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Structure-Activity Dependency of New Bacterial Tryptophanyl tRNA Synthetase Inhibitors

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Abstract: Analogues of the aminoacyl tRNA synthetase inhibitor, indolmycin, have been synthesised in which the side chain methyl group is replaced by a wide range of substituents. Their antibacterial and enzyme inhibitory potency is related to steric properties and conformational preferences. Copyright © 1996 Elsevier Science Ltd

The pivotal metabolic role played by bacterial aminoacyl tRNA synthetase enzymes makes them potential targets for antimicrobial strategies.¹ Compounds which can selectively inhibit specific bacterial aminoacyl tRNA synthetases, without affecting their mammalian counterparts, are therefore candidates for therapeutic antibiotics.² This has been an area of intense interest and several antibacterial aminoacyl tRNA synthetase inhibitors have been reported, including the natural product indolmycin 1, a potent and selective competitive inhibitor of the bacterial tryptophanyl enzyme.²³ It has also been demonstrated that indolmycin can compete with L-tryptophan 2 in the bacterial amino acid uptake mechanism.³ While the two molecules have obvious structural similarities, in indolmycin the α -amino acid functionality is replaced by an uncharged 2-methylaminooxazolin-4-one ring, with a 5'-methyl substituent corresponding to the amino acid β -position. Several studies have reported variants of this inhibitor, produced by either substitution on the oxazolinone ring or directed biosynthesis.⁴⁵ In this communication, the effect of modifying the methyl substituent at the carbon atom bridging the two heterocyclic rings is reported.



Indolmycin is known to compete for the tryptophan binding site in the synthetase-catalysed process in which tryptophan reacts with ATP to form tryptophanyl adenylate 3.⁶ The methyl group on the carbon atom separating the two rings could exert an effect in several ways: stabilising the particular indolmycin conformation which binds to the enzyme; interacting with a specific enzyme binding site; or it may be aligned with the bound amino acid backbone of tryptophan. In this last case, the oxazolinone moiety does not mimic the amino or carboxylic acid groups. To test these theories, analogues were synthesised in which the 5' substituent was replaced by larger or smaller groups, and by polar species including amino and carboxylic acid functionalities. Adjacent steric crowding is also introduced. The general strategy for their formation is shown in the retrosynthetic analysis of Scheme 1.



The indolmycin analogues were prepared by the condensation of the lithium enolate of an oxazolinone with a suitably protected indole electrophile. This strategy was used by Dirlam and co-workers in their synthesis of indolmycin; they demonstrated that 2-dimethylaminooxazolin-4-one so introduced could be transaminated to the bioactive 2-methylamino compound using methylamine.⁵ The 5-H and the 5-methyl oxazolinones 4 and 5 were synthesised (Scheme 2) by the reaction of dimethylcyanamide with either ethyl glycolate 6 or ethyl lactate 7 in the presence of base. This process afforded the corresponding crystalline oxazolinones in 55-65% yield. Deprotonation with lithium diisopropylamide furnished the appropriate oxazole lithium enolates 8 and 9 respectively.



In the first instance, a range of alkyl substituted analogues was prepared. All of the compounds described were synthesised as racemates; a comparison of the racemic and resolved enantiomers of indolmycin indicated that essentially all the enzyme inhibitory and antibacterial properties resided in the 5(S), 5'(R)-isomer 1. Both indolmycin diastereomers 1 and 10, its ethyl counterpart 11ab and the 5-methyloxazolinone 12ab were prepared by variants of the same route (Scheme 3). Indole-3-carboxaldehyde 13 was N-protected with a Cbz group to give 14 then treated with one equivalent of the methyl or ethyl Grignard reagent to afford the corresponding alcohols 15 and 16 in 72% and 79% overall yield respectively. These reacted with thionyl chloride to give intermediate chlorides, which on treatment with the oxazolinone lithium enolates 8 and 9 formed the corresponding diastereomeric adducts 17, 18 and 19 in overall yields of between 25% and 70%. The diastereomers were separated and each transaminated without racemisation by exposure to methylamine to afford respectively indolmycin 1, isoindolmycin 10 and both diastereomers of the two analogues 11ab and 12ab, in yields of between 25% and 80%.



Scheme 3 a) NaH, THF, then PhCH2OCOCi; b) RMgBr, THF, c) SOCi2, then 8 or 9 / THF; d) NH2Me, sealed tube

The gemdimethyl compound 20 could not be synthesised by the method of Scheme 3. Whereas the Nprotected acetylindole 21, prepared from acetyl indole 22 in an analogous manner to 14, reacted successfully with methyl magnesium bromide to give the tertiary alcohol 23 in 62% overall yield, subsequent attempts to prepare the corresponding chloride proved fruitless. However, the fluoride 24 could be formed by reaction of 23 with diethylaminosulfurtrifluoride, and although the coupling of 24 with 8 proceeded in only low yield, the adduct 25 could be isolated and transaminated quantitatively to the desired product 20 by exposure to methylamine (Scheme 4). The desmethyl analogue 26 was prepared from gramine 27 without resort to Nprotection. Selective quaternisation of the exocyclic nitrogen was achieved by treatment with iodomethane in ethanol⁷ and this product 28 reacted directly with the oxazolinone lithium enolate 8 to give the displacement product 29, in 10% overall yield. As before, transamination with methylamine afforded 26 in excellent yield.



Scheme 4 a) NaH, THF, PhCH₂OCOCI; b) MeMgBr, THF; c) DAST, CH₂Cl₂; d) 8, THF; e) NH₂Me, sealed tube; f) MeI, EtOH

The introduction of an ester side chain required a different strategy (Scheme 5). The *tert*butyl ester of carboxymethylenetriphenylphosphorane condensed with indole-3-carboxaldehyde 13 to give the *trans*-alkene

30 in 92% yield. The indole nitrogen was deprotonated with sodium hydride and protected by silylation to give 31. This compound reacted with the oxazole lithium enolate 8 to give the two diastereomeric Michael adducts 32 in 20% overall yield. These reacted with methylamine and caesium fluoride to afford the indolmycin analogues 33ab in 85% yield. The corresponding acids 34ab were formed in 80% yield by subsequently removing the *tert*butyl groups of 33ab with trifluoroacetic acid in the presence of ethane-1,2-dithiol.



Scheme 5 a) Ph₃P=CHCO₂tBu, 1,4-dioxane; b) NaH, THF, ClSiMe₂tBu; c) 8, THF; d) NH₂Me, sealed tube then CsF, MeOH, wet SiO₂; e) TFA, HSCH₂CH₂SH

Analogues containing nitrogen in the side chain were formed by a similar procedure (Scheme 6). In this case it was preferable to protect the indole nitrogen prior to olefination. The reaction of 13 with SEM chloride gave 35 which condensed with nitromethane in the presence of an ammonium acetate buffer to permit the construction of 36. This $\alpha\beta$ -unsaturated compound reacted with enolate 8 to give the diastereometric adducts 37 in 71% overall yield. Removal of the SEM group to form 38 was effected in 65% yield using acidified lithium tetrafluoroborate.



Scheme 6 a) NaH, THF then CICH₂OCH₂CH₂OSiMe₃; b) CH₃NO₂, NH₄⁺ ⁻OAc; c) 8, THF; d) LiBF₄, HSCH₂CH₂SH, TFA; e) NH₂Me sealed tube; f) Bu₃SnH, AIBN, Toluene; g) H₂, PtO₂, HOAc

The separated diastereomers of 38 were transaminated as before to afford 39ab in 50% yield. Reduction of the nitro group of 39a (the better inhibitor, *vide infra*) using prehydrogenated platinum oxide in acetic acid afforded the amino analogue 40a. By contrast, radical reduction of 39a with tributyltin hydride gave the oxime 41a, also in good yield.



Scheme 7 a) NaH, THF then Me2tBuSiCI; b) 8, THF; c) NH2Me, sealed tube then CsF, wet SiO2, MeOH

Finally, two groups of compounds were prepared by the direct reaction of the oxazole lithium enolate 8 with N-silylated indole-3-carboxaldehyde 42 and 3-acetylindole 43. Despite a potentially favourable reverse aldol reaction, both diastereomers of 44 and 45 could be isolated chromatographically in 60-65% yield. The structure of 45a was determined by X-ray crystallography. Transamination and removal of the silyl protection gave the hydroxyl analogues 46ab and 47ab in 45% and 35% yield respectively over the two steps.

R ₁	R ₂	R ₃	Structure	MIC (µg/ml)	I _{so} (ng/ml)
Me	Н	Н	1	0.125	16 (mean)
Н	Ме	н	10'	8	500
Et	Н	н	11a	64	250
Н	Et	Н	11b	>512	22000
Ме	н	Me	12a	64	1200
Н	Me	Me	12b	>512	>100,000
Me	Me	Н	20	32	580
Н	Н	Н	26	64	1550
CH,CO,'Bu	Н	H	33a	>512	>100,000
Н	CH,CO,'Bu	Н	33b	>512	>100,000
CH,CO,H	н	н	34a	>512	>100,000
Н	СН,СО,Н	Н	34b	>512	>100,000
CH,NO,	Н	Н	39a	64	2300
Н	CH,NO,	Н	39b	>64	>100,000
CH,NH,	H	Н	40a	>512	>100,000
CHNOH, H	Н	Н	41a	>512	>100,000
Н	OH	Н	46a	>64	44000
HO	Н	Н	46b	>64	4000
Me	ОН	Н	47a	0.25	46
НО	Me	н	47h	564	>100.000

Standard Deviation for I_{50} of racemic indolmycin (run as a standard) = 18. Estimate of accuracy for individual I_{50} values = +/- 20% based upon curve fitting.



Table 1: Comparison of minimum inhibitory concentration (MIC) and ${\rm I}_{50}$ values for 5-and 5'-substituted analogues.

The MIC values against *Staphylococcus aureus* Oxford and the I_{so} values against the isolated enzyme from this bacterium are indicated in **Table 1**.⁹ The relative stereochemistry of these indolmycin analogues was provisionally assigned by relating their physical and spectroscopic properties to indolmycin or to the hydroxyl analogue **45a**, for which the relative stereochemistries are known. In pairs of diastereomers, the better inhibitors were chromatographically the less polar isomers, and possessed the relative stereochemistry of indolmycin.¹⁰ The ~33 fold variation in the ratio MIC / I_{so} may reflect differences in penetration associated with either altered lipophilicity, or affinity for the tryptophan active transport mechanism (*vide supra*). The lack of activity of the

carboxylic acid and amino analogues 34ab and 40 militates against the theory that the methyl group represents the amino acid backbone. The ineffective nature of the hydroxy compounds 46ab, those with groups significantly larger than methyl 33ab, 39ab, or compounds with adjacent steric bulk 12ab, indicates that the methyl group binds in a small, essentially hydrophobic binding pocket. Equally, the weak inhibitory properties of the desmethyl compound 26 support the hypothesis that the methyl group does stabilise a particular conformation of the indole and oxazole moieties. This analysis is borne out in molecular modelling studies.

A comparison of the Ramachandran plots of calculated *CHARMm* energy¹¹ for rotation about the two bonds connecting the oxazole and indole moieties, supports an indolmycin conformation in which the hydrogen on C-5' lies broadly in the plane of the indole, pointing towards the benzenoid ring. The good inhibitor 47a adopts very similar dihedral angles to 1 while the dimethyl analogue 20, also disubstituted on C-5' but a much poorer inhibitor, does not. The conformation of the oxazole group relative to the bridging carbon is less clear; the two most energetically favoured minima for 1 are shown below.¹¹ Compounds such as 10, 11b or 12b, for which these conformations represent a relatively high energy state, are poor inhibitors. The diagrams show the E-2-methylaminooxazolinone but the conclusions are equally valid for the Z geometry.



In order to determine whether one of these structures represents the binding conformation of indolmycin, our results have encouraged us to synthesise constrained analogues of indolmycin in which the C-4 position of the indole is fused onto the bridging carbon atom. Preliminary results of this study have been reported elsewhere.¹² The effects of independently modifying the aromatic and oxazole groups are currently under investigation.

References and Notes

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