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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Squalamine Analogues as Potential Anti-Trypanosomal and Anti-Leishmanial Compounds

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Abstract—This paper concerns the synthesis of various simplified analogues of the novel anti-microbial agent, squalamine. The compounds were then investigated for activity against *Trypanosoma brucei*, the causative agent of African trypanosomiasis, *Trypanosoma cruzi*, the causative agent of Chagas disease and *Leishmania donovani*, the causative agent of visceral leishmaniasis. Several compounds showed in vitro activity, especially against *T. brucei* and *L. donovani*. However, one compound showed poor in vivo activity.

Introduction

Squalamine (**1**, Fig. 1) was first isolated in 1993 from the dogfish shark, *Squalus acanthias*.[1] It was shown to have potent anti-microbial activity against Gram positive and Gram negative bacteria and fungi. In addition, squalamine has been shown to have anti-angiogenic properties in several experimental tumour models.[2,3] The toxicity of squalamine has also been investigated.[1] It has been shown to cause haemolysis of red blood cells, although this occurs at higher levels than that required for anti-microbial activity, suggesting that there is a therapeutic window.

Squalamine itself can only be obtained in small quantities from the dogfish shark. Although it has been prepared synthetically,[4,5] the synthesis is long and not viable for detailed structure activity studies. Therefore a number of simplified analogues of squalamine have been prepared, for example by Sadownik et al.[6] who placed the sulphate group on the 3-position of the steroid ring and the polyamine on the steroid side chain to give compound **2** (Fig. 1), which was prepared in just three synthetic steps. This compound retained some of the anti-microbial activity of squalamine. A further set of analogues were prepared by Jones et al.[7] and Kikuchi et al.[8] Some of these analogues showed similar anti-bacterial activity to the parent compound squalamine. Variations in the structure of the analogues led to changes in the spectrum of activity against a variety of bacteria and yeasts. A number of conclusions can be drawn from these studies.

- The precise structure of the polyamine is not important.
- The sulphate group can be replaced by a carboxylate or even removed altogether.
- The structure of the steroid can also be varied.

The mode of action of squalamine and its derivatives has not yet been determined, although a number of suggestions have been made,[7,8] including membrane disruption.[9] Squalamine may achieve this by acting as an ionophore.[10] It has been shown experimentally that squalamine recognises negatively charged phospholipid membranes,[11] and also has been implicated in disruption of a Na^+/H^+ antiport.[12] Squalamine shows a novel mechanism of action and it is important to assess the extent of its potential as an antimicrobial agent.

Squalamine and its analogues have not been tested against the parasitic protozoa responsible for leishmaniasis, Chagas disease and African trypanosomiasis. As part of a programme to derive novel agents against these diseases we prepared a number of analogues of squalamine to test against the causative parasites of the

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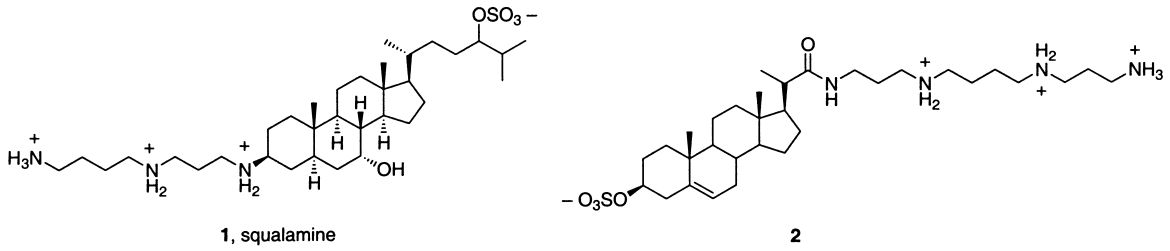


Figure 1.

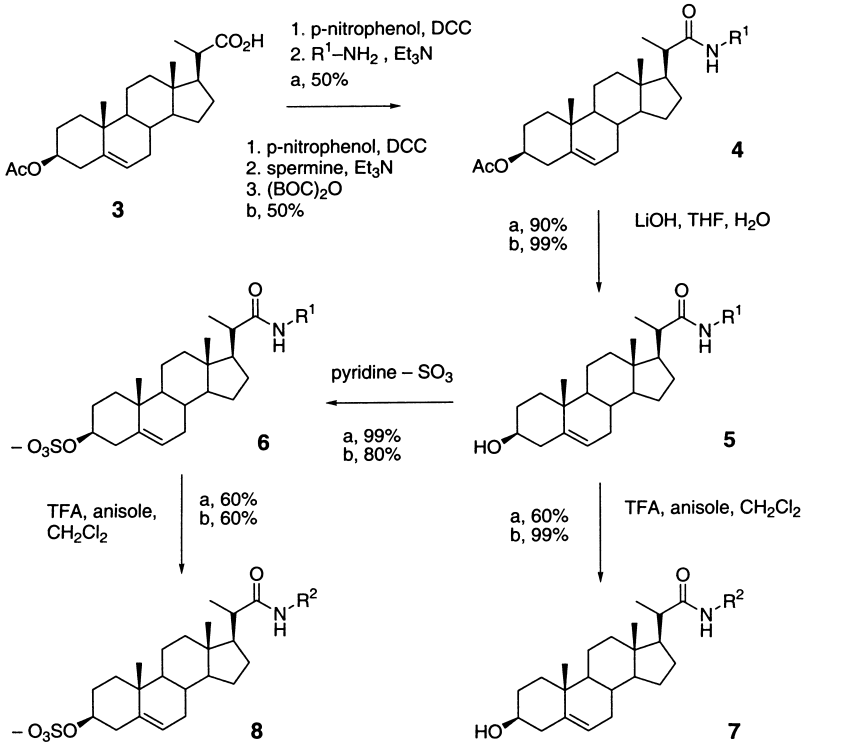
diseases: *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei* respectively. We were interested to determine the role of the sulphate group, so compounds were prepared with and without this group. We were also interested to see the role of the amines in the polyamine side chain, so protected and un-protected compounds were prepared and tested.

Chemistry

The squalamine analogues were prepared by a modification of the literature methods (Scheme 1).[6,8] The starting steroid, 3β-acetoxybisnor-5-cholenic acid, was activated as the *p*-nitrophenol ester and then reacted either with BOC-protected spermidine or unprotected spermine. In the case of the latter, the reaction mixture was then reacted with di-*tert*-butyl dicarbonate to give the fully protected compound **4b**. The acetate on the 3-position was then removed using lithium hydroxide to give **5**, followed by sulphation with pyridine–sulphur trioxide complex to give **6** and then removal of the BOC protecting groups with trifluoroacetic acid to give **7** and **8**.

Biological assays

Compounds were investigated for activity against the clinically relevant forms of *T. brucei* (bloodstream trypomastigote form), *T. cruzi* (amastigote form cultured in macrophages) and *L. donovani* (amastigote form cultured in macrophages) using established protocols.[13] The data is presented in Table 1.



a $R^1 = (CH_2)_3N(BOC)(CH_2)_4NHBOC$ $R^2 = (CH_2)_3NH(CH_2)_4NH_2$

b $R^1 = (CH_2)_3N(BOC)(CH_2)_4N(BOC)(CH_2)_3NHBOC$ $R^2 = (CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

Scheme 1.

Table 1. ED_{50} (μM) values for activity of compounds against trypanosomes and leishmania

Compound	*L. donovani*[b]	*T. cruzi*[b]	*T. brucei*[b]	Toxicity (KB cells)
Spermidine series				
4a	17.3	>42	2.6, 0.55[c]	111
5a	25.7	>15 (toxic)[a]	4.7, 1.5[c]	65
6a	4.9	>36 (toxic)	>36	>360
7a	44.7	>63 (toxic)	1.9, 0.63[c]	3.3
8a	39.9	13.5	>53	>526
Spermine series				
4b	4.7	>34 (toxic)	1.1, 0.57[c]	31
5b	12.6	>36	0.60	27
6b	>30	>30	5.4	1.5
7b	5.0	>57 (toxic)	0.56, 1.1[c]	15
8b	20.5	>48	>48	<0.47

[a]Toxic = toxic effects seen on the macrophages.
[b]Standard Drugs: *L. donovani*, Pentostam, ED_{50} = 6.16 μgSb^V/ml; *T. cruzi*, benznidazole ED_{50} = 7.45 μg/ml; *T. brucei*, pentamidine = 100% inhibition at 1 μg/mL.
[c]Results of two experiments.

Trypanosoma brucei

Compounds show the greatest activity against *T. brucei*, with five compounds showing ED_{50} values at or around 1 μM. A number of points can be made.

- The spermidine and spermine series show similar activity.
- The presence of the BOC protection on the polyamine or the acetate on the hydroxyl seemed to have minimal effect on the activity (compare compounds **4** and **5** with **7**).
- The sulphated compounds (**6** and **8**) were the least active compounds. This may be due to poor uptake into the parasites.

Leishmania donovani

Compounds **4b**, **6a** and **7b** showed significant activity against *L. donovani*. In general the spermine series (**b**) was more active than the spermidine series (**a**).

- Again the fully protected compounds (**4**) showed similar or greater activity than the partially (**5**) or fully deprotected (**7**) compounds.
- Sulphation reduced the activity of compounds (**6** and **8**) with the exception of **6a** which is also BOC protected.

Trypanosoma cruzi

In general compounds showed much poorer activity against *T. cruzi* with higher concentrations giving rise to toxic side effects on the host macrophages in which the *T. cruzi* was cultured.

In vivo tests

Compound **7b** was also investigated against rodent models of African trypanosomiasis and leishmaniasis. There was no effect against mice infected with *T. b. rhodesiense* (dosed at 50 mg/kg for 4 days intraperitonally) and only a 16% reduction in parasite load of mice infected with *L. donovani* (dosed at 50 mg/kg for 5 days intraperitonally).

Conclusion

A series of analogues of squalamine have been prepared. Several showed significant in vitro activity against *T. brucei* and *L. donovani* and little activity against *T. cruzi*. At this stage it is unclear why there is such a marked difference in activity between the species. This may have something to do with the nature of the membrane. Interestingly three compounds were active against *L. donovani* which is found in the phagolysosomal vacuole in the macrophage, whereas all were inactive against *T. cruzi* amastigotes in the macrophage cytoplasm.

In general the presence of the BOC or acetate protecting groups had little effect on activity, whilst the presence of the sulphate group appeared to decrease activity.

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