Bioorganic & Medicinal Chemistry Letters 18 (2008) 4294–4297

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationships of 2-aryl-1*H*-indole inhibitors of the NorA efflux pump in *Staphylococcus aureus*

Joseph I. Ambrus [a], Michael J. Kelso [a], John B. Bremner [a,*], Anthony R. Ball [b], Gabriele Casadei [b], Kim Lewis [b]

[a] School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia
[b] Department of Biology and Antimicrobial Discovery Center, Northeastern University, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 23 April 2008
Revised 25 June 2008
Accepted 26 June 2008
Available online 2 July 2008

Keywords:
2-Arylindoles
NorA efflux pump inhibitors
Antibacterial

ABSTRACT

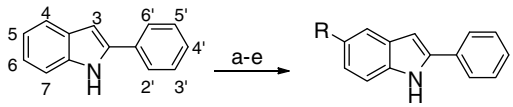
The synthesis of 22 2-aryl-1*H*-indoles, including 12 new compounds, has been achieved via Pd- or Rh-mediated methodologies, or selective electrophilic substitution. All three methods were based on elaborations from simple indole precursors. SAR studies on these indoles and 2-phenyl-1*H*-indole in *Staphylococcus aureus* as NorA efflux pump inhibitors indicated 5-nitro-2-(3-methoxycarbonyl)phenyl-1*H*-indole was a slightly more potent inhibitor than the lead INF55. A promising new antibacterial lead compound against *S. aureus* (2-phenyl-1*H*-indol-5-yl)-methanol, was also found.

Efflux pumps compromise the efficacy of a wide range of antibiotics by actively extruding them from bacterial cells.[1,2] The pumps can be expressed in many different forms in both Gram-positive[3] and Gram-negative bacteria,[4] and for some species a variety of pumps may be present with different or overlapping substrates. For the important community and nosocomially acquired human pathogen *Staphylococcus aureus* a number of pumps have been identified, including NorA, which has been shown to play a role in the development of clinical multidrug resistance (MDR) by this organism.[5] One promising strategy for combating MDR in *S. aureus* is to treat infections with a combination of a NorA efflux pump inhibitor and a conventional antibiotic, with the pump inhibitor serving to restore the antibiotic's potency by reducing its efflux from bacterial cells.[6]

Reported classes of NorA inhibitors include flavones and flavonolignans,[7] pyrroloquinoxalines,[8] 4-arylpyridine-3,5-dicarboxylate esters,[9] *N*-aryl ureas,[10], and indoles.[11]

From the indole class, 5-nitro-2-phenylindole (**2**, INF55) (Scheme 1) represents a promising lead structure capable of producing a 4-fold increase in *S. aureus* susceptibility to ciprofloxacin when co-administered with the antibiotic at a concentration of 1.5 μg/mL.[11]

Structure–activity relationships for INF55 are only just starting to emerge. In one theoretical COMFA (3D-QSAR) analysis, it was suggested that replacement of the indole 5-nitro group with other electron-withdrawing substituents should be favorable for activity.[10] A series of 2-arylbenzo[*b*]thiophenes related to INF55 were recently reported as NorA inhibitors[12] suggesting that the indole-NH is not essential for activity. In 2006, we reported preliminary structure–activity data showing that various substituents around the 2-aryl ring of INF55 improved NorA inhibitory potency.[13] For example, 5′-benzyloxy-2′-hydroxymethyl-5-nitroindole was found to potentiate the activity of the mild antibacterial agent berberine (a known substrate of NorA) more than 15-fold against the NorA overexpressing *S. aureus* strain K2361 at a concentration of 0.8 μg/mL (cf. INF55 potentiates berberine activity to the same degree at the higher concentration of 3.0 μg/mL).



1 **2-6**

Method	Compound	R	
a	**2**	NO_2	(INF55)
b	**3**	SO_3H	
c	**4**	SO_2NH_2	
d	**5**	SO_2OCH_3	
e	**6**	SO_2On-Pr	

Scheme 1. Reagents and conditions: (a) $NaNO_3$, H_2SO_4 (concd), −20 °C; [17] (b) i—$ClSO_3H$, 0 °C to rt, 30 min; ii—NaOH, H_2O, rt and then 1 M HCl; (c) (i) followed by (ii) NH_3/THF, rt; (d) (i) followed by (ii) MeOH, pyridine, rt; (e) (i) followed by (ii) *n*-PrOH, pyridine rt.

* Corresponding author. Tel.: +61 2 42214255; fax +61 2 42214287.
E-mail address: john_bremner@uow.edu.au (J.B. Bremner).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.093

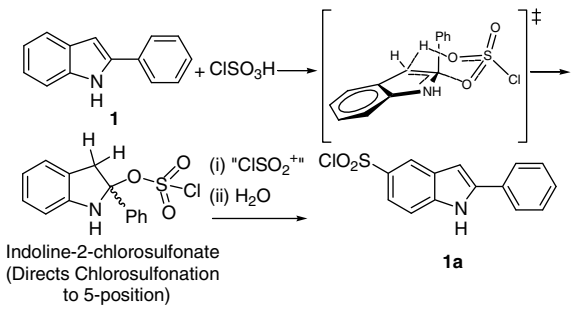
The current work discloses our latest systematic SAR exploration around the 2-aryl ring of INF55 and details the first experimental description of the effects of substituting the indole 5-nitro group. The goal of this study was to obtain a deeper understanding of the SAR of INF55 and analogs with a view to increasing potency against NorA in *S. aureus*. Furthermore, we sought to broaden our knowledge of substituent tolerance around the INF55 nucleus in order to advance our long-term goals of developing potent dual-action antimicrobials,[14] including both non-cleavable hybrid molecules[15] and cleavable "mutual" prodrugs[16] which combine antibacterial agents and NorA-inhibiting moieties into single molecules.

INF55 **2** was synthesized in one step (Scheme 1) from commercially available 2-phenylindole **1** using the known regioselective nitration procedure.[17] Compounds **3–6** were each synthesized in two steps starting from **1** and proceeding via a 5-chlorosulfonyl-2-phenylindole intermediate **1a** (Scheme 2). The intermediate was cleanly accessed using a previously unreported, highly regioselective 5-chlorosulfonation of 2-phenylindole. Briefly, **1** was stirred with neat chlorosulfonic acid at 0 °C and allowed to warm to rt with stirring over 30 min. The reaction mixture was then poured slowly onto crushed ice and the product filtered, washed with water, and dried under vacuum (80%). As **1a** degraded when left at rt over a period of several days, the freshly prepared crude 5-chlorosulfonyl-2-phenylindole (**1a**) was then reacted with appropriate nucleophiles to furnish the 5-sulfonyl-2-phenylindole derivatives **3–6** (Scheme 1) in good yields (**3** = 72%, **4** = 70%, **5** = 57%, **6** = 76%).

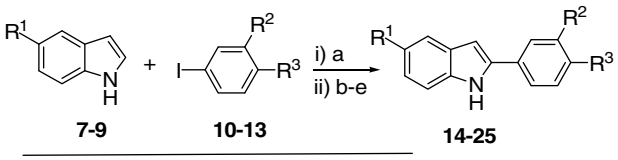
We propose that the regioselective 5-chlorosulfonation of 2-phenylindole proceeds via the electrophilic aromatic substitution mechanism shown in Scheme 2. Under strongly acidic conditions (e.g., neat $ClSO_3H$) indoles are known to protonate at the indole C3 position which serves to protect this normally reactive site from electrophiles. C3 protonation of 2-phenylindole would yield a 2-phenylindolinium cation that could potentially react directly with a weak nucleophile like $ClSO_3^-$ to form an indoline-2-chlorosulfonate adduct.

A related indoline-2-sulfonate adduct has been reported as a participant in the regioselective formation of 5-substituted indoles.[18] What is perhaps more likely is that the indoline-2-chlorosulfonate is formed in a concerted process that proceeds via the cyclic 6-membered transition state shown in Scheme 2. The resulting indoline-2-chlorosulfonate, which contains an *ortho*-substituted aniline ring, should direct a $ClSO_2^+$ electrophile to the indole C5 position (i.e., *para* to the aniline nitrogen), with indole **1a** returned after quenching with water.

Compounds **14**, **15**, **18**, and **22** were prepared by direct C2 arylation of commercially available indoles **7–9** with the respective aryl iodides **10–13** using the recently reported Rh-catalyzed coupling procedure (Scheme 3).[19] Yields for the couplings were disappointing (14–43%) but nevertheless provided sufficient quantities of pure materials for use in our study. 2D NOESY spectra of compounds **18** and **22** confirmed that indole C2 arylation had occurred in preference to arylation at C3 (i.e., NOEs were observed between the indole H3 and H4 signals for both **18** and **22**).



Scheme 2. Proposed mechanism for the regioselective 5-chlorosulfonation of 2-phenylindole in neat chlorosulfonic acid.



Compound	R^1	Compound	R^2	R^3
7	CN	**10**	H	H
8	CO_2Me	**11**	H	H
9	H	**12**	CO_2Me	H
		13	H	CO_2Me

Compound/Method	R^1	R^2	R^3
14	CN	H	H
b → **15**	CO_2Me	H	H
c → **16**	CO_2H	H	H
17	CH_2OH	H	H
d → **18**	H	CO_2Me	H
e → **19**	H	CH_2OH	H
d → **20**	NO_2	CO_2Me	H
d → **21**	NO_2	CH_2OH	H
d → **22**	H	H	CO_2Me
e → **23**	H	H	CH_2OH
d → **24**	NO_2	H	CO_2Me
d → **25**	NO_2	H	CH_2OH

Scheme 3. Reagents and conditions: (a) $[Rh(coe)_2Cl_2]_2$, $[p\text{-}CF_3\text{-}C_6H_4]_3P$, $Cs(OPiv)_2$, 1,4-dioxane, 120 °C;[19] (b) 1 M LiOH, THF, 75 °C; (c) $LiAlH_4$, THF, rt; (d) $LiBH_4$, THF, rt; (e) $NaNO_3$, H_2SO_4 (concd), −20 °C.[17]

The methyl ester **15** was smoothly hydrolyzed to the carboxylic acid **16** under standard conditions using LiOH/THF, and **15** was also reduced without incident to the hydroxymethyl derivative **17** using $LiAlH_4$ (Scheme 3). Esters **18** and **22** were reduced in essentially quantitative yield to their respective hydroxylmethyl derivatives **19** and **23** using $LiBH_4$. The same esters **18** and **22** could be regioselectively nitrated at −20 °C in $NaNO_3/H_2SO_4$ mixtures[17] to provide the 5-nitro-2-arylindole derivatives **20** and **24** in 80% and 81%, yields respectively. Compounds **22** and **24** were comprehensively characterized by 2D NMR spectroscopy, with NOESY spectra confirming that mononitration had occurred selectively at the indole C5 position for both compounds (i.e., observed NOEs included NH/H7, H6/H7, and H3/H4). Esters **20** and **24** were subsequently reduced to their respective hydroxymethyl derivatives **23** and **25** in high yields (90% and 79%, respectively) using $LiBH_4$ (Scheme 3).

A Pd-mediated intramolecular cyclization approach was used to access the 2′-substituted-2-arylindoles **31-35**. All attempts to access these analogs via Rh-catalyzed 2-arylation of indoles with *ortho*-substituted aryl iodides failed. *N*-benzoylindoles **26** and **27**