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Click chemistry decoration of amino sterols as promising strategy to developed new leishmanicidal drugs

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ABSTRACT

A series of 1,2,3-triazolylsterols was prepared from pregnenolone through reductive amination and copper(I)-catalyzed azide-alkyne cycloaddition (click chemistry). The newly generated stereocenter of the key propargylamino intermediate provided a mixture of diastereomers which were separated chromatographically, and the configuration of the *R* isomer was determined by X-ray crystallography. Ten triazolyl sterols were prepared, and the products and intermediates were screened *in vitro* against different parasites, with some compounds presenting IC_{50} values in the low micromolar range against *Leishmania donovani*.

1. Introduction

Parasitic diseases have burdened the world since the early days of mankind and several herbal and mineral extracts were used to treat such maladies until the 19th century. The development of new chemical processes allowed for the isolation of natural compounds and preparation of chemical entities displaying antiparasitic properties, culminating with the introduction of a great number of new drugs in the middle of the 20th century to treat many infectious diseases, including malaria and leishmaniasis [1]. However, the pace with which new drugs were introduced in the market was not kept over the rest of the century, especially in poorer countries, where diseases like malaria, trypanosomiasis and leishmaniasis are still responsible for millions of deaths [2–5]. Current treatments present many disadvantages, such as undesirable side effects and development of resistant parasite strains, creating an urgent need for new drugs [6].

Among many natural substances potentially useful for the development of new antiparasitic drugs, sterols are an important and ubiquitous class of compounds, constantly isolated from new natural sources and modified synthetically on the polycyclic system and side chain [7–9]. These modifications modulate their interactions with molecular targets, producing a wide spectrum of biological activities, and generating compounds showing antibacterial [10], antitubercular [11], and antiprotozoal [12] activities. Parasites, like any other organism, require sterols for survival, and are capable of salvaging their host's sterols to survive if their sterol biosynthetic pathways are inhibited [13]. Fungi and protozoa, such as trypanosomatids, produce and use ergosterol, in contrast to the mammalian cells' use of cholesterol. Ergosterol is biosynthesized by a sequence of enzymes that diverge in some points from the mammals' counterpart, offering a convenient target for new antifungal and antiparasitic agents [14]. Sterols isolated from natural sources have also shown activity against *Leishmania* sp. [15] and *Plasmodium* sp. [16]. Demethylase *Erg11* and methyl transferase *Erg 6* have been targeted by sterol derivatives with heteroatoms or heterocycles on the side chain, and some of these modified sterols have exhibited activity against *Leishmania* spp. and *Trypanosoma cruzi* [17–19].

The design of strategies to explore the chemical space through chemical diversity and construction of new chemical libraries are key steps on the search for new active compounds, especially when the specific target is unknown [20]. When the search starts with a validated target the best strategy is to prepare focused libraries [21], an approach that has been greatly facilitated by the application of click chemistry in medicinal chemistry [22,23], which has been commonly associated to the preparation of 1,2,3-triazoles through Cu(I) catalysis [24]. Due to the simplicity of this reaction,

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libraries of 1,2,3-triazoles have been prepared as selective enzyme inhibitors [25–27], and against parasites [27–30], tuberculosis [31,32], and cancer [33,34]. Recently, this versatile reaction has been used to make libraries of heterocyclic steroids [34–36].

In search of new chemical entities (NCE) with antiparasitic properties, we designed a simple strategy to prepare new heterocyclic steroids. Based on the literature, we hypothesized a synergistic effect of nitrogen on the lateral chain along with a heterocyclic ring, improving biological activity. To test this hypothesis, we introduced a propargylamine unit on the side chain of pregnenolone through reductive amination, which is both the source of the amino group and of the scaffold to build heterocycles through click chemistry (Fig. 1). The compounds prepared were assayed for antiparasitic activity and cytotoxicity.

2. Experimental section

2.1. General

Chemical reagents were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents (hexanes, ethyl acetate, CH_2Cl_2, Et_2O) were distilled prior to use. CH_2Cl_2 was dried over P_2O_5. DMF was distilled from BaO. Reactions were monitored on precoated silica gel G or GP TLC plates. Spots were visualized under 254 nm UV light and/or by TLC staining [37]. All reactions were performed under an atmosphere of nitrogen using oven-dried glassware and standard syringe/septa techniques. Column chromatography was performed with silica gel 60 (230–400 mesh). Yields were calculated for material judged homogeneous by thin layer chromatography (TLC) and nuclear magnetic resonance (^{1}H NMR).

2.2. Characterization of the products

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance II 300 MHz (75.13 MHz) using $CDCl_3$ as solvent. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane as internal standard and coupling constants are in hertz (Hz). Assignment of proton resonances was confirmed by correlated spectroscopy. High-resolution mass spectra (ESI-HRMS) were recorded on a Micromass spectrometer with lock spray source or on a Bruker MicroTOF II. IR spectra were obtained using an FT-IR Shimadzu spectrometer and only partial spectral data are listed. Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected.

2.3. Chemical synthesis

2.3.1. Synthesis of propargylamino intermediate **1** by reductive amination of pregnenolone

To a solution of pregnenolone (1.0 g, 3.16 mmol) in 20 mL of THF, propargylamine (1.05 g, 19 mmol), $NaBH(AcO)_3$ (1.34 g, 6. 32 mmol) and finally 4 Å molecular sieves (100 mg) were added in this order and the reaction mixture was stirred at room temperature. Additional molecular sieves were added every 48 h until the reaction was completed in 6 days. Then, the reaction was quenched by addition of 5% $NaHCO_3$ (50 mL) and the layers were separated and filtered to remove the molecular sieves. The aqueous phase was extracted with ether (4 × 20 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated. The products were purified by column chromatography in silica gel with increasing ethyl acetate/hexane gradient to yield a less polar fraction composed by **1*R***, 552 mg, 49%, and a more polar fraction containing **1*S***, 544 mg, 48%.

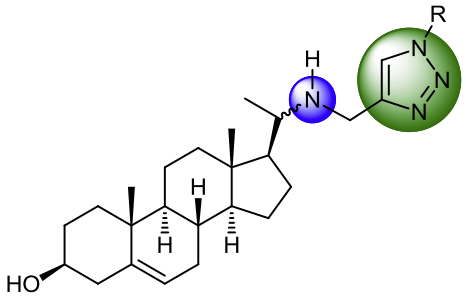

Fig. 1. Side chain functionalized steryl analogues designed.

*2.3.1.1. (20R)-20-(prop-2-yn-1-ylamino)pregn-5-en-3β-ol (**1R**).* Light yellow solid (552 mg, yield 49%, reaction time 6d). **Mp:** 148–149 °C **IR (KBr):** υ_{max} (cm^{-1}) = 3475, 3363, 3279, 2936, 2882, 2361, 1729, 1450, 1376. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.33 ppm (d, 1H, *J* = 5.2 Hz, C6–**H**); 3.50 (m, 1H, C3–**H**); 3.48, 3.35 (dd, 2H, *J* = 17.2 Hz, 2.4 Hz, –NH–C**H$_2$**–); 2.84 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 2.18 (t, 1H, *J* = 2.4 Hz, —C≡C**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.30 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.00 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); 0.96 (m, 3H, *J* = 6.0 Hz, C21–**H**) and 0.76 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C5**); 121.5 (**C6**); 82.5 (—**C**≡CH); 71.7 (**C3**); 71.0 (—C≡**C**H); 56.4 (**C14**); 56.1 (**C17**); 53.8 (**C20**); 49.9 (**C9**); 42.3 (**C4**); 42.0 (**C13**); 40.2 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 34.9 (NH—CH_2—CH≡); 31.8 (**C2**); 31.8 (**C8**); 31.6 (**C7**); 26.7 (**C15**); 24.1 (**C16**); 21.1 (**C11**); 19.4 (**C19**); 18.4 (**C21**) and 12.3 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 356.2953; found, 356.2953.

*2.3.1.2. (20S)-20-(prop-2-yn-1-ylamino)pregn-5-en-3β-ol (**1S**).* Light yellow solid (544 mg, yield 48%, reaction time 6d). **Mp** 145–146 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3283, 3268, 2894, 2885, 1646, 1443, 1421, 1375, 1382, 1249, 1097, 1070, 718. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.35 ppm (d, 1H, *J* = 4.7 Hz, C6–**H**); 3.52 (m, 1H, C3–**H**); 3.50, 3.34 (dd, 2H, *J* = 17.2 Hz, 2.4 Hz, –NH–C**H$_2$**–); 2.74 (m, 1H, C20–**H**); 2.27 (d, 2H; *J* = 7.5 Hz, C4–**H**), 2.19 (t, 1H, *J* = 2.4 Hz, —C≡C**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.06 (d, 3H, *J* = 6.1 Hz, C21–**H**); 1.06 (s, 3H, C19–**H**) 1.02 (m, 1H, C9–**H**) and 0.72 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C5**); 121.6 (**C6**); 82.6 (—NH—CH_2—**C**≡); 71.8 (**C3**); 71.0 (—**C**≡CH); 56.6 (**C17**); 56.3 (**C14**); 54.6 (**C20**); 50.0 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 35.1 (—NH—**C**H_2—C≡); 31.8 (**C8**); 31.7 (**C7**); 31.6 (**C2**); 26.9 (**C15**); 24.2 (**C16**); 20.9 (**C11**); 19.4 (**C19**); 18.6 (**C21**) and 12.3 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 356.2953; found, 356.2968.

2.3.2. General procedure for the Cu(I) mediated 1,3-dipolar cycloaddition

Alkyne (1 eq) and the azide (1.1 eq) were suspended in 10 mL/eq of ^{t}BuOH:H_2O (1:1) and then 1 M $CuSO_4$ solution and finally 1 M sodium ascorbate solution were added and the mixture stirred overnight at room temperature. Brine was added and the solution was extracted with dichloromethane. Combined organic extracts were dried over sodium sulfate and evaporated. Products were purified by column chromatography in silica gel with increasing ethyl acetate/methanol gradients.

*2.3.2.1. (20R)-20-(((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amino)-pregn-5-en-3β-ol (**2A-R**).* Light yellow solid (47 mg, yield 89%,