4th International Electronic Conference on Medicinal Chemistry
1-30 November 2018
chaired by Dr. Jean Jacques Vanden Eynde
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# Synthesis and Biological Screening of Analogues of Bioactive Acid Constituents from the Traditional Chinese Medicinal Plant *Liquidambar Formosana*

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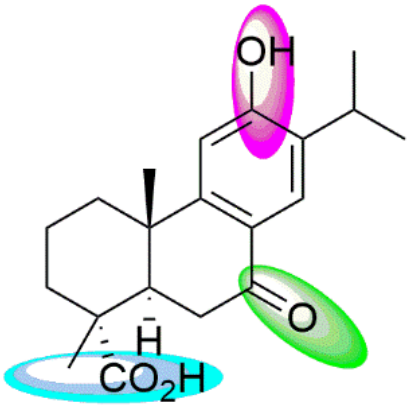
# Synthesis and Biological Screening of Analogues of Bioactive Acid Constituents from the Traditional Chinese Medicinal Plant Liquidambar Formosana




**(+)-liquiditerpenoic acid A**

$GI_{50}$(solid tumor) 4,4-25 μM; Potentiation $I_{GABA}$(%,100μM)= 12-378
$EC_{50}$ (leukemia) 8,8-81,5 μM ; $IC_{50}$ (*Leishmania*)=1,7-41,5 μM

**Abstract:** *Liquidambar formosana* Hence (also known as maple) is a tall deciduous tree widely distributed in various regions of the south of the Qinling Mountains and Huaihe River in China, and also found in northern Vietnam, Laos and South Korea. *L. formosana* is a famous ornamental plant for leaves are green in spring and summer, and red in autumn. Different plant parts of *L. formosana*, such as the leaf, fruit, bark, and resin, are proved to be the treasures as natural medicinal plant resources. Among the bioactive constituents, several diterpenoid acids of the abietane family have been identified.

Medicinal chemists have studied derivatives of two readily available abietane-type materials such as dehydroabietic acid and dehydroabietylamine (DHAA). To date, there is only one commercial drug, Ecabet® (ecabet sodium), based on abietanes, which is used for the treatment of reflux esophagitis and peptic ulcer disease.

These biological reports and the simultaneous isolation (in 2014) by Hua and co-workers, of the new abietane liquiditerpenoic acid A, a sugiol analogue, from the resin of *Liquidambar formosana* and from *Pinus massoniana,* by Kuo and co-workers named independently as abietopinoic acid, prompted us to synthesize it and study its biological properties along with some analogues.

**Keywords:** *abietane; semisynthesis; antitumor; leukemia; antileishmania*

# Introduction

## Privileged structure

NPR

REVIEW

View Article Online
View Journal

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Cite this: DOI: 10.1039/c4np00110a

**Aromatic abietane diterpenoids: their biological activity and synthesis**

Miguel A. González*

European Journal of Medicinal Chemistry 87 (2014) 834–842

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

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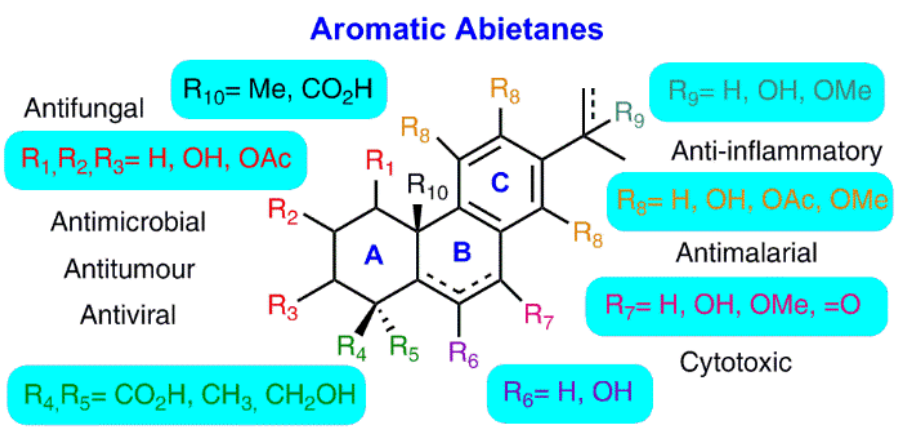
Mini-review

**Synthetic derivatives of aromatic abietane diterpenoids and their biological activities**

CrossMark

Miguel A. González




After a large number of scientific evidence (~250 references), the tricyclic system of the abietane skeleton with an aromatic C ring can be considered a privileged structure able to interact with a number of biological targets.

# Introduction

## Semisynthesis and Total synthesis

Tetrahedron 71 (2015) 1883–1908

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet


Tetrahedron report number 1072

### Aromatic abietane diterpenoids: total syntheses and synthetic studies

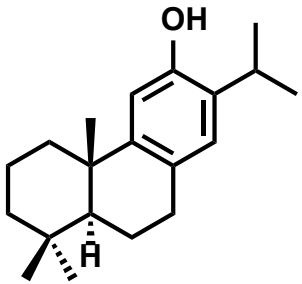
Miguel A. González *

*Departamento de Química Orgánica, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain*

Around 70 references on this subject.

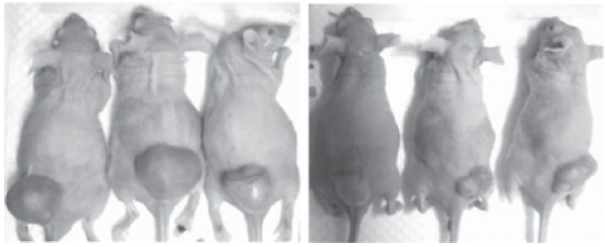
# Introduction

## Ferruginol and analogues

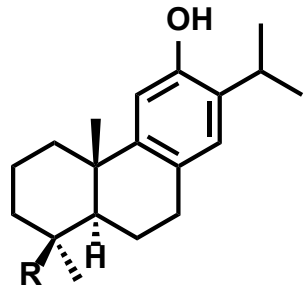



(+)-Ferruginol

Antitumor activity *in vitro* in prostate cancer, pancreas and leukemia (apoptosis).
Antitumor activity *in vivo* in lung cancer xenografts in mouse.

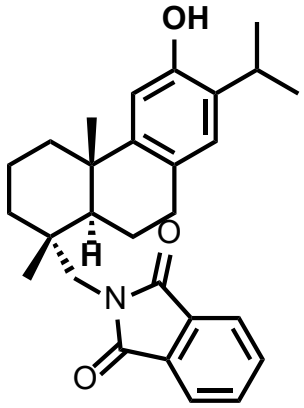


Control Ferruginol




R= $CH_2OH$, $CO_2Me$

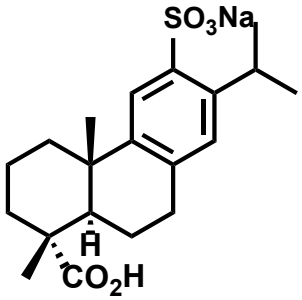
Antileishmania activity *in vitro* (ferruginol and C-19 derivatives)




Antiviral activity *in vitro* against Herpes and Dengue viruses

# Introduction

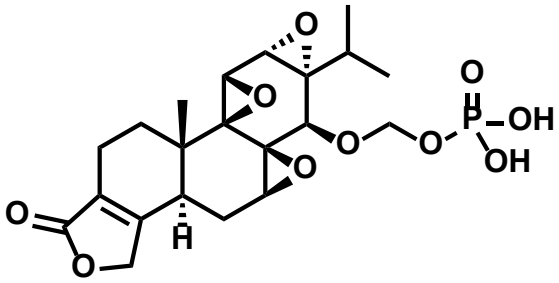
## Drugs based on abietane diterpenoids




Ecabet®

Sodium Ecabet is commercially available in Japan and China for the treatment of reflux oesophagitis and peptic ulcer disease.

Antibacterial against *Helicobacter pylori*

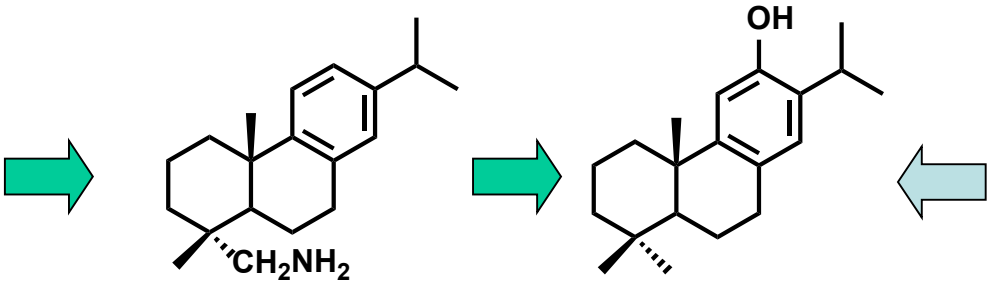



Minnelide™

In Clinical development for anticancer agent for pancreas carcinoma.

# Introduction

## Synthesis of bioactive Terpenoids in Valencia: from dehydroabietylamine




(+)-Dehydroabietylamine

60%, ~100 euros/100 grams

(+)-Ferruginol

Pine resins

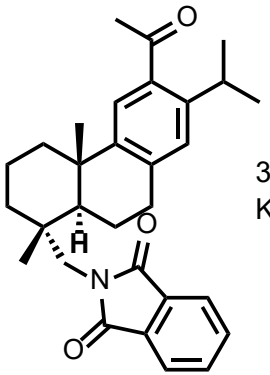
Conifers

The natural product Ferruginol has been isolated from plants of the families *Podocarpaceae, Cupressaceae, Lamiaceae* and *Verbenaceae* and has shown a wide range of biological activity such as antimicotic, antimicrobial, cardioprotective, antioxidant, antimalarial, antileishmania, antiinflammatory and antitumor.

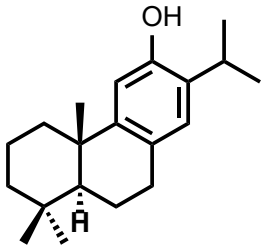
González, M. A.; Pérez-Guaita, D. *Tetrahedron* **2012**, *68*, 9612-9615.

# Introduction

## Biological activity of Ferruginol and analogs: Antimalarial and Antiviral

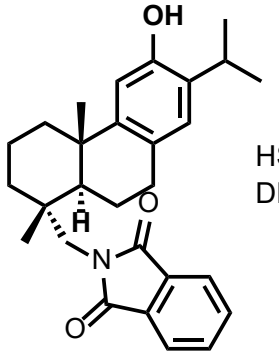


3D7 $EC_{50}$= 86nM
K1 $EC_{50}$= 201 nM

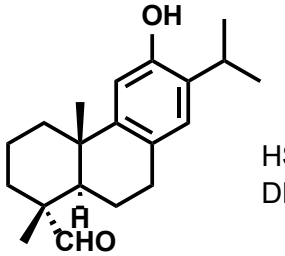


3D7 $EC_{50}$= 2.47 μM
K1 $EC_{50}$= 1.33 μM

**(+)-Ferruginol**



HSV-2 $EC_{50}$ (IS) = 19.2 μM (10)
DENV-2 $EC_{50}$ (IS) = 1.4 μM (58)!!!



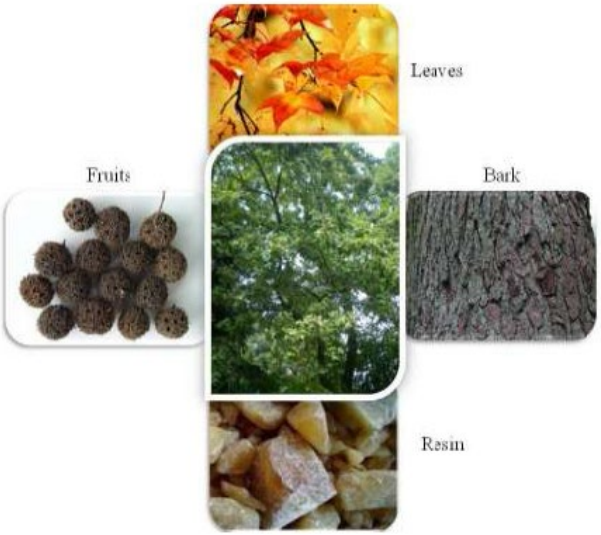
HSV-2 $EC_{50}$ (IS) = 16.6 μM (6)
DENV-2 $EC_{50}$ (IS) = 5.0 μM (10)

González, M. A.; Clark, J.; Connelly, M.; Rivas, F. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5234-5237.

Roa-Linares, V.; Betancur-Galvis, L.; González, M. A. *et al. Eur. J. Med. Chem.* **2016**, *108*, 79-88.

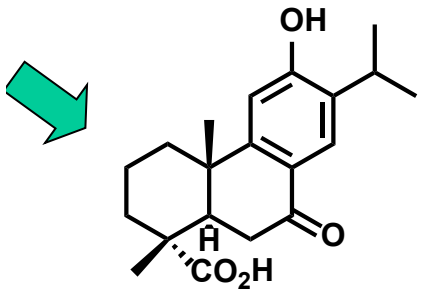
# Introduction

## Discovery of liquiditerpenoic acid A or abietopinoic acid




*Liquidambar formosana* and its organs

The resin and fruits of *Liquidambar formosana* Hance, a plant mainly distributed in South China, are indexed in Chinese Pharmacopoeia. The extracts of the resin, leaves and fruits showed antibacterial and antioxidant activities as well as effects on the cardiovascular system.




**(+)-Liquiditerpenoic acid A**

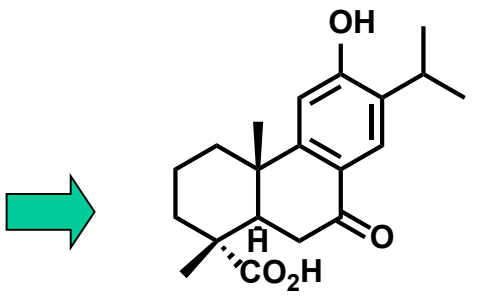
The resin is an expensive Traditional Chinese Medicine (TCM) has been used traditionally for the treatment of flutter injury, ulcer throat, vomiting, and trauma bleeding in China.

Chinese researchers: Three new diterpenoids from the resin of *Liquidambar formosana* Hua, H.-M. *et al. Nat. Prod. Res.* **2014**, *28*, 1-6. sent April 2013

# Introduction

## Discovery of liquiditerpenoic acid A or abietopinoic acid

*Pinus massoniana*
*distributed mainly in Taiwan*




**(+)-Abietopinoic acid**

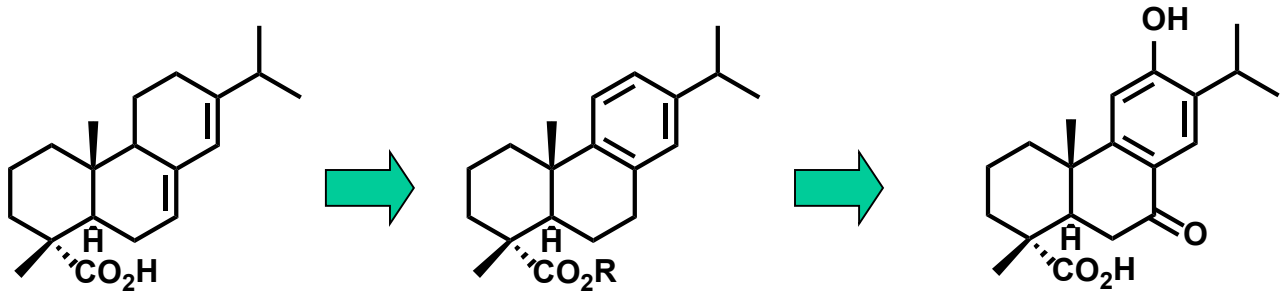
The resin exuded from the trunk of *Pinus massoniana* has been a traditional medication. The acidic fraction of the resin has been proven to exhibit antithrombotic and antiplatelet-aggregation activities.

**Semisystematic nomenclature:** 12-hydroxy-7-oxoabieta-8,11,13-trien-18-oic acid

Taiwanese researchers: Two New Acidic Diterpenoids from the Heartwood of *Pinus massoniana* Kuo, Y.-H. *et al. Helv. Chim. Acta.* **2014**, *97*, 1146-1151 sent December 2013

## Results and discussion

## Semisynthesis of liquiditerpenoic acid A or abietopinoic acid



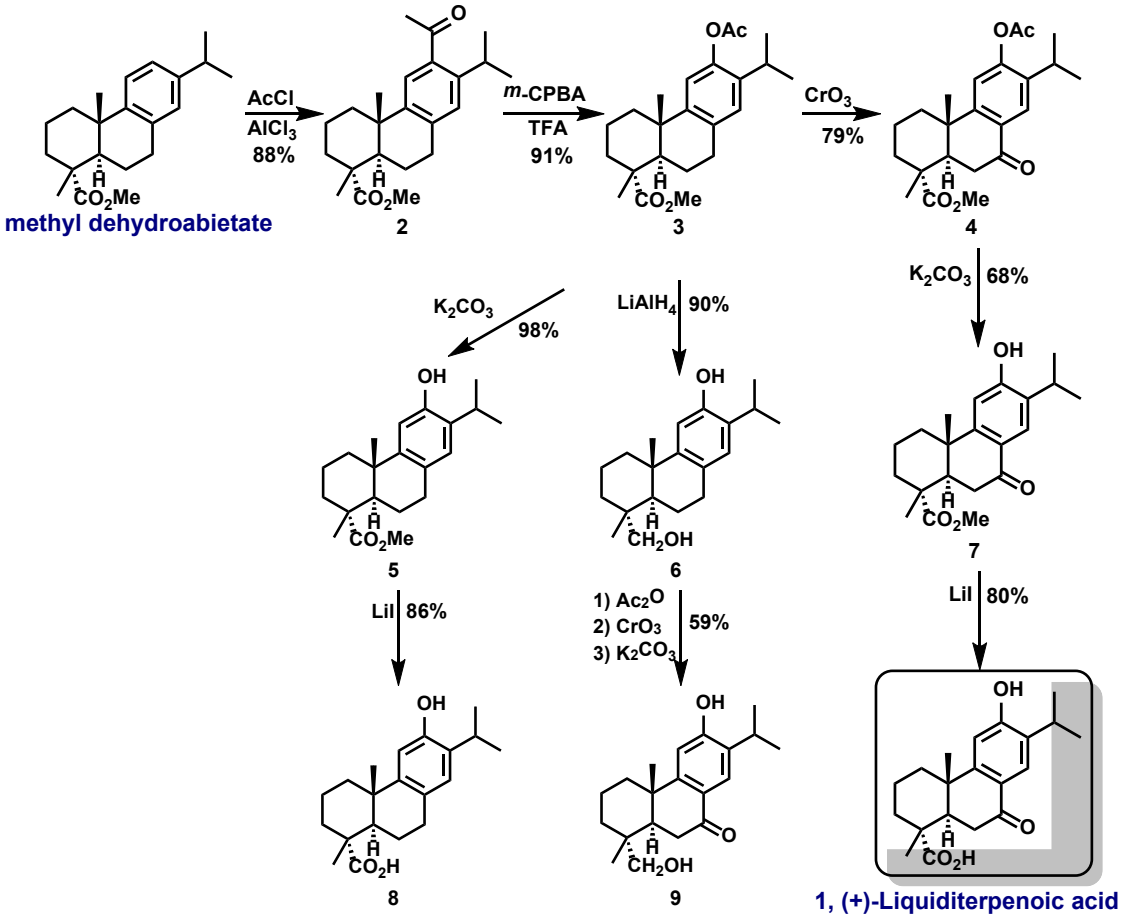

**(-)-Abietic acid** **R= H (+)-Dehydroabietic acid** **(+)-Liquiditerpenoic acid A**
**R= Me methyl dehydroabietate**

González, M. A. *et al.*
*Eur. J. Med. Chem.* **2010**, *45*, 811-816.

Planned semisynthesis starting from commercially available abietic acid, convertible in methyl dehydroabietate following our procedure published in 2010 via methylation and aromatization by heating with Pd/C as catalyst.

# Results and discussion

## Semisynthesis of liquiditerpenoic acid A and analogues




**Scheme 1. Synthesis of liquiditerpenoic acid (1) and related tested compounds 2-9 from methyl dehydroabietate.**

## Results and discussion

# Antiproliferative activity of liquiditerpenoic acid A and analogues

**Table 1.** Antiproliferative activity ($GI_{50}$) of liquiditerpenoic acid (**1**) and analogues **2-9** against human solid tumor cells.[a]

| | Cell line (origin) | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | A549 (lung) | HBL-100 (breast) | HeLa (cervix) | SW1573 (lung) | T-47D (breast) | WiDr (colon) |
| **DHA[b]** | **25 ± 5.2** | **39 ± 7.3** | **25 ± 9.1** | **47 ± 17** | **40 ± 5.9** | **26 ± 8.4** |
| **Methyl DHA[b]** | **15 ± 2.6** | **19 ± 0.2** | **15 ± 3.0** | **22 ± 6.2** | **16 ± 4.7** | **10 ± 3.6** |
| **1** | **>100** | **>100** | **85 ± 21** | **>100** | **>100** | **>100** |
| **2** | **18 ± 2.3** | **15 ± 1.2** | **11 ± 1.0** | **11 ± 4.6** | **10 ± 1.5** | **17 ± 2.4** |
| **3** | **11 ± 1.8** | **15 ± 3.9** | **4.4 ± 1.1** | **12 ± 3.2** | **14 ± 2.9** | **16 ± 2.5** |
| **4** | **14 ± 0.4** | **16 ± 1.5** | **13 ± 2.3** | **17 ± 1.9** | **20 ± 2.9** | **19 ± 1.7** |
| **5** | **9.4 ± 2.6** | **9.7 ± 2.7** | **5.8 ± 0.5** | **11 ± 2.0** | **19 ± 3.8** | **23 ± 4.4** |
| **6** | **12 ± 1.9** | **16 ± 2.0** | **12 ± 1.8** | **15 ± 2.8** | **16 ± 0.9** | **17 ± 1.3** |
| **7** | **>100** | **>100** | **>100** | **>100** | **>100** | **>100** |
| **8** | **70 ± 6.3** | **>100** | **96 ± 5.5** | **93 ± 9.3** | **>100** | **>100** |
| **9** | **19 ± 3.3** | **16 ± 2.0** | **16 ± 2.4** | **23 ± 0.7** | **20 ± 2.7** | **19 ± 2.1** |
| **etoposide** | **1.5 ± 0.3** | **1.4 ± 0.1** | **3.3 ± 1.6** | **15 ± 1.5** | **22 ± 5.5** | **23 ± 3.1** |
| **cisplatin** | **4.9 ± 0.2** | **1.9 ± 0.2** | **1.8 ± 0.5** | **2.7 ± 0.4** | **17 ± 3.3** | **23 ± 4.3** |

[a] Values are given in μM and represent the mean ± standard deviation of at least two independent experiments.
[b] Values taken from reference: Stadler, M. et al. *Planta Med. Int. Open 2017*, 4, e89-e92.

## Results and discussion

# Antiproliferative activity of liquiditerpenoic acid A and analogues

**Table 2.** Antiproliferative activity ($EC_{50}$) of liquiditerpenoic acid (**1**) and analogs **2-9** against leukemia cellular models.[a]

| | Cell line (origin) | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | Nalm06 | BCR-ABL | KOPN-8 | SUP-B15 | UoCB1 | BJ |
| **1** | **N.A** | **N.A** | **N.A** | **N.A** | **N.A** | **N.A** |
| **2** | **N.A** | **>40** | **>40** | **>40** | **>40** | **N.A** |
| **3** | **8.8** | **14.3** | **21.0** | **20.2** | **>40** | **N.A** |
| **4** | **10.7** | **12.2** | **18.5** | **21.7** | **>40** | **N.A** |
| **5** | **77.8** | **15.9** | **>40** | **>40** | **>40** | **N.A** |
| **6** | **26.3** | **N.A** | **>40** | **>40** | **>40** | **N.A** |
| **7** | **N.A** | **N.A** | **>40** | **>40** | **17.8** | **N.A** |
| **8** | **81.5** | **17.4** | **N.A** | **N.A** | **N.A** | **N.A** |
| **9** | **65.7** | **19.4** | **N.A** | **>40** | **>40** | **N.A** |

[a] Values are given in μM, N.A.: no activity at the tested concentrations.
Negative control: vehicle, positive control staurosporine dose response and kill at 2 μM.

## Results and discussion

# GABAA receptor modulating activity of liquiditerpenoic acid A and analogues

**Table 3.** Potentiation of $I_{GABA}$ in α1β2γ2S receptors by compounds **1** and **2-9**.

| Compound | Conc.; μM | $I_{GABA}$ potentiation (%) | S.E.M |
|---|---|---|---|
| DHA | 10 | 186.9 | 9.6 |
| | 100 | 841.3 | 52.9 |
| 1 | 10 | -2.0 | 4.4 |
| | 100 | -21.1 | 7.1 |
| 2 | 10 | 2.7 | 0.9 |
| | 100 | -2.9 | 5.1 |
| 3 | 10 | 5.1 | 2.1 |
| | 100 | 26.3 | 8.9 |
| 4 | 10 | -1.0 | 2.8 |
| | 100 | 46.7 | 8.3 |
| 5 | 10 | 23.7 | 9.6 |
| | 100 | 378.0 | 65.6 |
| 6 | 10 | 29.3 | 10.6 |
| | 100 | 154.1 | 34.2 |
| 7 | 10 | 37.4 | 12.7 |
| | 100 | 242.4 | 46.0 |
| 8 | 10 | 8.9 | 2.3 |
| | 100 | 12.1 | 2.9 |
| 9 | 10 | 4.5 | 5.8 |
| | 100 | -30.3 | 9.5 |

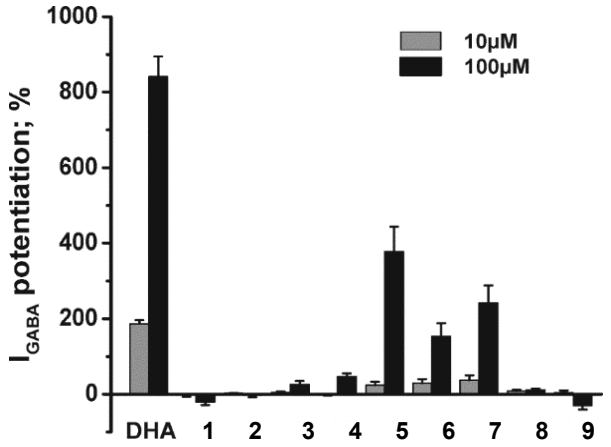



**Figure 1.** Potentiation of GABA-evoked currents ($I_{GABA}$) through α1β2γ2S receptors by indicated compounds at 10 and 100μM. Data represent mean ± SEM from at least three different oocytes and two oocyte batches.

## Results and discussion

# Antileishmanial activity of liquiditerpenoic acid A and analogues

**Table 4.** $IC_{50}$ Leishmanicidal and cytotoxic effects (in μM) of C7- and C12-functionalized dehydroabietic acid derivatives on *in vitro* promastigote assay.

| Compound | *L. amazonensis* | | *L. guyanensis* | | *L. donovani* | | *L. infantum* | | *Macrophages J774* |
|---|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$[a]±SD | SI[b] | $IC_{50}$±SD | SI | $IC_{50}$±SD | SI | $IC_{50}$±SD | SI | $CC_{50}$[c] |
| 1 | NA[d] | | NA | | NA | | NA | | 219.43±14.98 |
| 2 | 11.64±0.63 | 11.13 | 14.20±0.39 | 9.12 | 14.79±0.92 | 8.76 | 2.47±0.62 | 52.49 | 129.57±8.86 |
| 3 | 3.24±0.33 | 8.20 | 5.51±0.7 | 4.82 | 8.12±0.73 | 3.27 | 1.32±0.11 | 20.18 | 26.55±2.71 |
| 4 | 0.65±0.03 | 27.26 | 1.34±0.15 | 13.23 | 4.38±0.46 | 4.06 | 0.67±0.18 | 26.42 | 17.78±2.8 |
| 5 | 3.92±0.13 | 8.74 | 5.95±1.1 | 5.75 | 9.21±0.06 | 3.72 | 5.05±0.33 | 6.77 | 34.23±3.15 |
| 6 | 7.75±0.84 | 3.03 | 12.49±2.2 | 1.88 | 13.93±0.13 | 1.68 | 0.69±0.1 | 33.79 | 23.46±4.61 |
| 7 | ND[e] | | ND | | ND | | ND | | 153.89±49.28 |
| 8 | 54.10±6.53 | 1.59 | 71.40±1.5 | 1.20 | 58.10±0.67 | 1.48 | 43.65±3.41 | 1.97 | 85.82±28.78 |
| 9 | 10.23±0.65 | 1.4 | 16.46±0.69 | 0.87 | 19.7±0.39 | 0.73 | 9.69±0.77 | 1.49 | 14.37±2.83 |
| Miltefosine | 47.7 ± 5.0 | 2.9 | 18.2 ± 0.6 | 7.5 | 0.15 ± 0.02 | 909 | 3.4 ± 0.6 | 40.1 | 136.4 ± 1.4 |

[a] $IC_{50}$, concentration of the compound that produced a 50% reduction in parasites; SD: standard deviation. [b] Selectivity index, SI = $CC_{50}/IC_{50}$; [c] $CC_{50}$, concentration of the compound that produced a 50% reduction of cell viability in treated culture cells with respect to untreated ones; [d] NA, no activity; [e] ND, not determined.

# Results and discussion

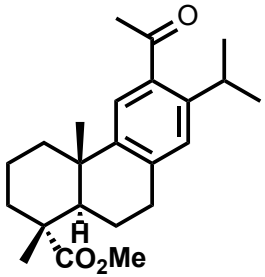
**Table 5.** Calculated molecular properties (drug-likeness) by molinspiration online software for compounds **1**, and **2-9**.[a]

| Compound | miLog P | MW | n-HBA | n-HBD | TPSA | Lipinski's violation |
|---|---|---|---|---|---|---|
| **1** | 4.21 | 330.42 | 4 | 2 | 74.60 | 0 |
| **2** | 5.64 | 356.51 | 3 | 0 | 43.38 | 1 |
| **3** | 5.29 | 372.50 | 4 | 0 | 52.61 | 1 |
| **4** | 4.39 | 386.49 | 5 | 0 | 69.68 | 0 |
| **5** | 5.73 | 330.47 | 3 | 1 | 46.53 | 1 |
| **6** | 5.24 | 302.46 | 2 | 2 | 40.46 | 1 |
| **7** | 4.83 | 344.45 | 4 | 1 | 63.60 | 0 |
| **8** | 5.11 | 316.44 | 3 | 2 | 57.53 | 1 |
| **9** | 4.33 | 316.44 | 3 | 2 | 57.53 | 0 |
| **Rule of five** | not >5 | <500 | not >10 | not >5 | | 1 violation allowed |

[a] Values were calculated using Molinspiration Cheminformatics software (Molinspiration, Slovensky Grob, Slovak Republic, 2015, http://www.molinspiration.com)

# Conclusions

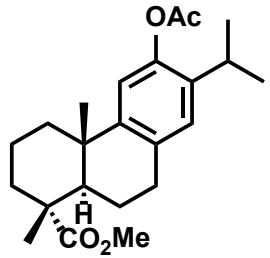
## Biological activity of Liquiditerpenoic acid A and analogues




2

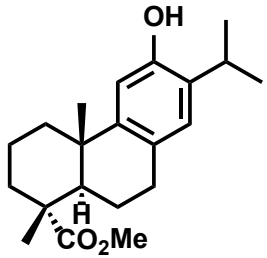
SW1573 (lung) $GI_{50}$= 11 μM
T-47D (breast) $GI_{50}$= 10 μM
WiDr (colon) $GI_{50}$= 17 μM

L. amazonensis $IC_{50}$= 11.6 μM
L. guyanensis $IC_{50}$= 14.2 μM
L. donovani $IC_{50}$= 14.8 μM
L. infantum $IC_{50}$= 2.5 μM



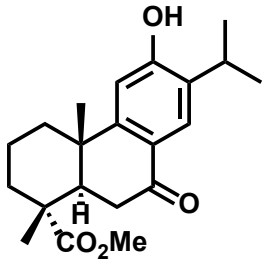

3

HeLa (cervix) $GI_{50}$= 4.4 μM
T-47D (breast) $GI_{50}$= 14 μM
WiDr (colon) $GI_{50}$= 16 μM
Nalm06 (leukemia) $EC_{50}$= 8.8 μM




5

$I_{GABA}$ potentiation (%) = 378 at 100 μM
L. amazonensis $IC_{50}$= 3.9 μM
L. guyanensis $IC_{50}$= 5.9 μM
L. donovani $IC_{50}$= 9.2 μM
L. infantum $IC_{50}$= 5.0 μM
but cytotoxic to J774 macrophages




7

$I_{GABA}$ potentiation (%) = 242.4 at 100 μM

The combined findings indicate that these abietane-diterpenoid natural product analogs offer a source of novel bioactive molecules with promising pharmacological and drug-likeness properties.

## Acknowledgments

[Spanish National Research Council grant 201680I008].

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**Thank you for your attention!**