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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 log P, 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, 3, 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, 17, 18, 19, 20, 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, 24, 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, 28, 29, 30, 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 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 log P (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 anidulalfungin,[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 cLog P, 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 ≤ 5, HBA ≤ 10, MW ≤ 500 and Log P ≤ 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-propenyl-amine (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 H-19/H-20/H-21/H-22/H-23 was the second. The last spin system was placed between the two methylene protons at H_2-16/H_2-17, and NH, with HMBC correlations from H_2-16 (δ_H 3.30) to C-12 (δ_C 168.6), C-17 (δ_C 35.2), and C-18 (δ_C 139.7), and from H_2-17 (δ_H 2.74) to C-16 (δ_C 40.7), C-18 (δ_C 139.7), and C-19/23 (δ_C 128.6). The amide proton also showed HMBC correlations to C-16 (δ_C 40.7) and C-12 (δ_C 168.6). These HMBC correlations in conjunction with the ROESY correlations (Fig. 2) confirmed the formation of the desired product, as well as the conservation of the configuration of the molecule.

These data enabled the chemical structure of **2** to be unambiguously assigned. The relative configuration of compound **2** was shown to be identical to the NP scaffold following the analysis of the ROESY and ^{1}H-^{1}H coupling constant data, and the magnitude of the ^{1}H NMR chemical shifts.

Slow evaporation of solutions (*n*-hexane/EtOAc) of **8** and **11** resulted in crystals suitable for X-ray diffraction. The absolute configuration of valerenic acid has been previously determined using circular dichroism,[43] and this was consistent with the X-ray data of compounds **8** and **11**, which were determined using a Cu source (Fig. 3).

All library members (**2**−**12**) together with the NP scaffold (**1**) were submitted to LEO Pharma's Open Innovation platform,[44] which is an open collaborative initiative to explore skin inflammation-related science by allowing external partners access to advanced *in vitro* assays.[45] As part of this initiative, the library was tested in two separate *in vitro* anti-inflammatory assays. One assay was looking for inhibitors of lipopolysaccharide (LPS)-induced tumor necrosis factor alpha (TNF-α) release from human primary peripheral blood mononuclear cell (PBMC), while the other assay was searching for small molecules that inhibit interleukin-8 (IL-8) release from interleukin-17 (IL-17) and TNF-α-induced primary human keratinocytes.

Psoriasis is a chronic, immune-mediated inflammatory skin disorder that occurs in approximately 3% of the world's population,[46] with 20% of patients suffering from moderate-to-severe disease.[47, 48] Symptoms include skin lesions, inflammatory plaques and silvery scaling due to keratinocyte hyperproliferation. Key disease mediators include TNF-α, and IL-17 which induces an inflammatory response measured as an increase in IL-8 secretion.[49] All compounds were tested for their ability to inhibit this inflammatory response in primary human keratinocytes. In addition, to distinguish a specific inhibition from a cytotoxic effect, a secondary readout, cell viability, was measured to determine possible general cytotoxic mechanisms.

Several library compounds (**2**–**8**) showed moderate ability to inhibit IL-17 and TNF-α induced IL-8 release from primary human keratinocytes, but only at the highest test concentrations. Furthermore, compounds **2**–**4** exhibited only a very low effect on cell

viability, strengthening the relevance of the effect on IL-8 release and indicating a possible scaffold for further development of early compounds for psoriasis treatment. All *in vitro* biological data are presented in Table 1. The well-known and clinically effective anti-inflammatory compound, betamethasone was the positive control (see Table 1), and has an effect at sub-micromolar levels without any detectable effect on cytotoxicity. However due to the mode of action, only about 50% of the IL-8 levels are inhibited at 10 μM. Some of the analogues exhibited a higher level of efficacy, but this could be due to cytotoxicity.

In regards to structure-activity relationships, the aliphatic amides, **11** and **12** showed low activity in inhibiting IL-8 release from primary human keratinocytes. Substitution of a cyclic side chain such as that in the morpholinoethyl amide derivative, **10** further reduced the biological effect. However, introduction of an aromatic ring into the side chain, such as those found in **2**–**8**, typically improved the activity. For instance, the benzyl amide series (**5**–**8**) showed the greatest inhibition of IL-8 release, but all molecules displayed a very high effect on cell viability, compared to the phenethyl amides **2**–**4**, which showed lower toxic effects.

Some of the more pertinent SAR observations are described below. The benzyl amide **5** and *ortho*-fluorobenzyl amide **6** have similar activites, 2.9 and 2.8 μM, respectively. However, moving fluorine from the *ortho*- to the *meta*- or *para*- positions of compounds **7** and **8**, respectively, decreased the activities by 1.6-fold and 1.7-fold. Interestingly, *para*-fluorophenethyl amide **3** was inactive when compared with *para*-fluorobenzyl amide **8**, indicating that the additional -CH_2- unit in the amide side chain is not required for IL-8 inhibition. Introducing nitrogen into the aromatic ring [i.e the pyridylethyl amide (**9**)] resulted in a loss of activity. Furthermore, substituting the phenethyl amide unit in **2** with a benzyl amide motif in **5**, resulted in a 2.6-fold increase in IL-8 activity, but a 6.5-fold decrease in cell viability. The same trend was observed when fluorine was introduced into the *para*-positions of **3** and **8**. This indicates that the benzyl amides showed better activity than the

phenethyl amides in inhibiting IL-17 and TNF-α induced IL-8 release from primary human keratinocytes; but the later series showed better selectivity.

Table 1. Biological Activity of Compounds **1**–**12**

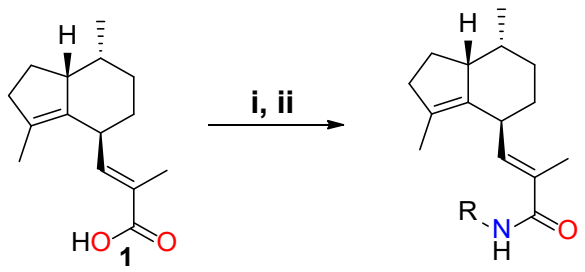
Compound	KC-IL-8 [a]		KC-Cytotoxicity	
	Abs. IC_{50} (μM)	Max.[c] (%)	Abs. IC_{50} (μM)	Max.[c] (%)
1	- [b]	4	N/A	15
2	7.5	64	N/A	11
3	N/A	44	N/A	17
4	8.3	60	N/A	24
5	2.9	84	9.0	72
6	2.8	85	8.7	72
7	4.7	79	9.6	67
8	4.9	70	9.6	61
9	N/A	14	N/A	20
10	N/A	17	N/A	12
11	N/A	22	N/A	9
12	N/A	30	N/A	7

[a] Assay control was betamethasone which showed 49% inhibition of IL-8 release at 10 μM, and an absolute IC_{50} of 300 nM in the cytotoxicity assay.
[b] IC_{50} value was not calculated.
[c] Max. denotes the maximal inhibition of either the IL-8 release or the cell viability, where the higher number represent a more efficacious biological effect. Typically, the Max. effect is achieved at the highest test concentration which was 10 μM.
N/A = Not active at 10 μM.
KC = human keratinocytes.

The endotoxin LPS is one of the most potent inducers of inflammatory response. LPS activates the Toll-like Receptor 4 (TLR4), which is responsible for activating the innate

immune system, resulting in the release of the pro-inflammatory cytokine TNF-α.[50] All compounds were tested for the ability to inhibit LPS-induced TNF-α release from primary human peripheral blood mononuclear cells (PBMC), as well as their general effect on cell viability. None of the tested compounds showed any significant effect (< 15% inhibition) on reducing the LPS-induced release of TNF-α from primary human PBMC.

In summary, 11 amide derivatives based on the valerenic acid scaffold were synthesized and evaluated for their *in vitro* anti-inflammatory activity in IL-8 and TNF-α assays. 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 TNF-α.



Scheme 1. Reagents and conditions. (**i**) anhydrous CH_2Cl_2, 1-Chloro-*N*,*N*,2-trimethylpropenylamine, 0 °C → rt, 4 h (**ii**) amine, 0 °C → rt, 16 h (yield = 32−99%).

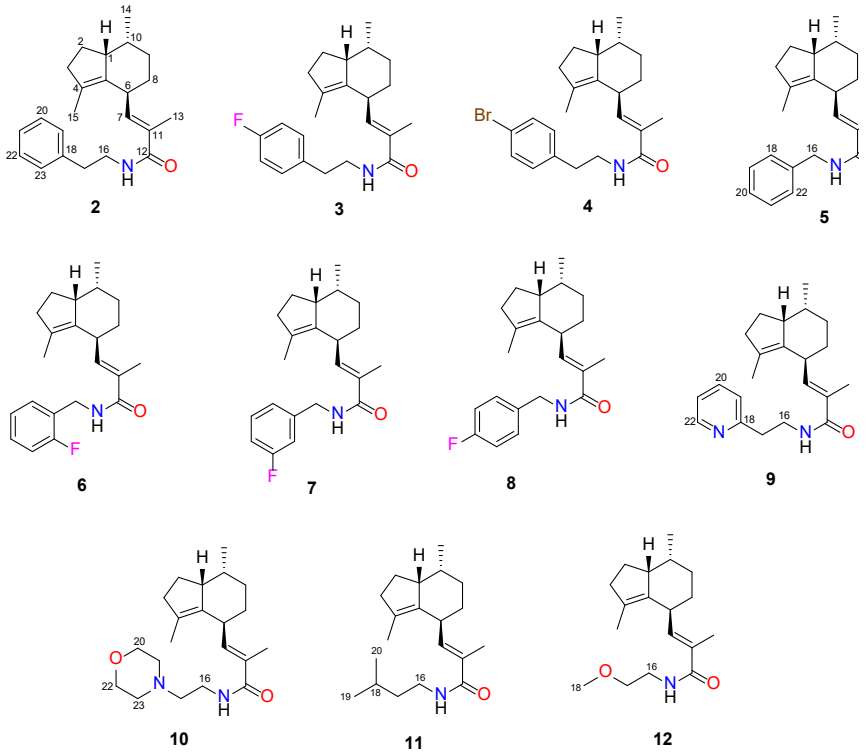


Fig. 1 Chemical structures of the amide library **2**–**12**.

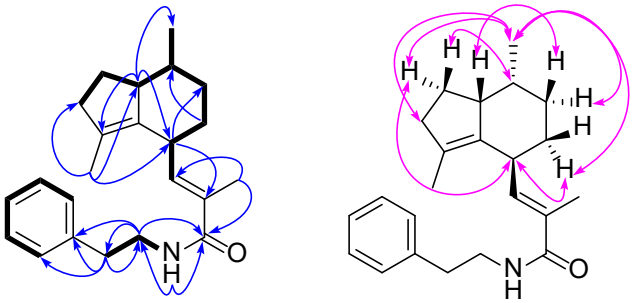


Fig. 2 COSY (bold line), key HMBC (→), and ROESY (↔) correlations for compound **2**.

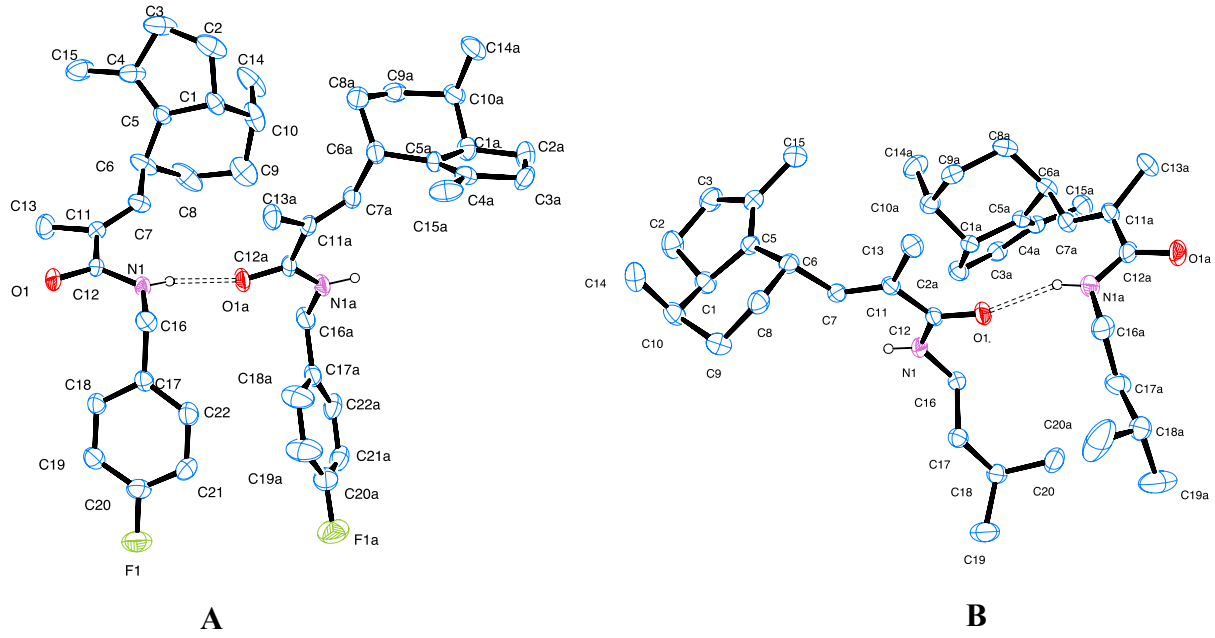


Fig. 3 A, ORTEP of **8** showing two independent molecules held together by an N-H..O hydrogen bond. The minor component disordered atoms of the bicyclic ring systems have been omitted for clarity. **B**, ORTEP of **11** showing two independent molecules of **11** held together by an N-H..O hydrogen bond.

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A. Supplementary material

Full details of the data collection and refinement and tables of atomic coordinates, bond lengths and angles, and torsion angles have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1488223 - 1488224). Copies can be obtained free of charge on application at the following address: http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/

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