

Short Communication

**INHIBITION OF TUMOUR NECROSIS FACTOR (TNF) ACTIVITY BY A NEW ERGOSTANE STEROID FROM *CLEOME DROSERIFOLIA***

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Received: 24 Jan 2018 Revised and Accepted: 02 Apr 2018

**ABSTRACT**

**Objective:** To isolate steroidal compound from *Cleome droserifolia* and explore its activity against tumour necrosis factor (TNF) using molecular docking studies.

**Methods:** Extraction of the plant material was carried using two successive solvents and three compounds were isolated using column chromatography. The isolated compounds were identified using different spectroscopic data such as 1D, 2D nuclear magnetic resonance (NMR), ultraviolet-visible spectroscopy (UV-Visible), fourier transform infrared spectroscopy (FT-IR) and electrospray ionization mass spectroscopy (ESI-MS). Molecular docking was carried in order to investigate the activity of the ergostane derivative as tumour necrosis factor inhibitor.

**Results:** Two steroidal compounds were isolated and identified as ergost-5,7,9,24 (28)-tetraen-3-one (1) and  $\beta$ -sitosterol glucoside (3) by means of spectroscopic measurement. 1-dodecanol (2) was also isolated. The ergostane derivative showed several binding sites with TNF- $\alpha$

**Conclusion:** The new ergostane derivative isolated from the aerial parts of *Cleome droserifolia* is introduced to serve as a potential tumour necrosis factor inhibitor and may be used as a lead compound for the treatment of rheumatoid arthritis.

**Keywords:** Cleome, Droserifolia, Ergosteroid, Dodecanol, TNF inhibitor, Autoimmune disease

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DOI: <http://dx.doi.org/10.22159/ijpps.2018v10i5.24945>

*Cleome droserifolia* is traditionally used in Egypt as a hypoglycaemic healing remedy as proven via several *in vivo* and clinical studies [1, 2]. The phytochemical investigation of the plant had revealed that it is rich in diverse chemical entities; as several research studies described the isolation of different classes of terpenes mainly, sesquiterpenes, diterpenes and triterpenes [1-4]. The literature also reported the isolation of a number of flavones (methoxylated ones as well as flavone glycosides). Furthermore, volatile oil, sterols, hydrocarbons and a glucosinolate (glucocapparin) were also reported [1, 3].

Our earlier work on *C. droserifolia* has described the isolation of sesquiterpenes, dolabellane diterpenes and flavonoids [2, 4]. The paucity of information on the chemical constitution of this plant extract has encouraged us to explore other chemical entities giving emphasis on phytochemical analysis of the medium polar fractions where additional chemical structures may reside.

About 3% of the world population suffer from autoimmune diseases and their conventional treatment always relied on immune-suppressive therapies. However, advances in understanding the cellular activity pathways have led to new therapeutic treatments targeting defined pathways of the adaptive immune response such as tumour necrosis factor- $\alpha$  inhibitors (TNF- $\alpha$ ) [5].

TNF- $\alpha$  is a protein involved in inflammation processes and immune response. Its overproduction leads to the development of chronic autoimmune diseases. Its role seems to be immune-regulatory that can change the balance of T regulatory cells and regulate acute immunological responses [5]. Inhibition of TNF production or function by small molecules has become a major focus in the pharmaceutical industry for the treatment of rheumatoid arthritis. But till date, only six drugs have been approved by FDA and they are all large protein monoclonal antibodies. Several natural products have been reported to inhibit TNF either *in vitro* or by computational docking studies. For instance, Ginseng saponins and 14-dehydroergosterol [6-8]. This has encouraged us to investigate the activity of the isolated steroidal compound as a possible TNF inhibitor.

*C. droserifolia* was collected from Wady Gnai; coordinates (28.453501 °, 34.448650 °); Dahab, Sinai, Egypt and kindly identified by Professor Loutfy boulos, National Research Centre, Dokki, Cairo, Egypt. A voucher specimen (CD106) has been deposited in the Pharmacognosy department, Faculty of Pharmacy, Alexandria University, Egypt

The air-dried powdered aerial parts of *C. droserifolia* (1 kg) were soaked in light petroleum, to give a dark green semisolid residue (52 g). The residue was re-dissolved in light petroleum and then extracted with 90% methanol (MeOH) which afforded a brown aggregate (12g). The later residue was subjected to column chromatography using silica gel and eluting with hexane with increasing concentrations of methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>)

The defatted powdered plant material was air dried then extracted with acetone to give a semisolid brown residue (70 g). The residue was then fractionated according to a modified Kupshan fractionation scheme [4] which enabled us to isolate dolabellane diterpenes, methoxylated flavones, a flavan and flavonoidal glycosides in our previous studies [2,4]. The carbon tetrachloride fraction (30 g) was chromatographed on a silica gel column and the fraction eluted with 50% ethyl acetate (EtOAc) in CH<sub>2</sub>Cl<sub>2</sub> (4 g) was re-chromatographed on a silica gel C18 column using 80% acetonitrile in water to isolate 10 mg of (2). While 50 mg of (3) were precipitated from the fraction eluted with 5% MeOH in EtOAc.

The 1D and 2D NMR analyses were obtained using a Jeol 500MHz spectrometer for 1H-and 125MHz for <sup>13</sup>CNMR. Residual peaks of the deuterated solvents were used to reference the spectrum. ESIMS was carried on a Finnigan LCQ. IR spectra were recorded as a film using a FTIR-8400S Fourier transform infrared spectrophotometer (Shimadzu). UV-visible spectra were carried on a Helios \_ thermo-spectronic, England, supported with Vision 32 software.

NMR examination (table 1) revealed that compound 1 is characterised by having a steroidal nucleus (19 carbons atoms) in

addition to three doublet methyl signals at  $\delta$ 0.93 (H21), 0.83 (H26) and 0.74 part per million (H27), designated to side chain methyls. Besides, additional two singlet methyl signals at  $\delta$ 0.90 (H18) and 0.88 (H19) were attached to the sterol nucleus.

The UV spectrum ( $\lambda_{\max}$  350 nm in MeOH) revealed that it possessed a conjugated triene structure composed of  $\Delta$ [5,6] and  $\Delta$ [7,9], with an extra conjugated double bond which could be located in either position  $\Delta^9$ [11] or  $\Delta$ [14,15]. Comparing its spectral data with dehydroergosterol (DHE) and 14-DHE revealed that the position of the additional double bond was suggested to be located at C-9 as in dehydroergosterol. This suggestion was further confirmed by the presence of a correlation between the proton at 5.56 with C12 and C9 with H1 (HMBC). The resonance of the two double bond were evident in the IR spectrum as C=C stretching band at 1649  $\text{cm}^{-1}$  in accordance with the value 1650-1655  $\text{cm}^{-1}$  Kasal et al mentioned for 5,7 diene steroidal structure. The B and C ring proton signals appeared at 5.56, 5.40, and 5.47 ppm in the  $^1\text{H}$ NMR. Two additional

olefinic proton signals, resonating as two broad singlets at 4.5 and 4.7 ppm were observed in the  $^1\text{H}$ NMR and were assigned to the unsaturated alkyl side chain.

The mass spectrum reveals a molecular ion peak  $M^+$  at  $m/z$  392 agreeing with formula  $\text{C}_{28}\text{H}_{40}\text{O}$ . Additional peaks at  $m/z$  348 (M-isopropyl moiety), and a peak at  $m/z$  265 corresponds to [M-side chain-2H].

The IR spectrum indicated the absence of a hydroxyl group, as there was no peak observed at the region of 3300-3400  $\text{cm}^{-1}$ . Meanwhile, the presence of a sharp peak detected at 1729  $\text{cm}^{-1}$  implicating the possibility that position 3 is occupied by a carbonyl carbon in accordance to the value 1720  $\text{cm}^{-1}$  Kasal et al mentioned in their book for 3 non conjugated ketone ester sterol [9]. This was further supported by the absence of peaks involving the loss of water in the mass spectrum. The structure of the compound was thus suggested to be ergost-5,7,9,24 (28)-tetraen-3-one (fig. 1).

Table 1:  $^1\text{H}$ ,  $^{13}\text{C}$ NMR spectral data for compounds (1-3)

Carbon	Compound 1		Compound 3'		Compound 2	
	$\delta$ C	$\delta$ H	$\delta$ C	$\delta$ H	$\delta$ C	$\delta$ H
1	25.6	1.99 (2H,m)	37.5	1.69 (2H, m)	67.9	3.79 (2H, m)
2	30.4	1.9 (1H, m)	30.3	1.71 (2H, m)	25.6	1.72 (2H, m)
3	21.3	-	78.1	3.97 (1H, m)	22, 25, 34	1.2-1.5 (18 H,m)
4	30.4	1.9 (1H, m)	39.3	2.4 (dt,j=11.4,2) 2.6 (dd, j=10.7,2)	14	0.85 (t, J=7,3H)
5	136.3	-	140.9	-	-	-
6	124.1	5.47 (d, J=1.6)	121.9	5.35 (d, 5.2)	-	-
7	122.3	5.40 (d, J=1.6)	32.2	1.30 (2H,m)	-	-
8	134.6	-	32.1	1.30 (1H, m)	-	-
9	134.3	-	50.4	0.9 (1H, m)	-	-
10	40.9	-	37.0	-	-	-
11	121.4	5.56 (br s)	21.3	1.1 (2H, m)	-	-
12	24.4	1.7 (1H, m)	40.0	1.9 (2H,m)	-	-
13	42.5	-	42.5	-	-	-
14	44.1	1.6 (1H, m)	57.0	1.1(1H, m)	-	-
15	23.7*	1.72(1H, m)	24.5	1.3 (2H, m)	-	-
16	29	1.3 (1H, m)	28.5	1.7(2H, m)	-	-
17	46.8	1.21(1H, m)	56.3	1.1 (1H, m)	-	-
18	21.6	0.90 (S,3H)	12.0	0.70 (3H, s)	-	-
19	21.5	0.88 (S,3H)	19.4	1.00 (3H, s)	-	-
20	39.0	1.72 (1H, m)	35.8	1.3 (1H,m)	-	-
21	21.2	0.93 (d, J=6.9,3H)	18.9	0.84 (3H, d)	-	-
22	36.2	2.36(1H, m) 2.39(1H, m)	34.0	1.7 (2H, m)	-	-
23	26.1	2.18 (1H, m) 2.16(1H, m)	29.7	1.7 (2H, m)	-	-
24	153.3	-	49.8	1.10 (1H, m)	-	-
25	36.6	2.05 (1H, m)	28.7	-	col	ed
26	15.7	0.83 (d, J=6.9,3H)	18.0	0.83 (3H,S)	iat	l l
27	15.0	0.74 (d, J=6.9,3H)	17.9	0.87 (3H,s)	-	-
28	103.1	4.55 (d, J=1.6,1H) 4.66 (d, J=1.6,1H)	22.8	1.3 (2H,m)	-	-
29	-	-	12.3	0.85 (3H,s)	-	-
Sugar cabons C1	-	-	102.6	5.05 (d, J=7)	-	-
C2	-	-	75.3	4.05 (t, J=7.8)	-	-
C3	-	-	78.6	4.27 (t, J=7.8)	-	-
C4	-	-	71.7	4.30 (t, J=7.8)	-	-
C5	-	-	78.4	3.97	-	-
C6	-	-	62.8	4.56 (dd, J=11.7, 2.5) 4.41 (dd, J=11.7, 2.5)	-	-

\*in pyridine-d6

NMR spectra of compound 2 showed signals that clearly indicated the presence of fatty alkyl chain as it contained a methyl signal at  $\delta$  0.85, (3H, t), Methylene protons at  $\delta$  1.2-1.5 (18H, br s) in addition to a methylene signal at (3.75) ppm.

Apt spectrum showed the presence of carbon signals in the aliphatic region (10-40 ppm), in addition to an oxygenated methylene at 67.9 ppm. ESIMS showed a prominent peak at  $m/z$  203 equivalent to

$[\text{M}+\text{NH}_3]^+$  suggesting a molecular formula of  $\text{C}_{12}\text{H}_{26}\text{O}$ . The presence of a peak at 167 [M-OH] indicate loss of OH suggesting that it contained an alcoholic function group which was observed as broad peak at 3437  $\text{cm}^{-1}$  and C-O stretching band at 1363  $\text{cm}^{-1}$  in the IR spectrum. The identity of the compound was ascertained to be dodecanol (lauryl alcohol) upon comparing its spectra with the previously reported one. It is worth mentioning that 3,7,11-trimethyldodeca-1,6,10-triene was previously reported from *C. Droserifolia* [3].

ESIMS spectra of compound 3 showed a peak at  $m/z$  599 ( $576+Na$ ) suggesting a molecular formula  $C_{35}H_{60}O_6$  and identified as  $\beta$ -sitosterol glucoside by Comparing its 1D,2D NMR spectra and mass spectra with the previously reported [10]. The compound was also subjected to acid hydrolysis and the aglycone was identified as  $\beta$ -sitosterol by means of spectroscopic data (NMR and ESIMS) as the carbon C-3 was shifted to 71.7 ppm and H-3 to 3.4 ppm indicating a free hydroxyl group. The sugar identity was confirmed to be glucose using TLC against reference standard. Compound 3 was also subjected to acetylation using pyridine and acetic anhydride and 4 methyl groups were observed in the  $^1H$ NMR spectra in the region between (1.9-2.1 ppm) corresponding to 4 acetyl groups of the sugar moiety.

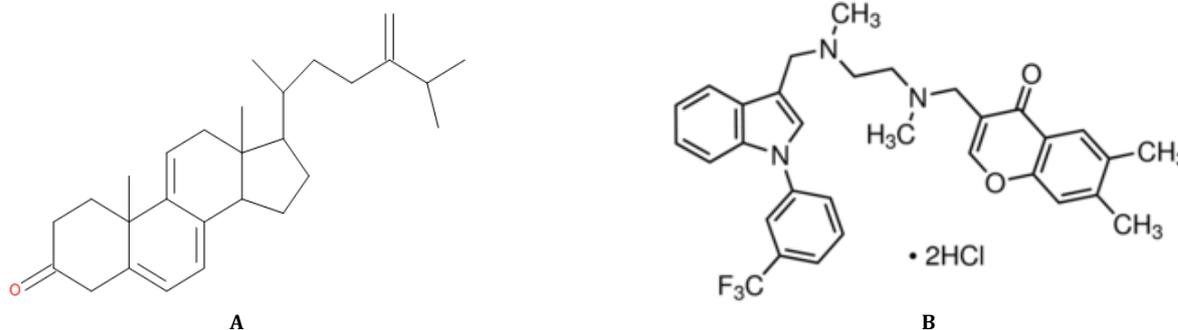
Molecular docking was carried as follows, The X-ray crystallographic structure of TNF complexed with a small molecule inhibitor (PDB Code: 2az5) was obtained from the RCSB Protein Data Bank. The three dimensional structure of ergostane 1 and TNF inhibitor marketed by sigma-Aldrich®[11] was derived by Avogadro software. The accurate docking was performed using the docking tool iGEMDOCK v2.0 based on the binding energy in kcal/mol. Numbers of runs taken are 80 and maximum interactions were 2000 with population size 200 and with an energy threshold of 100. Also at each step least 'minimum' torsions/translations/rotations are tested and the one giving lowest energy is chosen. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was identified at a distance 8 Å.

The steroidal skeleton reported previously for ginsenosides can have hydrophobic interaction with the residues located inside the pore of TNF at Cys69, Pro100, Cys101, Ala109, Pro113 and Trp114 [7]. Compound 1 binding energy was -86.9, it showed hydrogen bond with six amino acid residues, TYR 110, LEU 111, GLY 112, GLY 45, LEU 46 and GLN116 (fig. 1).



**Fig. 1: Predicted binding model of compound 1 to 2AZ5 and binding models of 2AZ5 was shown as a ribbon drawing. The side chains of the active site residues and compound 1 are represented as stick model**

In comparison with the compound "6,7-Dimethyl-3-[[methyl[2-[methyl[[1-[3-(trifluoromethyl)phenyl]-1H-indol-3-yl]methyl]amino]methyl]-(4H-1-Benzopyran-4-one dihydro-chloride)" marketed by sigma-Aldrich® as TNF inhibitor [10], (fig. 2) which showed binding energy -117.3 with only one hydrogen bond with amino acid residue SER 86, and VAN DER WAALS forces with GLU 44, TYR78, GLN 116, GLU 118, VAL 82, ASN 83, SER 86 and ILE 88. Thus, although the compound marketed by sigma showed lower energy, but actually it binds with only one amino acid residue compared to six amino acid residues in the case of compound 1.



**Fig. 2: Structure of; A: Ergost-5,7,9,24(28)-tetraen-3-one, B: 6,7-Dimethyl-3-[[methyl[2-[methyl[[1-[3-(trifluoromethyl)phenyl]-1H-indol-3-yl]methyl]amino]methyl]-(4H-1-Benzopyran-4-one dihydrochloride**

## CONCLUSION

A new ergostane was isolated from *Cleome droserifolia* and identified as ergost-5,7,9,24(28)-tetraen-3-one. Computational studies have proven its TNF inhibition effect which indicates its potential use in the development of novel drugs for the treatment of autoimmune disease.

## ACKNOWLEDGMENT

The author would like to express her deepest gratitude to Prof. Dr. Mohamed Aboushoer, Faculty of Pharmacy, Alexandria University, Egypt for critically and carefully revising the manuscript and for his fruitful discussion concerning the interpretation of the NMR data of the compounds.

## AUTHOR CONTRIBUTION

All the work have been carried out by me

## CONFLICT OF INTERESTS

Declared none

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