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Synthesis and biological evaluation of heterocyclic analogues of pregnenolone as novel anti-osteoporotic agents

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ABSTRACT

The structural modifications of pregnenolone have been described via the introduction of heterocyclic moieties at C-17 position by limiting the acyl group. Novel heterocyclic analogues of pregnenolone have been synthesized by using Friedlander and Claisen-Schmidt reactions, and the synthesized compounds were evaluated for their osteogenic activity. Among the synthesized derivatives, four compounds showed significantly increased ALP activity. Among all four active compounds, the novel compound **3a** has shown significant bone matrix mineralization and mRNA expressions of osteogenic marker genes, BMP2, RUNX-2 and OCN at 1 pM concentration.

The skeleton is extremely dynamic tissue where bone remodeling is ongoing and activated osteoclasts resorb bone while osteoblasts generate bone matrix.[1] This process serves to repair microdamage and ensure skeletal strength.[2] Numerous systemic mediators regulate bone cell activity, including endogenous parathyroid hormone, vitamin D metabolites, prostaglandins, cortisol, and hormones. Disruption in the growth and maintenance of skeleton can result in bone disorders like osteoporosis.[3] Osteoporosis is characterized by low bone mass and loss of structural integrity resulting in increased bone fragility and susceptibility to fracture.[4] Osteoporosis affects nearly 75 million people in Europe, USA and Japan.[5] In India, osteoporosis has emerged as a serious problem. One out of eight males and one out of three females in India suffers from osteoporosis, making India one of the largest affected countries in the world.[6] Approximately 80% of the urban Indian population is vitamin D deficient and hip fractures occur about a decade earlier than in Western nations.[7]

Therapeutic options for osteoporosis have greatly expanded over the past few decades. These include agents like nitrogen-containing bisphosphonates, which act by suppressing bone resorption.[8,9] Parathyroid hormone (PTH) and teriparatide, the only FDA-approved anabolic drug, also help to prevent fractures, but are generally reserved for patients with severe disease because of multiple factors, including cost and the inconvenience of daily injections.[10] Besides there are reports of osteosarcoma risk in patients taking PTH due to which its therapy is restricted to two years.[11] Other treatments include Denosumab, a monoclonal antibody that potently blocks the binding of osteoblast-derived RANKL to its osteoclast-derived receptor (RANK), an interaction that is required for osteoclast formation, activation, and survival. By blocking this receptor binding, denosumab potently inhibits osteoclast mediated bone resorption.[12] Romosozumab is another monoclonal antibody targeted against sclerostin, inhibitor of Wnt signaling pathway that is developed for osteoporosis treatment.[13]

Despite these advances, there is need for newer therapies as the above mentioned therapeutic options are associated with some or the other side effects. For instance, anti-resorptive therapies though prevent bone loss but do not increases bone mass. On the other hand, anabolic therapies like PTH are associated with osteosarcoma risks and also do not inhibit bone resorption. Thus, our efforts are to discover new potential agents that promote osteoblast differentiation and may have a role in management of bone related disorders.

The core structure of the steroids is a seventeen carbon arrangement of four fused rings, in which three rings are six membered as cyclohexane and the fourth ring as five membered skeleton of cyclopropane.[14] Steroids are well known for their chemical and

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therapeutical importance as they have played major role in drug discovery and development for different diseases such as cancer, inflammatory diseases, and asthma.[15–21]

Pregnenolone (**1**), a naturally occurring endogenous steroid and is a well-known precursor for the biosynthesis of various steroidal hormones like estrogen, progesterone, testosterone, glucocorticoids and mineralocorticoids.[22,23] Chemically, it consists of four interconnected cyclic hydrocarbon rings with a hydroxyl group at C-3 position, a double bond between C-5 & C-6 and an acyl group at C-17 position.

The unique structural features and the characteristic biological properties of the compound **1** (Fig. 1), attracted several research groups and several possible chemical modifications have been reported.[24,25] The literature surveys have demonstrated that the synthesis of chalcone derivatives with aromatic aldehydes by Claisen-Schmidt reaction is very common whereas very few reports are available on the introduction of heterocyclic groups by modifications of the acyl group in pregnenolone. The C-20 hydroxylated derivatives of pregnenolone have been found effective in calcium-dependent processes and hence affect the degree of depolarization of smooth muscle.[26] The previously pyrazoline,[27] pyrazole,[27,28] triazole,[29] epoxide[30,31] and chalcone[28] analogues of pregnenolone have shown significant effect against cancer cells. Similarly, Guo et al. have synthesized a series of 21-aylidenepregnenolone and their epoxide analogues of pregnenolone and evaluated their neuroprotective effect against beta-amyloid and hydrogen peroxide (H_2O_2)-induced neurotoxicity in PC12 cells, and oxygen-glucose deprivation (OGD)-induced neurotoxicity in SH-SY5Y cells.[31]

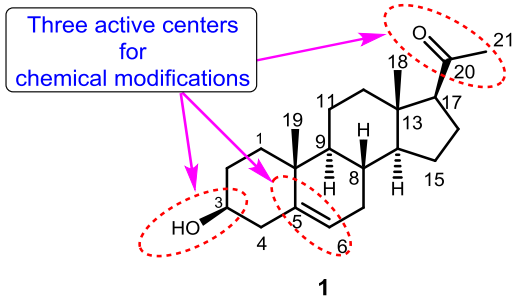

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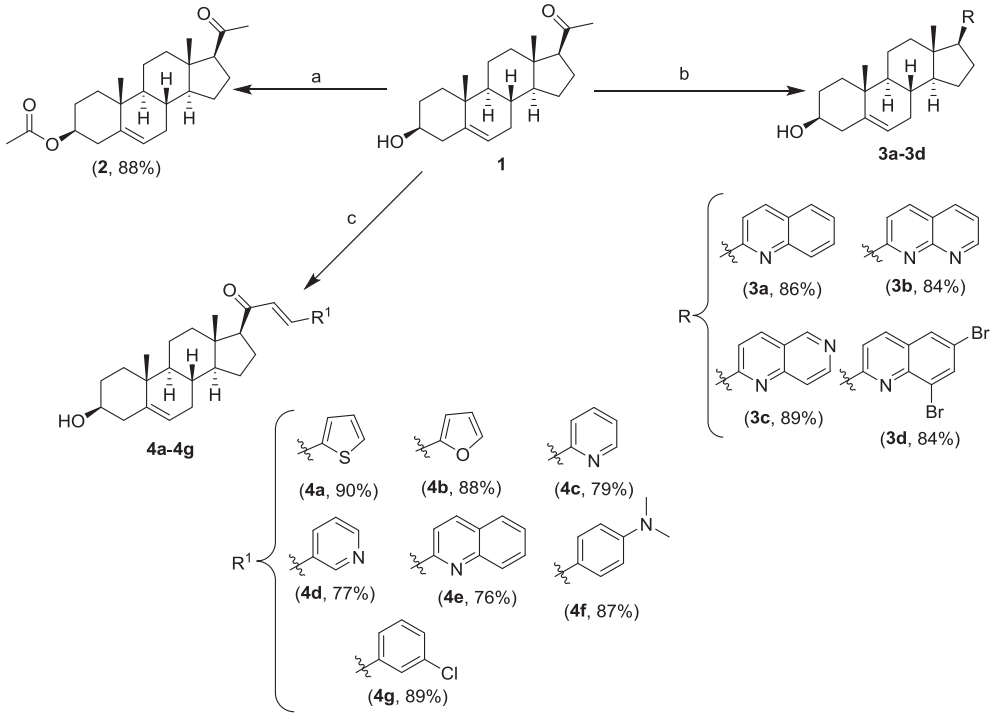
Fig. 1. Structure of pregnenolone with possible active centers for chemical modifications.

As per our aim of drug discovery from natural products and their semi-synthetic analogues for anti-osteoporotic activity, we have isolated and/or synthesized several natural product analogues for the management of osteoporosis.[32] Three active centers are present in pregnenolone **1** which could facilitate and attracted us for the possibility of new drug discovery and development from this steroidal molecule.

In order to achieve our aim, we designed and synthesized the heterocyclic analogues of pregnenolone by using Friedlander and Claisen-Schmidt reactions to introduce the heterocyclic moiety in pregnenolone skeleton. The details of syntheses of these compounds are shown in Schemes 1–3. Initially, the compound **1** was acetylated with Ac_2O/pyridine to afford pregnenolone acetate **2**. The presence of an acyl group at C-17 provided us an opportunity to employ the Friedlander reaction to generate heterocyclic core from the application of aromatic amino aldehydes. To achieve the synthesis of novel heterocyclic analogues (**3a**-**3d**) of pregnenolone by Friedlander reaction, we stirred the solution of compound **1** and 2-amino benzaldehyde or 2-amino pyridine carboxaldehyde derivatives in ethanol and KOH (aq.) at room temperature for 24 h in good yields. Similarly, the heterocyclic chalcone analogues (**4a**-**4e**) of pregnenolone were synthesized by employing the Claisen-Schmidt reaction, in which compound **1** was stirred with heteroaromatic aldehydes in ethanol and KOH (aq.) at room temperature for 24–48 h (Scheme 1).

As our approach relies on the modification of acyl group present at C-17 of pregnenolone, hence we further synthesized the imine



Scheme 1. Reaction conditions: (a) Ac_2O (1.1 eq.), pyridine, dry CH_2Cl_2, 0 °C-rt, 2 h, (b) 2-amino benzaldehyde or 2-amino pyridine carboxaldehyde derivatives (1.0 eq.), 50% KOH (aq.), ethanol, rt, 24–30 h, (c) R^1CHO (1.0 eq.), 50% KOH (aq.), ethanol, rt, 24–48 h.