Accepted Manuscript

Bioactive products from singlet oxygen photooxygenation of cannabinoids

Ahmed Galal, Khaled M. Elokely, Vivek K. Yadav, Paulo Carvalho, Mohamed Radwan, Desmond Slade, Waseem Gul, Shabana Khan, Olivia R. Dale, Afeef S. Husni, Michael L. Klein, Stephen J. Cutler, Samir A. Ross, Mahmoud A. ElSohly

PII: S0223-5234(17)30946-7

DOI: 10.1016/j.ejmech.2017.11.043

Reference: EJMECH 9918

To appear in: European Journal of Medicinal Chemistry

Received Date: 5 July 2017

Revised Date: 14 October 2017

Accepted Date: 17 November 2017

Please cite this article as: A. Galal, K.M. Elokely, V.K. Yadav, P. Carvalho, M. Radwan, D. Slade, W. Gul, S. Khan, O.R. Dale, A.S. Husni, M.L. Klein, S.J. Cutler, S.A. Ross, M.A. ElSohly, Bioactive products from singlet oxygen photooxygenation of cannabinoids, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.11.043.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



ACCEPTED MANUSCRIPT



Bioactive products from singlet oxygen photooxygenation of cannabinoids

Ahmed Galal[#]*, Khaled M. Elokely^{0,0,⊥}, Vivek K. Yadav^{0,0}, Paulo Carvalho^{||}, Mohamed Radwan[#], Desmond Slade[&], Waseem Gul^{#,§}, Shabana Khan^{##}, Olivia R. Dale[#], Afeef S. Husni[#], Michael L. Klein^{0,0}, Stephen J. Cutler^{#†}, Samir A. Ross^{#,#}, Mahmoud A. ElSohly^{§, Ω}.*

[#] National Center for Natural Products Research, the University of Mississippi, University, MS 38677

^o Institute for Computational Molecular Science, Temple University, Philadelphia, PA 19122

⁶ Department of Chemistry, Temple University, Philadelphia, PA 19122

[⊥] Department of Pharmaceutical Chemistry, Tanta University, Tanta 31527, Egypt

Feik School of Pharmacy, University of the Incarnate Word, San Antonio, TX, 78209

[&] MRI Global, 425 Volker Boulevard, Kansas City, Missouri

[§] ElSohly Laboratories, Inc., 5 Industrial Park Drive, Oxford, MS 38655

[#] Department of of BioMolecular Sciences, University of Mississippi, University, MS 38677

^Ω Department of Pharmaceutics, University of Mississippi, University, MS 38677

[†]Current affiliation: University of South Carolina College of Pharmacy, Columbia, SC 29208

* Corresponding authors

KEYWORDS Cannabinoids, Photooxygenation, CB₁ agonists, Antimicrobial, Anticancer, Antileishmanial.

ABSTRACT: Photooxygenation of Δ^8 tetrahydrocannabinol (Δ^8 -THC), Δ^9 tetrahydrocannabinol (Δ^9 -THC), Δ^9 tetrahydrocannabinolic acid (Δ^9 -THCA) and some derivatives (acetate, tosylate and methyl ether) yielded 24 oxygenated derivatives, 18 of which were new and 6 were previously reported, including allyl alcohols, ethers, quinones, hydroperoxides, and epoxides. Testing these compounds for their modulatory effect on cannabinoid receptors CB₁ and CB₂ led to the identification of **7** and **21** as CB₁ partial agonists with Ki values of 0.043 µM and 0.048 µM, respectively and **23** as a cannabinoid with high binding affinity for CB₂ with Ki value of 0.0095µM, but much less affinity towards CB₁ (Ki 0.467µM). The synthesized compounds showed cytotoxic activity against cancer cell lines (SK-MEL, KB, BT-549, and SK-OV-3) with IC₅₀ values ranging from 4.2 to 8.5 µg/mL. Several of those compounds showed antimicrobial, antimalarial and antileishmanial activities, with compound **14** being the most potent against various pathogens.

Introduction:

Cannabinoids, from *Cannabis sativa* L., have been the focus of extensive chemical and biological research due to their unique behavioral, psychotropic and other pharmacological effects. The discovery that some of their biological activity could be translated into treatments for a number of serious illnesses, such as glaucoma, depression, neuralgia, multiple sclerosis, Alzheimer's disease, alleviation of symptoms of HIV/AIDS and cancer [1-4] have given momentum for further exploration of their chemical and biological properties. The discovery of cannabinoid receptors CB₁ and CB₂ (with other possible receptors currently under investigation) [5, 6] opened new possibilities for the design and exploration of cannabinoid structures. CB₁ agonists exhibit analgesic properties, whereas CB₁ antagonists and inverse agonists have shown the potential to act as therapeutic agents against diabetes, drug dependence, and obesity. CB₂ agonists have exhibited cytotoxicity and demonstrated potential for treatment of neuropathic pain [7-9], suppression of inflammation [10] and attenuation of the severity of disease in animal models of multiple sclerosis [11] and age-related illnesses [12-14].

In search of compounds with affinity for CB₁ and CB₂ cannabinoid receptors our group decided to explore oxygenated derivatives of Δ^9 -THC and its isomer Δ^8 -THC. One of the earliest references reporting the photooxygenation of Δ^8 -THC acetate via irradiation with UV light in the presence of oxygen and using rose bengal as a photosensitizer [15] yielded three hydroperoxides: (-)-8 α - and (-)-8 β -hydroperoxido- $\Delta^{9,11}$ -THC acetate, and (-)-9 α -hydroperoxido- $\Delta^{7,8}$ -THC acetate. More recently, other oxygenated derivatives of Δ^9 -THC and Δ^8 -THC have been prepared, showing antibacterial [16] and anticancer effects [17-20], as well as demonstrating some degree of affinity to cannabinoid receptors CB₁ and CB₂ [21, 22]. This article is a continuation of our work [23] and describes the photooxygenation of Δ^9 tetrahydrocannabinol (Δ^9 -THC - 1), Δ^8 -tetrahydrocannabinol (Δ^8 -THC - 2), Δ^9 tetrahydrocannabinolic acid (Δ^9 -THCA - 8) and some of their derivatives (Figure 1) under different conditions. *Meso*-tetraphenylporphine was used as a photosensitizer in presence of oxygen and irradiation with incandescent light, generating singlet oxygen (1O_2*), which reacted with the trisubstituted olefinic moiety to form oxygenated products. Six of those compounds (15, 23, 10, 12, 14 and 31) have been previously reported as minor oxygenated cannabinoids from cannabis, serum metabolites of Δ^9 -THC, or products from non-photooxygenation reactions [15, 24-29].

These compounds were screened for various biological activities, including antimicrobial (*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* [MRSA]), antifungal (*Cryptococcus neoformans*, *Candida glabrata*, and *Candida krusei*), anticancer (cell lines SK-MEL, KB, BT-549, and SK-OV-3), antimalarial (*Plasmodium falciparum*, D6 clone - chloroquine-sensitive - and W2 clone - chloroquine-resistant) and antileishmanial (*Leishmania major*), as well as their binding affinity towards cannabinoid receptors CB₁ and CB₂.



Figure 1: Structures of Δ^9 -THC (1), Δ^8 -THC (2), Δ^9 -THC tosylate (3), Δ^8 -THC tosylate (4), Δ^8 -THC methoxy (5), Δ^9 -THC acetate (6), Δ^8 -THC acetate (7), and Δ^9 -THCA (8).

Results and discussion

Chemistry

Photooxygenation of 1, 2, tosylates 3 and 4 [25], methoxy- Δ^8 -THC 5, Δ^9 -THC acetate 6, Δ^8 -THC acetate 7, and Δ^9 -THCA 8 using *meso*-tetraphenylporphine as a photosensitizer [30] resulted in the formation of 24 derivatives. Six of them (15, 23, 10, 12, 14 and 31) have been previously reported [15, 24-29] and the other 18 are, to the best of our knowledge, novel compounds. One of our goals was to generate a large array of oxygenated derivatives in order to correlate the position and nature of those functionalities with their biological activity. Initial studies revealed that changes in polarity of the reactional solvent system led to the formation of products with different patterns of oxygenation, and this knowledge was used to guide the choice of solvents with variable polarities for our reactions. Reaction conditions and corresponding products are summarized on Table 1,

Starting material	Solvent system	Reaction time	Product(s)	Scheme
1	CH ₂ Cl ₂ /EtOH	11 h	9-12	
2	hexanes/ CH_2Cl_2 (4:1)	8 h and 30 min	13	1
2	CH ₂ Cl ₂ /propanol (1:1)	4 h and 30 min	14	
3	CH_2Cl_2	4 h and 15 min	15, 16, 17, and 18	2
3	CH ₂ Cl ₂ /EtOH	11 h and 30 min	17, 18, 19* and	
X			20	
4	CH ₂ Cl ₂ /EtOH	4 h and 15 min	21, 22 and 23**	3
5	CH ₂ Cl ₂ /EtOH	6 h	23-25	4
6	hexanes/ CH ₂ Cl ₂	4 h	26-30	5
7	hexanes/ CH ₂ Cl ₂	8 h and 15 min. followed	27	
		by reduction with NaBH ₄		
8	$CH_2Cl_2/MeOH(1:1)$	3 h	28	
*product of redu	action of 18. ** product	of hydrolysis of 71		

Table 1. Reaction time, solvent systems and products of photooxygenation of THC derivatives.

product of reduction of 18; product of hydrolysis of 21

ACCEPTED MANUSCRIPT

Some of the products were subjected to further treatment under different conditions.

Compound **18**, submitted to reduction with dimethyl sulfide for 22 hours, yielded the allylic alcohol **19** (Scheme 2); epoxide **27**, reduced with NaBH₄ or NaHCO₃/H₂O/Adogen 464, afforded compound **29**, while reduction with Pd/C yielded compound **30** (Scheme 5). Attempted reduction of compounds **21**, **22** and hydroperoxides **24**, **25**, resulted in decomposition. Alkaline hydrolysis of **21** yielded the known tertiary allylic alcohol **23** (Scheme 3), while attempted hydrolysis of **22** resulted also in decomposition.

Scheme 1. Synthesis of compounds 9-14.



Reagents and conditions: (a) *meso*-tetraphenylporphine, O₂, light, (b) CH_2Cl_2 /anhydrous EtOH (1:2), 11 h; (c) hexanes/CH₂Cl₂ (4:1), 8 h and 30 min; (d) CH_2Cl_2 /n-propanol (1:1), 4 h and 30 min.

Scheme 2. Synthesis of compounds 15-20.



Reagents and conditions: (a) *meso*-tetraphenylporphine, O₂, light, (b) CH₂Cl₂, 4 h and 15 min; (c) Me₂S/22 h; (d) CH₂Cl₂/abs EtOH (1:1), 11 h and 30min.

X-ray analysis of compound **20** [31] (CCDC reference: 1442416), crystallized from ethyl acetate: hexanes 1:9 producing needle-like crystals, allowed for confirmation of structure and the establishment of its relative configuration (Figure 2).





Figure 2. Plot of the molecular structure of compound 20, Displacement ellipsoids are drawn at the 50% probability level

9

Scheme 3. Synthesis of compounds 21-23.



Reagents and conditions: (a) meso-tetraphenylporphine, O_2 , light, (b) CH_2Cl_2 /anhydrous EtOH

(1:1), 4 h; (c) hydrolysis, KOH/EtOH, 75 min.

10



Scheme 4. Synthesis of compounds 32, 24-26.

Reagents and conditions: (a) *meso*-tetraphenylporphine, O₂, light, (b) CH₂Cl₂/abs EtOH (1:1), 6 h, (c) CH₂Cl₂/MeOH (1:1), 3 h.

Compounds **25** and **26** (Scheme 4) were obtained as separated compounds and exhibited different chromatographic behavior on TLC, $R_f = 0.42$ (Hexanes- DCM-MeOH, 9:9:0.8) and $R_f = 0.36$ (Hexanes - DCM-MeOH, 9:9:0.8) respectively. They also display different specific rotation values and different ¹H and ¹³C NMR shifts. However, none of the spectroscopic methods used was capable of assigning with certainty the configuration of each isomer and we were unable to obtain a crystalline sample for X-ray analysis.

Scheme 5. Synthesis of compounds 27 - 31



Reagents and conditions: (a) *meso*-tetraphenylporphine, O₂, light; (b) hexanes/CH₂Cl₂ (1:1), 10° C, 3 h, 45 min; (c) hexanes/CH₂Cl₂ (1:1), 7.7° C, 8 h; (d) NaBH₄/MeOH, 6 h; (e) NaBH₄/MeOH, 2 h, 30 min; (f) NaHCO₃/H₂O/MeOH, Adogen 464, (g) Pd/C, H₂, MeOH, 10 h.

Stereochemical assignments

Stereochemical assignments for derivatives **9**, **13**, **17**, **18**, **19**, **21**, **24** were determined on the basis of NOESY correlations, as seen on Figure 3 and Table 2. The assignments for compound **23** were confirmed by comparison with published NMR data [15, 32].

ACCEPTED MANUSCRIPT

The orientation of the hydroxyl and ethoxyl functionalities at C-9 and C-10 of compound **11** were determined by comparison with the 13 C values of the two diastereomers previously reported [33].



Figure 3. Relative configuration of compounds 9, 13, 17, 18, 19, 21, and 24, based on NOESY correlations.

CER

Tabl	e 2. Stereochemical	orientation	of derivatives	9, 13, 17	7, 18, 19 , 2	21, and 24.,	as determined
on th	e basis of NOESY c	orrelations.					

Compound	Group analyzed	Orientation	Correlation
9	OEt at C-9	α -oriented	between Me-11 (δ 1.41) at C-9 and Me-13 (δ 1.04 - previously established as β -oriented)
13	OH at C-8	α-oriented	between H-8 (δ 4.14) and the α -oriented Me-12 (δ 1.52).
17	HOO at C-10	β -oriented	between H-10 (δ 4.63) and the α oriented H-10a (δ 2.07)
18	HOO at C-10	α -oriented	between H-10 (δ 4.14) and the β -oriented H-6a (δ 1.79)
19	OH at C-10	α -oriented	between H-10 (δ 4.06) and the β -oriented H-6a (δ 1.74).
21	HOO at C-9	α -oriented	between the proton of HOO (δ 9.28) at C-9 and the α -oriented H-10 (δ 3.52)
	H-10	α-oriented	with H-10a (δ 2.85)
24	HOO at C-9	α-oriented	between Me-11 (δ 1.55 at C-9) and the β -oriented H-6a (δ 2.77)

2.2. Biological activity

2.2.1. Affinity to cannabinoid receptors

The control used in both binding and functional assays was the non-traditional cannabinoid, CP 55,940[34]. The binding Ki for CP 55,940 at CB₁ is 0.5 to 5nM, and the binding Ki for CP 55,940 at CB₂ is 0.69 to 2.8nM. The functional Ki for agonism for this control at the CB₁ receptor is 0.07 to 4nM, and 0.2 to 7.4nM for CB₂.

Derivatives **13**, **18**, **19**, **21**, **27** and **29** have shown affinity for cannabinoid receptors in the low micromolar and nanomolar range.

Emax of compound 21 at 100 μ M was 20% stimulation for CB₁ and 90% for CB₂. Compound 29, also at 100 μ M, exhibited an Emax of 20% stimulation for CB₁ and 40% for CB₂. Table 3 presents the most representative values of binding affinity to CB₁ and CB₂ and Table 4 presents the most representative results from functional assays.

Table 3: Results of CB₁ and CB₂ binding assays for compounds 7, 14, 18, 19, 21, 22, 23, 27, 29, and 30. Error was monitored for each concentration point and displayed on the graphics (supplementary information) with error bars.

Compound	CB ₁ *	CB ₂ *
7	0.088/0.044	0.316/0.158
14	1.84/0.919	4.07/2.034
18	1.024/0.512	0.851/0.426
19	1.28/0.642	1.11 (0.552)
21	0.275/0.137	0.421/0.211
22	5.70/2.85	2.06/1.03
23	0.93/0.47	0.019/0.0095
27	0.573/0.286	0.927/0.464
29	1.077/0.538	0.599/0.300
30	2.80/1.40	1.98/0.99
CP 55,940	-/0.0005-0.005	-/0.00069-0.0028

*Values are expressed as IC₅₀/Ki in µM

ACCEPTED MANUSCRIPT

Table 4: Results of CB_1 and CB_2 functional assays for compounds **7**, **21**, and **23**. The radioligand used was [35S]-GTP- Υ S, from Perkin Elmer. Error was monitored for each concentration point and displayed on the graphics (supplementary information) with error bars.

Compound	CB ₁ *	CB ₂ *
7	0.087/0.043	0.518/0.259
21	0.097/0.048	1.75/0.876
23	0.387/0.193	11.42/5.70
CP 55,940	-/0.0007-0.004	0.0002-0.0074

*Values are expressed as IC_{50} / Ki in μM

Compounds **18** (C1 tosylate, C10 hydroperoxide), **19** (C1 tosylate, C10 hydroxyl) and **29** (C9 methoxy, C1 and C10 hydroxyl) showed a relatively high affinity for CB₂ receptors, with IC₅₀ of the order of 0.5-0.6 μ M whereas their affinity for CB₁ receptors was at and slightly above 1 μ M.

Addition of hydroxyl groups to C9 seems to improve affinity for both cannabinoid receptors, but the state of the hydroxyl group at C1 (tosylated or free) seems to make a difference in selectivity. Compound **21**, a C9 hydroperoxide tosylate derivative, displayed good affinity for the CB₁ receptors with IC₅₀ lower than 100 nM, and lower affinity for CB₂. Compound **23**, a diol with a hydroxyl group at C9 and free hydroxyl at C1, displayed marked and selective binding affinity for CB₂ receptor with an IC₅₀ lower than 20 nM, and lower affinity for CB₁, with an IC₅₀ of the order of 1 μ M. Compound **27**, a C9-C10 epoxide with the hydroxyl group at C-1 masked by an acetate, had the opposite profile, showing higher affinity for the CB₁ receptors with IC₅₀ of the order of 0.5 μ M and lower affinity for CB₂ receptors, with IC₅₀ of the order of 1 μ M.

Functional assays on cannabinoid receptors

Cannabinoid receptor assays led to identification of derivatives 7, and 21 (Table 4) as CB_1 partial agonists, with affinity values in the nanomolar level, and marginal affinity to CB_2 .

As previously mentioned, Emax of 21 (100 μ M) was 20% for CB1 and 90% for CB2 and 29, also at 100 μ M, exhibited an Emax of 20% for CB1 and 40% for CB2.

The aforementioned results revealed that, in contrast to a previous study [35] reporting complete loss of activity when the phenolic hydroxyl group at C-1 of THC is blocked, photooxygenation of acetate and tosylate derivatives yielded oxygenated derivatives with masked hydroxyl groups at C-1 which were found to retain affinity towards the cannabinoid receptors.

1,4-Quinones **24-26** did not exhibit any level of affinity towards CB_1 and CB_2 , presumably indicating that this functionality may hinder receptor binding due to steric effect.

17

2.2.2. Anticancer activity

Quinones 9, 10 [36], 14, and 25 exhibited anticancer activity against cell lines SK-MEL, KB, BT-549, and SK-OV-3 with IC₅₀ values ranging from 4.2 μ g/mL (14, against BT-549) to 8.65 μ g/mL (25, against SK-MEL) (Table 5). It is noteworthy to mention that cannabinoid quinone derivatives prepared through KOH/EtOH oxidation [17] have been previously reported to possess antitumor activity, with HU-331 [19] exhibiting its anticancer effect through a novel mechanism of action as topoisomerase II inhibitor.

Table 5. Anticancer activity of compounds 9, 10, 14, 25 and 26, expressed as IC_{50} of growth inhibition (μ g/mL).

Compound		Cancer	Noncancer Cells			
_	SK-MEL	KB	BT-549	SK-OV-3	VERO	LLC-PK ₁
9	6.2 <u>+</u> 0.28	NA	5.3 <u>+</u> 0.70	NA	5.95 <u>+</u> 0.78	5.4 <u>+</u> 0.42
10	7.6 <u>+</u> 0.85	NA	6.05 <u>+</u> 0.49	NA	NT	5.65 <u>+</u> 0.07
14	NT	5.25 + 0.35	4.2 + 0.28	4.35 + 0.21	4.1 + 0.42	2.25 + 0.07
25	8.65 <u>+</u> 0.49	NA	NA	NA	NT	9.9 <u>+</u> 0.14
26	NA	NA	NA	NA	NT	9.95 <u>+</u> 0.07

SK-MELHuman melanomaKBHuman epidermal carcinoma, oralBT-549Ductal carcinoma, breastSK-OV -3Human ovary carcinomaVeroMonkey kidney fibroblastsLLC-PK1Pig kidney epithelial cells

Values are average of two determinations <u>+</u> std dev NA = no activity up to $10 \ \mu g/mL$ NT = not tested

2.2.3. Antimicrobial activity

Compounds 9, 10, 11, 13, 14, 24-26, and 31 exhibited antimicrobial activity against pathogenic bacteria *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and pathogenic fungi *Candida glabrata*, *Candida krusei*, and *Cryptococcus neoformans* (Table 6). Compound 14, a quinone derivative of Δ^8 -THC, was found to be the most active anticryptococcal agent and also the strongest antibacterial agent against MRSA with IC₅₀ of 1.36 µg/mL and MIC 2.50 µg/mL. Compound 25 was the most potent agent against *S. aureus* with IC₅₀ 0.91 µg/mL and MIC 2.50 µg/mL. Compounds 9, 10, 11, 13, 14, and 31 exhibited considerable activity against *C. neoformans*, *S. aureus*, and MRSA, without any effect on both *Candida* species tested. Compound 24, despite not being the most active compound against bacterial strains, exhibited inhibitory activity against all the organisms tested and was the most active against both species of *Candida*.

Compound	Candida	Candida	Cryptococcus	Staphylococcus	MRSA					
	glabrata	krusei	neoformans	aureus						
9	-/-	-/-	0.88/-	2.04/-	2.04/-					
10	-/-	-/-	4.44/20.0	>20/-	Q -/-					
11	-/-	-/-	4.57/-	4.86/-	17.07/-					
13	-/-	-/-	1.84/5.00	2.03/2.50	5.53/10.0					
14	-/-	-/-	0.70/2.50	1.35/2.50	1.36/2.50					
24	6.54/10.0	5.77/10.0	0.93/2.50	1.30/2.50	2.63/5.0					
25	-/-	17.0/-	1.40/2.50	0.91/2.50	5.78/10.0					
26	-/-	20.0/-	2.05/5.0	2.41/5.0	15.3/-					
31	-/-	-/-	8.34/-	10.71/-	_/_					
Amphotericin B	-/-	-/-	1.36/2.50	-/-	_/_					
Ciprofloxacin	-/-	-/-	-/-	0.11/0.25	0.12/0.25					

Table 6. Antimicrobial activity of compounds 9, 10, 11, 13, 14, 24, 25, 26 and 31 expressed as IC_{50} / MIC (µg/mL)

2.2.4. Antimalarial activity

Among the compounds tested against *Plasmodium falciparum* D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains, compound **14** exhibited the highest activity with IC₅₀ of 0.16 μ g/mL for D6 and IC₅₀ of 0.20 μ g/mL for W2 (Table 7).

Table 7. Antimalarial activity of compounds 1-10, 17, 19-21, represented as IC₅₀ (µg/mL)

Compound	9	11	14	20	25	28	30	31	Chloroquine	Artemisinin
P. falciparam (D6 strain)	4.76	3.6	0.160	22	1.0	4.50	3.3	2.4	0.016	0.013
P. falciparam (W2 strain)	4.50	3.7	0.20	1.8	0.90	3.20	3.0	1.7	0.140	0.014

D6: chloroquine-sensitive strain

W2: chloroquine-resistant strain

2.2.5. Antileishmanial activity

Compound **14**, in addition to its antimicrobial and antimalarial effects, also displayed pronounced antileishmanial effect against promastigotes of *Leishmania donovani* with IC₅₀0.06 μ g/mL and IC_{90 of} 0.13 μ g/mL (Table 8). Those inhibitory concentrations are almost three times lower than the standard compound Amphotericin B, placing compound **14** as a good candidate for further studies of its antileishmanial properties.

Compound	9	11	13	14	17	20	24	25	26	29	Pentamidine	Amphotericin B
IC ₅₀	0.5	3.0	0.6	0.06	3.1	4.5	0.7	2.1	3.1	35	1.0	0.16
IC ₉₀	3.0	6.0	1.3	0.13	6.5	22	1.2	11	8	>40	2.0	0.33
							\checkmark					
		~										

Table 8 Antileishmanial activity of compounds 9, 11, 13, 14, 17, 20, 24, 25, 26 and 29 presented as IC_{50} and IC_{90} (μ g/mL)

2.3. Molecular Modeling

The two known subtypes of cannabinoid (CB) receptors CB_1 and CB_2 share approximately 44% identity throughout the entire protein sequence and roughly 74% of the seven transmembrane (TM) regions. The structural similarities, principally in the ligand binding cavity, led to non-selective behaviors of many CB modulators. Experimental crystal structure is not available for CB_2 receptor and homology models were built to be used in the study.

3D models were validated by inspecting dihedral angles, bond length, planarity and other criteria of structural quality assessment. Molecular docking was performed to investigate the binding pattern of our compounds. The effects of structural modifications of the phenolic hydroxyl group at C1, aliphatic chain at C3, and aliphatic hydroxylation at C9 of classical CB modulators are thoroughly studied showing their importance for CB activity. Several of the active compounds lack some of these structural elements, and therefore we tried to understand how these compounds interact with CB receptors.

Compounds **18**, **19** and **29** showed better fitting in the active site of CB_2 compared to CB_1 as implied by lower docking scores. Compounds **7**, **21** and **23** showed docking scores of -7.4, -10 and -9.1 kcal/mol in CB_1 , and -7, -5.1 and -7.1 kcal/mol in CB_2 .

The interaction models of compound **21** in CB1 (Figures 4 and 5) demonstrated H-bonds with Ser383 and His178, π - π stacking with Phe170, and hydrophobic contacts with the surrounding amino acids in the binding pocket.

Compound **7** presented π - π stacking with Phe170 and multiple hydrophobic interactions with the amino acid residues of CB₁, while compound **23** displayed strong H-bonding with Ser285, π - π stacking with Phe183 and Phe87, and several hydrophobic contacts with the surrounding amino acids of CB₂ (Figures 4 and 5).

ACCEPTED MANUSCRIPT

We explored the stability of the docking poses of **7**, **21** and **23** with molecular dynamics (MD) simulations. The protein-ligand interactions were investigated throughout the course of MD simulations. Protein structures were converged after a short MD period as calculated by the root mean square deviation (RMSD) of backbone, side chains and heavy atoms (Figure 7), indicating that the production stage was reached. The RMSD values, over 40 ns, showed a fluctuation within 1-2 Å after the equilibration period confirming system stability.





CB2-23



ACCEPTED MANUSCRIPT

Figure 4. 3D interaction models of compounds **7** (CB1-7) and **21** (CB1-21) with CB₁, and compound **23** (CB2-23) with CB₂. The protein is displayed as orange α -helices and green loops. The ligands are shown as white sticks, and surrounding amino acids as lines.



Figure 5. 2D interaction models of compounds **7** (CB1-7), **21** (CB1-21) and **23** (CB2-23). Hbonds are shown as purple lines. π - π stacking is shown as green dashed lines with green spheres at the ends. Hydrophobic interactions are displayed as solid green lines.

Compound **21** demonstrated hydrophobic contacts with the surrounding amino acid residues in the binding pocket of CB₁. His178 forms a well-preserved H-bond with the sulfonyl oxygen of the tosylate group (~56% of the simulation time) and the peroxy group shows intramolecular hydrogen bond with the same sulfonyl oxygen (~50%). His178, Phe170 and Phe288 make π - π stacking (~23%, 44% and 18%, respectively) with the aromatic rings. Blocking the phenolic hydroxyl group at C1 with toyslate group did not abolish the CB activity of compound **21**. The toyslate group offered favorable molecular region for CB interactions. The sulfonyl group acted in part as the free phenolic hydroxyl group and formed strong H-bond with His178 and Ser383, and the tosylate aromatic group formed π - π stacking with His178.

Hydrophobic contacts are very important for compound **7** with CB₁. Phe108, Phe170 and Leu193 display strong hydrophobic interactions with ligand atoms. Compound **23** strongly binds to CB₂ through H-bonds with His95 (~94%) and Ser285 (~99%), and hydrophobic contacts with Phe87 (~19%) and Phe183 (~73%).

3. Conclusion

Photooxygenation of Δ^9 -THC, Δ^8 -THC, Δ^9 - THCA and derivatives resulted in the formation of 24 oxygenated products with diversified functionalities, some of them previously reported as minor constituents in *Cannabis* or its metabolites [37]. Change of reaction time and solvent systems led to the formation of different products. Compounds **7** and **21** were recognized as selective CB₁ partial agonists, demonstrating that blockade of the C-1 hydroxyl function of the cannabinoid structure does not necessarily abolish affinity towards cannabinoid receptors.

Cannabinoid receptor binding and functional assays also demonstrated that the introduction of the 1,4-quinone moiety (compounds **9**, **10**, **14** and **24-26**) led to loss of affinity towards

cannabinoid receptors CB₁ and CB₂. Those same quinone derivatives, however, were the only derivatives exhibiting anticancer and marked antimicrobial activity. Quinone **14** was the most potent anti-cryptococcal and anti-MRSA agent, **25** was the best agent against *S. aureus* and quinones **24-26** showed anticandidal activity. **9**, **10**, **11**, **13**, **14** and **31** showed antimicrobial activity against *C. neoformans*, *S. aureus*, and MRSA, without any effect on the *Candida* species tested.

Compounds 21, 23 and the quinone derivatives 9, 10, 14 and 24-26 bear promising bioactivities warranting further pursuit focusing on improving yields and increasing selectivity of the reactions.

4. Experimental protocols

4.1. Chemistry

Starting materials Δ^9 -THC, Δ^8 -THC, and Δ^9 -THCA were isolated from *Cannabis sativa*[38] grown in the Medicinal Plant Garden at the University of Mississippi, Mississippi, USA and authenticated by Dr. Suman Chandra[39]. 1D and 2D NMR spectra were recorded in CDCl₃ as a solvent on a Bruker Avance DPX-400 spectrometer and on a Varian AS 400 spectrometer. HRESIMS was obtained using a Bruker Bioapex FTMS in ESI mode. LRESIMS was obtained using a 3200 Q Trap LC/MS/MS (Applied Biosystems MDS Sciex, Foster City, CA). TLC was carried out on aluminum-backed plates precoated with silica gel F₂₅₄ (20 x 20 cm, 200 µm, 60 Å, Merck). Visualization was accomplished by spraying with fast blue or p-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and H₂SO₄ (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (40-63 µm, 60 Å, Silicycle) and SiliaBond C18 silica gel (40-63 µm, 60 Å, 17% carbon loading, Silicycle) were used for column chromatography.

General experimental conditions

 Δ^9 -THC, Δ^8 -THC were converted to the tosylate [25], acetate [40, 41] esters or methyl ether [42] prior to photooxygenation, In addition, free cannabinoids and Δ^9 -THCA were also subjected to photooxygenation. For the photooxygenation reactions, *meso*-tetraphenylporphine (1.0 mg) was added to the appropriate THC derivative dissolved in a solvent or mixture of solvents. The reaction mixture was irradiated with 500 W incandescent light for the appropriate amount of time, with oxygen being gently bubbled into the solution and the temperature of the reaction bath maintained at 10-13°C. At the end of the reaction, the solvent was removed and the mixture purified by column chromatography, unless otherwise specified.

Progress of the reactions was monitored by TLC. Free cannabinoids on the TLC plates were visualized with fast blue, while tosylate derivatives were detected with p-anisaldehyde/H₂SO₄. The identity of these compounds was deduced from spectral analysis including specific rotation, NMR (1D and 2D), and HRESMS.

Compounds **10**, **12**, **14**, **15**, **23** and **31** along with their spectral data have been previously published [15, 24-26].

(6aR,10aS)-10-ethoxy-9,10a-dihydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10ahexahydro-6H-benzo[c]chromen-1-yl 4-methylbenzenesulfonate (16),

Following the general experimental conditions, **2** (800 mg, 1.17 mmol) dissolved in dichloromethane (50 mL), was irradiated for 4h and 45 min to afford compound **16** (70 mg, 7.8%) as a resinous matter; $R_f = 0.72$ (Hexanes- EtOAc, 7:3); $[\alpha]_{D}^{26} = 15.5$ (*c* 0.11, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.88 (distorted t, 3H, Me-5'), 1.34 (s, 3H, Me-12), 1.26 (brs, Me-15), 1.32 (s, 3H, Me-11), 1.37 (s, 3H, Me-13), 2.43 (s, 3H, Me-4''), 3.57 (m, 2H, H-14a and

H-14b), 3.69 (brs, 1H, H-10), 6.46 (brs, 1H, H-2), 6.54 (brs, 1H, H-4), 7.31 (brs, 2H, H-3^{''}, H-5^{\''}), 7.90 (brs, 2H, H-2^{\''}, H-6^{\''}); ¹³C NMR (Table 1); HRESIMS *m*/*z* 546.2785 [M]⁻ (calcd for $C_{30}H_{42}O_7S$, 546.2651).

(6aR,10S,10aR)-10-hydroperoxy-6,6-dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10ahexahydro-6H-benzo[c]chromen-1-yl 4-methylbenzenesulfonate (17)

Following the general experimental conditions, **2** (800 mg, 1.17 mmol) dissolved in dichloromethane (50 mL), was irradiated for 4h and 45 min to afford compound **17** (118 mg, 14.1%) as a resinous matter; $R_f = 0.36$ (Hexanes- EtOAc, 80:20); $[\alpha]^{26}_{D} = -22.0$ (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87(t, *J* =7 Hz, 3H, Me-5′), 0.81(s, 3H, Me-13), 1.31 (s, 3H, Me-12), 1.46 (1H, m, H-6a), 2.07 (1H, dd, J = 4 and 12.8 Hz, H-10a), 2.41 (s, 3H, Me-4′′), 5.00 (s, 1H, H-11a), 5.10 (s, 1H, H-11b), 4.63 (d, *J* = 2.4 Hz, 1H, H-10), 6.32 (d, *J* = 1.6 Hz, 1H, H-2), 6.49 (d, 1.2 Hz, 1H, H-4), 7.257(d, *J* = 8.4 Hz, 2H, H-3′′, H-5[×]), 7.69 (d, *J* = 8.4 Hz, 2H, H-2^{××}, H-6^{××}), 8.12 (s, 1H, HOO-10); ¹³C NMR (Table 1); HRESIMS *m*/*z* 501.2311 [M+H]⁺ (calcd for C₂₈H₃₇O₆S, 501.2278).

(6aR,10R,10aR)-10-hydroperoxy-6,6,9-trimethyl-3-pentyl-6a,7,10,10a-tetrahydro-6Hbenzo[c]chromen-1-yl 4-methylbenzenesulfonate (18)

Following the general experimental conditions, **2** (800 mg, 1.17 mmol) dissolved in dichloromethane (50 mL), was irradiated for 4h and 45 min to afford compound **18** (87 mg, 10.4%) as a resinous matter; $R_f = 0.50$ (Hexanes- EtOAc, 80:20); $[\alpha]_{D}^{26}$ -39.2 (*c* 0.125, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.74 (s, 3H, Me-13), 0.87(t, *J* =7.2Hz, 3H, Me-5'), 1.31(s, 3H, Me-12), 1.79 (br dd, *J* = 8.6 Hz, 1H, H-6a), 1.89 (s, 3H, Me-11), 2.40 (s, 3H, Me-4^{*}), 3.05

(t, J = 8.4 Hz, 1H, H-10a), 4.14 (d, J = 8.8 Hz, 1H, H-10), 5.75 (d, J = 6.0, 1H, H-8), 6.29 (d, J = 1.60 Hz, 1H, H-2), 6.52 (s, 1H, H-4), 7.26 (d, J = 8.0 Hz, 2H, H-3^{**}, H-5^{**}), 7.66 (d, J = 8.4 Hz, 2H, H-2^{**}, H-6^{**}), 9.20 (s, 1H, HOO-10); ¹³C NMR (Table 1); HRESIMS *m*/*z* 483.2227 [M-OH] + (calcd for C₂₈H₃₅O₅S, 483.2205).

(6aR,10S)-10-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,10,10a-tetrahydro-6H-

benzo[c]chromen-1-yl 4-methylbenzenesulfonate (19)

13 mg, 0.026mmol, of **18** was added to 1 mL of Me₂S and the mixture was stirred for 22h at room temperature. The reaction mixture was concentrated under *vacuum* to yield 11 mg (87.3%) of compound **19** as a viscous brownish yellow oil; R_f = 0.40 (Hexanes- EtOAc, 80:20); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.80 (s, 3H, Me-13), 0.92 (t, J = 8.6 Hz, 3H, Me-5'), 1.34 (s, 3H, Me-12), 1.81 (s, 3H, Me-11), 2.43 (s, 3H, Me-4^{\circk}), 2.62 (overlapped with DMSO signal (1H, H-10a), 4.06 (d, J = 6.3 Hz, 1H, H-10), 5.60 (d, J = 5.5, 1H, H-8), 6.48 (br d 1H, H-2), 6.50 (br d, 1H, H-4), 7.28 (d, J = 8.3 Hz, 2H, H-3^{\circk}, H-5^{\circk}), 7.71 (d, J = 8.3 Hz, 2H, H-2^{\circk}, H-6^{\circk}), ; ¹³C NMR (Table 1); HRESIMS *m/z* 483.2227 [M-OH] ⁺ (calcd for C₂₈H₃₅O₅S, 483.2205).

(9S,10S)-10-ethoxy-9-hydroxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6Hbenzo[c]chromen-1-yl 4-methylbenzenesulfonate (20)

Following the general experimental conditions, **3** (1.5 g, 2.19 mmol) dissolved in a mixture of dichloromethane (25 mL) and anhydrous ethanol (25 mL) was irradiated for 11 ½ h to afford compound **20** (291 mg, 17.2%) as an amorphous solid; R_f = 0.35 (Hexanes- EtOAc, 70:30); $[\alpha]^{26}_{D}$ = -24.0 (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.84 (t, *J* =6.4 Hz, 3H, Me-5'), 0.88 (s, 3H, Me-12), 1.03 (t, *J* = 6.8 Hz, Me-15), 1.23 (s, 3H, Me-11), 1.37 (s, 3H, Me-13),

2.35 (s, 3H, Me-4^{''}), 3.66 (m, 1H, H-14a), 3.90 (m, 1H, H-14b), 4.5 (s, 1H, H-10), 6.37 (d, J = 1.2, Hz, 1H, H-2), 6.53 (d, J = 1.6 Hz, 1H, H-4), 7.22 (d, J = 8.0 Hz, 2H, H-3^{''}, H-5^{\\\)}), 7.64 (d, J = 8.0 Hz, 2H, H-2^{\\\\)}, H-6^{\\\}); ¹³C NMR (Table 1); HRESIMS *m*/*z* 527.2484 [M-H]⁻ (calcd for $C_{30}H_{39}O_6S$, 527.2481).

(6aR,10aR)-9-hydroperoxy-6,6,9-trimethyl-3-pentyl-6a,9,10,10a-tetrahydro-6Hbenzo[c]chromen-1-yl 4-methylbenzenesulfonate (21)

Following the general experimental conditions, **4** (1.0 g, 2.14 mmol) dissolved in a mixture of dichloromethane (30 mL) and anhydrous ethanol (15 mL) was irradiated for 4h and 15 min to afford compound **21** (117 mg, 13.7%) as a resinous matter; $R_f = 0.44$ (Hexanes- EtOAc, 80:20); $[\alpha]^{26}_{D}$ - 33 (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87(t, *J* =6.0 Hz, 3H, Me-5'), 0.87(s, 3H, Me-13), 1.43 (s, 3H, Me-11), 1.43 (s, 3H, Me-12), 2.09 (brd, J = 10.8Hz, 1H, H-6a), 2.40 (s, 3H, Me-4''), 2.85 (brt, J = 10.0 Hz, H-10a), 5.62 (brd, J = 9.6 Hz, 1H, H-7), 5.90 (brd, J = 10Hz, 1H, H-8), 6.11 (brs, 1H, H-2), 6.53 (brs, 1H, H-4), 7.28 (d, J = 8.4 Hz, 2H, H-3'', H-5``), 7.71 ((d, J = 8.8 Hz, 2H, H-2``, H-6``), 9.28 (s, 1H, HOO-9); ¹³C NMR (Table 1); HRESIMS *m*/*z* 523.2118 [M+Na]⁺ (calcd for C₂₈H₃₆O₆SNa, 523.2233).

(6aR,10aR)-8-hydroperoxy-6,6-dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10ahexahydro-6H-benzo[c]chromen-1-yl 4-methylbenzenesulfonate (22)

Following the general experimental conditions **4** (1.0 g, 2.14 mmol), dissolved in a mixture of dichloromethane (30 mL) and anhydrous ethanol (15 mL), was irradiated for 4h and 15 min to afford compound **22** (249 mg, 29.1%) as a resinous matter; $R_f = 0.25$ (Hexanes- EtOAc, 80:20); $[\alpha]_{D}^{26} = -61.9$ (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.852(t, *J* =6.8 Hz, 3H,

Me-5'), 0.78(s, 3H, Me-13), 1.43 (s, 3H, Me-11), 1.31 (s, 3H, Me-12), 1.47 (m, 1H, H-6a), 2.37 (s, 3H, Me-4''), 3.48 (dd, J = 13.4, 3.6 Hz, H-10a), 3.48 (dd, J = 3.6, 13.4 Hz, 1H, H-10a), 4.40 (m, 1H, H-8), 4.94 (s, 1H, H-11'), 5.01 (s, 1H, H-11), 6.44 (d, J = 6.4 Hz, 1H, H-2), 6.50 (brs, 1H, H-4), 7.25 (d, J = 7.6 Hz, 2H, H-3'', H-5``), 7.66 (d, J = 8.0 Hz, 2H, H-2``, H-6``), 9.28 (s, 1H, HOO at C9); ¹³C NMR (Table 1); HRESIMS *m*/*z* 501.2302 [M+H] ⁺ (calcd for C₂₈H₃₇O₆S, 501.2266).

(6aR,9R,10aR)-6,6,9-trimethyl-3-pentyl-6a,9,10,10a-tetrahydro-6H-benzo[c]chromene-1,9-diol (23)

Compound **21** (47 mg, 0.094 mmol), dissolved in 5 mL of 10% KOH in ethanol, was refluxed for 75 min, affording compound **23** (25 mg, 80.6%) as a resinous matter; $R_f = 0.17$ (Hexanes-EtOAc, 75:25); $[\alpha]^{26}_{D}$ -17 (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.88(t, *J* =7.2 Hz, 3H, Me-5'), 0.95(s, 3H, Me-13), 1.38 (s, 3H, Me-11), 1.44 (s, 3H, Me-12), 2.14 (d, *J* = 10.8 Hz, 1H, H-6a), 3.50 (d, *J* = 13.6Hz, 1H, H-10a), 5.78 (m, 2H, H-7, H-8), 6.20 (brs, 1H, H-2), 6.21 (brs, 1H, H-4); ¹³C NMR (Table 2).

(6aR,9S)-9-ethoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9-tetrahydro-1H-benzo[c]chromene-1,4(6H)-dione (9)

Following the general experimental conditions, **1** (800 mg, 2.55 mmol) dissolved in a mixture of dichloromethane (25 mL) and anhydrous ethanol (50 mL) was irradiated for 11h to yield compound **9** (102 mg, 11%) as a resinous matter; $R_f = 0.46$ (Hexanes- EtOAc, 85:15); $[\alpha]^{26}_{D}$ -60.9 (*c* 0.16, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87(distorted t, 3H, Me-5'), 1.04(s, 3H, Me-13), 1.097 (t, J = 7.2 Hz, 3H, Me-2''), 1.22 (s, 3H, Me-12), 1.41 (s, 3H, Me-11), 2.27 (

1H, H-6a), 3.38 (q, J = 6.8 Hz, 2H, CH₂-1^{''}), 6.25 (s, 1H, H-2), 7.10 (s, 1H, H-10); ¹³C NMR (Table 2); HRESIMS m/z 371.2368 [M+H]⁺ (calcd for C₂₃H₃₃O₄, 371.2222).

10-ethoxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-benzo[c]chromene-1,9-diol (11).

Following the general experimental conditions, **1** (800 mg, 2.55 mmol) dissolved in a mixture of dichloromethane (25 mL) and anhydrous ethanol (50 mL) was irradiated for 11h to yield compound **11** (48 mg, 5.0%) as a resinous matter; $R_f = 0.30$ (Hexanes- EtOAc-MeOH, 10:10:0.2); $[\alpha]^{26}{}_D = -20.0$ (*c* 0.11, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.84 (t, J = 6.8 Hz, 3H, Me-5'), 1.42 (s, 3H, Me-13), 1.15 (t, J = 7.2 Hz, 3H, Me-15), 1.34 (s, 3H, Me-11), 1.24 (s, 3H, Me-12), 3.50 (m, 2H, CH₂-14), 4.2 (s, 1H, H-10), 6.21 (s, 1H, H-2), 6.29 (s, 1H, H-4); ¹³C NMR (Table 2); HRESIMS *m*/*z* 373.2393 [M-H]⁻ (calcd for C₂₃H₃₃O₄, 373.2379).

(6aR,8R,10aR)-8-hydroxy-6,6-dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10a-hexahydro-1H-benzo[c]chromene-1,4(6H)-dione (13)

Following the general experimental conditions, **2** (260 mg, 0.83 mmol) dissolved in a mixture of 40 ml of hexanes and 10 mL of dichloromethane was irradiated for 8 h affording compound **13** (15.7 mg, 5.5%). ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.88(t, *J* = 6.2 Hz, 3H, Me-5'), 1.12 (s, 3H, Me-13), 1.52 (s, 3H, Me-12), 1.72 (m, 1H, H-6a), 2.28 (m, H, H-10a), 4.14(m, 1H, H-8), 5.01 (s, 2H, CH₂-11), 6.36 (s, 1H, H-2); ¹³C NMR (Table 2); HRESIMS *m*/*z* 359.1994 [M-H]⁻ (calcd for C₂₁H₂₈O₄, 359.1858).

10-ethoxy-1,9-dihydroxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-

benzo[c]chromene-2-carboxylic acid (32)

Following the general experimental conditions, **8** (360 mg, 1.0 mmol) dissolved in a mixture of 40 mL of equal parts of MeOH and CH₂Cl₂ was irradiated for 3 1/2 hours, resulting in the formation of **32** (94 mg, 22.4%) as a resinous matter; $R_f = 0.15$ (Hexanes- EtOAc, 70:30); $[\alpha]^{26}_{D} = -43.8$ (*c* 0.105, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87(brt, 3H, Me-5'), 1.05 (t, *J* = 7.0 Hz, 3H, Me-2''), 1.22(s, 3H, Me-12), , 1.35 (s, 3H, Me-11), 1.45 (s, 3H, Me-13), 3.68 (t, J= 7.4 Hz, 1H, H-1''a), 3.80 (t, J = 7.4 Hz, 1H, H-1''b), 5.0 (s, 1H, H-10), 6.29 (s, 1H, H-4), 12.6 (brs, 1H, OH-3''); ¹³C NMR (Table 2); HRESIMS *m/z* 417.2219 [M-H]⁻ (calcd for C₂₄H₃₃O₆, 417.2277).

(6aR,10aR)-9-hydroperoxy-6,6,9-trimethyl-3-pentyl-6,6a,10,10a-tetrahydro-1Hbenzo[c]chromene-1,4(9H)-dione (24)

Following the general experimental conditions, **2** (260 mg, 0.83 mmol) dissolved in a mixture of 40 ml of hexanes and 10 mL of dichloromethane was irradiated for 8 h affording compound **24** (34 mg, 11.4%). Under the same conditions, compound **5** (methylated Δ^{8} -THC[42] - 800 mg, 2.44 mmol) was dissolved in a mixture of 30 mL of dichloromethane and 20 mL of absolute ethanol and irradiated for 6 h, also forming product **24** (12.5 mg, 14.1%) as a resinous matter; R_f = 0.48 (Hexanes- DCM-MeOH, 9:9:0.8); $[\alpha]^{26}_{D}$ = -2.3 (*c* 0.110, MeOH); ¹H NMR (400 MHz, CHCl₃ TMS) δ : 0.86(t , *J* = 6.2 Hz, 3H, Me-5'), 1.10(s, 3H, Me-13), 1.34 (s, 3H, Me-12), 1.55 (s, 3H, Me-11), 2.03 (d, J= 10 Hz, 1H, H-6a), 2.74 (m, 1H, H-10a), 5.68 (d, J=10Hz, H-7), 5.82 (d, J = 10, 1H, H-8), 6.37 (s, 1H, H-2); ¹³C NMR (Table 2); HRESIMS *m/z* 383.1779 [M+Na]⁺ (calcd for C₂₁H₂₈O₅Na, 383.1834).

(6aR,10aR)-8-hydroperoxy-6,6-dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10ahexahydro-1H-benzo[c]chromene-1,4(6H)-dione (25)

Following the general experimental conditions, **5** (800 mg, 2.44 mmol) was dissolved in a mixture of 30 mL of dichloromethane and 20 mL of absolute ethanol and irradiated for 6 h, forming product **25** (11mg, 13.6%) as a resinous matter; $R_f = 0.42$ (Hexanes- DCM-MeOH, 9:9:0.8); $[\alpha]^{26}_{D} = -15.4$ (*c* 0.13, MeOH); OR = - 0.020, 2.6 mg/2 mL MeOH; ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.88(t , *J* = 6.0 Hz, 3H, Me-5'), 1.08 (s, 3H, Me-13), 1.47 (s, 3H, Me-12), 1.83 (m, 1H, H-6a), 2.35 (m, 1H, H-10a), 4.54 (brt, 1H, H-8), 5.12 (s, 3H, Me-11b), 5.22 (s, 3H, Me-11a), 5.68 (d, J=10Hz, H-7), 5.82 (d, J = 10, 1H, H-8), 6.36 (s, 1H, H-2); ¹³C NMR (Table 2); HRESIMS *m*/*z* 327.2012 [M-HOO] ⁺ (calcd for C₂₁H₂₇O₃, 327.1960).

(6aR,10aR)-8-hydroperoxy-6,6-dimethyl-9-methylene-3-pentyl-6a,7,8,9,10,10ahexahydro-1H-benzo[c]chromene-1,4(6H)-dione (26)

Following the general experimental conditions, **5** (800 mg, 2.44 mmol) was dissolved in a mixture of 30 mL of dichloromethane and 20 mL of absolute ethanol and irradiated for 6 h, forming product **26** (10 mg, 12.4%) as a resinous matter; $R_f = 0.36$ (Hexanes- DCM-MeOH, 9:9:0.8); $[\alpha]^{26}_{D} = -24.0$ (*c* 0.10, MeOH); OR = -0.024, 2.0 mg/2 mL MeOH; ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87(t, J = 6.2 Hz, 3H, Me-5′), 1.12 (s, 3H, Me-13), 1.52 (s, 3H, Me-12), 5.04 (brs, 2H, CH₂-11), 1.74 (m, 1H, H-6a), 2.33 (m, 1H, H-10a), 4.49 (dd, 4.8, 10.8 Hz, 1H, H-8), 6.37 (s, 1H, H-2); ¹³C NMR (Table 3); HRESIMS *m*/*z* 359.1994 [M-H]⁻ (calcd for C₂₁H₂₇O₅, 359.1858).

1a,4,4-trimethyl-7-pentyl-2,3,4,9c-tetrahydro-1aH-oxireno[2',3':3,4]benzo[1,2-c]chromen-9-yl acetate (27). Following the general experimental conditions, **6**[41] (740 mg, 2.08 mmol) was dissolved in 60 mL of a mixture of hexanes/dichloromethane (1:1) and irradiated for 3 h, 45 min, resulting in the formation of derivative **27** as a resinous matter; $[\alpha]^{26}{}_{D} = 4.0$ (*c* 0.10, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ: 0.88 (t, *J* = 6.0 Hz 3H, Me-5'), 1.28 (s, 3H, Me-12), 1.38 (s, 3H, Me-13), 1.45 (s, 3H, Me-11), 2.30 (s, 3H, Me-15), 3.77 (s, 1H, H-10), 6.47 (s, 1H, H-4), 6.61 (s, 1H, H-2); ¹³C NMR (Table 3); HRESIMS *m/z* 371.2354 [M+H]⁺ (calcd for $C_{23}H_{31}O_{4}$, 371.2222).

9,10-dihydroxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-benzo[c]chromen-1-yl acetate (28). Following the general experimental conditions, 6 (740 mg, 2.08 mmol) was dissolved in 60 mL of a mixture of hexanes/dichloromethane (1:1) and irradiated for 3 h, 45 min, resulting in the formation of derivative 28 (15.7 mg, 2.0%) as a resinous matter; $R_f = 0.40$ (Hexanes- EtOAc, 80:20); $[\alpha]^{26}_{D} = -9.5$ (*c* 0.21, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.86 (t, J = 6.0 Hz 3H, Me-5'), 1.20 (s, 3H, Me-12), 1.33 (s, 3H, Me-13), 1.39 (s, 3H, Me-11), 2.13 (s, 3H, Me-15), 4.19 (s, 1H, H-10), 6.25 (brd, 1H, H-2), 6.29 (brd, 1H, H-4); ¹³C NMR (Table 3); HRESIMS *m/z* 387.2194 [M-H]⁻ (calcd for C₂₃H₃₁O₅, 387.2250).

9-methoxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-benzo[c]chromene-1,10-diol (29)

Compound **27** (95 mg, 0.26mmol) was dissolved in 6.0 mL of MeOH and treated with 180 mg of NaBH₄ for 2 1/2h, diluted with water and extracted with dichloromethane. Removal of solvent and purification on prep TLC afforded **29** (21.8 mg, 23.6%). Treatment of **27** (41 mg, 0.11

mmol) with NaHCO₃ (42 mg) in 2 mL of water, MeOH (4 mL), dichloromethane (3mL) and Adogen® 464 (26 mg), mixed and stirred for 2h also yielded compound **29** (13 mg, 32.6%) as a resinous matter; $R_f = 0.45$ (Hexanes- EtOAc, 80:20); $[\alpha]^{26}_{D} = -15.4$ (*c* 0.175, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87 (t, J = 7.0 Hz 3H, Me-5′), 1.30 (s, 3H, Me-12), 1.39 (s, 3H, Me-11), 1.49 (s, 3H, Me-13), 2.31 (s, 3H, Me-3''), 3.33 (s, 3H, OMe-1'), 4.26 (s, 1H, H-10), 6.29 (s, 1H, H-2), 6.36 (s, 1H, H-4); ¹³C NMR (Table 3); HRESIMS *m*/*z* 385.2479 [M-OH]⁺ (calcd for C₂₄H₃₃O₄, 385.2379).

10-hydroxy-9-methoxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-

benzo[c]chromen-1-yl acetate (30)

Pd/C (5 mg) was added to a solution of **27** (55 mg, 0,15 mmol) in MeOH. The reaction mixture was stirred while hydrogen was gently bubbled for 10 h, then diluted with water and extracted with dichloromethane. Removal of solvent and purification on prep TLC afforded compound **30** (16 mg, 26.8%); R_f = 0.46 (Hexanes- EtOAc, 80:20); $[\alpha]^{26}_{D}$ = -17.8 (*c* 0.09, MeOH); ¹H NMR (400 MHz, CHCl₃, TMS) δ : 0.87 (t, *J* = 7.0 Hz 3H, Me-5'), 1.22 (s, 3H, Me-12), 1.30 (s, 3H, Me-11), 1.45 (s, 3H, Me-13), 2.31 (s, 3H, Me-3''), 3.32 (s, 3H, OMe-1'), 4.29 (s, 1H, H-10), 6.42 (d, *J* = 1.6 Hz, 1H, H-2), 6.61 (d, *J* = 1.6 Hz, 1H, H-4); ¹³C NMR (Table 3); HRESIMS *m*/*z* 385.2479 [M-OH]⁺ (calcd for C₂₄H₃₃O₄, 385.2379).

4.2. Biological evaluation

Anticancer, antimicrobial, antimalarial, and antileishmanial evaluations were conducted in accordance with published procedures[43].

4.2.1.Cell lines and cell culture

Cell culture. HEK293 cells (ATCC #CRC-1573) were stably transfected via electroporation with full-length human recombinant cDNA for cannabinoid receptor subtypes 1 and 2 (obtained from Origene). These cells were maintained in a Dulbecco's modified Eagles's medium/ F-12 (50/50) nutrient mixture supplemented with 10% fetal bovine serum and either 1% penicillin/streptomycin or 1% G418 sulfate (Geneticin), depending on the cell line. Both cannabinoid cell lines were kept at 37°C and 5% CO₂. Membranes were prepared by scraping the cells in a 50 mM Tris-HCl buffer, homogenized via sonication, and centrifuged for 40 min at 13650 rpm at 4°C. The isolated membranes were kept at -80°C and brought up to room temperature for binding and functional assays. Protein concentration was determined via Bio-Rad protein assay[44].

4.2.2. Radioligand binding for cannabinoid receptor subtypes.

In the primary bioassay screen, compounds were tested at a final concentration of 10 μ M for competitive binding to the respective receptor. The compounds were added to a 96-well plate followed by 0.6 nM [³H]CP-55,940 and 10 μ g of cannabinoid membrane resuspended in 50 mM Tris (pH 7.4), 154 mM NaCl, and 20 mM Di-Na-EDTA supplemented with 0.02% BSA. The cannabinoid assay was incubated at 37°C for 90 min. The reaction was then terminated by rapid filtration using GF/C (presoaked in 0.3% BSA) and washed with the buffer. Dried filters were then covered with scintillant and measured for the amount of radioligand retained using a Perkin-

Elmer Topcount (Perkin-Elmer Life Sciences Inc., Boston, MA, USA). Nonspecific binding, which was determined in the presence of 1 μ M CP-55,940 for cannabinoid receptors, was subtracted from the total binding to yield the specific-binding values. Compounds showing competitive inhibition of the labeled ligand to bind to the receptor at 50% or greater were tested in a dose-response curve with concentrations of the test compound ranging from 300 μ M to 1.7 nM.

4.2.3. $[^{35}S]$ -GTP-YS binding. For the functional assay, membranes (20µg/ well) were incubated with the test compound, 0.5 nM $[^{35}S]$ -GTP-YS in 50mM Tris-HCl, 0.2mM EGTA, 9mM MgCl₂, 150mM NaCl, 50µM GDP, and 1.4mg ml⁻¹ BSA. The reaction was incubated for 2 h at 30°C and was terminated by rapid vacuum filtration with cold 10mM Tris-HCl in a Perkin Elmer harvester through GF/B filters. Nonspecific binding was determined by 40µM of GTP-YS.

4.3. Molecular modeling study

4.3.1. Homology modeling

Amino acid sequences of CB₂ was retrieved from the UniProt database (http://www.uniprot.org). Prime[41, 45, 46] was used for 3D model construction and refinement steps. The models were then validated using BioLuminate suite[27, 47-49]. BLAST homology search was run against the non-redundant database of the national center for biotechnology information (NCBI) to identify the highest homologous experimental protein structures from the protein databank (PDB) repository (<u>http://www.rcsb.org</u>). The alignment score of sequence alignment was calculated with the BLOSUM62 similarity matrix (BLOcks Substitution Matrix that is built using sequences with no more than 62% similarity). We used 11.0 for the gap

ACCEPTED MANUSCRIPT

opening cost (penalty) if a gap is introduced in the sequence alignment and 1.0 penalty score for each gap extension. BLAST homology search was carried out for maximum of three iterations at an inclusion threshold of 0.005. The globally conserved residues in the query sequences were examined to aid in selecting the homologous experimental structures.

The crystal structure of CB_1 (PDB accession code: 5XR8 [50]) was used as the template structure for modelling studies of CB_1 . Secondary structure prediction was established by SSPro. We used Prime STA GPCR-specific alignment for sequence alignment and knowledge-based model building method was employed to construct 10 models in each run. We refined the loops using a VSGB solvation model with OPLS 2005 force field and charges. 3D models were then subjected to energy minimization using OPLS2005 force field to remove atomic clashes. The refined models were evaluated by checking the ϕ - ψ angles, chirality, bond lengths, close contacts and also the stereo chemical properties using BioLuminate suite.

4.3.2. Protein preparation

Protein structures were prepared prior to docking by the protein preparation wizard of Schrödinger[51, 52]. The original hydrogen atoms were replaced with new ones followed by adjustment of bond orders. Hydrogen bonding network was corrected by adjusting the orientations of the amide groups (Asn and Gln), hydroxyl groups (Tyr, Thr and Ser), and relevant states of imidazole ring (His). The protein structures were then refined by restrained energy minimization using OPLS2005 force field with convergence of heavy atoms to an RMSD of 0.3 Å.

4.3.3. Ligand preparation

Ligands were prepared through LigPrep[53] with OPLS2005 force field and charges with only the lowest energy conformer for each ligand being kept. 2D structures of the compounds were sketched in Maestro and converted into 3D structures to produce corresponding low energy 3D output. Structures were included without performing pre-docking filtering.

4.3.4. Induced fit docking (IFD)

Induced fit Docking (IFD) protocol[54, 55] of Schrödinger was used for ligand docking to predict binding modes and associated effects on structural changes of the receptor.

The docking receptor grids were prepared using cavity occupied by the native ligand of CB₁. The CB1 ligand coordinates was copied into the binding pocket of CB₂ to be used in the IFD protocol. Ligand conformational sampling was performed with an energy window of 20.0 kcal/mol. A maximum of 20 poses for each ligand was retained. The poses were required to have a Coulomb-vdW score of <100 and an H-Bond score of <0.05. To attain better binding domain flexibility, Prime Molecular Dynamics module[45] was used to refine all amino residues which fell with 5 Å of each pose. Then, the best 20 poses within 30 kcal/mol were re-docked using Glide[56] SP.

4.3.5. MD simulations

Three MD simulation runs were carried out for CB_1 complexes with compounds 7 and 21, and CB_2 complex with compound 23. We used DESMOND [57-60] employing OPLS-2005 force field in all MD runs. The proteins were solvated, immersed in membrane (POPC 300K) and energy minimized for 5000 iterations. The minimized structures were subjected to six relaxation steps and protein-ligand contacts were calculated using simulation interactions diagram before

the MD production process. The production step was achieved using NPT ensemble. RMSD and RMSF.

ASSOCIATED CONTENT

Appendix A. Supplementary data

Supplementary data related to this article such as the amino acid sequences of $CB_1 \& CB_2$, graphics expressing receptor binding and functional assays, NMR (1D and 2D, NOESY), mass spectra and ¹³C tables can be found at (link to be added)

Author contribution

AG, SC, ME designed the experiments; AG, AH, WG, MR, DS, SR isolated starting cannabinoids, synthesized and confirmed structures; KE, VY, MK developed computational studies; SK and OD ran enzymatic assays and helped writing the manuscript; PC helped writing, organized and formatted the manuscript.

Acknowledgments

This research was partially funded by the National Institute on drug Abuse (NIDA) contract # N01DA-15-7793. The authors also thank the United States Department of Agriculture, Agriculture Research Service Specific Cooperative Agreement No. 58-6408-7-012, for partial support of this work; Dr. Bharathi Avula for helping with the HRESIFTMS analyses; Dr. Melissa for conducting antimicrobial assays and Dr. Babu Takwani for conducting the antileishmanial assay.

References:

- A.M. Galal, D. Slade, W. Gul, A.T. El-Alfy, D. Ferreira, M.A. Elsohly, Naturally occurring and related synthetic cannabinoids and their potential therapeutic applications, Recent Pat. CNS Drug Discovery, 4 (2009) 112-136.
- [2] B.J. Cridge, R.J. Rosengren, Critical appraisal of the potential use of cannabinoids in cancer management, Cancer Manage. Res., 5 (2013) 301-313, pp.
- [3] S. Pisanti, A.M. Malfitano, C. Grimaldi, A. Santoro, P. Gazzerro, C. Laezza, M. Bifulco, Use of cannabinoid receptor agonists in cancer therapy as palliative and curative agents, Best Pract. Res., Clin. Endocrinol. Metab., 23 (2009) 117-131.
- [4] S. Sarfaraz, V.M. Adhami, D.N. Syed, F. Afaq, H. Mukhtar, Cannabinoids for Cancer Treatment: Progress and Promise, Cancer Res., 68 (2008) 339-342.
- [5] I. Svizenska, P. Dubovy, A. Sulcova, Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures--a short review, Pharmacology, biochemistry, and behavior, 90 (2008) 501-511.
- [6] F.R. Kreitzer, N. Stella, The therapeutic potential of novel cannabinoid receptors, Pharmacology & therapeutics, 122 (2009) 83-96.
- [7] P. Diaz, J. Xu, F. Astruc-Diaz, H.-M. Pan, D.L. Brown, M. Naguib, Design and Synthesis of a Novel Series of N-Alkyl Isatin Acylhydrazone Derivatives that Act as Selective Cannabinoid Receptor 2 Agonists for the Treatment of Neuropathic Pain, J. Med. Chem., 51 (2008) 4932-4947.
- [8] M.P. Davis, Cannabinoids in pain management: CB1, CB2 and non-classic receptor ligands, Expert Opin. Invest. Drugs, 23 (2014) 1123-1140.
- [9] J. Guindon, A.G. Hohmann, Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain, Br. J. Pharmacol., 153 (2008) 319-334.
- [10] G.A. Cabral, L. Griffin-Thomas, Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation, Expert Rev Mol Med, 11 (2009) e3.
- [11] A.M. Malfitano, C. Laezza, A. D'Alessandro, C. Procaccini, G. Saccomanni, T. Tuccinardi, C. Manera, M. Macchia, G. Matarese, P. Gazzerro, M. Bifulco, Effects on immune cells of a new 1,8-naphthyridin-2-one derivative and its analogues as selective CB2 agonists: implications in multiple sclerosis, PLoS One, 8 (2013) e62511.
- [12] A. Dhopeshwarkar, K. Mackie, CB2 cannabinoid receptors as a therapeutic target what does the future hold?, Mol. Pharmacol., 86 (2014) 430-437, 438 pp.
- [13] A. Kauppinen, T. Nevalainen, M. Hytti, A. Salminen, K. Kaarniranta, T. Parkkari, CB2 receptor as a potential target in age-related diseases, J. Biochem. Pharmacol. Res., 2 (2014) 33-43, 11.
- [14] T. Nevalainen, Recent Development of CB2 Selective and Peripheral CB1/CB2 Cannabinoid Receptor Ligands, Curr. Med. Chem., 21 (2014) 187-203.
- [15] T. Petrzilka, M. Demuth, Hashish constituents. 7. Synthesis of (-)-11-hydroxy-Δ8-6a,10a-trans-tetrahydrocannabinol, Helv. Chim. Acta, 57 (1974) 121-150.
- [16] G. Appendino, S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, E. Smith, M.M. Rahman, Antibacterial Cannabinoids from Cannabis sativa: A Structure-Activity Study, J. Nat. Prod., 71 (2008) 1427-1430.
- [17] N.M. Kogan, R. Rabinowitz, P. Levi, D. Gibson, P. Sandor, M. Schlesinger, R. Mechoulam, Synthesis and Antitumor Activity of Quinonoid Derivatives of Cannabinoids, J. Med. Chem., 47 (2004) 3800-3806.

- [18] N.M. Kogan, M. Schlesinger, M. Peters, G. Marincheva, R. Beeri, R. Mechoulam, A cannabinoid anticancer quinone, HU-331, is more potent and less cardiotoxic than doxorubicin: a comparative in vivo study, J. Pharmacol. Exp. Ther., 322 (2007) 646-653.
- [19] N.M. Kogan, M. Schlesinger, E. Priel, R. Rabinowitz, E. Berenshtein, M. Chevion, R. Mechoulam, HU-331, a novel cannabinoid-based anticancer topoisomerase II inhibitor, Mol. Cancer Ther., 6 (2007) 173-183.
- [20] G. Pryce, C. Visintin, S.V. Ramagopalan, S. Al-Izki, L.E. De Faveri, R.A. Nuamah, C.A. Mein, A. Montpetit, A.J. Hardcastle, G. Kooij, H.E. de Vries, S. Amor, S.A. Thomas, C. Ledent, G. Marsicano, B. Lutz, A.J. Thompson, D.L. Selwood, G. Giovannoni, D. Baker, Control of spasticity in a multiple sclerosis model using central nervous system-excluded CB1 cannabinoid receptor agonists, FASEB J., 28 (2014) 117-130, 110.1096/fj.1013-239442.
- [21] A.S. Husni, C.R. McCurdy, M.M. Radwan, S.A. Ahmed, D. Slade, S.A. Ross, M.A. El Sohly, S.J. Cutler, Evaluation of phytocannabinoids from high-potency Cannabis sativa using in vitro bioassays to determine structure-activity relationships for cannabinoid receptor 1 and cannabinoid receptor 2, Med. Chem. Res., 23 (2014) 4295-4300.
- [22] J.W. Huffman, J. Liddle, S. Yu, M.M. Aung, M.E. Abood, J.L. Wiley, B.R. Martin, 3-(1',1'-Dimethylbutyl)-1-deoxy-Δ8-THC and related compounds: synthesis of selective ligands for the CB2 receptor, Bioorg. Med. Chem., 7 (1999) 2905-2914.
- [23] A. Galal, M. Radwan, S. Ahmed, D. Slade, W. Gul, S. Khan, S. Ross, M. ElSohly, Bioactive Products from Singlet Oxygen Photooxygenation of Δ9-THC, Δ8-THC, And Δ9-THC-Acid-A, Planta medica, 78 (2012) P_26.
- [24] N.M. Kogan, C. Blazquez, L. Alvarez, R. Gallily, M. Schlesinger, M. Guzman, R. Mechoulam, A cannabinoid quinone inhibits angiogenesis by targeting vascular endothelial cells, Mol. Pharmacol., 70 (2006) 51-59.
- [25] J.R. Duchek, Preparation of crystalline cannabinoid esters for cannabinoid purification, in, Mallinckrodt Inc., USA . 2004, pp. 22 pp.
- [26] C.G. Pitt, M.S. Fowler, S. Sathe, S.C. Srivastava, D.L. Williams, Synthesis of metabolites of delta-9-tetrahydrocannabinol, Journal of the American Chemical Society, 97 (1975) 3798-3802.
- [27] A.P. Brogan, L.M. Eubanks, G.F. Koob, T.J. Dickerson, K.D. Janda, Antibody-catalyzed oxidation of delta(9)-tetrahydrocannabinol, Journal of the American Chemical Society, 129 (2007) 3698-3702.
- [28] A.T. McPhail, H.N. ElSohly, C.E. Turner, M.A. ElSohly, Stereochemical Assignments for the Two Enantiomeric Pairs of 9,10-Dihydroxy-Δ6a(10a)-Tetrahydrocannabinols. X-Ray Crystal Structure Analysis of (±) Trans-cannabitriol, Journal of natural products, 47 (1984) 138-142.
- [29] A.J. Ganz, P.G. Waser, [Testing the pharmacological activity of some synthetic cannabinoids in mice (author's transl)], Arzneimittel-Forschung, 30 (1980) 471-477.
- [30] H.H. Wasserman, T.J. Lu, I.A. Scott, The total synthesis of tetracycline, J. Am. Chem. Soc., 108 (1986) 4237-4238.
- [31] W. Gul, A. Galal, M.A. ElSohly, P. Carvalho, Crystal structure of (9S,10S)-10-eth-oxy-9hy-droxy-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetra-hydro-6H-b enzo[c]chromen-1-yl 4-methylbenzene-sulfonate, Acta Crystallogr E Crystallogr Commun, 71 (2015) o1082-1083.
- [32] A.J. Ganz, P.G. Waser, Testing the pharmacological activity of some synthetic cannabinoids in mice, Arzneim.-Forsch., 30 (1980) 471-477.

- [33] M. Carbone, F. Castelluccio, A. Daniele, A. Sutton, A. Ligresti, V. Di Marzo, M. Gavagnin, Chemical characterization of oxidative degradation products of Δ9-THC, Tetrahedron, 66 (2010) 9497-9501.
- [34] J.M. Franklin, G.A. Carrasco, Cannabinoid receptor agonists upregulate and enhance serotonin 2A (5-HT(2A)) receptor activity via ERK1/2 signaling, Synapse, 67 (2013) 145-159.
- [35] J.W. Huffman, S.A. Hepburn, N. Lyutenko, A.L.S. Thompson, J.L. Wiley, D.E. Selley, B.R. Martin, 1-Bromo-3-(1',1'-dimethylalkyl)-1-deoxy-Δ8-tetrahydrocannabinols: New selective ligands for the cannabinoid CB2 receptor, Bioorg. Med. Chem., 18 (2010) 7809-7815.
- [36] HPLC, analyzed with a Waters 486 Tunable Absorbance Detector with Semi-Prep Flow Cell, at 350 nm, eluted isocratically with 0.5% anhydrous ethanol in hexanes at a flow rate of 15 mL/min.
- [37] M.M. Radwan, M.A. ElSohly, A.T. El-Alfy, S.A. Ahmed, D. Slade, A.S. Husni, S.P. Manly, L. Wilson, S. Seale, S.J. Cutler, S.A. Ross, Isolation and Pharmacological Evaluation of Minor Cannabinoids from High-Potency Cannabis sativa, Journal of natural products, 78 (2015) 1271-1276.
- [38] M. Elsohly, S. Ross, Method of preparing delta-9-tetrahydrocannabinol US 20020086438 A1, in, U.S. Patent and Trademark Office <u>http://www.uspto.gov/</u>, 2002.
- [39] Senior Research Scientist in the National Center for Natural Products Research, http://pharmacy.olemiss.edu/ncnpr/team/dr-suman-chandra/
- [40] M. Starks, Marijuana Chemistry: Genetics, Processing & Potency, Ronin Pub., 1990.
- [41] R.A. Archer, W.B. Blanchard, W.A. Day, D.W. Johnson, E.R. Lavagnino, C.W. Ryan, J.E. Baldwin, Cannabinoids. 3. Synthetic approaches to 9-ketocannabinoids. Total synthesis of nabilone, The Journal of organic chemistry, 42 (1977) 2277-2284.
- [42] D.R. Compton, W.R. Prescott, Jr., B.R. Martin, C. Siegel, P.M. Gordon, R.K. Razdan, Synthesis and pharmacological evaluation of ether and related analogues of delta 8-, delta 9-, and delta 9,11-tetrahydrocannabinol, Journal of medicinal chemistry, 34 (1991) 3310-3316.
- [43] M. Ilias, M.A. Ibrahim, S.I. Khan, M.R. Jacob, B.L. Tekwani, L.A. Walker, V. Samoylenko, Pentacyclic ingamine alkaloids, a new antiplasmodial pharmacophore from the marine sponge Petrosid Ng5 Sp5, Planta medica, 78 (2012) 1690-1697.
- [44] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem, 72 (1976) 248-254.
- [45] M.P. Jacobson, D.L. Pincus, C.S. Rapp, T.J. Day, B. Honig, D.E. Shaw, R.A. Friesner, A hierarchical approach to all-atom protein loop prediction, Proteins: Structure, Function, and Bioinformatics, 55 (2004) 351-367.
- [46] M.P. Jacobson, R.A. Friesner, Z. Xiang, B. Honig, On the role of the crystal environment in determining protein side-chain conformations, Journal of molecular biology, 320 (2002) 597-608.
- [47] K. Zhu, T. Day, D. Warshaviak, C. Murrett, R. Friesner, D. Pearlman, Antibody structure determination using a combination of homology modeling, energy-based refinement, and loop prediction, Proteins: Structure, Function, and Bioinformatics, 82 (2014) 1646-1655.
- [48] N.K. Salam, M. Adzhigirey, W. Sherman, D.A. Pearlman, Structure-based approach to the prediction of disulfide bonds in proteins, Protein Engineering Design and Selection, 27 (2014) 365-374.

- [49] H. Beard, A. Cholleti, D. Pearlman, W. Sherman, K.A. Loving, Applying physics-based scoring to calculate free energies of binding for single amino acid mutations in proteinprotein complexes, PloS one, 8 (2013) e82849.
- [50] T. Hua, K. Vemuri, S.P. Nikas, R.B. Laprairie, Y. Wu, L. Qu, M. Pu, A. Korde, S. Jiang, J.-H. Ho, G.W. Han, K. Ding, X. Li, H. Liu, M.A. Hanson, S. Zhao, L.M. Bohn, A. Makriyannis, R.C. Stevens, Z.-J. Liu, Crystal structures of agonist-bound human cannabinoid receptor CB1, Nature, 547 (2017) 468-471.
- [51] G.M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, W. Sherman, Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments, Journal of computer-aided molecular design, 27 (2013) 221-234.
- [52] Schrödinger Suite 2015-3 Protein Preparation Wizard; Epik version 3.3, Schrödinger, LLC, New York, NY, 2015; Impact version 6.8, Schrödinger, LLC, New York, NY, 2015; Prime version 4.1, Schrödinger, LLC, New York, NY, 2015, in.
- [53] LigPrep, version 3.5, Schrödinger, LLC, New York, NY, 2015, in.
- [54] R. Farid, T. Day, R.A. Friesner, R.A. Pearlstein, New insights about HERG blockade obtained from protein modeling, potential energy mapping, and docking studies, Bioorganic & medicinal chemistry, 14 (2006) 3160-3173.
- [55] Schrödinger Suite 2015-3 Induced Fit Docking protocol; Glide version 6.8, Schrödinger, LLC, New York, NY, 2015; Prime version 4.1, Schrödinger, LLC, New York, NY, 2015, in.
- [56] R.A. Friesner, R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T.A. Halgren, P.C. Sanschagrin, D.T. Mainz, Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes, Journal of medicinal chemistry, 49 (2006) 6177-6196.
- [57] K.J. Bowers, E. Chow, H. Xu, R.O. Dror, M.P. Eastwood, B.A. Gregersen, J.L. Klepeis, I. Kolossvary, M.A. Moraes, F.D. Sacerdoti, Scalable algorithms for molecular dynamics simulations on commodity clusters, in: SC 2006 Conference, Proceedings of the ACM/IEEE, IEEE, 2006, pp. 43-43.
- [58] D. Shivakumar, J. Williams, Y. Wu, W. Damm, J. Shelley, W. Sherman, Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field, Journal of chemical theory and computation, 6 (2010) 1509-1519.
- [59] Desmond Molecular Dynamics System, version 4.3, D. E. Shaw Research, New York, NY, 2015. Maestro-Desmond Interoperability Tools, version 4.3, Schrödinger, New York, NY, 2015., in.
- [60] Z. Guo, U. Mohanty, J. Noehre, T.K. Sawyer, W. Sherman, G. Krilov, Probing the α-Helical Structural Stability of Stapled p53 Peptides: Molecular Dynamics Simulations and Analysis, Chemical biology & drug design, 75 (2010) 348-359.

Highlights

- Photooxygenation of cannabinol derivatives yielded 18 new oxygenated compounds.
- Products exhibited anticancer, antimicrobial, and antileishmanial effects.
- One derivative showed better antileishmanial activity than standard drugs.
- Two derivatives are CB1 partial agonists, one shows high CB2 binding affinity.
- Photooxygenation may be a practical way to synthesize cannabinoid metabolites.

CER MAN