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Synthesis of novel lupane triterpenoid-indazolone hybrids with oxime ester linkage

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ABSTRACT

An efficient protocol for the synthesis of novel lupane triterpenoid-indazolone hybrids with oxime ester linkage has been developed from naturally accessible precursor betulin. For the first time a series of betulonic acid-indazolone hybrids have been synthesized *via* an acylation of corresponding 6,7-dihydro-1*H*-indazol-4(5*H*)-one oximes with betulonic acid chloride. Diastereoselective reduction of the obtained betulonic acid conjugates with $NaBH_4$ resulted in a formation of betulinic acid-indazolone hybrids in excellent yields. The configuration of the key compounds has been fully established by X-ray and 2D NMR analysis.

1. Introduction

The hybridization of bioactive natural and unnatural compounds is one of the most promising for the design of new leading structures and the discovery of new and potent drugs in the field of medicinal chemistry [1–4]. Hybrid systems (sometimes also referred as conjugates) are defined as assembly of diverse molecular entities (in general two), natural or synthetic, to afford functional molecules, which intrinsically enhance or modulate the biological properties of individual components or, may exhibit new properties. The most widely applied definition of "hybrid molecule" defines a hybrid as a molecule that covalently connects two parent molecules that independently act at two distinct pharmacological target structures and the hybrid consequently is designed in a manner to maintain their activities at these two targets. In terms of drug design these two pharmacological actions are supposed to act in an overall synergistic manner concerning the disease to be targeted. This is why the hybrid approach gained special attention in recent times, since the complexity of several severe diseases, such as cancer and neurodegenerative disorders, can hardly be effectively targeted by one [5]. While tremendous efforts have been made over the past decades to improve the available therapeutic options, and a large number of potent chemotherapeutic anticancer agents have been identified and successfully used in clinical practice, cancer still remains a major cause of disease and death in most countries. Therefore, development of potent and specific anticancer agents is well-timed.

Triterpenoids represent a varied class of natural products which constitute the major components of some medicinal plants [6–8]. Among these, some pentacyclic triterpene members have been exploited in recent past owing to their significant role in various biological activities [9,10]. Betulin, betulonic and betulinic acids (Fig.1), naturally occurring pentacyclic lupane triterpenoids, are common secondary metabolites of plants, primarily from Betula species (Betulaceae), that exhibits a variety of biological activities including an inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, antitumor and other activities [11–14]. A large number of betulinic and betulonic acid derivatives have been developed to increase its therapeutic activity [15,16]. One well-tolerated betulinic acid derivative is NVX-207, which showed significant antitumor activity in clinical studies in canine cancer patients with treatment-resistant malignancies [17]. Some lupane triterpene hybrids with bioactive compounds showed interesting biological properties [18–21]. Previous studies suggested that an introduction of the nitrogen-containing

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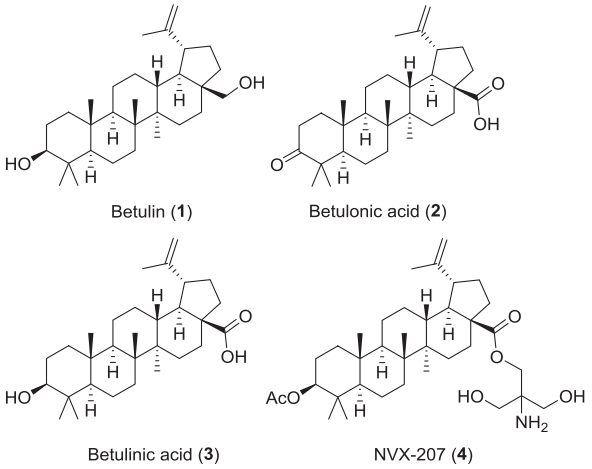

Fig. 1. Naturally occurring lupane triterpenoids **1**–**3** and an example of their semisynthetic derivative **4**.

heterocyclic rings to the pentacyclic triterpenoids can significantly improve the biological activities [15,22–24].

On the other hand, indazole skeleton is an attractive structural scaffold in medicinal chemistry and various indazole derivatives have been described to possess useful levels of antiinflammatory, antipyretic, analgesic, antimicrobial, anticancer, sodium channel blocker, antitubercular, antiviral, antihypertensive, antiglaucoma, antioxidant, antidepressant, anxiolytic, neuroprotective and antidiabetic activities [25–28].

In particular, variously substituted tetrahydroindazolones were recently recognized as important anticancer drug candidates. Depending on structural peculiarities they were proved to act either as excellent inhibitors of heat-shock protein 90 [29,30] (Fig. 2) or regulators of the mitotic motor protein Eg5 [31]. The specific inhibition of the latter prevents uncontrollable division of malignant cells. Additionally, some of tetrahydroindazolone derivatives were shown to be active against various carcinomas [32] but at the same time being less toxic than other available antitumor drugs [33].

In the light of these facts, synthetic methodologies towards tetrahydroindazole analogs continue to develop [25]. Thus, we have recently developed an efficient one-step procedure for the synthesis of fluorine-containing 6,7-dihydro-1*H*-indazol-4(5*H*)-ones [34–37], which made these compounds accessible. We have also reported synthesis of triazolyl-substituted indazolones [38] and functionalized amino-tetrahydroindazolones, including enantiomerically pure analogs and their conjugation with various carbohydrates [39–41].

The present work deals with synthesis of novel lupane triterpenoid-indazolone hybrids. Thus, in order to search for agents with potential antitumor activity and selectivity, a series of lupane triterpene hybrids were synthesized by introducing variously substituted indazolone moieties at C28 position of betulonic and betulinic acid *via* oxime ester linkage. It should be noted that introduction of an oxime function into an appropriate skeleton is a reasonable approach to the preparation of potent cytotoxic agents and many oxime derivatives have exhibited potent inhibition activities against human tumors [42–44].

2. Experimental

2.1. General

^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker AVANCE 500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C and 470 MHz for ^{19}F) or Bruker AVANCE 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shift values are given in δ (ppm) relative to the residual solvent signals for δ_H 7.26 ppm ($CDCl_3$), 2.05 ppm (acetone-d_6), 7.16 ppm (C_6D_6) and δ_C 77.16 ppm ($CDCl_3$), 29.84 ppm (acetone-d_6), 128.06 ppm (C_6D_6). α,α,α-Trifluorotoluene was used as an external standard for ^{19}F NMR spectra; the chemical shifts were converted from α,α,α-trifluorotoluene to CCl_3F. COSY, HSQC, HMBC, and NOESY experiments were carried out with the use of the standard Bruker program package. Melting points were measured on Boetius apparatus and are uncorrected. IR spectra were recorded in KBr discs on a FT-IR Perkin Elmer Spectrum 100. High-resolution mass spectra (HRMS) were recorded on a 6550 iFunnel Q-TOF LC/MS (Agilent Technologies) micromass spectrometer by electrospray ionization (ESI). Column chromatography was performed on 70–230 mesh silica gel. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel F_{254} plates with a UV indicator. Chemicals were purchased from Aldrich or Acros and used as received. Solvents were dried and freshly distilled according to common practice.

Betulonic acid (**2**) with technical product quality was obtained by reported procedure [45]. Indazolones 7**a**–**7n** were synthesized by a condensation of 2-acylcyclohexane-1,3-diones with phenyl hydrazines by described procedure [34–37]. The analytical data of indazolones 7**a**–**7f**,**7h**,**7i** were identical to those described in [34–37]. Indazolone oximes **8k**,**l** [46] and **8m**,**n** [33] were obtained according to previously published procedures.

2.2. Synthesis of the compounds

2.2.1. Synthesis of betulonic acid (*2*) from its cyclohexylammonium salt

5% Aqueous solution of phosphoric acid (185 mL) was added to a stirred suspension of cyclohexylammonium betulonate (**5**) (29.8 g, 0053 mol, 98.7% purity by HPLC) in DCM (900 mL). The layers were separated and the DCM layer was successively washed with 5% aqueous solution of phosphoric acid (6 × 60 ml), water (7 × 120 ml; until pH 5–6) and brine (150 ml). The organic layer was dried over anh. Na_2SO_4, filtered and evaporated to provide betulonic acid with 97.8% purity by HPLC (23.7 g, 96% calculated on 100% cyclohexylammonium betulonate; or 88% calculated on the initial technical betulonic acid). Mp 245–247 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.55–1.18 (m, 15H, CH, CH_2), 1.06 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.92 (s, 3H CH_3), 1.76–1.58 (m, 2H, CH, CH_2) 1.69 (s, 3H, CH_3), 2.07–1.84 (m, 3H, CH, CH_2), 2.34–2.15 (m, 2H, CH, CH_2), 2.56–2.34 (m, 2H, CH, CH_2), 3.07–2.94 (m, 1H, CH), 4.61 (brs, 1H, H_{vinyl}), 4.74 (brs, 1H, H_{vinyl}), 11.68–11.00 (brs, 1H, COOH). ^{1}H NMR data correspond to those reported in reference [47]. Elemental analysis, %: Found: C, 79.12; H, 10.29. $C_{30}H_{46}O_3$. Calcd: C, 79.25; H, 10.20.

2.2.2. Cyclohexylammonium betulonate (*5*)

A solution of cyclohexylamine (6.62 mL, 0.058 mol; 1 equiv.) in TBME (90 mL) was added to a vigorously stirred solution of technical betulonic acid [45] (92.8% purity by HPLC) (28.3 g, 0.058 mol; 1 equiv.) in TBME (260 mL) at room temperature. The resulting suspension was stirred for 40 min at room temperature, filtered and washed on the filter with TBME (5 × 20 mL). The precipitate consists of cyclohexylammonium betulonate with 96.1% purity by HPLC (31.3 g, 94%, calculated on 100% betulonic acid). The resulting technical salt was crystallized from a mixture of EtOAc/EtOH (400 mL:400 mL) and the product (29.8 g, 89% calculated on 100% betulonic acid) with 98.7% purity by HPLC was obtained. Mp 208–210 °C. IR (KBr), ν_{max}: 2940 (CH), 2865 (CH), 2755–2610 (RN^+H_3), 2215, 1710 (CO), 1635, 1525, 1455,