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The design, synthesis, and anti-inflammatory evaluation of a drug-like library based on the natural product valerenic acid

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ABSTRACT

The plant natural product, valerenic acid (**1**) was chosen as a desirable scaffold for the generation of a novel screening library due to its drug-like physicochemical parameters (such as LogP, hydrogen bond donor/acceptor counts, and molecular weight). An 11-membered amide library (**2**–**12**) was subsequently generated using parallel solution-phase synthesis and Ghosez's reagent. The chemical structures of all semi-synthetic analogues (**2**–**12**) were elucidated following analysis of the NMR, MS, UV and IR data. The structures of compounds **8** and **11** were also confirmed by X-ray crystallographic analysis. All library members were evaluated for their ability to inhibit the release of IL-8 and TNF-α. Six analogues showed moderate activity in the IL-8 assay with IC_{50} values of 2.8–8.3 μM, while none of the tested compounds showed any significant effect on inhibiting TNF-α release.

Nature has played an important role in drug discovery and development,[1] yet it still remains an under-investigated source of unique chemical scaffolds for semi-synthetic library generation, and subsequent bioassay screening for hit or lead molecules.[2–4] Natural products (NPs) are a pre-validated source of small molecules that can be used for the design of unique biologically active compounds, because they have been optimized via natural evolution for maximum interactions with biosynthetic enzymes.[2,5,6] For many decades, NPs have impacted pharmaceutical research and development by providing unique chemical diversity and complexity that was exploited as either NP drugs or as starting points for lead identification and optimization programs (i.e. hits/leads).[7,8] Between 1981 and 2014, 387 NP-based drugs were approved for use worldwide, 67 of which were unaltered NP, while 320 were NP-derived drugs.[1]

The genus *Valeriana* is made up of about 200 species, some of which are endemic to Europe and Asia, others to North America and South America.[9,10] In many cultures, the roots and rhizomes of various species are used traditionally for the treatment of insomnia and anxiety.[11] This genus has been shown to display a wide range of biological effects including anti-HIV,[12] cytotoxicity,[13] anti-convulsant,[14] and anti-hypertensive activities.[15] Previous investigations of this genus showed the presence of iridoids, sesquiterpenoids, flavone glycosides, lignans, and alkaloids.[16–21]

Valeriana officinalis is a well-known source of the bioactive sesquiterpene, valerenic acid (**1**). Pharmacological studies have shown that valerian extracts allosterically modulate $GABA_A$ receptors, with valerenic acid (**1**) described as one of the active principles underlying this observed effect.[22] Several investigations have revealed various biological activities observed for valerenic acid and its derivatives, namely cytotoxicity,[13] anxiolytic,[14,23–25] and anti-inflammatory activities.[26] *V. officinalis* has been widely researched, with the aim of understanding the activity, which has been observed *in vivo* and *in vitro*. Yet, this plant and its chemistry are still the focus of considerable investigation, aimed at finding new biological targets and effects e.g. anticoronaryspastic and antibronchospastic activities.[15]

Our research focuses on the design and semi-synthesis of drug discovery libraries based on unique NP scaffolds from various biota sources, such as fungi, plants, and marine invertebrates.[27–31] The ultimate goal of such libraries is to assist in the identification of hit or lead compounds that impact the NP drug discovery process. The use of NP scaffolds as the starting point for focused library synthesis is proving to be a powerful tool for NP-based drug discovery.[4,32]

As part of our continuing efforts to contribute to knowledge in this area of research, valerenic acid (**1**) was chosen for library

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generation and medicinal chemistry studies. Our strategy involved the use of commercially available valerenic acid (**1**), which has the advantages of saving both time and cost, as it bypasses the *de novo* synthesis for scaffold production. This sesquiterpenoid was an attractive NP scaffold for synthetic studies since it was commercially available, has a low molecular weight (MW) (234 Da), multiple stereogenic centers (n = 3) that confers a unique 3D shape, favourable calculated LogP (cLogP, 3.21), and a functional group (carboxylic acid) that is amenable to chemical modification.

Herein we report the design, parallel solution-phase synthesis and the anti-inflammatory evaluation of a library of semi-synthetic derivatives based on the NP scaffold, valerenic acid (**1**).

The amide functional group plays a major role in the composition of biological systems.[33] Being the essential chemical bond found in peptides and proteins,[34] this moiety is ubiquitous in life, as proteins are significant in many biological processes such as enzymatic catalysis, transport/storage, immune protection, and mechanical support.[33] This functionality also plays a key role in medicinal chemistry, widely occurring in biologically active compounds and pharmaceuticals, such as the antifungal drug anidulafungin,[35] the anticancer drugs flutamide and bicalutamide,[1] the multikinase inhibitor and antitumor agent sorafenib,[2] and the antibiotic tigecycline.[35]

Prior to commencing synthesis on scaffold **1**, 26 commercially available amines were initially selected, and a virtual analogue (VA) library generated (VA1–VA26), which was subsequently analyzed using ChemDraw Ultra (See Supplementary material S66 for chemical structures of VA1–VA26).[36] Physicochemical parameters such as cLogP, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and molecular weight (MW) of these VAs were determined *in silico*. The molecules with desirable physicochemical properties, as described by Lipinski's "Rule of Five" for orally bioavailable drug-like compounds (HBD $\leq$ 5, HBA $\leq$ 10, MW $\leq$ 500 and LogP $\leq$ 5),[37] were subsequently chosen for synthesis (see Supplementary material Table S65). VA1–VA11 were prioritized for synthesis, since they all had minimal or no "Rule of Five" violations.

Literature reports have shown the usage and advantages of coupling agents in amide bond formation.[33,34,38,39] However, making an appropriate choice of coupling reagent is often demanding, as a result of the plethora of reagents available.[33,34] The phenethylamine derivative (**2**) was chosen as the first synthetic target for coupling optimization studies. Trial reactions were initially attempted using the commercially available valerenic acid and three coupling reagents which included, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI),[27] *N,N,N′,N′*-tetramethylchloroformamidinium hexafluorophosphate (TCFH),[40] and 1-chloro-*N,N*,2-trimethyl-1-propenylamine (Ghosez's reagent),[41] with yields of 6%, 12%, and 91% respectively. Due to the superior yield associated with Ghosez's reagent, this was chosen as the coupling reagent of choice for all other amidation reactions undertaken on scaffold **1**.

Treatment of NP scaffold **1** with 11 primary amines, and Ghosez's reagent afforded the secondary amides (**2**–**12**) (Fig. 1 and Scheme 1) in moderate to excellent yields (32–99%). The structures of all the amide analogues were determined following 1D/2D NMR and (+)-HRESIMS data analysis (see Supplementary material). While the synthesis of some valerenic acid amide analogues have been previously reported by other researchers,[41,42] this is the first reported synthesis and characterization of amides **2**–**12**.

An example of the NMR characterization of compound **2** is given below. Briefly, the ^{1}H NMR spectrum in DMSO-d_6 indicated the presence of two methylenes [δ_H 2.74 (H-17), and 3.30 (H-16)], five aromatic protons [δ_H 7.18 (H-21), 7.20 (H-19 and H-23) and 7.28 (H-20 and H-22)], and an amide proton (δ_H 7.86); these data were consistent for amidation of the valerenic acid scaffold. Analysis of the COSY spectrum (see Fig. 2 and Supplementary material Fig. S4) of this compound identified three spin systems. Fragment H-1/H_2-2/H_2-3/H-6/H-7/H_2-8/H_2-9/H-10/H_3-14 was the first spin system identified, while the aromatic fragment

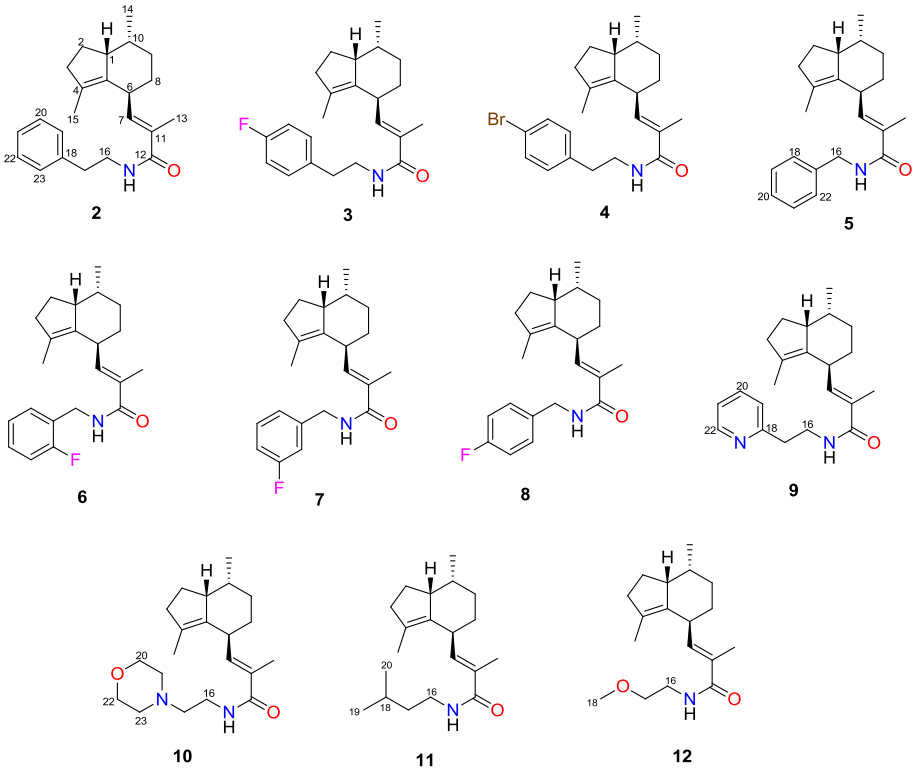

Fig. 1. Chemical structures of the amide library **2**–**12**.