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Research paper

Synthesis of α-santonin derivatives for diminutive effect on T and B-cell proliferation and their structure activity relationships☆

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ABSTRACT

A new library of 20 compounds from α-santonin was synthesized and tested against Con-A induced T-cell proliferation and LPS-induced B-cell proliferation *via* MTT assay. The study resulted in the identification of potent immunosuppressant molecules, which were further screened along with α-santonin for Tumor Necrosis Factor Alpha (TNF-α) inhibitory activity. One of the molecules (**7**) at 10 μM showed equipotency to that of dexamethasone (1 μM conc.) used as a standard. Structure activity relationships of the synthesized compounds along with our earlier reported α-santonin derivatives have been studied. Inferences from the modifications carried out at all the three sites of α-santonin have been elaborated. Computational study of the active compounds shows TNF-α protein as its preferable target rather than Inosine Monophosphate Dehydrogenase (IMPDH).

1. Introduction

Natural products (NPs) and their analogs/derivatives have been used as therapeutic agents beside their roles in the service of mankind in several other ways. In many cases, NPs have been used directly as drugs [1], and in other cases, as their structural analogs and/or mimics. Natural product modification has led to a drug with optimized or minimized toxicities with better drug delivery or higher potency, and examples include artemether, arteether, sodium artesunate, and fluoroanilide derivatives of artemisinin [2,3]. Betulinic acid modified derivative as potent anti-HIV drug 3-*O*-(3′, 3'-dimethylsuccinyl)-betulinic acid (bevirimat, DSB, PA-457) [4] and oleanolic acid derivative bardoxolone methyl for diabetes mellitus type 2 [5]. Camptothecin derivatives topotecan (Hycamtin) and irinotecan (Camptosar) are used as water soluble analogs [6]. There are several other examples related to structural modification of NPs for the development of lead molecules, including CNB-001 from curcumin, and colforsin from forskolin [7–10]. Therefore, the usage of natural product entities shall continue to play significant role in the drug discovery and development for regimen of the human ailments.

Immunosuppressant drugs aroused for the organ transplantation (is the optimal treatment for many patients with end-stage organ failure) and autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis, and psoriasis). Presently FDA approved immunosuppressive drugs are associated with severe side effects such as nephrotoxicity, neurotoxicity, hirsutism, skin rash, diabetogenic, diarrhoea, hepatotoxicity, and marrow suppression [7–9]. Keeping in view the unwanted properties associated with these drugs, be worth in finding new drug candidates with new mechanism of action, reduced toxicities and improved selectivity. Recently,

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sesquiterpenes have gained attention in drug discovery and development due to vast biological activities and these include development of EBC-46, irofulven, artemisinin, thapsigargin (G-202), and parthenolide (dimethyl amino parthenin, melampomagnolide B) which are under clinical trials for the treatment of cancer.

α-Santonin, a sesquiterpene isolated by Kahler in 1830 from *Artemisia santonica* [11], and known for centuries as "worm-seed" [10], has remarkable efficacy in ridding both human beings and animals of incapacitating round worms (Nematodes). Apart from anthelmintic properties, α-santonin derivatives/analogs are also reported to possess several other activities such as anticancer [10,12–17], antioxidant [18], antitrichomonal [19], antimalarial [20], and 5-lipoxygenase inhibitory activity [21,22]. In addition to the above said activities, we also reported immunosuppressive activity of α-santonin and its derivatives recently [23,24]. In continuation of our interest in structural modification of natural products, a new set of α-santonin derivatives were synthesized for study of immunosuppressive activity as well as their structure activity relationships study including the α-santonin derivatives reported by us. Further, computational study of the active compounds on TNF-α protein and Inosine Monophosphate Dehydrogenase (IMPDH) has also been carried out for target (Protein) identification.

2. Result and discussion

2.1. Chemistry

α-Santonin (**1**) was isolated from the aerial part of *Artemisia laciniata* and used as a starting material for chemical modification at three different reactive sites encompassing ring-A, B, and C of the molecule (Fig. 1).

2.1.1. Modification at ring-A, B, and C

The natural product (**1**) was subjected to Thiele reaction (rearrangement) with Ac_2O/H_2SO_4 to get acetyl α-desmotroposantonin (**2**) which on deacetylation afforded α-desmotroposantonin (**3**), the latter on nitration using nickel nitrate (II) hexahydrate in acetone/p-TSA resulted in the formation of 2-nitro α-desmotroposantonin (**4**) (Scheme 1). The presence of nitro group in **4** was confirmed by appearance of IR band at 1557 cm^{-1}, and disappearance of the aromatic proton signal (observed at δ 6.65 in **3**) in ^1H NMR. Observance of $[M^+]$ *m/z* at 291 in mass spectrum further confirms the structure of **4**. Hydrogenation of **4** with $NiCl_2/NaBH_4$ afforded 2-amino-α-desmotroposantonin (**5**) which showed disappearance of IR band at 1557 cm^{-1} and appearance of band at 3601 cm^{-1} (NH) in IR spectrum, and displayed $[M^+]$ *m/z* at 262 in the mass spectrum.

$DMF\text{-}POCl_3$ reaction of **1** at 0 °C resulted in the formation of 8-chloro-3, 5, 9-trimethyl-tetrahydronaphtho furan-2(3H)-one (**6**) involving modification in the ring A and B of α-santonin. ^1H NMR of **6** showed signals for olefinic proton at δ 5.81 along with disappearance of typical IR band at 1680 cm^{-1} for enone carbonyl group

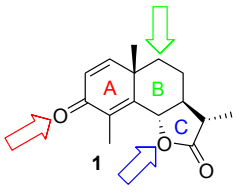
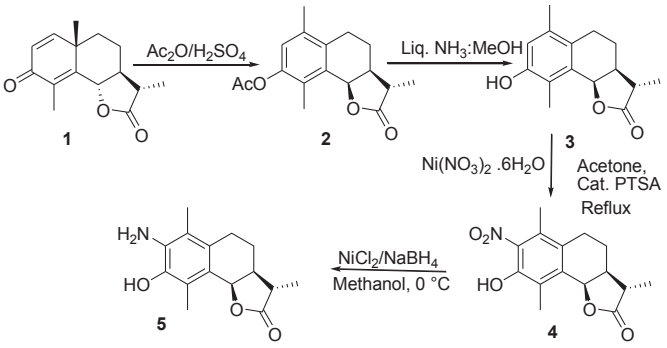


Fig. 1. Structural modification of α-santonin (**1**) involving ring-A, B, and C.



Scheme 1. Structural modification of ring A: Preparation of α-santonin derivatives **2**–**5**.

of **1**. Further confirmation was obtained by mass spectrum showing *m/z* $[M^+]$ at 265 and 267. Reaction of α-santonin with acetyl bromide in DCM resulted in the formation of a rearranged product 3,5,9-trimethyl-2-oxo-hexahydronaphtho-furan-8-yl acetate (**7**). Compound **7** showed signals for three methyl groups as singlet at δ 2.0, 1.89 and 1.15 respectively in ^1H NMR spectrum and disappearance of band due to enone carbonyl in ring A and observance of band at 1753 cm^{-1} for carbonyl group of $-O\text{-}CO\text{-}CH_3$ in IR spectrum. Further, $[M^+]$ *m/z* at 289 in mass spectrum and carbon signals in ^{13}C NMR supported the assigned structure of **7**. The reaction sequence for the preparation of **6** and **7** is shown in Scheme 2. Modification was carried out in the lactone part (ring C) of compound **1** by refluxing α-santonin with HCl/MeOH which led to the formation of **8** (Scheme 2). The product **8** in its ^1H NMR spectrum showed signal for olefinic proton at δ 6.47, in IR spectrum a band for ester carbonyl at 1725 cm^{-1} and in mass spectrum $[M^+]$ *m/z* at 274. Spectral data including ^{13}C NMR confirmed the assigned structure of **8** as methyl-2-(7-methoxy-5,8-dimethyl-3,4-dihydronaphthalen-2-yl) propanoate. The product was identified as 2-(aryl) propanoic acid and belongs to the NSAID group of compounds [25].

Further structural modification of compound **3** was carried out by LAH reduction to afford a mixture of **9** (diol) and **10** (triol) (Scheme 3) which after purification and subsequent acetylation afforded respective di- and tri-acetylated derivatives **11** and **12**. The structure of products (**9**–**12**) was confirmed by IR, ^1H, ^{13}C NMR and mass spectral data. Hydrogenation of α-desmotroposantonin (**3**) in methanol-Pd/C resulted in the formation of product **13**, identified as 2-(7-hydroxy-tetrahydronaphthalen-2-yl) propanoic acid. Treatment of **13** with thionyl chloride in dry benzene followed by coupling with appropriate amines in dry DCM, afforded the respective amides (**14**–**18**) (Scheme 3). The structures of the resulting amides were confirmed by IR (bands for amide carbonyl observed at 1685-1660 cm^{-1}), NMR and mass spectra.

Refluxing of **3** in ethanolic hydrochloric acid solution after usual workup afforded ethyl (7-hydroxy-dihydronaphthalen-2-yl) propanoate (**19**). The structure of the product was confirmed by spectral data and X-ray crystallography (Fig. 2). To find out the role of saturation/unsaturation in ring B of the 2-(tetrahydro naphthalene) propanoic acid (**13**), unsaturated 2-(substituted naphthyl) propanoic acids/esters (**19**–**23**) were prepared by following Scheme 4.

Reaction of **19** with NBS in CCl_4 using AIBN as a radical initiator afforded ethyl 2-[7-(hydroxy-dimethylnaphth-2-yl)] propanoate (**20**), and its methylated product (**21**). The latter on saponification and subsequent acidification afforded [2-(7-methoxy-5,8-dimethyl-1-napthyl)] propanoic acid (**22**) which is dimethyl substituted positional isomer of naproxen [26] and in true sense a mimic of NSAID drug naproxen. The structure of **22** was confirmed