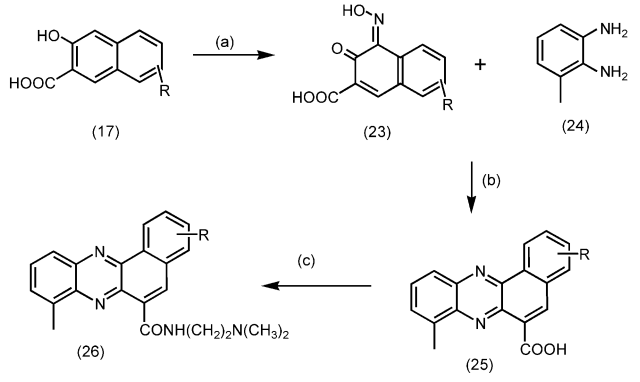


(15)

When a mixture of benzofuroxan (**16**) and 2-hydroxy-naphthoic-3-acid or its substituted analogues (**17**) was treated with 10% NaOH, the benzophenazine di-*N*-oxides (**18**) were formed in good yields. The reduction of the dioxides (**18**) was accomplished using sodium thiosulfate in 0.5 N NaOH, leading to the key intermediates (**19**). Subsequent coupling with the diamine (**11**) using the standard protocols then afforded a number of (substituted) amides (**20**). Further alkylations of the 3-hydroxy intermediates (**19**) with alkyl halides and subsequent amide formation gave products (**21**). The corresponding 3-hydroxy analogue (**20e**, R = 3-OH) was also reacted with (thio)isocyanates to give products (**22**).



Scheme 2. Reagents and conditions: (a) 10% NaOH, rt, 16 h, then HCl, 63–90%; (b) $Na_2S_2O_3$, 0.5 N NaOH, 100 °C, 15 min, then AcOH, 78%; (c) CDI, **11**; (d) NaOH, H_2O, EtOH, R′CH_2Br; (e) R″N=C=O(S),CH_3CN/DCM.



Scheme 3. Reagents and conditions: (a) NaOH, H_2O, then $NaNO_2$, 2 N HCl, 10 °C, 69%; (b) AcOH, concd HCl, 100 °C, 66%; (c) CDI, DMF, **11**.

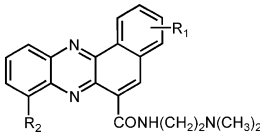
For 8-methyl substituted benzophenazine-6-carboxamides (**26**), the chemistry described in Scheme 3 was developed. Hence, treatment of 2-hydroxy-naphthoic-3-acids (**17**) with sodium nitrite and 2 N HCl gave the hydroxyimino-oxo acid (**23**)[23] in good yields. The subsequent condensation with 2,3-diaminotoluene (**24**) afforded the 8-methyl substituted phenazine acids (**25**) regioselectively, due to a combination of relative functional reactivity of **23** and steric effects of the nucleophilic amino groups in **24**. The formation of the amides was achieved using the standard protocols. A NOESY experiment was carried out on **26**, (R = H) and its regiochemistry was confirmed.

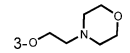
Table 2 shows some of the cytotoxic activity data on selected examples.

As the data in Table 2 illustrate, these compounds are much more cytotoxic than those in Table 1, as expected for agents likely to have tighter DNA binding (although the mechanism of this cytotoxicity has not been determined). The parent analogue (**20a**) shows almost equal activity against H69/P and H69/LX4 cell lines with potency around 500 nM; therefore it is not pumped by P-gp.

Nitro group substitution at the 1-position does not alter the activity considerably (**20b**), whereas the electron donating amino analogue (**20c**) gives slight better activity. The 3-methoxy substituted analogue (**20d**) is as active as **20c** in both cell lines, but the 3-hydroxy analogue (**20e**) shows more than two-fold enhancement in activity over **20d**, although a slight increase in resistance is observed (R_f = 2.12). This suggests that it may be

Table 2. Cytotoxic activity of benzo[*a*]-phenazine-6-carboxamide analogues



Compd	R_1	R_2	H69/P IC_{50} μM	H69/LX4 IC_{50} μM	R_f LX4/P
20a	H	H	0.406	0.511	1.26
20b	1-NO_2	H	0.458	0.584	1.30
20c	1-NH_2	H	0.259	0.345	1.33
20d	3-OMe	H	0.282	0.358	1.30
20e	3-OH	H	0.094	0.198	2.12
21a	3-OCH_2—≡	H	0.435	0.613	1.40
21b	3-$O(CH_2)_2OMe$	H	0.445	0.545	1.23
21c	3-$O(CH_2)_3OH$	H	0.523	2.026	3.87
21d	3-OBn	H	1.223	1.581	1.29
21e		H	0.506	0.592	1.17
22a	3-OCONHPh	H	0.104	0.252	2.42
22b	3-OC(S)NHEt	H	0.519	0.583	1.12
26	H	8-CH_3	0.417	0.320	0.80

pumped by P-gp to a small extent. It has been proposed by Vicker et al.[17] and Rewcastle[21] that biological activity in this class of compounds depends critically not only on intercalation of the chromophore but also on positioning of the carboxamide side chain. However, investigation for finding further potential binding interactions in 3,4-benzofused regions is relatively unknown. Therefore, a strategy was devised to derivatize the 3-hydroxy group (**20e**) with a number of functional groups with the aim of discovering any potential binding sites. These groups included the propargyl group (**21a**), a proton acceptor methoxy ethyl group (**21b**) and a proton donor group (**21c**), which all give decreased activity relative to **20e**. Notably, compound **21c** that bears a hydroxy group in the side chain, like **20e** shows some degree of resistance ($R_f = 3.87$). Introduction of the solublilizing morpholine group fails to show any improvement in activity (**21e**), while analogue **21d**, that bears a lipophilic benzyl group, results in a 10-fold loss of activity compared with **20e**. Different linker groups such as carbamate groups were also examined, with compound **22a** showing the same level of potency as **20e**. In contrast, the ethyl thio carbamate analogue (**22b**) showed a loss of potency.

Rewcastle has shown that introduction of a methyl group *peri* to the phenazine N gives enhancement of activity in their assay (P388 leukemia and Lewis lung carcinoma cell lines).[21] However, in the 3,4-benzofused series, no such effect is observed in our assays (**26**).

Although these data show good cytotoxic activity for analogues with the 3,4-benzofused phenazine chromophore, it is clear that the 8,9-benzofused series[17] displayed superior potency and less toxicity. This resulted in our main biochemical focus being on the latter series, culminating in the selection of the development candidate, XR11576.[17]

Acknowledgements

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References and Notes

1. Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.
2. Ferro, A. M.; Olivera, B. M. *J. Biol. Chem.* **1984**, *259*, 547.
3. Muller, M. T.; Spitzher, J. R.; Didonato, J. A.; Metha, V. B.; Tsutsui, K. *Biochemistry* **1988**, *27*, 8369.
4. Nelson, E. M.; Tewey, K. M.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361.
5. (a) Robinson, M. J.; Osheroff, N. *Biochemistry* **1991**, *30*, 1807. (b) Burden, D. A.; Kingma, P. S.; Froelich-Ammon, S. J..; Bjornski, M. A.; Patchan, M. W.; Thompson, R. B.; Osheroff, N. J. *J. Biol. Chem.* **1996**, *271*, 29238.
6. Capranico, G.; Binaschi, M.; Evelina Borgnetto, M.; Zunino, F.; Palumbo, M. *Tips* **1997**, *18*, 323.
7. Denny, W. A. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1845.
8. Dancey, J.; Eisenhauer, E. A. *Br. J. Cancer* **1996**, *74*, 327.
9. Husain, I.; Mohler, J. L.; Seigler, H. F.; Besterman, J. M. *Cancer Res.* **1994**, *54*, 539.
10. Holden, J. A.; Rolfson, D. H.; Wittwer, C. T. *Biochemistry* **1990**, *29*, 2127.
11. Heck, M. M. S.; Hittelman, W. N.; Earnshaw, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *85*, 1086.
12. Tan, K. B.; Mattern, M. R.; Eng, W.-K.; McCabe, F. L.; Johnson, R. K. *J. Natl. Cancer Inst.* **1989**, *81*, 1732.
13. Sugiomoto, Y.; Tsukahara, S.; Oh-Hara, T.; Isoe, T.; Tsu-Ruo, T. *Cancer Res.* **1990**, *50*, 6925.
14. Whiteacre, C. M.; Zborowska, E.; Gordon, N. H.; Mackay, W.; Berger, N. A. *Cancer Res.* **1425**, *997*, 57.
15. Poddevin, B.; Riou, J. F.; Lavelle, F.; Pommier, Y. *Mol. Pharmacol.* **1993**, *44*, 767.
16. Finlay, G. J.; Riou, J. F.; Baguley, B. C. *Eur. J. Cancer* **1996**, *32A*, 708.
17. Vicker, N.; Burgess, L.; Chuckowree, I.S.; Dodd, R.; Folkes, A.; Hardick, D. J.; Hancox, T. C.; Miller, W.; Milton, J.; Sohal, S.; Wang, S.; Wren, S. P.; Charlton, P.; Dangerfield, W.; Liddle, C.; Mistry, P.; Stewart, A.; Denny, W. A. *J. Med. Chem.* In press.
18. (a) Beck, W. T.; Cirtain, M. C.; Danks, M. K.; Felsted, R. L.; Safa, A. R.; Wolverton, J. S.; Suttle, D. P.; Trent, J. M. *Cancer Res.* **1987**, *47*, 5455. (b) Isaacs, R. J.; Davies, S. L.; Wells, N. J.; Harris, A. L. *Anti-cancer Drugs* **1995**, *6*, 195.
19. Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 396.
20. Saggiomo, A. J.; Kano, S.; Kikuchi, T.; Okubo, K.; Shinbo, M. *J. Med. Chem.* **1972**, *15*, 989.
21. Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. *J. Med. Chem.* **1987**, *30*, 843.
22. Ley, K.; Seng, F. *Synthesis* **1975**, 415.
23. Ullmann, H. *Chem. Ber.* **1909**, *42*, 4266.