OBC
www.rsc.org/obc

Structure-based design of carboxybiphenylindole inhibitors of the ZipA–FtsZ interaction

Alan G. Sutherland,*[a] Juan Alvarez,[b] Weidong Ding,[a] K. W. Foreman,[b] Cynthia Hess Kenny,[a] Pornpen Labthavikul,[c] Lidia Mosyak,[b] Peter J. Petersen,[c] Thomas S. Rush III,[b] Alexey Ruzin,[c] Desiree H. H. Tsao[b] and Karen L. Wheless[a]

[a] *Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, USA. E-mail: sutherag@wyeth.com; Fax: +01 845 602 5561; Tel: +01 845 602 3701*

[b] *Chemical and Screening Sciences, Wyeth Research, Cambridge, MA 02140, USA*

[c] *Infectious Diseases Biology, Wyeth Research, Pearl River, NY 10965, USA*

Received 29th September 2003, Accepted 21st October 2003
First published as an Advance Article on the web 29th October 2003

Structural features of two weak inhibitors of the ZipA–FtsZ protein–protein interaction which were found to bind to overlapping but different areas of the key binding site were combined in one new series of carboxybiphenylindoles with improved inhibitory activity.

As the incidence of infectious bacteria with resistance to multiple classes of drugs continues to increase[1,2] there is a continuing need to identify new antibiotics — particularly those that target essential pathogen specific processes through novel mechanisms of action. We are therefore investigating small molecule inhibitors of the binding of the proteins ZipA and FtsZ as this interaction is an essential component of the cell division process in Gram-negative bacteria.[3–6] Briefly, these proteins are essential components of the septal ring which forms at the site of cell division — inhibition of the interaction between the two results in inhibition of cell division, leading to filamentation and ultimately cell death. While ZipA or its orthologs have only been detected in Gram-negative organisms to date, FtsZ is universally present in bacteria. ZipA orthologs are therefore likely to exist in Gram-positive organisms[7] so the discovery of these inhibitors may lead to an antimicrobial agent with a broad spectrum of activity — including against organisms with resistance to a range of other drugs.

The structure of ZipA is well described[8,9] and, in particular, high quality X-ray and NMR structures demonstrating the key binding interactions at and between the ZipA and FtsZ C-termini have been solved.[10–12] These show that the FtsZ binding site on ZipA is a broad, flat, predominantly hydrophobic surface. Our initial leads were relatively weak inhibitors and included the indoles **1**[13] (IC_{50} 1170 μM)[14] and **2**[15] (IC_{50} 2060 μM), and the oxazole **3**[16] (IC_{50} 2750 μM). X-Ray analysis demonstrated that all three compounds span this hydrophobic region to some extent. While the crystal structures of the indole ring systems essentially overlay in the binding site, the oxazole occupies a largely distinct region (Fig. 1).

This raised the possibility that a chimeric molecule, incorporating structural features of both the indoles and oxazole, might have enhanced activity. Although the binding free energies would not be additive because parts of either **1** or **2** overlap with **3** in their bound orientations, the interaction of the combined fragments with distinct regions of the binding site would be expected to increase the potency.

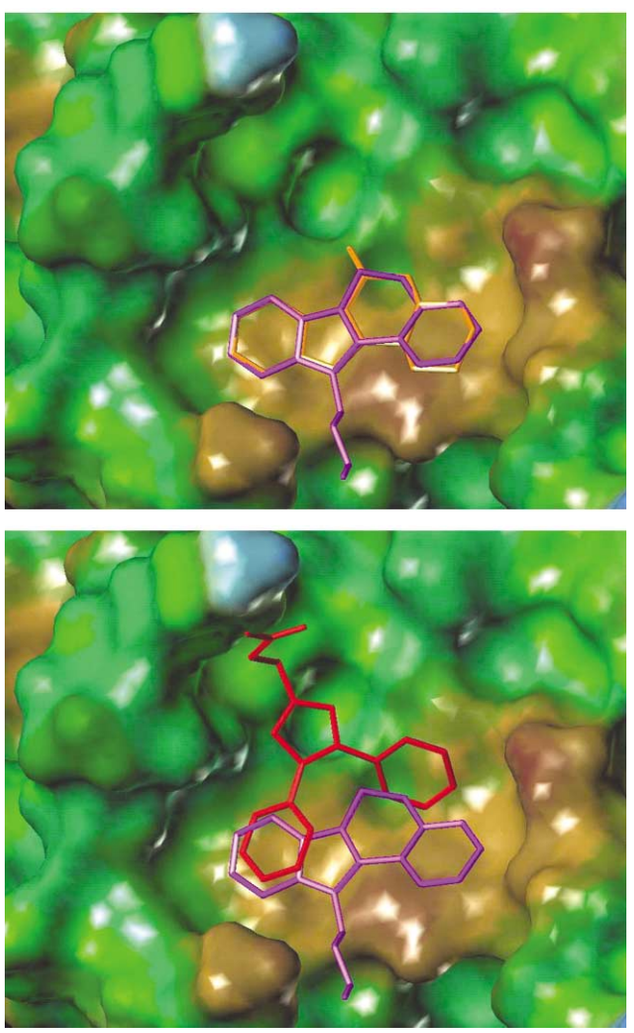
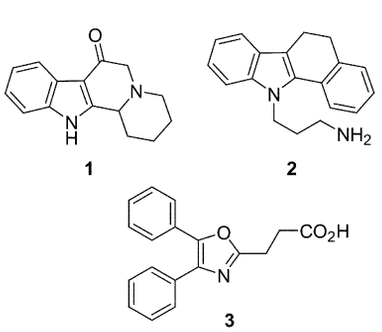


Fig. 1 Overlay of crystallographic orientations for indole **2** (violet) with indole **1** (orange) and oxazole **3** (red). The protein surface is colored according to lipophilicity, with brown indicating strong lipophilicity, blue indicating strong hydrophilicity, and shades of green indicating amphiphilicity.



We therefore modeled several options for a chimera in an effort to find the best starting point for potency optimization. Compounds **2** and **3** overlap in two places (see Fig. 1). One of

DOI: 10.1039/b312016c

the phenyl rings of the oxazole overlaps nearly completely with the phenyl in the indole ring while the other oxazole phenyl approaches the saturated carbons in the fused ring system. The clear choice was to eliminate not only the phenyl from the oxazole scaffold that overlaps with the indole but also the saturated carbons from the fused ring indole scaffold (Fig. 2).

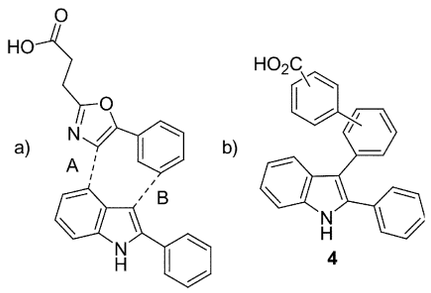
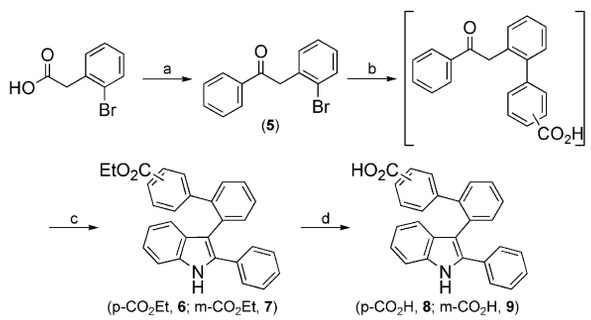


Fig. 2 a) Potential attachment points for the fragments from **2** and **3**. Connections were considered at either the indole 4-position (A) or the 3-position (B); b) a "proof of concept" analogue.

Two potential attachment points were available for connecting the resulting fragments: the indole 3- and 4-positions. Since the binding site features a largely non-specific hydrophobic interface, considering other (larger) rings besides the oxazole became desirable for increased potency. Modeling suggested that the indole 4-position is modestly close to the protein and that increasing bulk at that position (even simply substituting a phenyl for the oxazole) would perturb the binding mode considerably.[17] As expected from the crystal structures, a phenyl at the indole 3-position is well tolerated. Attachments at the indole C4 were thus lower in priority relative to the C3 position. We therefore set out to prepare a series of ligands with the general structure **4**.

Our initial synthetic strategy was to construct the 2,3-diarylindole ring system *via* a Fischer indole synthesis then introduce the benzoic acid moiety *via* a Suzuki coupling. In practice we ultimately prepared the *ortho*-substituted systems **8** and **9** by the reverse sequence (Scheme 1) as couplings to the 3-(2′-bromophenyl)indole system gave negligible yields of the required products. Thus, after preparation of the aryl benzyl ketone **5**,[18] Suzuki coupling (where use of aqueous 1-propanol as solvent proved particularly advantageous)[19] followed by subjecting the crude product to a one-pot hydrazone formation-Fischer cyclization provided rapid access to the esters **6** and **7**. Simple hydrolysis yielded the final compounds **8** and **9**.

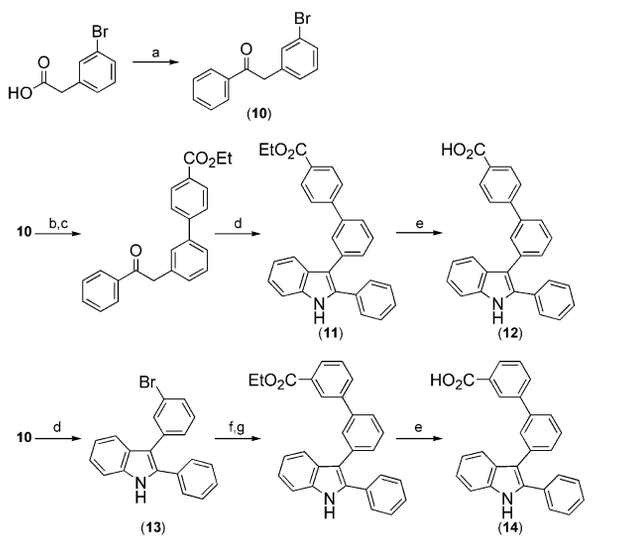


Scheme 1 *Reagents and conditions*: a) 1. PCl_3, 2. $AlCl_3$, benzene (87%); b) PPh_3, $Pd(OAc)_2$, Na_2CO_3, C_3H_7OH, H_2O, $HO_2CC_6H_4B(OH)_2$, Δ; c) $PhNHNH_2$, HCl, EtOH, Δ (overall yield **6**, 22%; **7**, 24%); d) NaOH, aq. EtOH, Δ (**8**, 87%; **9**, 98%).

The preparation of the *meta*-substituted systems (Scheme 2) proved more forgiving, allowing two possible approaches. Thus, starting from the common intermediate aryl benzyl ketone **10** we were able to use the same sequence as above to give target **12** *via* the intermediate ester **11**. Alternatively we could also return to our original plan of performing the Fischer cyclization first,

Table 1 Inhibition of the binding of ZipA and FtsZ[14]

	IC_{50}/μM
1	1170
2	2060
3	2750
8	1060
9	619
12	286
14	192



Scheme 2 *Reagents and conditions*: a) 1. PCl_3, 2. $AlCl_3$, benzene (61%); b) PPh_3, $Pd(OAc)_2$, Na_2CO_3, C_3H_7OH, H_2O, $HO_2CC_6H_4B(OH)_2$, Δ; c) HCl, EtOH, Δ (14% overall) d) $PhNHNH_2$, HCl, EtOH, Δ (**11**, 75%; **13**, 98%); e) NaOH, aq. EtOH, Δ (**12**, 96%; **14**, 97%); f), $Pd(PPh_3)_4$, Na_2CO_3, C_3H_7OH, H_2O, $HO_2CC_6H_4B(OH)_2$, Δ; g) HCl, EtOH, Δ (25% overall).

to give bromophenylindole **13**, followed by a Suzuki coupling as illustrated in the synthesis of **14** (although it proved pragmatic to insert an esterification with subsequent hydrolysis in this sequence to permit ready purification of the intermediate).

All the target compounds displayed an improved inhibition of the ZipA–FtsZ interaction relative to our initial hits (Table 1) and were demonstrated to be interacting with the binding site on ZipA by ^1H and ^{15}N NMR chemical shift perturbation analysis.[11,12] Although the *ortho*-substituted systems displayed a relatively small improvement, molecular modeling suggested that these systems were too sterically congested to achieve a more planar conformation that would allow better binding with the relatively flat protein surface. Such constraints play a far smaller role with the *meta*-substituted systems and here we saw a consequent marked improvement in activity.

The activity of the compounds against a variety of microorganisms (Table 2) also shows a marked improvement. Since the ultimate goal of this project was the design of broad-spectrum inhibitors of bacterial cell division, both Gram-negative and Gram-positive bacteria were used for determination of minimal inhibitory concentrations (MIC). A yeast, *C. albicans*, was used as a control to determine non-specific activity as a means of indicating general cytotoxicity of the compounds. Since none of the compounds inhibited growth of *E. coli* wt ("wild-type"), likely due to an inability to effectively penetrate the outer membrane, an outer membrane permeable strain *E. coli* imp was utilized to assess penetration of the compounds. Comparison of the data presented in Tables 1 and 2 shows that improvement in IC_{50} generally tracks with improvement in MICs. The exception is **2** where the activity against *C. albicans* suggests a general cytotoxicity likely ascribable to detergent effects stemming from the amine side chain. The inhibitory activity of compounds against Gram-positive

Table 2 Minimal inhibitory concentrations (μg mL^{-1}) against various microorganisms[20]

	S. aureus	*S. pneumoniae*	*E. faecalis*	*E. coli* wt	*E. coli* imp	*B. subtilis*	*C. albicans*
1	128–256	64–128	128–256	256	512	128	512
2	8–16	8	16	512	512	nd	8
3	512	256	512	512	512	256	512
9	16	4–64	16–32	128	16	8	128
10	16	16–32	32	128	8	nd	128
12	4–8	4–32	4–8	128	16	4	128
14	4–8	8–32	8–16	128	8	4	128
Piperacillin	1->128	2	2–4	2	<0.12	0.5	>128

bacteria might be considered as a pointer to the existence of ZipA ortholog(s) in these organisms. Alternatively it may suggest that the compounds are not entirely specific for ZipA–FtsZ interaction and may also be acting on other molecular targets. Further research is needed to explore these possibilities.

We have demonstrated that a chimeric strategy does offer an approach to improved inhibition of the ZipA–FtsZ interaction. Future publications will describe the characterization of the inhibitor–protein complex and the synthesis and activity of analogues substituted at the indole 4-position.

Acknowledgements

We would like to thank the members of the Discovery Analytical Chemistry department for their assistance in this work and Russell G. Dushin and Janis Upeslacis for helpful discussions.

Notes and references

1 C. W. Stratton, *Emer. Infect. Dis.*, 2003, **9**, 10–16.
2 A. P. Johnsonn and N. Woodford, *J. Antimicrob. Chemother.*, 2002, **50**, 621–623.
3 C. A. Hale and P. A. J. de Boer, *Cell*, 1997, **88**, 175–185.
4 C. A. Hale and P. A. J. de Boer, *J. Bacteriol.*, 1999, **181**, 167–176.
5 Z. Liu, A. Mukherjee and J. Lutkenhaus, *Mol. Microbiol.*, 1999, **31**, 1853–1861.
6 S. Pichoff and J. Lutkenhaus, *EMBO J.*, 2002, **21**, 685–693.
7 S. Haney, *PCT Int. Appl.*, 2002, WO 0251977.
8 S. A. Haney, E. Glasfeld, C. A. Hale, D. Keeney, Z. He and P. A. J. de Boer, *J. Biol. Chem.*, 2001, **276**, 11980–11987.
9 T. Ohashi, C. A. Hale, P. A. J. de Boer and H. P. Erickson, *J. Bacteriol.*, 2002, **184**, 4313–4315.
10 L. Mosyak, Y. Zhang, E. Glasfeld, S. Haney, M. Stahl, J. Seehra and W. S. Somers, *EMBO J.*, 2000, **19**, 3179–3191.
11 F. J. Moy, E. Glasfeld, L. Mosyak and R. Powers, *Biochem.*, 2000, **39**, 9146–9156.
12 F. J. Moy, E. Glasfeld and R. Powers, *J. Biomol. NMR*, 2000, **17**, 275–276.
13 P. Rosenmund, W. Trommer, D. Dorn-Zachertz and U. Ewerdwalbesloh, *Liebigs Ann. Chem.*, 1979, 1643–56.
14 All IC_{50} values were determined by a fluorescence polarization assay which is described in depth in C. H. Kenny, W. Ding, K. Kelleher, S. Benard, E. G. Dushin, A. G. Sutherland, L. Mosyak, R. Kriz and G. Ellestad, *Anal. Biochem.*, in press. Briefly, the assay determines the inhibition of the binding of a fluorescein-labeled analogue of the C-terminal residues (367–383) of FtsZ with the the C-terminal domain of ZipA (residues 185–328). This assay has proved robust for a wide variety of classes of inhibitor with close agreement upon repetition of analyses.
15 M. E. Freed, E. Hertz and L. M. Rice, *J. Med. Chem.*, 1964, **7**, 628–32.
16 K. Brown, J. F. Cavalla, D. Green and A. B. Wilson, *Nature (London)*, 1968, **219**, 164.
17 Typically we used constrained minimizations using the Tripos forcefield in Sybyl with Kollman charges on the protein, Gasteiger–Marsilli charges on the ligand, and a distance dependent dielectric of 4*r*. The X-ray conformation of the protein with **2** bound was fixed then **2** was then modified to an analog, which was allowed to relax in all degrees of freedom.
18 J. E. Mulvaney and L. J. Carr, *J. Org. Chem.*, 1968, **33**, 3286–3291.
19 B. E. Huff, T. M. Koenig, D. Mitchell and M. A. Staszak, *Org. Synth.*, 1997, **75**, 53–60.
20 *Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, Approved Standards: M7-A5, vol. 20, National Committee for Clinical Laboratory Standards, Wayne, PA.