DIVING DEEPER WITH MARINE STEROLS

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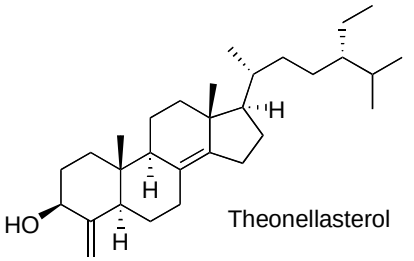
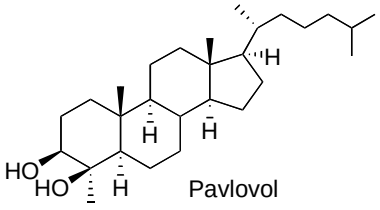
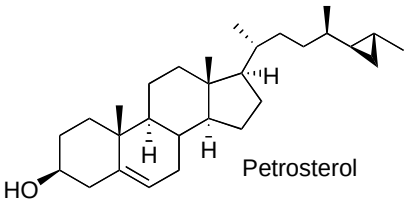
Conicasterol and theonellasterol are sterols from marine sponges that feature an exo-methylene group at the 4-position of ring-A. In 2011, they were reported to be the first known ligands to two "orphan" nuclear steroid receptors, human nuclear pregnane receptor (PXR) and farnesoid-X-receptor (FXR). Synthetic routes to these sterols will be presented, including a 5 step synthesis from ergosterol. The pavlovols are 4-methyl, 4-hydroxy sterols from marine algae that we have found to mimic the effects of the arthropod molting hormone ecdysterone. The identification of several new pavlovols from marine algae will be reported and different routes for their preparation will be presented. In addition, the synthesis of simplified analogs of petrosterol, a sponge sterol recently shown to inhibit phytosterol dealkylation in arthropods, will be shown.



Theonellasterol



Pavlovol



Petrosterol

IMMUNOMODULATORY PROPERTIES OF NATURAL SESQUITERPENE LACTONE TRILOBOLIDE AND ITS CONJUGATES

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Trilobolide (TB) is a sesquiterpene lactone of guaianolide type. Similarly to well-known thapsigargin, TB is a potent and selective inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) leading to rapid elevation of intracellular calcium. Recently we have shown another biological effect of TB, *i.e.* production of nitric oxide (NO) and secretion of interferon-γ (IFN-γ). These interesting immunomodulatory effects encouraged us to synthesize novel TB conjugates and analyze their biological properties. Trilobolide molecule isolated from *Thapsia garganica* L. and *Laser trilobum* (L.) Borkh. was modified with acyl chain linkers of various lenght at *O*-8 position. Moreover, the resulting derivatives were used for conjugation with fluorescent marker Bodipy. For evaluation of biological effects, primary peritoneal cells isolated from rats were used. Rat peritoneal cells ($2x10^6$/ml) were cultured 24 h in presence of TB, TB-linkers and TB conjugates in RPMI-1640 medium. Supernatants of cells were analyzed for NO production (Griess reagent). Concentrations of cytokines were determined by ELISA. Cytotoxicity of TB and its derivatives was analysed by WST-1 method. Cellular uptake of the test derivatives was performed by fluorescence microscopy in live cells. The effects of conjugates were not influenced by fluorescent Bodipy itself. TB-linkers induced NO production in dependence on a length of linker. We also found significant and dose-dependent (0.01- 100.0 μM) NO production induced by trilobolide conjugates. The potency of conjugates correlated with the cytokine expression (TNF-α, IFN-γ, IL-2). The high cytotoxic effect was found only for TB. Using the live cell microscopy, we confirmed intracellular target, *i.e.* endoplasmic reticulum for bioconjugates.

We can conclude that our original trilobolide-linkers conjugated with fluorescent Bodipy penetrate the macrophages and possess immunobiological activity. Trilobolide conjugates will serve as a good tool for further pharmacological characterization and implication in immunotherapy.

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NEW SYNTHETIC ANTI-INFECTIVE AGENTS BY SCAFFOLD PRENYLATION

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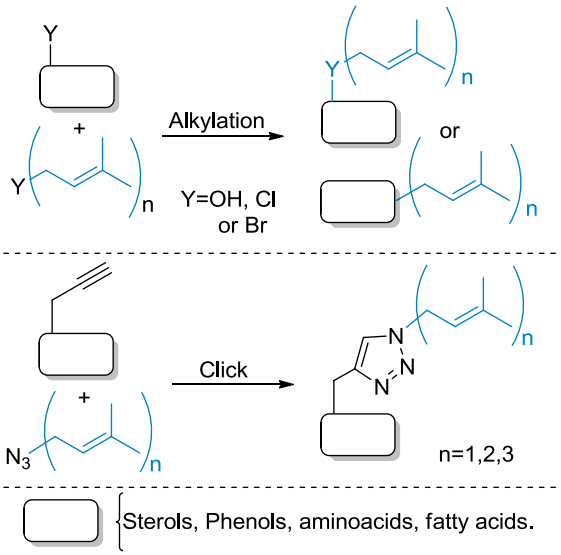
World most vulnerable population is in risk of being affected by Neglected Tropical Diseases (NTD). One out of seven people around the globe is affection by those diseases the forecast is not going to change in the near future[1]. The arsenal of present treatments are insufficient and inefficient leading to urgent need of new drugs and strategies to prevention. Within the universe of small organic molecules, the isoprenoids are the most important and numerous families of metabolites. It has been proposed that prenylated products are more potent than their unsubstituted counterpart[2]. Based on those concepts and as a part of an ongoing program to develop new drugs against NTD, we wanted to explore the scaffold prenylation as a novel strategy to find new chemical entities.

The implemented strategy requires the selection of adequate scaffold that were conveniently prenylated. The selected reaction for the scaffold decoration included the direct scaffold N, O or C alkylation and Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)[3] of previously functionalized clickable substrate . (Scheme 1)



Scheme 1. Scaffolds prenylation strategy.

The generated libraries were screened against ethiological agents of malaria (*P. falciparum* chloroquine sensitive and resistant strains), visceral leishmaniasis (*L. donovani*), HAT (*T. brucei*) and Chagas´ disease (*T. cruzi*). As result of our effort we have found very promising new hits to develop drugs against trypanosomiasis, malaria and other diseases.

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IDENTIFICATION AND FUNCTION OF A MALE TILAPIA SEX PHEROMONE

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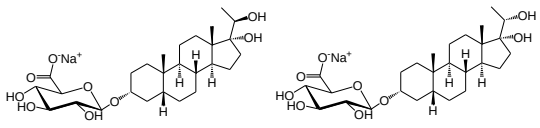
Pheromones are involved in the regulation of social interactions between conspecifics, for example, the sexual attraction of mates. Mozambique tilapia (*Oreochromis mossambicus*) males aggregate in breeding arenas where they fight out strict hierarchies. Dominant males have stronger urinary bladder muscles than subordinates and store larger volumes of urine which they release at high frequency in the presence of subordinate males or pre-ovulatory, but not post-spawned, females. The urine contains pheromones that reduce aggressive behaviour in rival males. The compounds also boost the production of the oocyte maturation-inducing steroid 17α,20β-dihydroxypregn-4-en-3-one (17,20β-P) in reproductively active females, which are attracted by the urinary pheromone to visit dominant males in their nests for mating.

An enantiomeric mixture of pregnane glucuronides was now isolated from the urine of male Mozambique tilapia (*Oreochromis mossambicus*) by a bioassay-guided fractionation procedure. The structures of the two enantiomers have been identified as 5ß-pregnane-3α,17α,20α-triol 3-glucuronate and 5ß-pregnane-3α,17α,20ß-triol 3-glucuronate by means of NMR spectroscopic methods and mass spectrometry. The two epimers have been synthesized and their analytical data match those isolated from fish urine.



5ß-Pregnan-3α,17α,20ß-triol 3-glucuronate and 5ß-pregnan-3α,17α,20α-triol 3-glucuronate

The Mozambique tilapia thus is one of the first fish species in which the chemical structure of sex pheromones has been identified and their biological relevance established. After carps, tilapias are the second most important edible fish in the world raised commercially. The pheromones identified in this study could help to improve aquaculture and to develop tools to reduce uncontrolled spread of tilapia as an invasive species in many tropical and subtropical water bodies.

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6- AND 7-SUBSTITUTED 19-CALCITRIOL ANALOGS: COMPARISON OF VDR BINDING ABILITY AND RESULTS OF DOCKING STUDIES

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Research conducted over the past decades indicate that the natural hormone, 1α,25-dihydroxyvitamin D_3 (calcitriol) is responsible not only for calcium and phosphorus homeostasis but also plays a critical role in many other physiological processes such as cellular differentiation, inhibition of cell proliferation and immunomodulation. Such a broad array of biological activities underscores its significant therapeutic potential and has stimulated intensive research effort towards the synthesis of calcitriol analogs characterized by dissociated physiological activities. Although a large number of vitamin D compounds have been obtained to date and biologically tested, analogs characterized by modification of the B-seco ring are exceptionally rare, mostly due to difficulties in synthesis of compounds substituted at intercyclic C(5)=C(6)-C(7)=C(8) moiety. As a continuation of structure-activity studies carried out in our laboratories we have synthesized new analogs of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxy-19-norvitamin D_3 with different substituents at C-6 and C-7. An introduction of substituents at these positions of the vitamin D skeleton results in steric interactions that cause deviation from planarity of the conjugated 5,7-diene fragment. The presented studies indicate that substitution of C-6 and C-7 affects the VDR binding ability of such modified calcitriol analogs and, consequently, influences the transcription of genes controlled by the nuclear receptor.