# Saponins as novel TNF-α inhibitors: isolation of saponins and a nor-pseudoguaianolide from *Parthenium hysterophorus*†

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Two novel saponins and a 13-nor-pseudoguaianolide designated as hysterolactone were isolated from *Parthenium hysterophorus*. The two saponins were found to be potent inhibitors of TNF-α. Their mode of inhibition was studied through molecular modeling. The wet lab results were in concordance with the data obtained from docking experiments.

## Introduction

Terpenoids are the largest group of natural products with more than 25 000 structures reported.[1] They have a variety of roles in mediating antagonistic and beneficial interactions among organisms.[2] *Parthenium hysterophorus* commonly known as the congress grass, an aggressive weed of American and Caribbean origin, belongs to the family Asteraceae, which is very well known for the large number of sesquiterpene lactones it possesses.[3] *P. hysterophorus* is known to have two major pseudoguaianolides namely parthenin and coronopilin along with some minor constituents, which are mostly sesquiterpene lactones.[4] Their cytotoxic and anti-cancer properties are well known in the literature.[5] Despite the fact that a number of phytochemicals have been identified from the plant in the past by different research groups, it still remains a source of novel molecules.

In this paper we describe the isolation and structure elucidation of two novel saponins and a nor-pseudoguaianolide, together with three known compounds, *i.e.* coronopilin,[6] 2β-hydroxy-coronopilin[7] and 3,7-dimethoxy-3′,4′,5,6-tetrahydroxy flavonoid from the methanolic extract of aerial parts of the *P. hysterophorus*.[8] The presence of saponins which are also new to the literature is being reported for the first time from the species. Besides, a nor-pseudoguaianolide (a new natural product) has not been previously reported from any source. The saponins which are present in comparatively higher quantities were tested for TNF-α inhibitory activity to assess their anti-inflammatory potential and their use as therapeutic agents. Also an attempt has been made to establish their mode of action through *in silico* modeling.

## Results and discussion

Saponins **1**, **2** and hysterolactone (**3**) were isolated from the methanolic extract of the aerial parts of *P. hysterophorus*. They were purified by column chromatography using a chloroform : MeOH mixture as eluant. Compound **1**, a saponin of oleanolic acid, was obtained as a white powder after purifying through column chromatography. Its molecular formula was determined to be $C_{48}H_{78}O_{18}$ from elemental analysis, and MS showed a pseudo molecular ion peak at $m/z$ 965 $[M + Na]^+$ corresponding to MW 942. The structure of compound **1** was established from its NMR studies including 2-D experiments. The aglycon part was identified as oleanolic acid and the sugar moieties as glucose by acid hydrolysis followed by acetylation. The presence of three sugar units was confirmed from the observation in the ^{1}H NMR spectrum of the signals for three anomeric protons δ 4.86 (d, $J = 7.5$ Hz), 5.20 (d, $J = 7.6$ Hz) and 6.30 (d, $J = 8.0$ Hz), using co-relations with three anomeric carbon atoms at δ 105.7, 104.2 and 91.0 respectively in the HSQC spectrum. The ^{1}H and ^{13}C NMR data assigned from COSY, HSQC and HMBC experiments allowed the identification of the three sugar units as a 3-substituted β-D-glucopyranosyl (Glc I), a terminal β-D-glucopyranosyl (Glc II) and a β-D-glucopyranosyl ester (Glc IV). The ^{13}C NMR spectrum indicated that one sugar unit attached to C-3 (δ 88.3) and another to C-28 (δ 179.1) of oleanolic acid. Sequencing of the glucosidic chain was achieved by analysis of HMBC and COSY experiments. Cross peak correlations were clearly depicted in the HMBC spectrum between H-1′ (δ 4.86) and C-3 (δ 88.3) of oleanolic acid, H-3 (δ 3.34) of oleanolic acid and C-1′ (δ 105.7), H-1″ (δ 5.2) and C-4′ (δ 80.6), H-4′ (δ 4.15) and C-1″ (δ 104.2) respectively. The 1→4 linkage was further established by COSY between H-1″ (δ 5.2) and H-4′ (δ 4.15) and confirmed by downfield shifting of the C-4′ carbon signal at δ 80.6. Furthermore, the linkage between the two sugar moieties was assigned as β due to $J = 7.6$ Hz for H-1″. Other HMBC correlations were observed between H-1′′′′ (δ 6.3) and C-28 (δ 179.1) implying an ester linkage between the Glc IV and oleanolic acid. Thus the structure of compound **1** was assigned as 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranosyl-oleanolic acid (Fig. 1).

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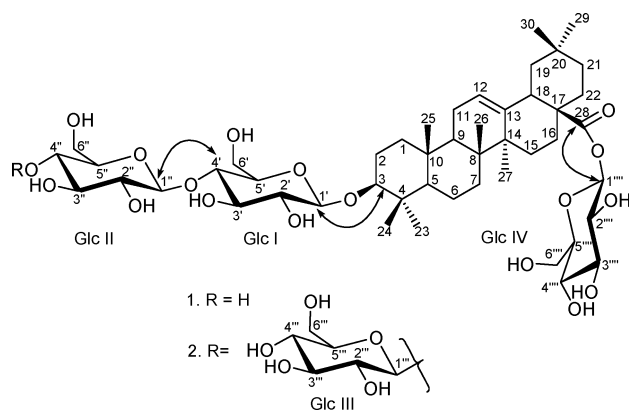
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**Fig. 1** Structure and important HMBC correlations of compounds **1** and **2**.

Compound **2** was also obtained as a white powder after purifying through column chromatography. Its molecular formula was determined to be $C_{54}H_{88}O_{23}$ from elemental analysis, and MS displayed a pseudo molecular ion peak at $m/z$ 1127 [M + Na]+ corresponding to MW 1104. The structure of compound **2** was also established through NMR studies including 2-D experiments. The aglycon part was again identified as oleanolic acid and the sugar moieties as glucose by acid hydrolysis followed by acetylation. The presence of four sugar units was confirmed from the observation in the ^{1}H NMR and HSQC spectra of four anomeric protons $\delta$ 4.82 (d, $J$ = 7.7 Hz), 5.08 (d, $J$ = 7.9 Hz), 5.25 (d, $J$ = 7.9 Hz) and 6.26 (d, $J$ = 8.1 Hz), and their correlations with four anomeric carbon atoms at $\delta$ 106.5, 104.8, 104.4 and 95.7 respectively. The ^{1}H and ^{13}C NMR data assigned from COSY, HSQC and HMBC experiments allowed the identification of the four sugar units as a 3-substituted β-D-glucopyranosyl (Glc I), another β-D-glucopyranosyl (Glc II), a terminal β-D-glucopyranosyl (Glc III) and a β-D-glucopyranosyl ester (Glc IV). The ^{13}C NMR spectrum indicated that one sugar unit attached to C-3 ($\delta$ 89.1) and another to C-28 ($\delta$ 178.5) of oleanolic acid. Sequencing of the glucosidic chain was achieved by HMBC and COSY experiments. Cross peak correlations were clearly depicted in the HMBC spectrum between H-1′ ($\delta$ 4.82) and C-3 ($\delta$ 89.1) of oleanolic acid, H-3 ($\delta$ 3.26) of oleanolic acid and C-1′ ($\delta$ 106.5), H-1″ ($\delta$ 5.08) and C-4′ ($\delta$ 80.6), H-4′ ($\delta$ 4.19) and C-1″ ($\delta$ 104.8), H-1‴ ($\delta$ 5.25) and C-4″ ($\delta$ 81.1), besides H-4″ ($\delta$ 4.29) and C-1‴ ($\delta$ 104.4) respectively. The 1→4 linkage between the three sugar moieties was further established by COSY, between H-1″ ($\delta$ 5.08) and H-4′ ($\delta$ 4.19), H-1‴ ($\delta$ 5.25) and H-4″ ($\delta$ 4.29) and confirmed by downfield shifting of the C-4′ and C-4″ carbon signals at $\delta$ 80.6 and 81.1 respectively. Furthermore, the linkage between the three sugar moieties was assigned as β due to $J$ = 7.9 Hz for both H-1″ and H-1‴. Other HMBC correlations were observed between H-1⁗ ($\delta$ 6.26) and C-28 (178.5) implying an ester linkage between the Glc IV and oleanolic acid. Thus the structure of compound **2** was assigned as 3-O-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-O-β-D-glucopyranosyl-oleanolic acid (Fig. 1). The complete ^{1}H and ^{13}C NMR analysis of **1** and **2** is presented in Table 1.

Hysterolactone (**3**) was isolated as a colorless solid and its molecular formula was determined to be $C_{14}H_{16}O_4$ from elemental

analysis, and MS showed a pseudo molecular ion peak at $m/z$ 271 [M + Na]+ corresponding to MW 248. The structure of **3** was established from its NMR spectra including 2-D experiments. The ^{1}H NMR of **3** displayed most of the proton signals related to parthenin. However, the two exocyclic α,β-unsaturated protons signals of the lactone moiety were conspicuously absent. The additional two doublets at $\delta$ 2.43 and 2.60 ($J$ = 18.5 Hz) integrating for one proton each were attributed to two geminal protons at C-11. Non-formation of acetate indicated the absence of a free hydroxyl group as also supported by the IR spectrum. A singlet at $\delta$ 4.52 was assigned to H-6, which otherwise appears as a doublet in parthenin, thus indicating the absence of a vicinal C-7 proton. The ^{13}C NMR spectrum of **3** revealed the presence of 14 carbons which also included two quaternary carbons at $\delta$ 95.9 and 90.4 for C-1 and C-7 respectively, both having an oxygen linkage and a third signal at $\delta$ 91.3 for C-6.

The stereo-chemical assignments of hysterolactone **3** were established on the basis of NOESY and HMBC experiments. The NOESY correlations between C-13 Me/C-14 Me and C-13 Me/H-6 unambiguously established a *trans* geometry at the γ-lactone intersection. The correlation between H$_{6\beta}$ and H$_{9\beta}$ further corroborated the above observations. The absolute stereochemical assignments in **3** are established in correlation with the absolute configuration of parthenin, based on a biogenetic principle that a class of chiral natural products in a given tissue must have the same (or derivable therefrom) absolute stereochemistry at a common reference point (unless each compound is formed by a one-step process, which is considered less likely).[9] This is a typical example of the isolation of a novel nor-pseudoguaianolide from a *Parthenium* species having a *trans* geometry at the lactone junction. Fig. 2 depicts some important correlations in compound **3**. Besides, compounds **1**, **2** and **3**, other molecules *i.e.* parthenin (**4**),[4] coronopilin (**5**),[6] 2β-hydroxy coronopilin (**6**)[7] and 3,7-dimethoxy-3′,4′,5,6-tetrahydroxyflavonoid (**7**)[8] were also isolated (Fig. 3).
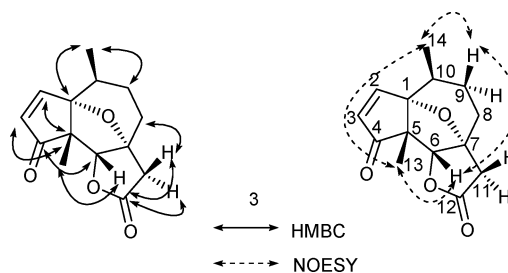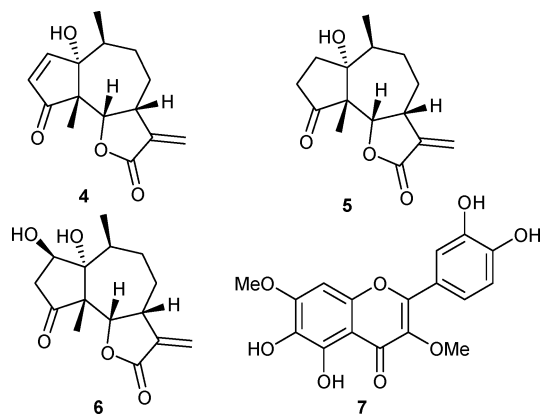


**Fig. 2** Some important HMBC and NOESY correlations of compound **3**.

## Biological activity

Triterpene saponins are known for a wide range of biological activities like antifungal,[10] anti-leishmanial,[11] antitumor,[12] immunomodulatory[13] and molluscicidal activity.[14] Furthermore, triterpene saponins having a cellobioside (β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl) moiety at C-3 as in saponin **1** have been shown to inhibit cholesterol absorption in animals.[15] In addition some alfalfa saponins have also been shown to inhibit

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**Table 1** $^1$H and $^{13}$C NMR chemical shifts (ppm) of aglycone and sugar moieties of compounds **1** and **2** in pyridine-$d_5$

| Chemical shifts (ppm) for aglycone moieties | | | | | | Chemical shifts (ppm) for sugar moieties | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | | **2** | | | | **1** | | | **2** | | |
| Carbon | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | | Carbon | $\delta_C$ | $\delta_H$ | Carbon | $\delta_C$ | $\delta_H$ | |
| 1 | 38.7 | 1.73, 1.21 | 37.0 | 1.72, 1.32 | C-3 | | | | | | | |
| 2 | 25.6 | 2.12, 1.78 | 26.4 | 2.09, 1.76 | Glc I | 1′ | 105.7 | 4.86 (d, 7.5) | 1′ | 106.5 | 4.82 (d, 7.7) | |
| 3 | 88.3 | 3.34 (dd, 11.6, 3.3) | 89.1 | 3.26 (dd, 11.5, 4.1) | | 2′ | 74.5 | 4.02 | 2′ | 75.2 | 4.01 | |
| 4 | 33.3 | — | 38.7 | — | | 3′ | 75.7 | 4.05 | 3′ | 76.3 | 4.06 | |
| 5 | 55.8 | 0.77 (d, 12) | 55.8 | 0.77 (d, 11.7) | | 4′ | 80.6 | 4.15 | 4′ | 80.6 | 4.19 | |
| 6 | 17.8 | 1.42 | 18.4 | 1.48 | | 5′ | 76.1 | 3.87 | 5′ | 76.8 | 3.87 | |
| 7 | 32.4 | 1.86, 1.71 | 32.4 | 1.84, 1.72 | | 6′ | 61.6 | 4.55, 4.30 | 6′ | 62.2 | 4.53, 4.28 | |
| 8 | 38.0 | — | 41.6 | — | GlcII | 1″ | 104.2 | 5.2 (d, 7.6) | 1″ | 104.8 | 5.08 (d, 7.9) | |
| 9 | 48.9 | 1.61 | 48.0 | 1.59 | | 2″ | 73.3 | 4.09 | 2″ | 74.2 | 4.02 | |
| 10 | 31.8 | — | 39.8 | — | | 3″ | 77.4 | 4.17 | 3″ | 76.2 | 4.09 | |
| 11 | 23.0 | 1.94 | 23.4 | 1.89 | | 4″ | 70.2 | 4.49 | 4″ | 81.1 | 4.29 | |
| 12 | 124.8 | 5.43 | 122.8 | 5.42 | | 5″ | 78.1 | 3.86 | 5″ | 76.5 | 3.89 | |
| 13 | 145.8 | — | 144.8 | — | | 6″ | 61.5 | 4.58, 4.36 | 6″ | 62.2 | 4.50, 4.30 | |
| 14 | 41.0 | — | 41.7 | — | GlcIII | | | | 1‴ | 104.4 | 5.25 (d, 7.9) | |
| 15 | 28.4 | 2.34 | 28.3 | 2.32 | | | | | 2‴ | 74.0 | 4.03 | |
| 16 | 23.9 | 2.16, 1.90 | 23.8 | 2.00, 1.81 | | | | | 3‴ | 78.1 | 4.15 | |
| 17 | 45.5 | — | 46.1 | — | | | | | 4‴ | 71.1 | 4.29 | |
| 18 | 40.8 | 3.18 (dd, 13.7, 3.8) | 39.9 | 3.15 (dd, 13.5, 3.8) | | | | | 5‴ | 78.8 | 3.98 | |
| 19 | 46.1 | 1.76, 1.26 | 47.0 | 1.73, 1.34 | | | | | 6‴ | 61.6 | 4.48, 4.38 | |
| 20 | 30.1 | — | 30.7 | — | C-28 | | | | | | | |
| 21 | 33.2 | 1.44 | 32.5 | 1.43 | Glc IV | 1‴′ | 91.0 | 6.3 (d, 8.0) | 1‴′ | 95.7 | 6.26 (d, 8.1) | |
| 22 | 32.1 | 2.14, 1.96 | 30.8 | 2.11, 1.93 | | 2‴′ | 74.1 | 4.21 | 2‴′ | 74.6 | 4.21 | |
| 23 | 27.8 | 1.31 | 28.2 | 1.28 | | 3‴′ | 77.6 | 4.26 | 3‴′ | 76.4 | 4.24 | |
| 24 | 16.3 | 1.04 | 16.9 | 0.98 | | 4‴′ | 70.4 | 4.36 | 4‴′ | 71.4 | 4.32 | |
| 25 | 14.9 | 0.85 | 15.6 | 0.85 | | 5‴′ | 78.5 | 4.03 | 5‴′ | 79.2 | 4.01 | |
| 26 | 16.8 | 1.11 | 17.4 | 1.03 | | 6‴′ | 62.6 | 4.45, 4.38 | 6‴′ | 62.4 | 4.44, 4.37 | |
| 27 | 25.4 | 1.27 | 26.1 | 1.25 | | | | | | | | |
| 28 | 179.1 | — | 178.5 | — | | | | | | | | |
| 29 | 22.9 | 0.92 | 23.7 | 0.92 | | | | | | | | |
| 30 | 32.5 | 0.89 | 33.2 | 0.89 | | | | | | | | |



**Fig. 3** Structures of compounds **4–7**.

cholesterol absorption in animals.[16] Besides, triterpene saponins have also been shown to represent a promising class of multiple-target anti-diabetic agents that exert hypoglycemic effects through glycogen phosphorylase inhibition.[17] The anti-inflammatory activity of saponins has also attracted the attention of chemists in the recent past.[18] We envisaged testing of the isolated saponins for their anti-inflammatory potential and consequently TNF-α inhibitory activity. TNF-α is a potent inflammatory mediator secreted from macrophages and has revealed its importance in

many macrophage associated reactions such as immune and inflammation processes.[19] Besides, TNF-α is also involved in insulin resistance through an inhibitory effect on insulin sensitivity and, hence, it may have implications as an anti-diabetic agent also.[20]

TNF-α inhibition studies were carried out on activated murine neutrophils. Neutrophils are involved in processes like phago-cytosis, release of proteolytic enzymes, generation of active free radicals, and synthesis of cytokines, chemokines and lipid media-tors causing inflammation. Recent studies have demonstrated that the recruitment of leukocytes into an inflamed area is mediated by various peptides and other factors which in turn amplify the inflammatory response by their effects on macrophages and lymphocytes.[21] Flow cytometric analysis of the saponins **1** and **2** were carried out to assess the TNF-α inhibitory potential in murine neutrophils. Flow cytometric studies of compound **3** could not be carried out due to the paucity of the molecule. TNF-α, a pro-inflammatory chemical in nature plays a major role in the pathogenesis of septic shock induced by LPS (lipopolysaccharide, Sigma Chemicals, USA) endotoxin injection.[22] LPS is an im-portant triggering factor for the systemic inflammatory response *in vivo*.[23] The intracellular expression of TNF-α was estimated in a gated population of neutrophils treated with graded doses of saponins **1** and **2** through flow cytometry, which is a powerful analytical tool that enables the characterization of cells and sub-cellular organelles as well as particles on the basis of size and
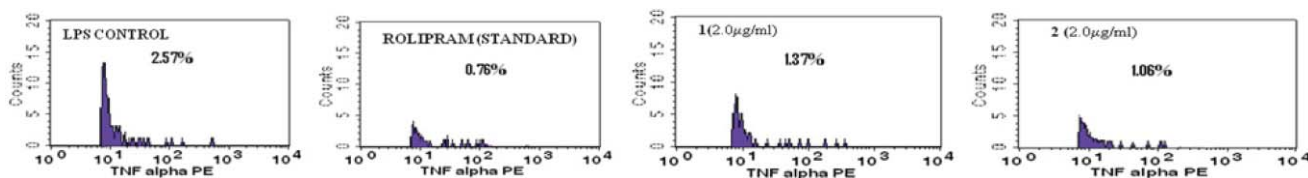
**Fig. 4** Histograms showing percentage inhibition of TNF-α expression by compounds **1** and **2** in LPS activated murine neutrophils.

granularity (light scattering characteristics) and a number of different parameters defined by fluorescent probes (including fluorescent antibodies and dyes).[24] Neutrophil separation was carried out by the Ficoll Hypaque gradient method.[25] Cells were stimulated with LPS and incubated with test compounds for 3 h, in a $CO_2$ incubator. For the permeabilisation of the cells, the permeabilising solution (BD-Biosciences) was added and then the cells were incubated for 10 min. These cells were then labeled with conjugated anti-mouse TNF-α monoclonal antibody (BD-Biosciences) and the incubation was carried out for 30 min in the dark. After washing with PBS (phosphate buffered saline, Sigma Chemicals, USA), the samples were acquired directly on a BD-LSR Flow Cytometer (BD-LSR, Beckton-Dickinson Biosciences, CA, USA). A fluorescence trigger was set on the PE (FL1) parameter of the gated neutrophil populations (10 000 events) and fluorescence compensation, data analysis, and data presentation were performed using Cell Quest Pro software (Beckton-Dickinson Biosciences, CA, USA).

The results illustrated that saponins **1** and **2** significantly decreased intracellular TNF-α expression in LPS activated neutrophils in a dose related manner with the maximum effect at the higher dose level of 2 µg ml⁻¹ where the percentage inhibition was 46.69% and 58.75% respectively. Whereas rolipram, a standard TNF-α inhibitor showed 70.42% inhibition at 100 µg ml⁻¹, when compared with LPS stimulated untreated neutrophils (Fig. 4). Saponin **2** also significantly suppressed the intracellular TNF-α expression level at a dose of 1 µg ml⁻¹ (% inhibition: 49.02%) (Table 2). These findings demonstrate saponins **1** and **2** to have a potent TNF-α inhibitory activity, indicating their possible anti-inflammatory potential, which is suggestive of further development for therapeutic usefulness.

**Table 2** Estimation of intracellular TNF-α expression in LPS activated murine neutrophils[a]

| Compound | Concentration/µg ml⁻¹ | Mean ± SE | % TNF-α estimation |
|---|---|---|---|
| LPS control | — | 2.57 ± 0.09 | — |
| **1** | 0.25 | 2.25 ± 0.01 | 12.45↓ |
| | 0.5 | 2.07 ± 0.06 | 19.45↓ |
| | 1.0 | 1.83 ± 0.05 | 28.79↓ |
| | 2.0 | 1.37 ± 0.05* | 46.69↓ |
| **2** | 0.25 | 2.09 ± 0.02 | 18.67↓ |
| | 0.5 | 1.95 ± 0.04 | 24.12↓ |
| | 1.0 | 1.31 ± 0.02* | 49.02↓ |
| | 2.0 | 1.06 ± 0.05** | 58.75↓ |
| Rolipram (standard) | 100 | 0.76 ± 0.06** | 70.42↓ |

[a] %↓: indicates suppression of TNF-α expression; no. of observations = 3; $p$-value: * <0.01; ** <0.001 Student's 't' test.

## In silico approach for the study of the mode of inhibition of TNF-α with 1 and 2

It was of interest to study the mode of inhibition and to have the mechanistic aspects explored. In this direction, ligand receptor docking studies were carried out using **1**, **2** and rolipram as ligands and TNF-α as the receptor (using Cerius2 LigandFit and Schrodinger Glide modules). In the docking studies, the ligands were docked on 2AZ5 (dimeric form) using the 3D-crystal structure of TNF-α co-crystallized with inhibitor **8** used by He *et al.*[26] (Fig. 5).
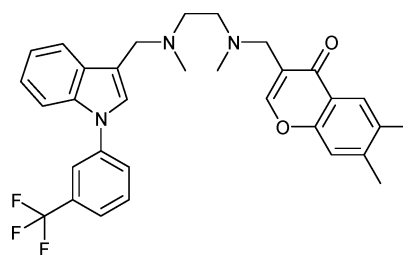


**Fig. 5** Structure of compound **8**.

The docking of **2** and rolipram on the TNF-α dimer 2AZ5 with LigandFit showed the docked scores which were found to be highest for **2** followed by **1** and rolipram respectively (dock scores being 115, 110 and 84 respectively). The consensus score was also found in the order **2** > **1** > rolipram with values of 29, 21 and 18 respectively. The same trend was also observed using the Glide module wherein the glidescores for the ligands **1**, **2** and rolipram were observed to be −10.83, −12.39 and −6.18 respectively. The results obtained from the dock experiments have been found to be in conformity with the wet lab results. He *et al.*[26] in their pioneering work on the mechanistic aspects of TNF-α inhibition reportedly demonstrated that TNF-α inhibition is more effectively achieved when the inhibitor binds to the dimer for the stabilization of the latter after the ligand forms a transient complex which triggers fast dissociation of one of the monomers, leading thereby to the stabilization of the dimer; the consequence of the operation being effective inhibition of the receptor. Moreover, the trimer (1TNF) is involved in hydrogen bond formation of the amino acid residues Arg6, Tyr59, Arg103 and Gly121 of the dissociable monomer with the residues Glu102, Gly148 and Tyr151 of monomer A, and Val123 and Gln125 of monomer B. Also, from our docking studies it was observed that saponin **2** displayed a strong hydrogen bond formation with Gln61, Gln149 and Tyr151 of monomer A (Table 3). The strong association of **2** with Tyr151 deprives partially the reattachment of the dissociable monomer C with the dimer again to its active form leading thereby to the stabilization of the dimer. In docking experiments, it was also

**Table 3** Interaction of AA's of 2AZ5 (dimer) with compounds **1**, **2** and rolipram

| Compound | Residues involved in binding | H bonds |
|---|---|---|
| **1** | Monomer A: Gln61 Gly121 Gly122 Ile155 Leu57 Leu120 Ser60 Tyr59 Tyr151 Tyr119 | 1 H bond with Lys98 (monomer B) |
| | Monomer B: Gly121 Leu57 Leu120 Lys98 Tyr59 Tyr119 Tyr151 | |
| **2** | Monomer A: Gln61 Gln149 Leu57 Leu120 Gly121 His15 Ile155 Tyr119 Tyr59 Tyr151 | 1 H bond with Gln149 (monomer A) |
| | | 1 H bond with Gln61 (monomer A) |
| | Monomer B: Gly121 Leu57 Tyr59 Tyr119 | 1 H bond with Tyr151 (monomer A) |
| Rolipram | Monomer A: Gln61 Gly121 Gly122 Leu57 Leu120 Ser60 Tyr59 Tyr119 Tyr151 | No H bonds |
| | Monomer B: Leu57 Ser60 Tyr59 Tyr119 Tyr151 | |

observed that the contact residues of **1**, **2** and rolipram with the dimer are almost the same as reported by He *et al.*[26] However, in the complex between the dimer and compound **2**, Gln61 and Tyr151 (from the reported residues) are involved in H-bond formation with the ligand, besides the involvement of Lys98 for an additional H-bond formation. This effect (H-bond formation) is not observed for **1** and rolipram, thereby explaining the better stabilization of the dimer by **2** than the other compounds (Fig. 6).
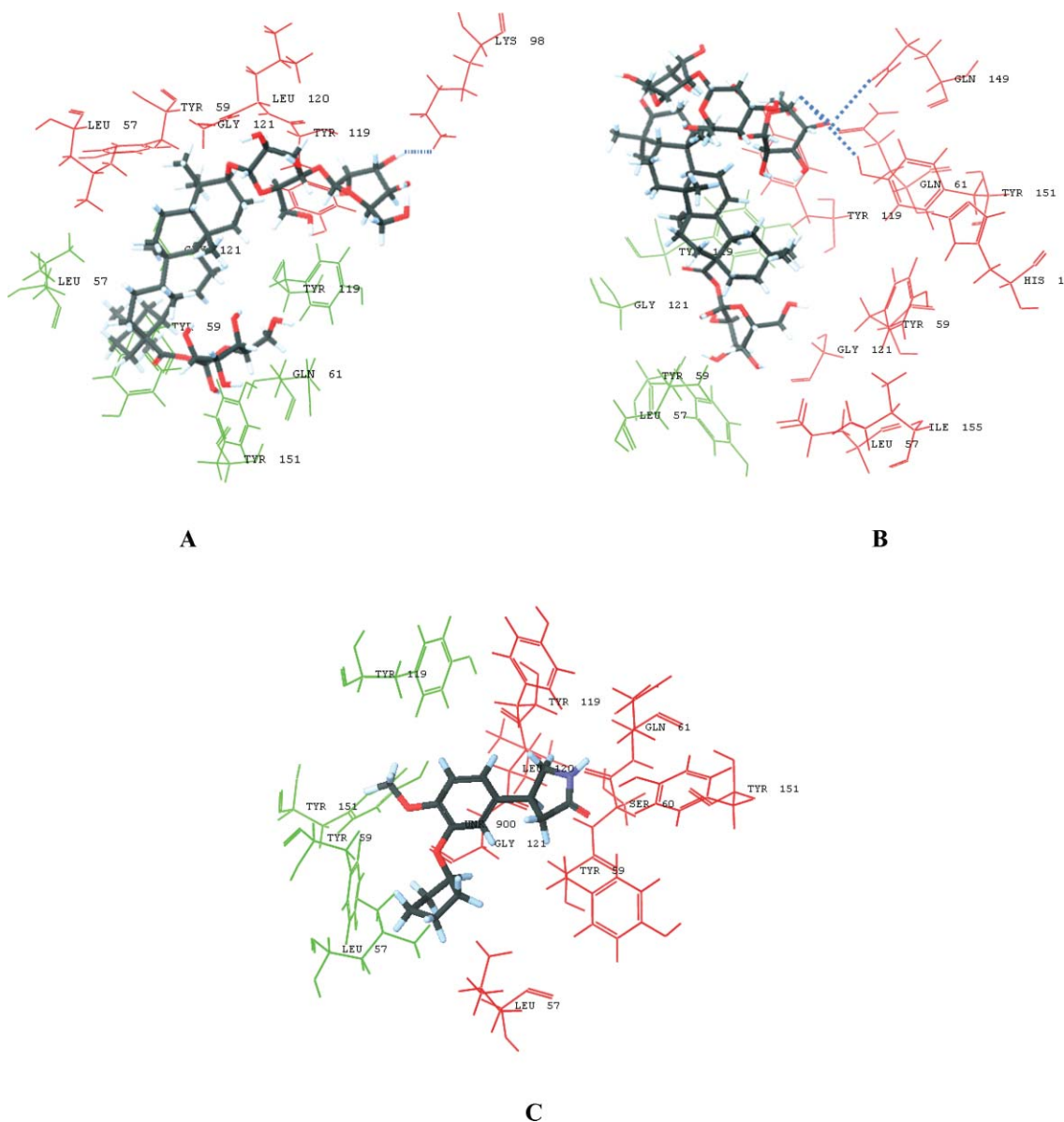


**Fig. 6** A = compound **1** with 2AZ5 (dimer); B = compound **2** with 2AZ5 (dimer); C = rolipram with 2AZ5 (dimer).

## Conclusions

In summary, three novel compounds (**1–3**) have been isolated from *P. hysterophorus* and their molecular structures established. It is also the first report of the isolation of triterpene saponins from *Parthenium* species including that of a nor-pseudoguaianolide skeleton having a tetrahydrofuran ring in its structure, though a partly resembling guaianolide has been reported earlier from a different source.[27] The TNF-α inhibitory study on human neutrophils has revealed that the isolated saponins have a great potential of being developed into a therapeutic agent. The comparatively higher TNF-α inhibition shown by compound **2** may be attributed to the better stabilization of the receptor dimer involving participation of Lys98 in an additional H-bond formation as corroborated by the docking study.

## Spectral Data

### 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranosyl-oleanolic acid (1)

White solid, mp 192–194 °C; [α]$_D$ +3.0 (*c* 0.25, MeOH); $^1$H and $^{13}$C NMR in Table 1. Anal calcd for $C_{48}H_{78}O_{18}$: C, 61.13; H, 8.34. Found: C, 61.34; H, 8.61%. ESI-MS (*m/z*): 965 [M + Na]$^+$.

### 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranosyl-oleanolic acid (2)

White solid, mp 257–260 °C; [α]$_D$ +6.2 (*c* 0.1, H$_2$O); $^1$H and $^{13}$C NMR in Table 1. Anal calcd for $C_{54}H_{88}O_{23}$: C, 58.68; H, 8.03. Found: C, 58.93; H, 8.29%. ESI-MS (*m/z*): 1127 [M + Na]$^+$.

### Hysterolactone (3)

Colorless solid, mp 192–195 °C; [α]$_D$ +6.0 (*c* 0.5, CHCl$_3$); IR: $\nu_{max}$ (neat) 2923, 2853, 2361, 1763, 1716 cm$^{-1}$. $^1$H NMR (500 MHz, CDCl$_3$): δ 7.39 (d, 1H, *J* = 5.9 Hz, H-2), 6.12 (d, 1H, *J* = 5.9 Hz, H-3), 4.52 (s, 1H, H-6), 2.43 & 2.60 (2×d, 2H, *J* = 18.5 Hz, H-11), 2.80 (m, 1H, H-7), 1.79–2.10 (m, 4H, H-8 & H-9), 1.51 (3H, s, H-15), 1.09 (d, 3H, *J* = 7.5 Hz, H-14). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 15.2 (C-13), 18.9 (C-14), 26.2 (C-9), 29.6 (C-8), 34.8 (C-10), 38.5 (C-11), 62.6 (C-5), 90.4 (C-7), 91.3 (C-6), 95.9 (C-1), 134.0 (C-3), 157.0 (C-2), 172.9 (C-12), 205.1 (C-4). Anal calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.50; H, 6.77%. ESI-MS (*m/z*): 271 [M + Na]$^+$.

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## Notes and references

1 *Dictionary of Natural Products*, ed. J. Buckingham, Chapman and Hall, London, 1994.

2 (*a*) J. Gershenzon and N. Dudareva, *Nat. Chem. Biol.*, 2007, **3**, 408; (*b*) B. A. Shah, G. N. Qazi and S. C. Taneja, *Nat. Prod. Rep.*, 2009, **26**, 72.

3 A. K. Picman, *Biochem. Syst. Ecol.*, 1986, **14**, 255.

4 (*a*) W. Herz, H. Watanabe, M. Miyazaki and Y. Kishida, *J. Am. Chem. Soc.*, 1962, **84**, 2601; (*b*) J. R. DelaFuente, L. Novara, S. R. Alarcon, O. J. Diaz, M. L. Uribura and V. E. Sosa, *Phytochemistry*, 1997, **45**, 1185; (*c*) C. Ramesh, N. Ravindranath, B. Das, A. Prabhakar, J. Bharatam, K. Ravikumar, A. Kashinatham and T. C. McMorris, *Phytochemistry*, 2003, **64**, 841; (*d*) B. A. Shah, S. C. Taneja, V. K. Sethi, P. Gupta, S. S. Andotra, S. S. Chimni and G. N. Qazi, *Tetrahedron Lett.*, 2007, **48**, 955; (*e*) B. A. Shah, R. Kaur, P. Gupta, A. Kumar, V. K. Sethi, S. S. Andotra, J. Singh, A. K. Saxena and S. C. Taneja, *Bioorg. Med. Chem. Lett.*, 2009, DOI: 10.1016/j.bmcl.2009.05.089.

5 (*a*) S. M. Kupchan, M. A. Eakin and A. M. Thomas, *J. Med. Chem.*, 1971, **14**, 1147; (*b*) D. Mew, F. Blaza, G. H. N. Towers and J. G. Levy, *Planta Med.*, 1982, **45**, 23.

6 A. K. Picman, G. H. N. Towers and P. V. S. Rao, *Phytochemistry*, 1980, **19**, 2206.

7 V. K. Sethi, S. K. Koul, S. C. Taneja and K. L. Dhar, *Phytochemistry*, 1987, **26**, 3359.

8 M. C. Shen, E. Rodriguez, K. Kerr and T. J. Marby, *Phytochemistry*, 1976, **15**, 1045.

9 S. Dev, *Pure Appl. Chem.*, 1979, **51**, 837.

10 P. Bowyer, B. R. Clarke, P. Lunness, M. J. Daniels and A. E. Osbourn, *Science*, 1995, **267**, 371.

11 N. Germonprez, L. Maes, L. V. Puyvelde, M. V. Tri, D. A. Tuan and N. D. Kimpe, *J. Med. Chem.*, 2005, **48**, 32.

12 (*a*) C. Zhu, P. Tang and B. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 5872; (*b*) H. Takouda, T. Konoshima, M. Kuzuka and T. Kimura, *Oncology*, 1991, **48**, 77.

13 (*a*) Y. J. Kim, P. Wang, M. N. Villalobos, B. D. Rohde, D. Berry and D. Y. Gin, *J. Am. Chem. Soc.*, 2006, **128**, 11906; (*b*) Y. J. Kim and D. Y. Gin, *Org. Lett.*, 2001, **3**, 1801.

14 O. Takeda, S. Tanaka, K. Yamasaki, H. Khoda, Y. Ywanaga and M. Tsuji, *Phytochemistry*, 1989, **32**, 1167.

15 (*a*) P. A. McCarthy, M. P. DeNinno, L. A. Morehouse, C. E. Chandler, F. W. Bangerter and T. C. Wilson *et al.*, *J. Med. Chem.*, 1996, **39**, 1935; (*b*) J. W. Clader, D. A. Burnett, M. A. Caplen, M. S. Domalski, S. Dugar, W. Vaccaro, R. Sher, M. E. Browne, H. Zhao, R. E. Burrier, B. Salisbury and H. R. Davis, *J. Med. Chem.*, 1996, **39**, 3684.

16 (*a*) M. R. Malinow, *Ann. N. Y. Acad. Sci.*, 1985, **454**, 23; (*b*) G. S. Sidhu, B. Upson and M. R. Malinow, *Nutr. Rep. Int.*, 1987, **35**, 615; (*c*) M. R. Malinow, W. H. Elliott, P. McLaughlin and B. Upson, *J. Lipid Res.*, 1987, **28**, 1.

17 X. Wen, H. Sun, J. Liu, K. Cheng, P. Zhang, L. Zhang, J. Hao, L. Zhang, P. Ni, S. E. Zographos, D. D. Leonidas, K.-M. Alexacou, T. Gimisis, J. M. Hayes and N. G. Oikonomakos, *J. Med. Chem.*, 2008, **51**, 3540.

18 (*a*) S. Zhu, Y. Li and B. Yu, *J. Org. Chem.*, 2008, **73**, 4978; (*b*) M. C. Recio, M. J. Just, R. M. Giner, S. Manez, J. L. Rios and K. Hostettmann, *J. Nat. Prod.*, 1995, **58**, 140.

19 F. Belardelli, *Acta Pathol. Microbiol. Immunol.*, 1995, **103**, 161.

20 P. Peraldi and B. Spiegelman, *Mol. Cell. Biochem.*, 1998, **182**, 169.

21 T. Oda, M. Katori, K. Hatanaka and S. Yamashina, *Mediat. Inflamm.*, 1992, **1**, 403.

22 D. G. Remick, R. M. Strieter, M. K. Eskandari, D. T. Nguyen, M. A. Genord and C. L. Raiford *et al.*, *Am. J. Path.*, 1990, **136**, 49.

23 R. C. Bone, *Chest*, 1991, **100**, 802.

24 (*a*) H. Shapiro, *Practical Flow cytometry*, Wiley, New York, 3rd edn, 1994; (*b*) T. R. Mosmann and T. A. T. Fong, *J. Immunol. Methods*, 1989, **116**, 151.

25 C. Bueno, C. A. Rodriguez, M. A. Garcia, A. Pandiella, J. Almeida and A. Orfao, *J. Immunol. Methods*, 2002, **264**, 77.

26 M. M. He, A. S. Smith, J. D. Oslob, W. M. Flangan, A. C. Braisted, A. Whitty, M. T. Cancilla and J. Wang *et al.*, *Science*, 2005, **310**, 1022.

27 H. Ghouila, A. Beyaoui, H. B. Jannet, B. Hamdi, A. B. Salah and Z. Mighri, *Tetrahedron Lett.*, 2008, **49**, 5721.