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Fine Tuning of Physico-Chemical Parameters to Optimise a New Series of Novobiocin Analogues

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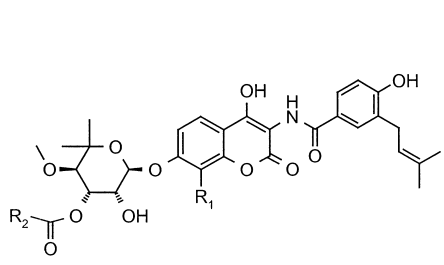
Abstract—A novel series of novobiocin analogues has been synthesised by removing the lipophilic aryl chain in novobiocin and introducing an amino substituent. The structural modifications have been dictated by the control of lipophilicity and the dissociation constant of the resulting compounds. Antibacterial activity of the new coumarin derivatives could be correlated with the amount of uncharged form in physiological conditions. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

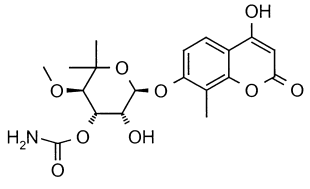
The naturally occurring antibiotics novobiocin **1** and clorobiocin **2** have attracted renewed attention because of their impressive activity against β-lactamase producing and methicillin-resistant *Staphylococcus aureus* (MRSA) strains.[1] These coumarin-containing antibacterial agents block the negative supercoiling of relaxed DNA (a process involved in gene expression) by inhibiting ATP hydrolysis in the B sub-unit of DNA gyrase.[2–5] We described in recent papers our extensive programme of chemical modifications aimed at establishing structure–activity relationships and identifying new candidates for pre-clinical and clinical evaluations.[6–10] We report here our rational approach relying on the control of physico-chemical parameters in the development of a new series of gyrase B coumarin inhibitors **4**.[11]

In structure **4**, the noviose derived moiety of clorobiocin has been retained since it is implicated in high energy hydrogen bonding with gyrase B amino-acid residues.[4] On the other hand, the C3-isopentenylhydroxybenzocarboxamide group has been removed in **4**. This



1 novobiocin : R_1 = Me, R_2 = NH_2

2 clorobiocin : R_1= Cl, R_2 = Me



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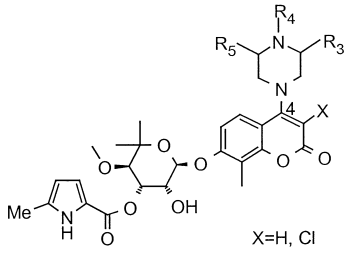

X=H, Cl

4

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lipophilic group has a limited contribution to the inhibition of DNA gyrase activity but facilitates transport of drugs **1** and **2** across the bacterial cytoplasmic membrane by passive diffusion[12] (compound **3** has no antibacterial activity while being a gyrase B inhibitor only 2-fold less potent than novobiocin). Consequently, novobiocin and clorobiocin are highly lipophilic derivatives exhibiting low water solubility and high binding to serum proteins.[13,14] Amine introduction in the coumarin moiety (C4 position) was expected to increase water solubility, reduce the negative serum effect on activity as well as develop additional interactions with gyrase B. Piperazine was selected because it provides a basic function that could potentially co-exist in charged (NH^+–R_4) and uncharged forms (N–R_4) at neutral pH. The charged species (more hydrophilic) could give aqueous solubility and electrostatic interactions at the membrane interface.[15] The uncharged species (more lipophilic) could be able to cross phospholipid bilayers providing that lipophilicity of the whole molecule **4** is appropriate for passive diffusion.[16] Several coumarin compounds bearing a differently substituted piperazine moiety (**4a**–**g**) were synthesised as well as closely related analogues (**4h**–**j**, Scheme 1). Their corresponding dissociation constants (pK_a) and lipophilicity values (log P) were measured and correlated with their respective potency against DNA gyrase supercoiling (IC_{50})[6] and antibacterial activity (MIC) determined in the absence and in the presence of serum proteins.

Synthesis

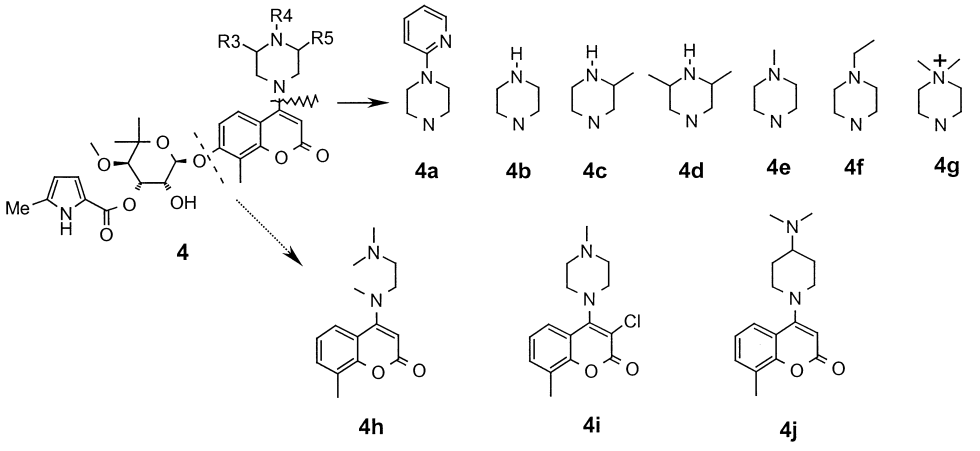
The 4-amino derived novobiocin analogues **4** that were synthesised are listed in Scheme 1.

Our general synthetic approach from **5**[7] relied on amination of triflate[17] intermediate **6** followed by a glycosylation reaction carried out under Mitsunobu conditions between 4-amino-7-hydroxycoumarin substrates and the noviose derivative **9**[6] (e.g., synthesis of **4e** and **4g** from **8**; Scheme 2). All amines were commercially available.

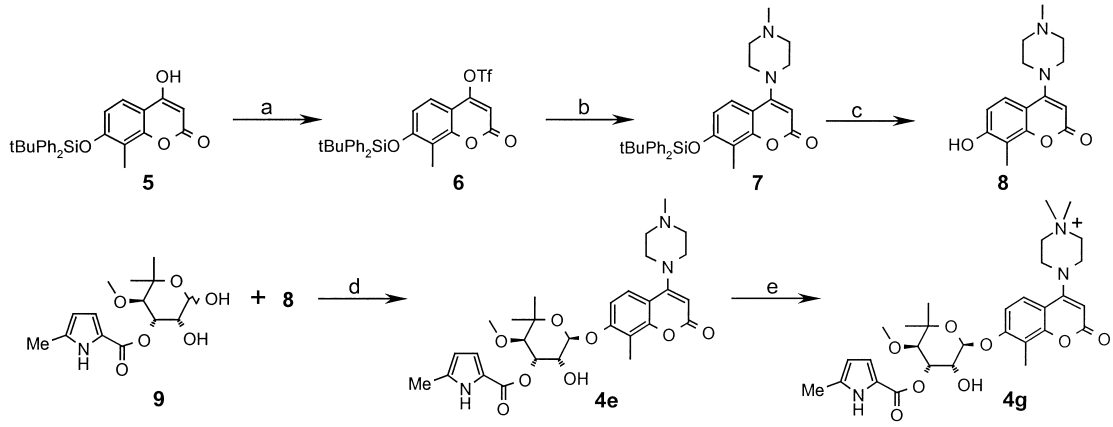
Limited yields were observed in the glycosylation of coumarin substrates bearing unprotected NH groups. Consequently additional protection-cleavage steps were required for the successful preparation of analogues such as **4b**, **4c** and **4d**. Chlorination of coumarin **7** at the C3 position was successfully achieved by addition of a chlorine solution in acetic acid at 0 °C to lead to the coumarin precursor of compound **4i** in 69% yield. To overcome the low yield observed in the glycosylation reaction to obtain **4j**, we had to develop an alternative strategy based on the terminal amination of tosylate intermediate **11**[18] (Scheme 3).

Results and Discussion

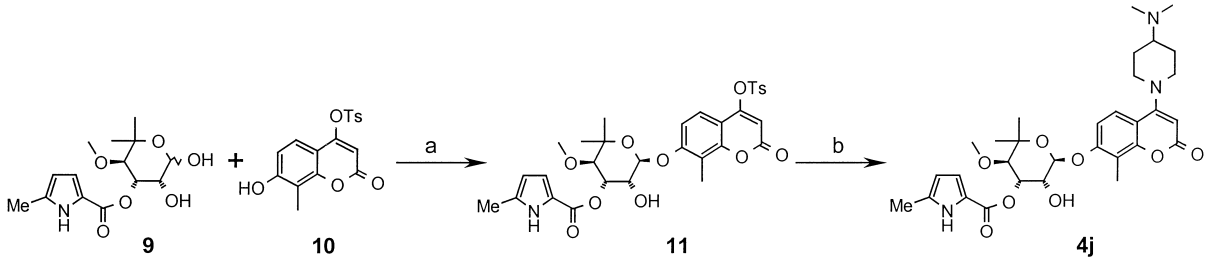
Lipophilicity of coumarin antibiotics **4** was estimated on reversed-phase thin layer chromatography (RP-TLC) plates and R_{Mw} values were determined (see Table 1).[19] The R_{Mw} parameter was shown to be consistent with the octanol/water partition coefficient for novobiocin ($R_{Mw} = 5.2$ vs $\log P_{o/w} = 5.5$).[20] Compound **4a** bearing a pyridine moiety, uncharged at neutral pH, is highly lipophilic and hence exhibited a marked reduced antibacterial activity against *S. aureus* in the presence of serum. Introduction of a secondary amine (**4b**–**d**) reduced lipophilicity, improved DNA gyrase inhibition but was detrimental to antibacterial activity when compared to novobiocin. The negative contribution of the NH function to permeability[21] could not be compensated by addition of two α-methyl groups (**4d** vs **4b**). On the other hand, the *N*-methyl piperazine derived compound **4e** displayed a noteworthy antibacterial activity against *S. aureus* (as well as other Gram-positive strains)[22] which was retained in the presence of serum. When compared to the secondary amines **4b**–**d**, the tertiary amine **4e** showed similar gyrase inhibition and lipophilicity but decreased basicity. Inactivity of **4g** (piperazinium derivative) and lower antibacterial activity of **4f** (*N*-ethyl piperazine derivative) when compared to **4e** confirm that the development of a positive charge in the molecule at physiological pH diminishes antibacterial activity. Chlorination of **4e** (**4i**) failed to decrease the basicity of the *N*-methyl piperazine group by remote electron-withdrawing induction. Moreover, the positive contribution of the chlorine atom to lipophilicity led to a more pronounced serum effect than for



Scheme 1.



Scheme 2. Reagents and conditions: (a) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2, 1 h, 0 °C, 87%; (b) 1-methylpiperazine, THF, 1 h, rt, 98%; (c) Bu_4NF, THF, 1 h, 0 °C, 69%; (d) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 6 h, 50%; (e) CH_3I, CH_3CN, 1 h, 60 °C, 83%.



Scheme 3. Reagents and conditions: (a) PPh_3, $EtO_2CN{=}NCO_2Et$, CH_2Cl_2, rt, 2.5 h, 42%; (b) 4-dimethylaminopiperidine, THF, 1.5 h, rt, 19%.

Table 1. Physico-chemical properties and biological activities of 4-aminocoumarin analogues **4**

Compound	R_{Mw}[a]	pK_a[b]	IC_{50}[c] (μg/mL)	MIC[d] (μg/mL) on *S. aureus*[e]	MIC (μg/mL) on *S. aureus* + 10% serum
Novobiocin	5.2	3.6	0.25	≤ 0.04	2.5
4a	6.4	5.5	1.1	0.30	10
4b	4.37	8.3	0.125	5.0	5.0
4c	4.44	8.3	0.125	2.5	2.5
4d	4.70	8.0	0.16	2.5	2.5
4e	4.48	7.3	0.3	0.15	0.30
4f	4.78	7.5	0.25	0.6	2.5
4g	—	—	0.14	> 40	> 40
4h	4.50	8.2	0.35	5.0	5.0
4i	4.71	7.4	0.5	1.2	20
4j	4.60	9.0	0.28	2.5	2.5

[a]Determined on RP-18 TLC plates in an incubator adjusted to 30 °C. Methanol–Tris buffer (pH 7.4) mixtures were used as eluent.
[b]Measured with the Sirius GLpKa instrument coupled to the Sirius D-PAS system.
[c]See ref 6.
[d]MIC, minimum inhibitory concentration, measured by using a 2-fold broth microdilution after a 24-h incubation.
[e]Strain 011HT3 with no particular phenotype of resistance.

4e. The very close analogues **4h** and **4j** of compound **4e** exhibited a limited antibacterial activity which can be attributed to their significantly higher basicity (these two tertiary amines are at least as basic as the secondary amines **4b–d**).

In conclusion, the novel series of novobiocin analogues **4** bearing different amino groups on the coumarin moiety displayed excellent inhibitory potency against DNA gyrase supercoiling. Antibacterial activity and hence permeability across the bacterial membrane was shown to be correlated predominantly with basicity over lipophilicity of the molecule. The control of lipophilicity was critical for maintaining the antibacterial activity in the presence of serum proteins. Among the derivatives synthesised, compound **4e** exhibited the best biological profile derived from optimised physico-chemical properties. This coumarin analogue was further investigated in vivo.

Acknowledgements

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