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Short communication

Synthesis and antifungal activity of cholesterol-hydrazone derivatives

Céline Loncle [a], Jean Michel Brunel [a,*], Nicolas Vidal [a], Michel Dherbomez [b], Yves Letourneux [a,*]

[a] *Laboratoire synthèse et étude de substances naturelles à activités biologiques (SESNAB), IMRN INRA 1111, faculté des sciences et techniques de St Jérôme, université Paul Cézanne, Aix-Marseille III, avenue Escadrille Normandie Niémen, 13397 Marseille cedex 20, France*
[b] *IUT La Rochelle, 15, rue Francois de Vaux de Foletier, 17026 La Rochelle, France*

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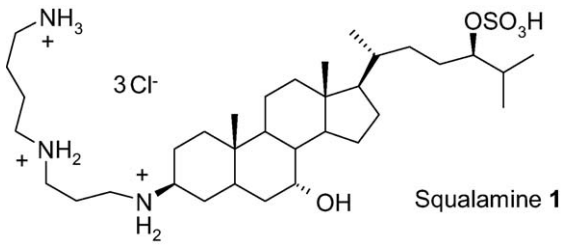
Abstract

A series of hydrazones synthesized from various cholesterol derivatives were evaluated for their in vitro antimicrobial properties against human pathogens. The activity was highly dependent on the structure of the different compounds involved. The best results have been obtained with tosylhydrazone cholesterol derivatives **8** and **9** exhibiting activities against *Candida albicans* (CIP 1663-80) at a concentration of 1.5 μg/ml.

Keywords: Hydrazone; Cholesterol derivatives; Antifungal activity; Antimicrobial activity

1. Introduction

Emergence of multidrug-resistant microorganisms such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and drug-resistant *Mycobacterium tuberculosis* has prompted efforts to develop new classes of antibiotics [1]. Recently, various polyamines conjugated to cholesterol, cholenic acid and bile acids have been reported [2]. Some of these sterol-polyamine conjugates exhibit antimicrobial [3–6], anti-trypanosomal [7] activities, and DNA binding affinity[8–11]. Squalamine **1**, a 5α-hydrido-7α-hydroxyl-24-sulfated cholestane steroid conjugated to spermidine at C-3β, isolated from the dogfish shark, *Squalus acanthias*, displays potent activity against both Gram-positive, Gram-negative bacteria [12–14] and yeast but also shows significant preclinical antitumor activity against human lung cancer by antiangiogenic effects [15–18] (Scheme 1). Recently, we [19] and others [3–6] synthesized and evaluated aminosterol and squalamine analogues exhibiting comparable antimicrobial activity against Gram-positive, Gram-negative bacteria and yeast. In this context, several aminosterol derivatives have been prepared in order to obtain inhibitors of sterol biosynthesis [20,21]. The compound having an allylamine moiety, 7-aminosterol, appeared to be the most powerful inhibitor of yeast cell growth. Schiff bases, and Mannich bases of isatin were reported to possess antibacterial [22,23], antifungal [24] and antiviral activities [25]. In the same area, recent results have been reported on the synthesis and antiproliferative, anti-HIV and antimicrobial properties of heterocyclic hydrazones [26,27]. Moreover, Siemann et al. [28] have identified in 2002 *N*-arylsulfonyl hydrazone derivatives presenting structural analogy with β-lactams and acting as inhibitors of IMP-1, a metallo-β-lactamase of increasing prevalence. In continuation of our work on biologically active aminosterol derivatives, we re-



Scheme 1. Structure of squalamine **1**.

* Corresponding authors.
E-mail addresses: bruneljm@yahoo.fr (J.M. Brunel), yvesletourneux@yahoo.fr (Y. Letourneux).

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port herein the synthesis of various new hydrazone cholesterol derivatives and their promising antibacterial properties.

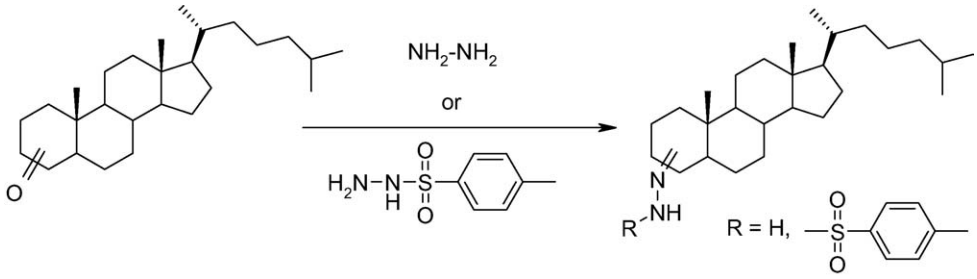
2. Chemistry

In the present study, various hydrazines were subjected to reaction with different ketosterol derivatives to afford the corresponding expected hydrazones (Scheme 2–3).

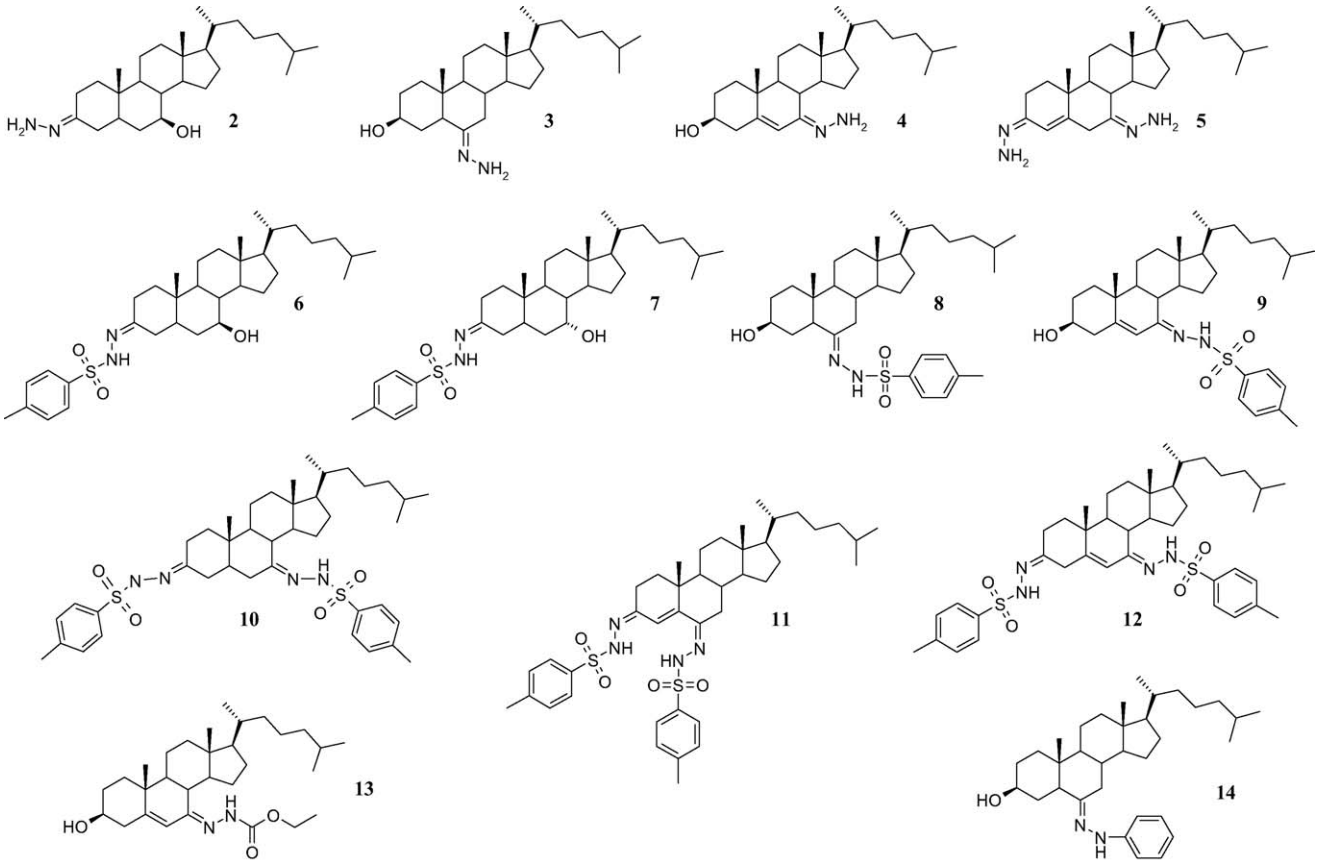
All these compounds were isolated in satisfactory yields (55–92%) except for product **13** which was obtained in 18% yield due to a low stability of the final product under the acidic conditions of the reaction. In all cases, chemical structures were consistent with both analytical and spectroscopic data (^{1}H and ^{13}C NMR) (Table 1).

Table 1
Physical properties of compounds **2–14**

Entry	Product	Isolated yield (%)	Mp (°C)
1	**2**	61	132
2	**3**	71	121
3	**4**	68	96
4	**5**	81	135
5	**6**	83	145
6	**7**	72	154
7	**8**	92	162
8	**9**	83	103
9	**10**	55	50
10	**11**	91	110
11	**12**	62	137
12	**13**	18	115
13	**14**	84	94



Scheme 2. General pathway for the synthesis of hydrazones **2–14**.



Scheme 3. Structure of hydrazones **2–14**.

Table 2
Antimicrobial activity of cholesterol-hydrazone derivatives **2–14**

Sample CIP	Antimicrobial activity (IC_{50}), μg/ml				
	S. cerevisiae (ATCC 28383)	*E. Coli* (54-127)	*S. aureus* (53-154)	*C. albicans* (1180-79)	*C. albicans* (1663-86)
Squalamine		ATCC 25922	ATCC 29213	ATCC 14053	–
1	–	**1–2**	**1–2**	**4–8**	
2	–	>50	>50	25	–
3	–	>50	>50	25	–
4	–	>50	>50	25	–
5	–	>50	25	>50	–
6	25	>50	–	>50	–
7	–	>50	>50	>50	–
8	0.8	–	–	25	1.5
9	3.1	–	–	12.5	1.5
10	–	>50	>50	>50	–
11	0.8	–	–	>50	25
12	–	–	–	>50	25
13	–	>50	6.2	12.5	–
14	–	–	6.2	12.5	12.5

3. Biological investigation and discussion

All the synthesized compounds were screened for antimicrobial activity against several yeast strains as well as Gram-positive and Gram-negative bacteria strains [29]. Seven out of 13 novel compounds tested in the present study were found to have no activity against the microorganisms listed in Table 2. Results of the remaining six compounds showed that they have some antifungal activities but no antibacterial activities. The best results were encountered using tosylhydrazone cholestane compounds **8–12**. Thus, compounds **8** and **9** exhibited activities against *Candida albicans* (CIP 1663-80) at a concentration of 1.5 μg/ml and against Amphotericine B and miconazole resistant strain *C. albicans* (CIP 1180-79) at a concentration of 25 and 12.5 μg/ml, respectively. In all other cases, moderate results were encountered even though an antibacterial activity against *S. aureus* at a concentration of 6.2 μg/ml was observed using compounds **13** and **14**.

4. Experimental section

All solvents were purified according to reported procedures, and reagents were used as commercially available. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl immediately prior to use. Ethyl acetate and petroleum ether (35–60 °C) were purchased from SDS and used without further purification. Column chromatography was performed on SDS silica gel (70–230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC 300 spectrometer working at 300 00 and 75 MHz, respectively (the usual abbreviations are used: s: singulet, d: doublet, t: triplet, q: quadruplet, m: multiplet). Tetramethylsilane was used as internal standard. All chemical shifts are given in ppm. All the products were prepared from well known ketones prepared according to classical methodology.

4.1. General procedure for the synthesis of hydrazones ***2–5***

4.1.1. Synthesis of hydrazone ***2***

To a solution of cholestan-3-one-7β-ol (50 mg, 1.24×10^{-4} mol) in ethanol (96%, 5 ml) were added hydrazine hydrate (50 μl, 1.3×10^{-4} mol) and NEt_3 (250 μl, 2.47×10^{-3} mol). The stirred mixture was heated at reflux for 24 h. After cooling to 25 °C, the mixture was concentrated under vacuum affording a yellow precipitate. The precipitate was taken into absolute ethanol then filtered on paper. The filtrate was concentrated providing a yellow solid which was washed with H_2O to give pure **2** in 61% yield.

4.1.2. Hydrazone ***2***

A 61% yield; yellow solid; mp: 132 °C; ^{1}H NMR: δ = 3.30–3.40 (m, 1H), 0.5–2.69 (m, 47H); ^{13}C: δ = 154.5, 74.6, 55.6, 55.5, 55.2, 55.1, 52.1, 43.5, 43.2, 42.1, 39.8, 39.4, 38.1, 37.5, 36.1, 35.6, 30.6, 28.7, 27.9, 26.9, 26.8, 23.8, 22.7, 22.5, 21.2, 18.7, 12.1; $C_{27}H_{48}N_2O$ (416.7): calcd. C 77.8, H 11.6, N 6.7, O 3.8; found C 77.1, H 10.9.

4.1.3. Hydrazone ***3***

A 71% yield; yellow solid; mp: 121 °C; ^{1}H NMR: δ = 3.51–3.55 (m, 1H), 2.76–2.82 (dd, 1H), 0.5–2.05 (m, 45H); ^{13}C: δ = 155.3, 72.7, 57.3, 56.9, 55.2, 51.7, 43.7, 40.4, 40.2, 39.6, 37.1, 36.8, 36.5, 33.1, 31.6, 30.2, 28.7, 24.9, 24.6, 23.3, 23.2, 22.2, 19.4, 13.3, 12.8; $C_{27}H_{48}N_2O$ (416.7): calcd. C 77.8, H 11.6, N 6.7, O 3.8; found C 77.5, H 11.6.

4.1.4. Hydrazone ***4***

A 68% yield; white solid; mp: 96 °C; ^{1}H NMR: δ = 6.1 (s, 1H), 3.51–3.55 (m, 1H), 0.5–2.5 (m, 44H); ^{13}C: δ = 160.7, 152.5, 115.2, 70.7, 54.7, 50.2, 50.0, 42.9, 42.6, 39.5, 38.6, 36.6, 35.8, 35.6, 31.3, 28.3, 27.9, 26.9, 25.1, 23.6, 22.7, 22.5, 20.8, 18.9, 18.1, 17.8, 14.5, 12.2; $C_{27}H_{46}N_2O$ (414.7): calcd. C 78.2, H 11.1, N 6.7, O 3.9; found C 78.5, H 11.6.

*4.1.5. Hydrazone **5***

An 81% yield; white solid; mp: 135 °C; ^{1}H NMR: δ = 6.4 (s, 1H), 0.5–2.62 (m, 45H); ^{13}C: δ = 149.4, 149.0, 145.8, 121.7, 56.7, 56.0, 50.4, 42.5, 39.4, 36.0, 35.6, 33.7, 32.4, 29.1, 28.0, 27.9, 24.0, 23.7, 22.7, 22.4, 21.3, 18.6, 17.8, 11.8; $C_{27}H_{46}N_4$ (426.6): calcd. C 76.0, H 10.8, N 13.1; found C 76.5, H 11.0.

*4.2. General procedure for the synthesis of tosylhydrazones **6–12***

*4.2.1. Synthesis of tosylhydrazone **9***

In a two necked round-bottom flask were placed under argon 550 mg (1.37 mmole) of 7-keto cholesterol and 306 mg (1.65 mmole) of tosylhydrazide in 15 ml of anhydrous methanol. Three drops of concentrated HCl were added and the mixture was stirred overnight at room temperature. A 10 ml of water were added and a white precipitate was obtained. After filtration and drying under vacuum tosylhydrazone **9** was obtained as a white solid in 83% yield.

*4.2.2. Tosylhydrazone **6***

A 83% yield; white solid; mp: 145 °C; ^{1}H NMR: δ = 7.80–7.83 (m, 2H), 7.25–7.28 (m, 2H), 3.25–3.47 (m, 1H), 0.6–2.60 (m, 49H); ^{13}C: δ = 175.8, 144.3, 129.9, 128.5, 75.0, 55.9, 55.5, 52.1, 43.9, 43.5, 39.9, 39.8, 36.5, 36.0, 35.9, 31.9, 31.2, 29.0, 28.4, 27.1, 24.2, 23.2, 22.9, 22.0, 19.1, 12.5; $C_{34}H_{54}N_2O_3S$ (570.80): calcd. C 71.5, H 9.5, N 4.9, O 8.4, S 5.6; found C 71.2, H 9.7, S 5.3.

*4.2.3. Tosylhydrazone **7***

A 72% yield; white solid; mp: 154 °C; ^{1}H NMR: δ = 7.80–7.85 (m, 2H), 7.30–7.34 (m, 2H), 3.25–3.49 (m, 1H), 0.5–2.50 (m, 49H); ^{13}C: δ = 180.9, 144.1, 135.5, 129.1, 128.0, 68.9, 56.0, 51.2, 45.1, 40.1, 39.6, 37.0, 36.2, 36.0, 35.9, 31.8, 31.2, 29.0, 28.2, 27.1, 24.2, 23.2, 22.9, 18.8, 17.4, 12.8; $C_{34}H_{54}N_2O_3S$ (570.80): calcd. C 71.5, H 9.5, N 4.9, O 8.4, S 5.6; found C 71.4, H 9.5, S 5.9.

*4.2.4. Tosylhydrazone **8***

A 92% yield; white solid; mp: 162 °C; ^{1}H NMR: δ = 7.83–7.86 (m, 2H), 7.28–7.33 (m, 2H), 3.51–3.55 (m, 1H), 0.5–2.60 (m, 49H); ^{13}C: δ = 180.9, 144.2, 135.5, 129.7, 128.6, 71.5, 56.7, 56.4, 54.5, 51.6, 43.3, 39.8, 36.7, 36.4, 36.0, 32.2, 32.0, 31.5, 28.4, 24.1, 23.2, 22.9, 21.9, 21.7, 19.0, 12.8, 12.4; $C_{34}H_{54}N_2O_3S$ (570.80): calcd. C 71.5, H 9.5, N 4.9, O 8.4, S 5.6; found C 71.4, H 9.1, S 5.1.

*4.2.5. Tosylhydrazone **9***

An 83% yield; white solid; mp: 103 °C; ^{1}H NMR: δ = 7.98 (s, 1H), 7.76–7.79 (d, 2H), 7.22–7.25 (d, 2H), 5.96 (s, 1H), 3.48 (m, 1H), 2.38 (s, 3H), 0.6–2.31 (m, 42H); ^{13}C: δ = 207.6, 157.2, 143.9, 136.0, 129.5, 128.9, 113.2, 71.2, 55.1, 50.4, 50.0, 43.0, 42.6, 39.8, 39.7, 38.8, 36.8, 36.5, 35.9, 31.3, 28.7, 28.3, 27.1, 24.1, 23.1, 22.8, 21.8, 21.0, 19.2, 18.2, 12.5; $C_{34}H_{52}N_2O_3S$ (568.87): calcd. C 71.7, H 9.2, N 4.9, O 8.4, S 5.6; found C 71.8, H 9.1, S 5.8.

*4.2.6. Tosylhydrazone **10***

A 55% yield; white solid; mp: 50 °C; ^{1}H NMR: δ = 10.1 (m, 2H), 7.74–7.9 (m, 4H), 7.38–7.43 (m, 4H), 0.62–2.61 (m, 50H); ^{13}C: δ = 179.7, 176.0, 144.1, 135.5, 129.1, 128.0, 55.6, 49.8, 45.5, 44.3, 39.6, 38.5, 36.8, 36.4, 34.0, 33.2, 32.2, 28.7, 24.1, 22.7, 21.4, 18.8, 14.6, 13.5; $C_{41}H_{60}N_4O_4S_2$ (737.07): calcd. C 66.8, H 8.2, N 7.6, O 8.6, S 8.7; found C 66.8, H 8.0, S 8.3.

*4.2.7. Tosylhydrazone **11***

A 91% yield; white solid; mp: 110 °C; ^{1}H NMR: δ = 7.85–7.91 (m, 4H), 7.28–7.38 (m, 4H), 6.27 (s, 1H), 3.45–3.49 (m, 1H), 0.64–2.52 (m, 48H); ^{13}C: δ = 155.2, 155.6, 150.0, 149.1, 146.0, 145.0, 136.0, 135.9, 135.8, 130.7, 130.5, 130.4, 128.8, 128.6, 124.3, 57.3, 57.2, 56.6, 50.8, 50.6, 46.7, 43.2, 43.1, 40.7, 40.2, 38.4, 36.8, 36.4, 34.0, 33.2, 32.2, 28.7, 24.5, 23.6, 23.4, 22.5, 22.4, 19.3, 18.3, 12.6; $C_{41}H_{58}N_4O_4S_2$ (735.07): calcd. C 66.9, H 7.9, N 7.6, O 8.7, S 8.7; found C 66.2, H 7.8, S 8.5.

*4.2.8. Tosylhydrazone **12***

A 62% yield; white solid; mp: 137 °C; ^{1}H NMR: δ = 9.8 (m, 2H), 7.85–7.91 (m, 4H), 7.39–7.41 (m, 4H), 5.94 (s, 1H), 0.61–3.01 (m, 47H); ^{13}C: δ = 172.8, 170.1, 152.7, 142.3, 135.7, 129.2, 128.0, 115.2, 55.2, 51.1, 44.7, 39.6, 38.5, 36.4, 36.3, 34.2, 33.2, 31.2, 28.7, 23.1, 22.7, 22.1, 21.4, 19.5, 12.3; $C_{41}H_{58}N_4O_4S_2$ (735.05): calcd. C 66.9, H 7.9, N 7.6, O 8.7, S 8.7; found C 66.8, H 7.8, S 8.3.

*4.3. Procedure for the synthesis of hydrazone **13***

In a two necked round-bottom flask were placed under argon 50 mg (1.24×10^{-4} mol) of 7-keto cholesterol, 15.8 mg (1.52×10^{-4} mol) of ethyl carbazate in 5 ml of anhydrous methanol. Three drops of concentrated HCl were added and the mixture was stirred overnight at reflux. After evaporation of the solvent, the product was purified by flash chromatography (eluent: EtOAc-petroleum ether (50/50)) affording the expected product in 18% yield as a white solid.

mp: 115 °C; ^{1}H NMR: δ = 5.7 (s, 1H), 5.35–5.36 (m, 1H), 4.66–4.72 (d, 2H), 3.44–3.57 (m, 1H), 0.61–2.37 (m, 45H); ^{13}C: δ = 175.4, 157.9, 150.5, 112.7, 72.0, 55.3, 50.9, 50.5, 43.2, 40.4, 40.3, 39.3, 37.2, 36.9, 36.3, 32.0, 29.1, 28.8, 27.4, 24.4, 23.5, 23.3, 20.6, 19.7, 18.9, 12.9; $C_{30}H_{50}N_2O_3$ (486.7): calcd. C 74.0, H 10.3, N 5.7, O 9.8; found C 74.2, H 10.2.

*4.4. Procedure for the synthesis of hydrazone **14***

In a two necked round-bottom flask were placed under argon 100 mg (2.48×10^{-4} mole) of 6-keto cholestanol and 32 mg (3×10^{-4} mole) of phenylhydrazine in 4 ml of anhydrous methanol. Two drops of concentrated HCl were added and the mixture was stirred overnight at room temperature. A 10 ml of water were added and a yellow precipitate was obtained. After filtration and drying under vacuum hydrazone **14** was obtained as a yellow solid in 84% yield.

mp: 94 °C; ^{1}H NMR: δ = 6.8–7.3 (m, 5H), 3.51–3.60 (m, 1H), 2.75 (d, 1H), 0.50–2.36 (m, 43H); ^{13}C: δ = 161.8, 143.3, 129.5, 120.0, 113.4, 71.8, 57.1, 56.5, 54.7, 51.7, 47.3, 39.9, 38.3, 36.7, 36.5, 36.1, 32.3, 31.0, 28.5, 28.4, 24.3, 24.2, 23.2, 22.9, 19.0, 13.5, 12.4; $C_{33}H_{50}N_2O$ (490.7): calcd. C 80.7, H 10.2, N 5.7, O 3.2; found C 79.9, H 10.6.

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