III SIMPÓSIO INTERNACIONAL EM INVESTIGAÇÕES QUÍMICO-FARMACÊUTICAS

I ENCONTRO IBERO-AMERICANO DE PLANTAS MEDICINAIS Dr. Mahabir Gupta

I CONGRESSO LUSO-BRASILEIRO DE CIÊNCIAS E TECNOLOGIAS EM SAÚDE

28 a 30 de setembro de 2022, Itajaí-SC-Brasil

UNIVALI
UNIVERSIDADE DO VALE DO ITAJAÍ

UNOCHAPECÓ
A COMUNIDADE DO CONHECIMENTO

Isobolographic analysis of the interaction between synthetic drugs and natural products: synergistic, additive or antagonistic effects?

Liz Girardi Müller
lizmuller@unochapeco.edu.br

Itajaí
September 29th 2022

ISOBOLOGRAPHIC ANALYSIS

ISOBOLOGRAPHIC ANALYSIS

Introduced by S. Loewe;

Published: July 1926

Über Kombinationswirkungen

Mitteilung: Hilfsmittel der Fragestellung

S. Loewe & H. Muischnek

Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie **114**, 313–326 (1926) | Cite this article

716 Accesses | **570** Citations | **19** Altmetric | Metrics

Effect of combinations: mathematical basis of problem.

ISOBOLOGRAPHIC ANALYSIS

Ronald Tallarida discussed the use and construction of a linear isobole;

› Pain. 2002 Jul;98(1-2):163-8. doi: 10.1016/s0304-3959(02)00041-6.

The interaction index: a measure of drug synergism

Ronald J Tallarida [1]

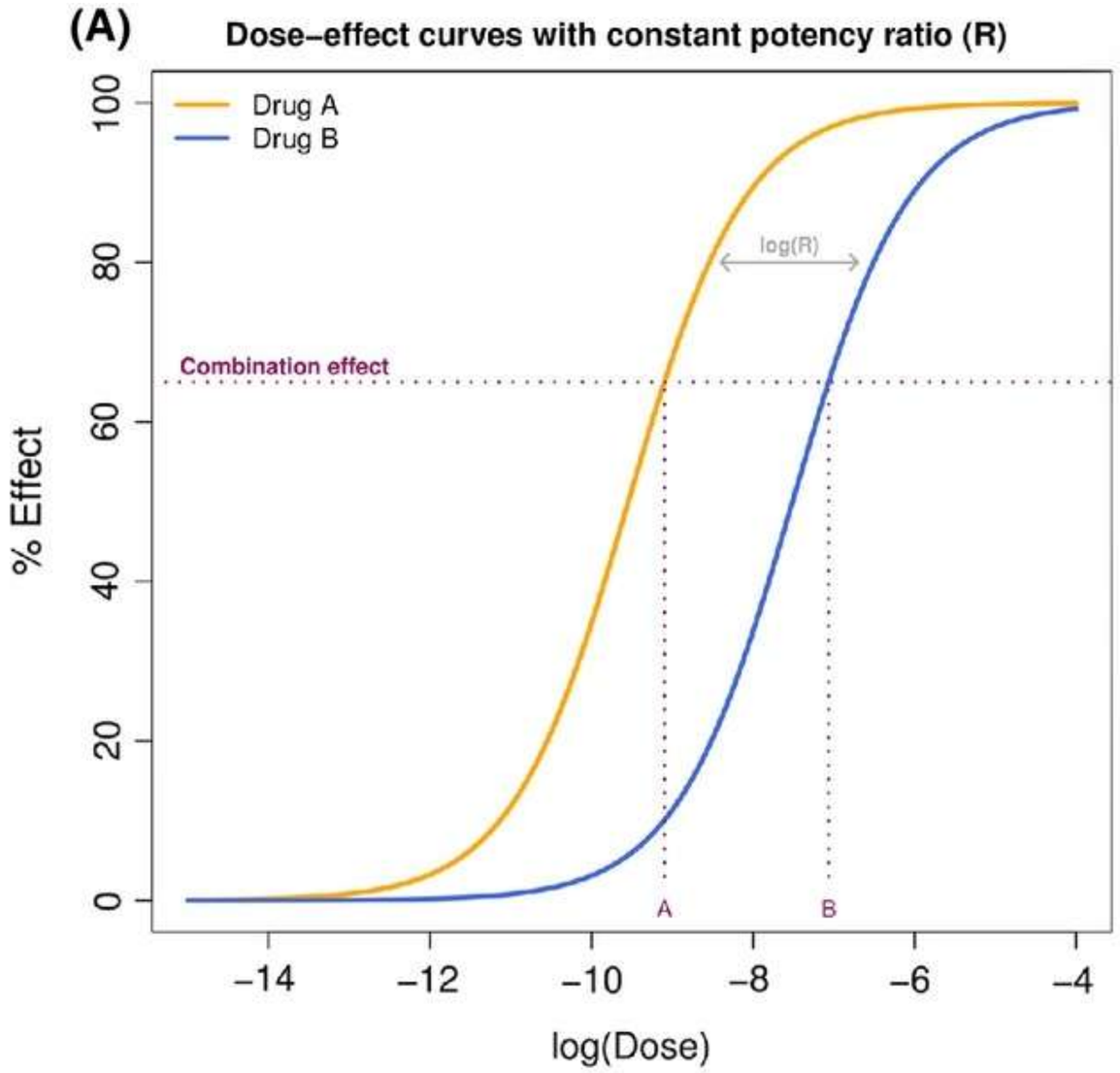
Affiliations + expand

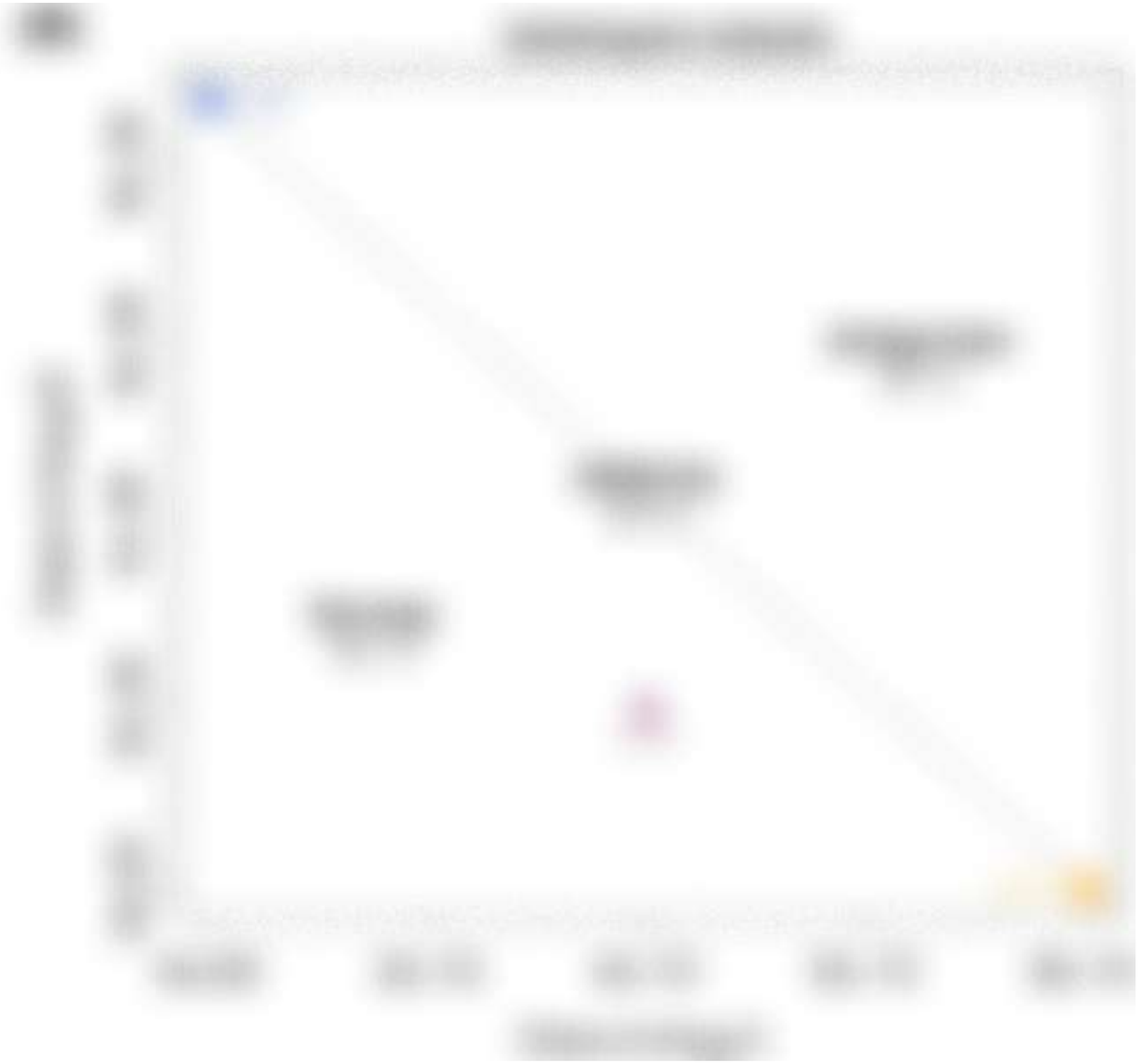
PMID: 12098628 DOI: 10.1016/s0304-3959(02)00041-6

A reliable strategy to evaluate the interactions between drugs.

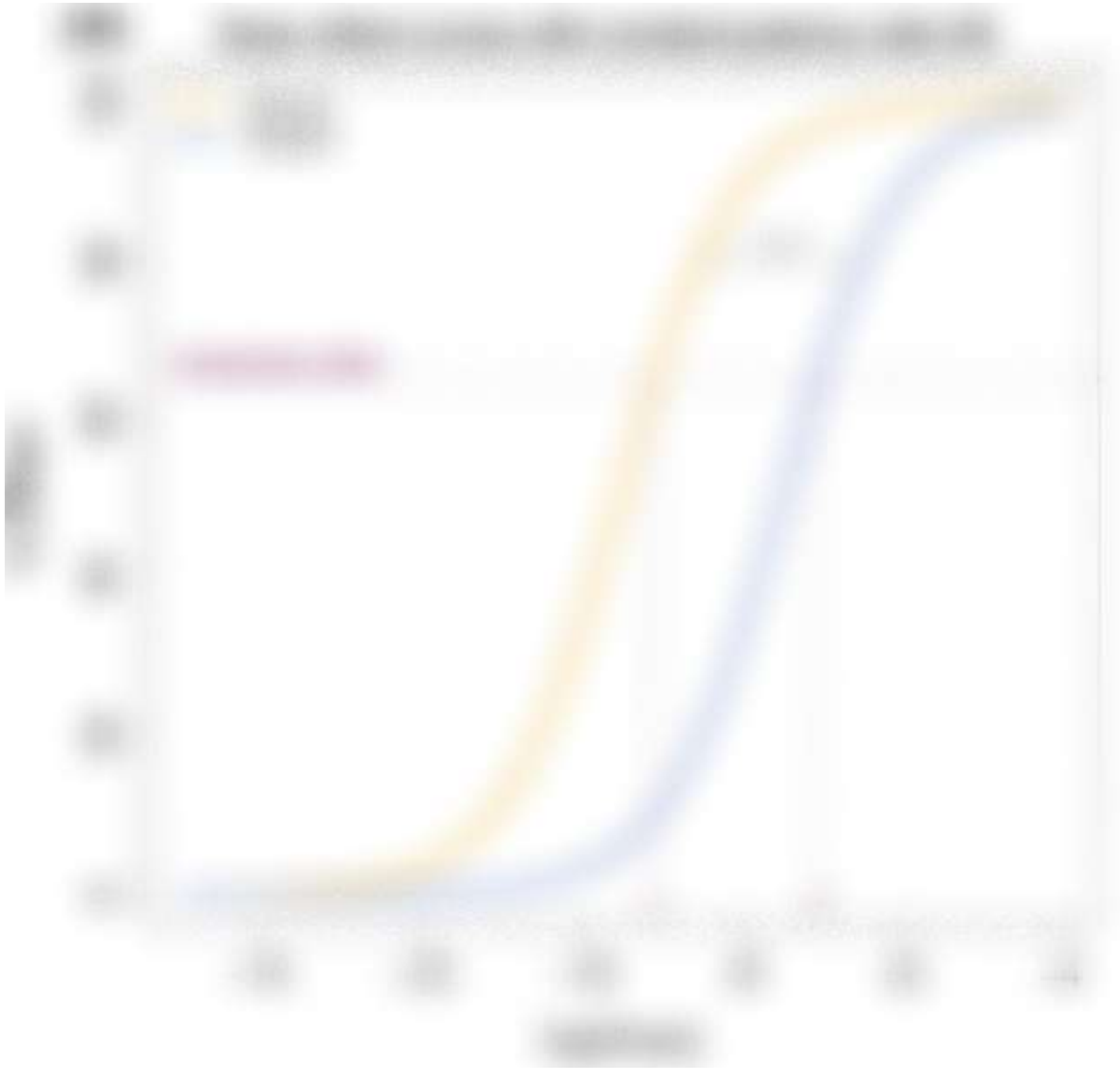
Isobologram analysis has been mathematically proven and widely used to evaluate drug interactions.

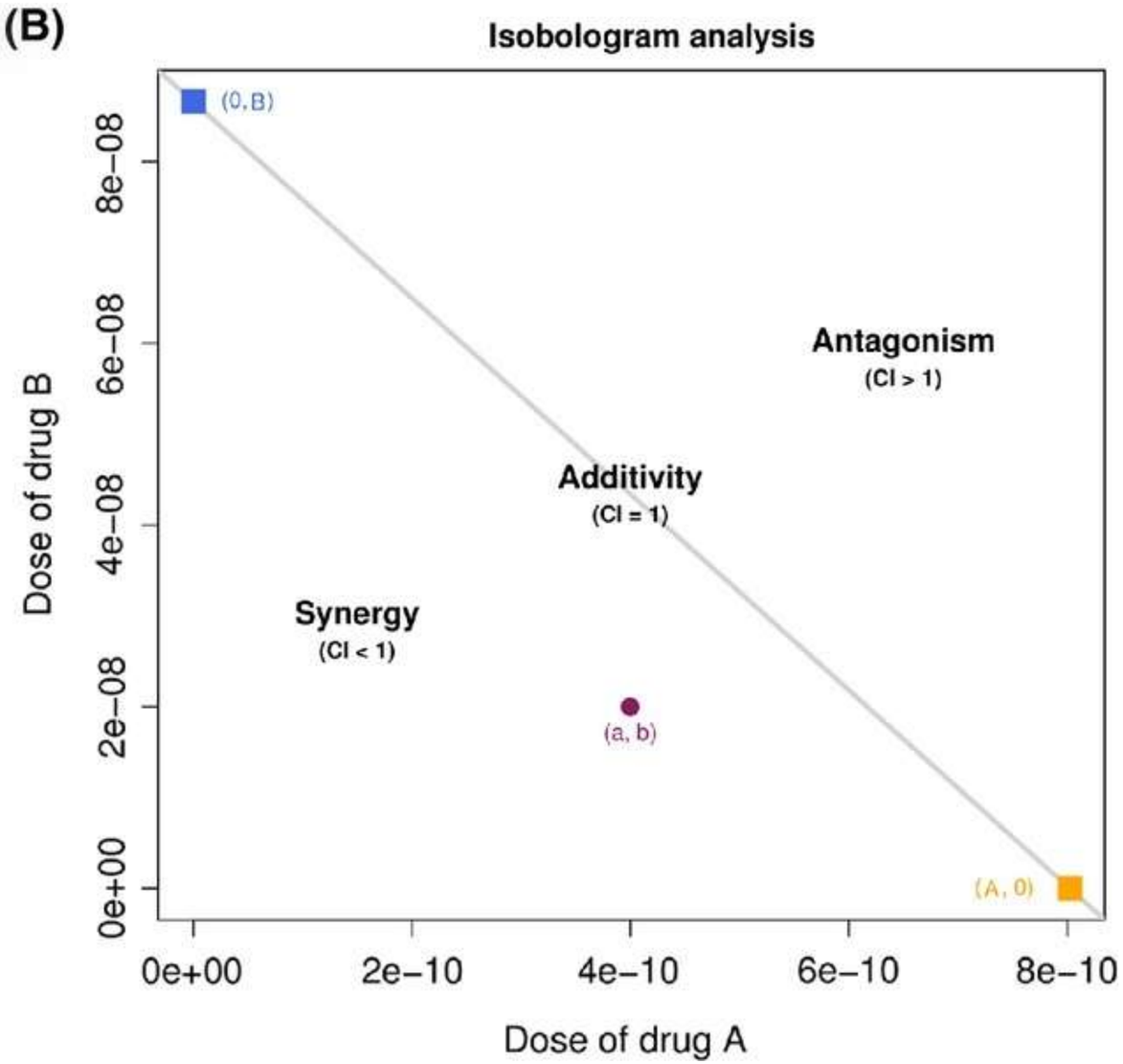
Antagonistic, additive or synergistic interactions





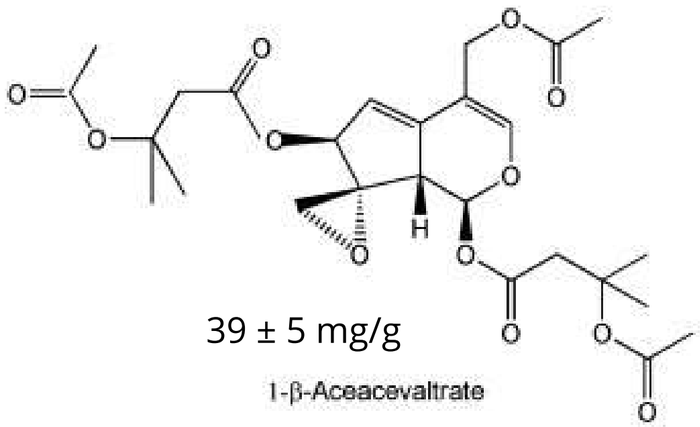
Foucquier and Guedj (2015)
10.1002/prp2.149





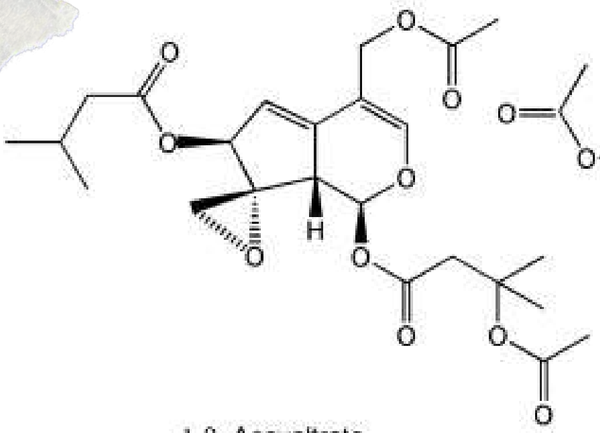
Foucquier and Guedj (2015)
10.1002/prp2.149

Valeriana glechomifolia Meyer



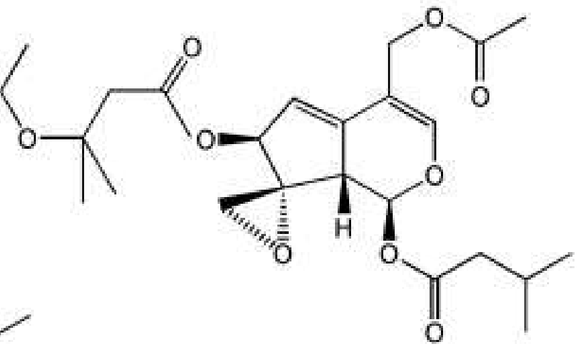
39 ± 5 mg/g

1-β-Aceacevaltrate



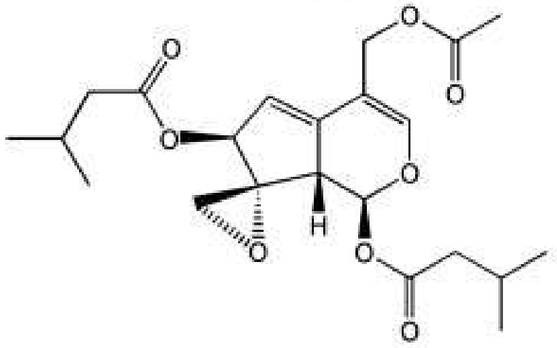
1-β -Acevaltrate

87 ± 9 mg/g

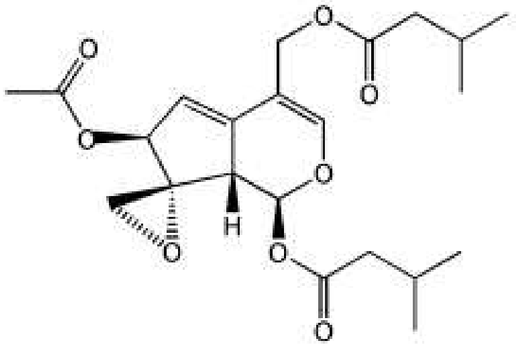


Acevaltrate

172 ± 34 mg/g



Valtrate 643 ± 56 mg/g



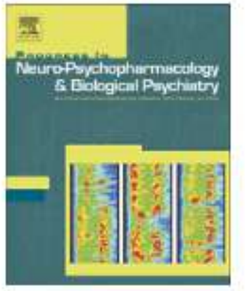
Isovaltrate 37 ± 6 mg/g

Progress in Neuro-Psychopharmacology & Biological Psychiatry 36 (2012) 101–109

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

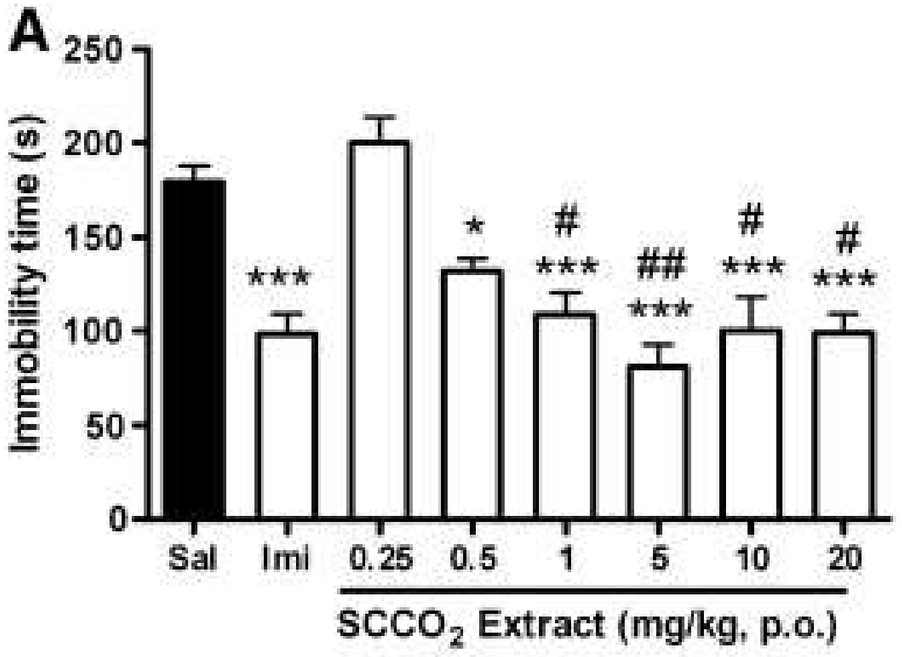
journal homepage: www.elsevier.com/locate/pnp

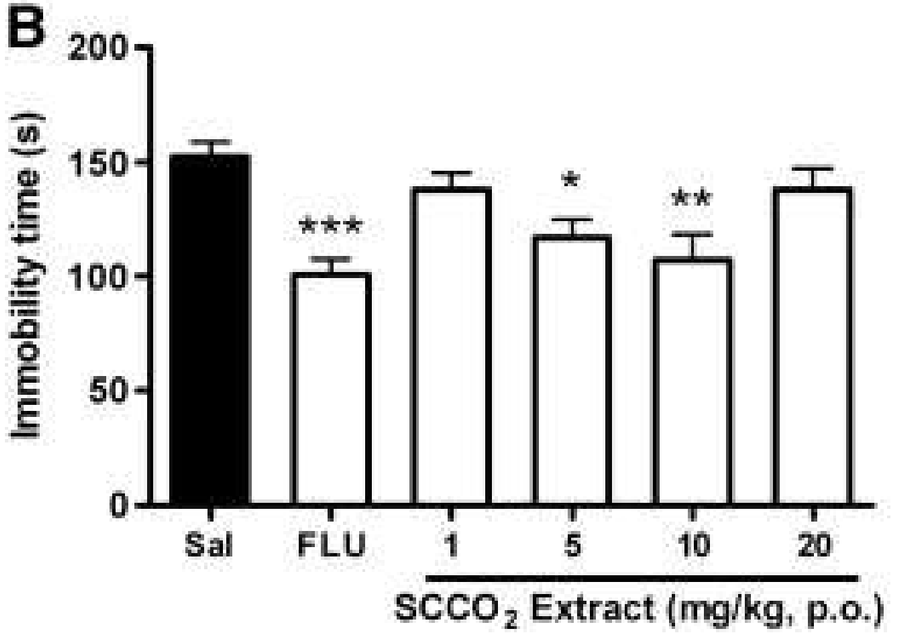


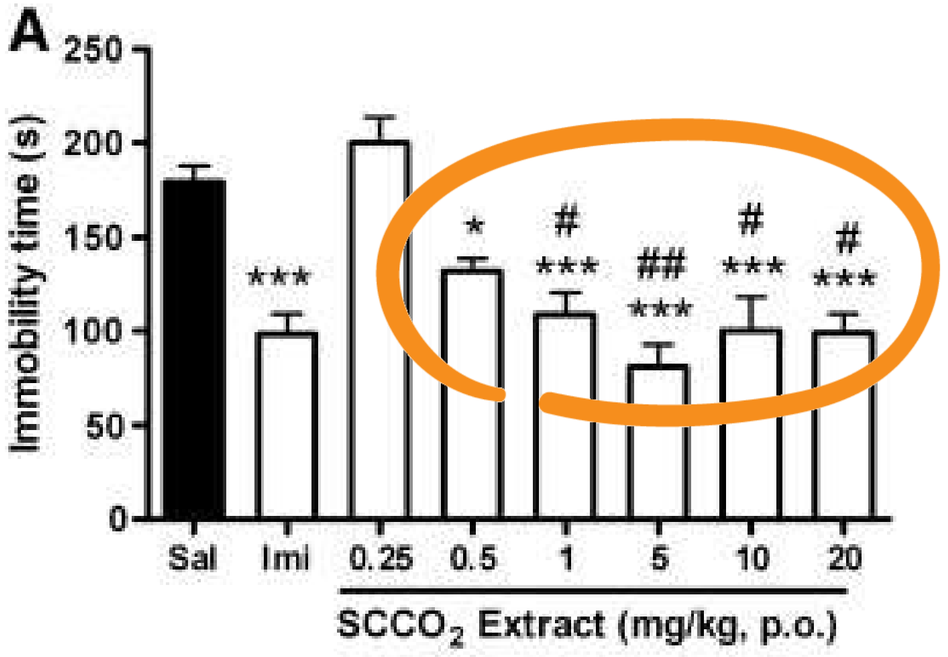
Antidepressant-like effect of *Valeriana glechomifolia* Meyer (Valerianaceae) in mice

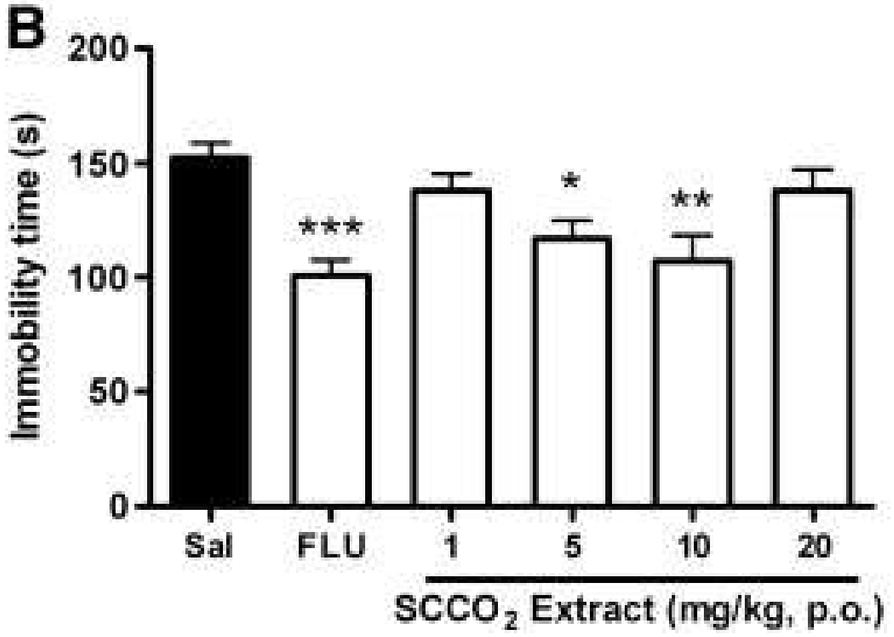
Liz G. Müller [a], Luisa A. Salles [a], Ana C. Stein [a], Andresa H. Betti [a], Satchie Sakamoto [a], Eduardo Cassel [b], Rubem Figueiró Vargas [b], Gilsane L. von Poser [a], Stela M.K. Rates [a,*]

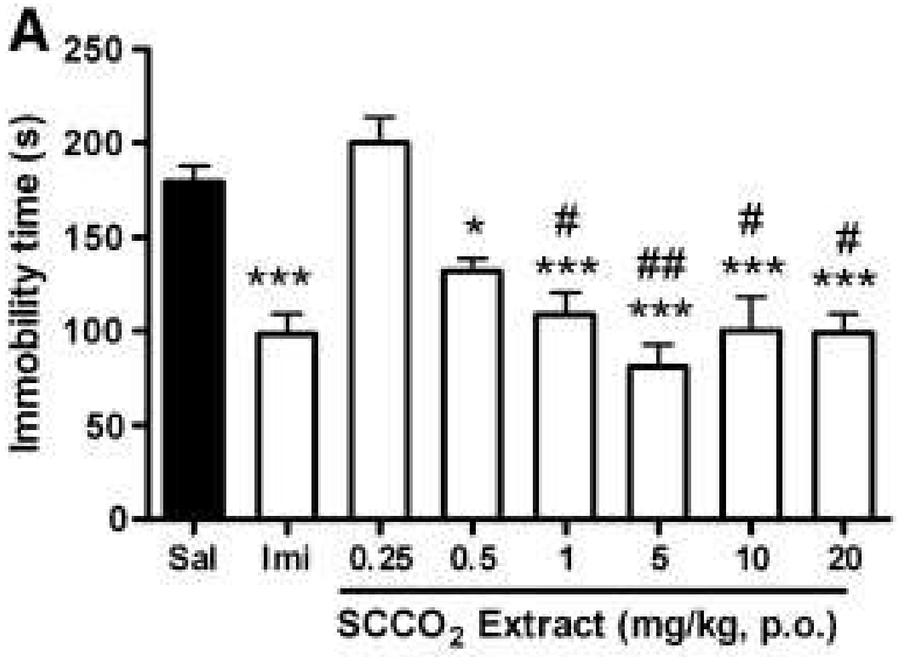
[a] *Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[b] *Faculdade de Engenharia, Departamento de Engenharia Química, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil*

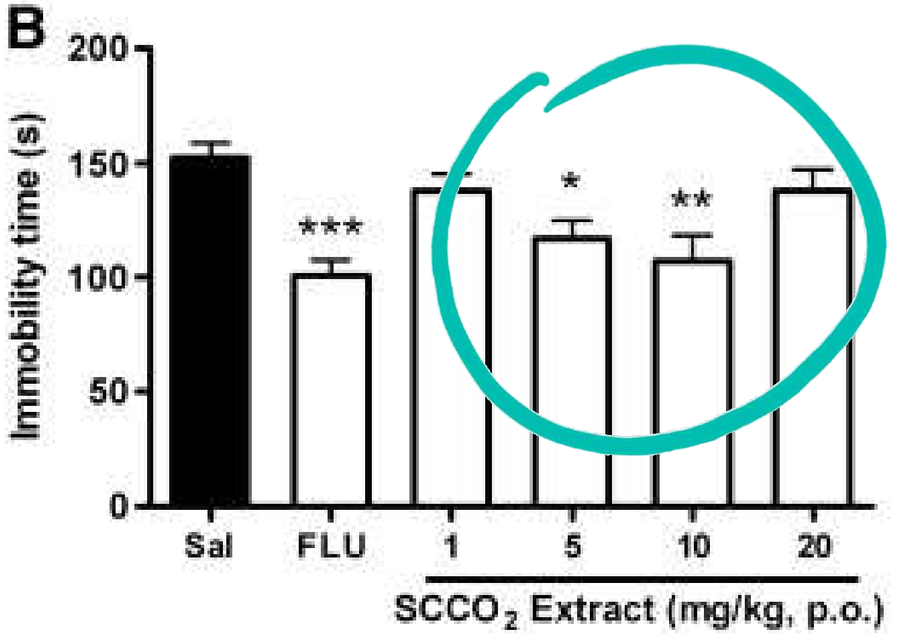

A
Immobility time (s)
250
200
150
100
50
0
Sal
Imi
0.25
0.5
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)

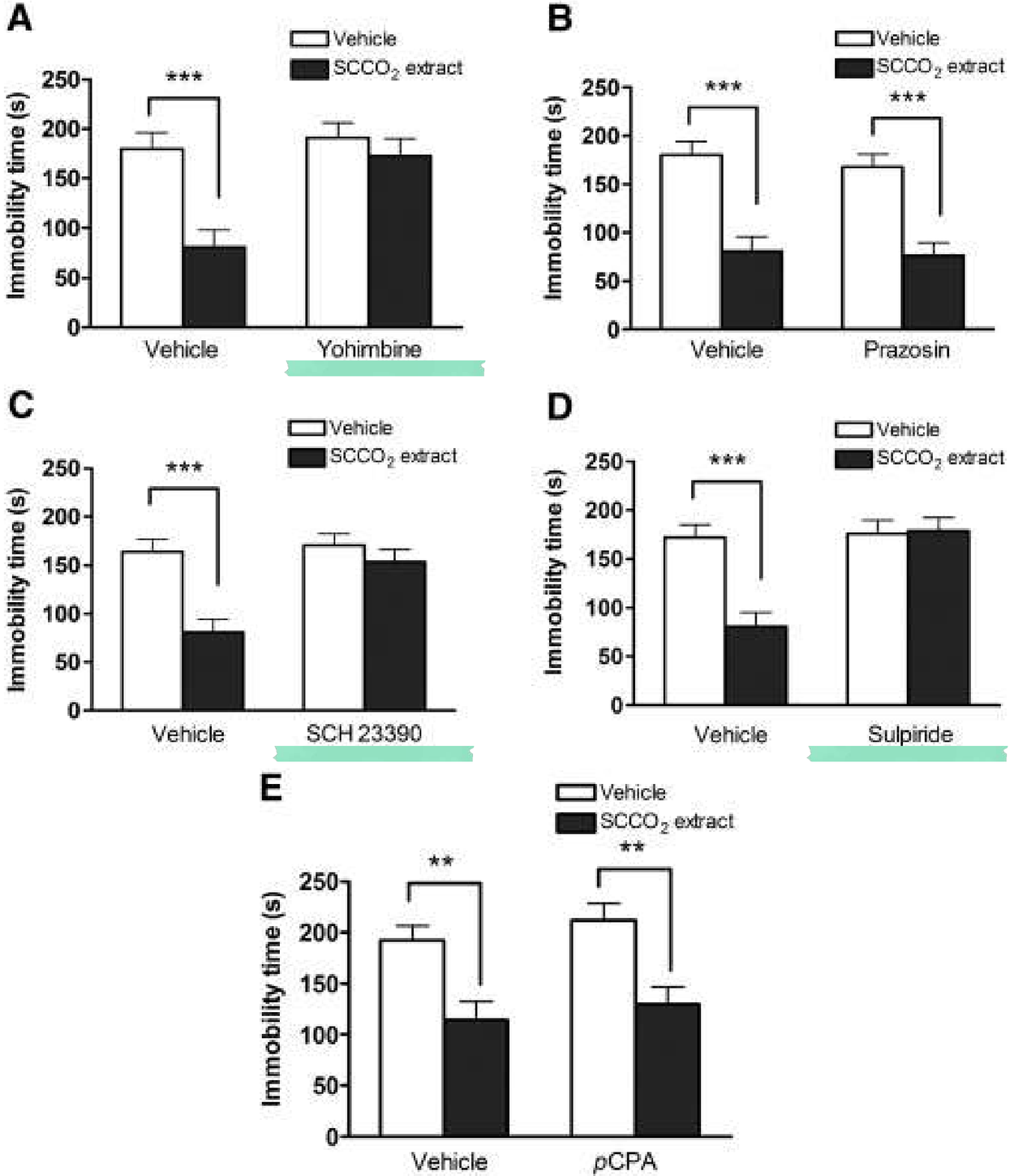

B
Immobility time (s)
200
150
100
50
0
Sal
FLU
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)

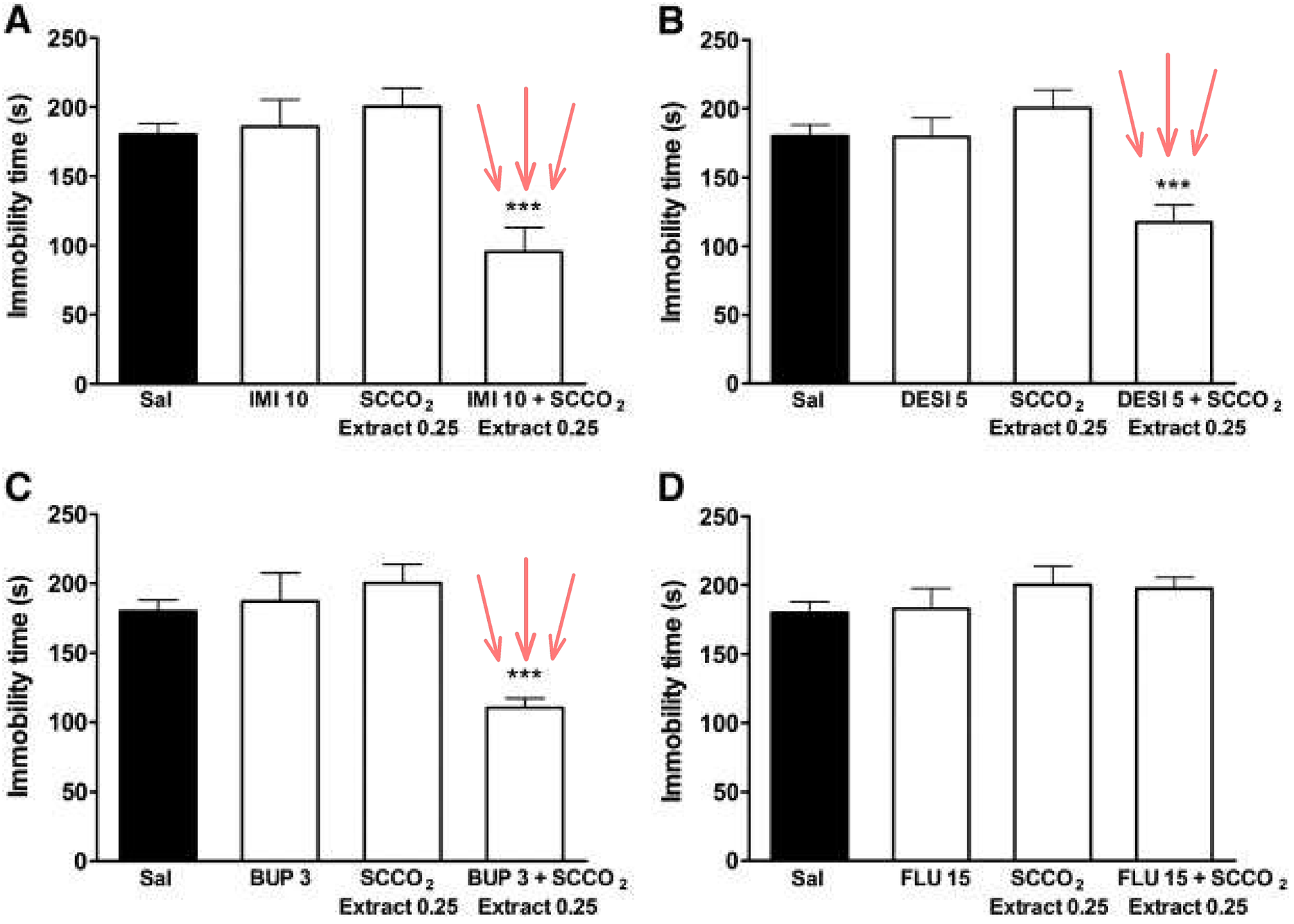

A
Immobility time (s)
250
200
150
100
50
0
***
*
***
#
***
##
***
#
***
#
***
Sal
Imi
0.25
0.5
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)


B
Immobility time (s)
200
150
100
50
0
***
*
**
Sal
FLU
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)


A
Immobility time (s)
250
200
150
100
50
0
***
*
#
***
##
***
#
***
#
***
Sal
Imi
0.25
0.5
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)


B
Immobility time (s)
200
150
100
50
0
***
*
**
Sal
FLU
1
5
10
20
SCCO2 Extract (mg/kg, p.o.)


A
Vehicle
SCCO2 extract
Immobility time (s)
250
200
150
100
50
0
***
Vehicle
Yohimbine
B
Vehicle
SCCO2 extract
Immobility time (s)
***
***
Vehicle
Prazosin
C
Vehicle
SCCO2 extract
Immobility time (s)
***
Vehicle
SCH 23390
D
Vehicle
SCCO2 extract
Immobility time (s)
***
Vehicle
Sulpiride
E
Vehicle
SCCO2 extract
Immobility time (s)
**
**
Vehicle
pCPA


A
Immobility time (s)
250
200
150
100
50
0
***
Sal
IMI 10
SCCO2 Extract 0.25
IMI 10 + SCCO2 Extract 0.25
B
Immobility time (s)
250
200
150
100
50
0
***
Sal
DESI 5
SCCO2 Extract 0.25
DESI 5 + SCCO2 Extract 0.25
C
Immobility time (s)
250
200
150
100
50
0
***
Sal
BUP 3
SCCO2 Extract 0.25
BUP 3 + SCCO2 Extract 0.25
D
Immobility time (s)
250
200
150
100
50
0
Sal
FLU 15
SCCO2 Extract 0.25
FLU 15 + SCCO2 Extract 0.25

The potentiation of the antidepressant effect following a single combination of sub-effective doses **does not provide any information** about the nature of the underlying pharmacological interactions!

JPP JOURNAL OF Pharmacy and Pharmacology

ROYAL PHARMACEUTICAL SOCIETY

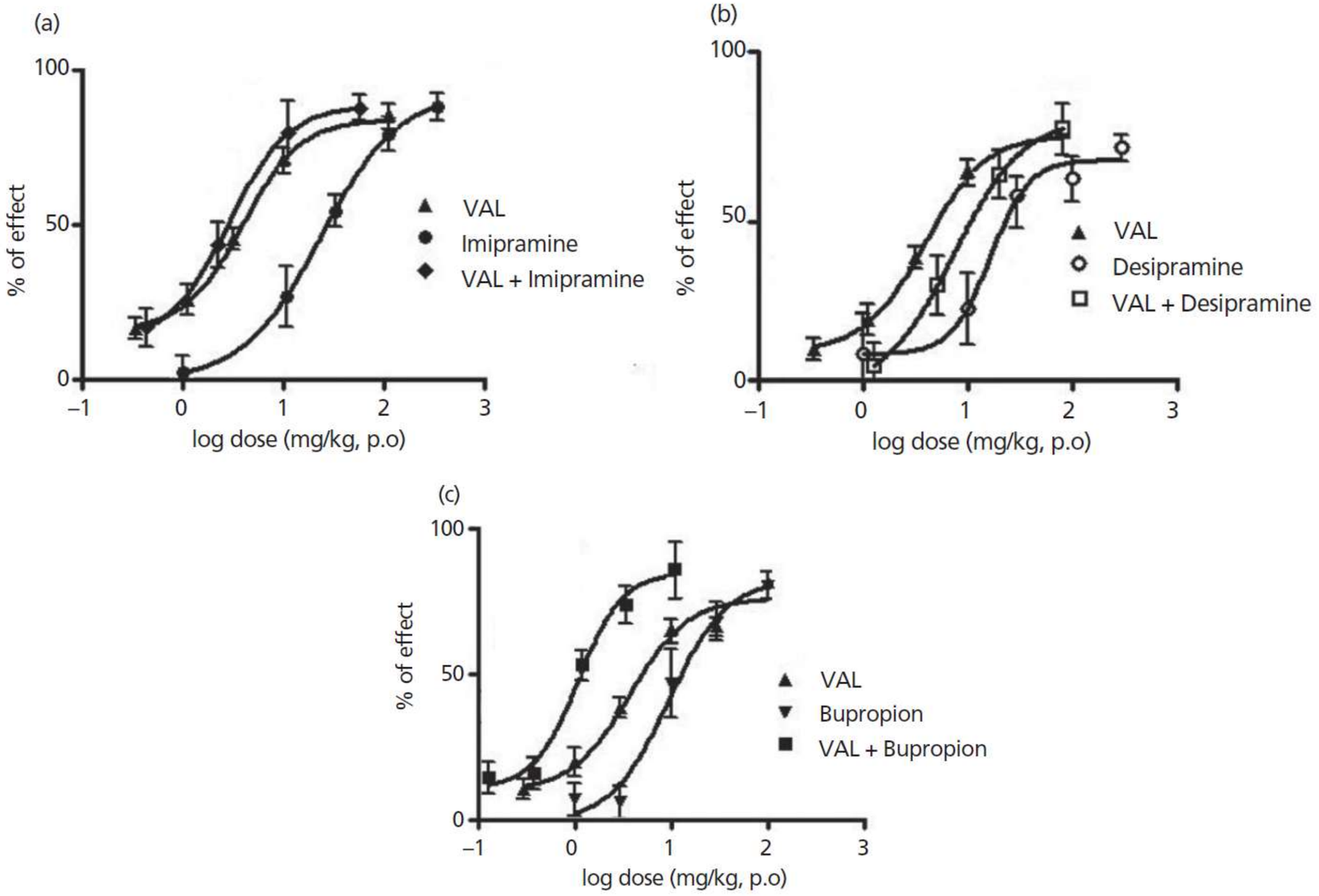
JPP
Journal of Pharmacy
And Pharmacology

Research Paper

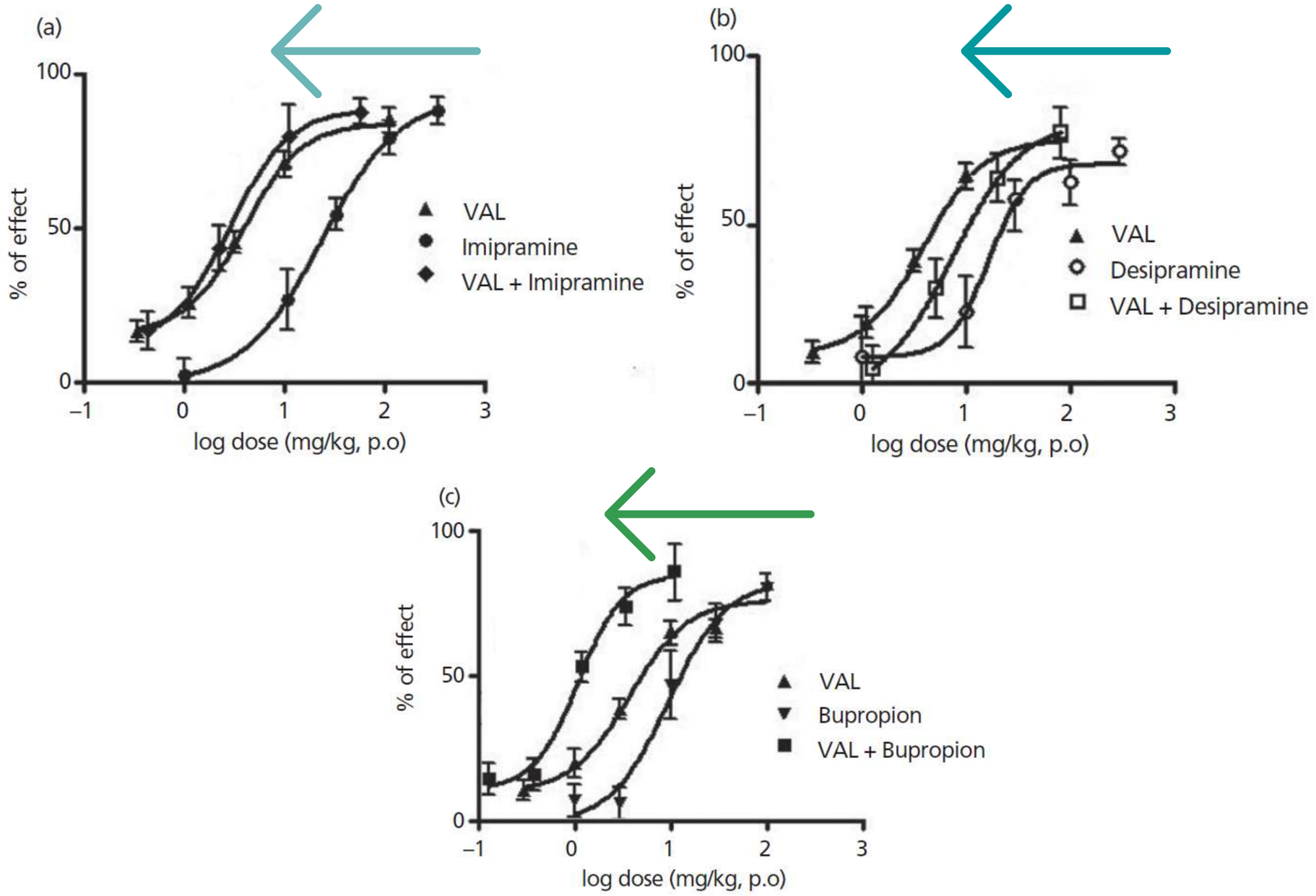
Synergistic interaction between diene valepotriates from *Valeriana glechomifolia* Meyer (Valerianaceae) and classical antidepressants: an isobolographic analysis

Liz G. Müller, Eveline D. Stolz, Andresa H. Betti, Vivian Herzfeldt and Stela M. K. Rates

Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

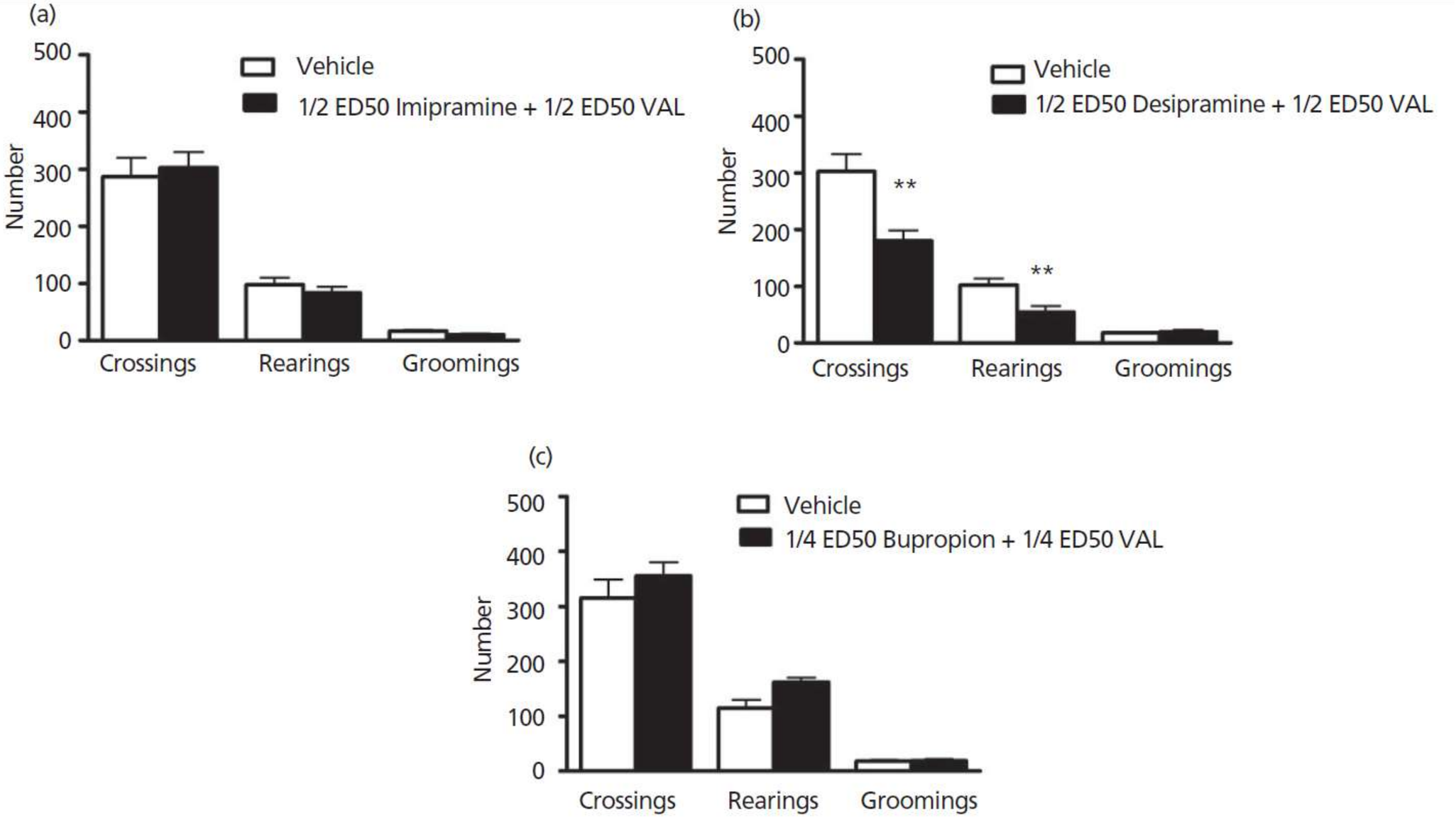


Hill coefficients and Emax were similar



Hill coefficients and Emax were similar

Drug/drug combination	ED50 (CI 95%)
VAL	2.34 (0.45–12.2)
Bupropion	9.12 (5.19–16.04)
Imipramine	25.34 (7.9–31.1)
Desipramine	16.47 (7.92–34.21)
	ED50 mix (CI 95%)
VAL + bupropion	0.8 (0.38–1.7)
VAL + imipramine	2.91 (2.04–4.15)
VAL + desipramine	3.83 (2.29–6.41)


(a)
500
400
300
200
100
0
Number
Vehicle
1/2 ED50 Imipramine + 1/2 ED50 VAL
Crossings
Rearings
Groomings
(b)
500
400
300
200
100
0
Number
Vehicle
1/2 ED50 Desipramine + 1/2 ED50 VAL
**
**
Crossings
Rearings
Groomings
(c)
500
400
300
200
100
0
Number
Vehicle
1/4 ED50 Bupropion + 1/4 ED50 VAL
Crossings
Rearings
Groomings

$$\mathrm{ED50\ add} = f(\mathrm{A}) + (1 - f)\,\mathrm{B}$$

ED50 of VAL

fraction of the corresponding ED50 in the drug mixture (f = 0.5)

ED50 of each antidepressant alone

$$\mathrm{ED50\ add} = f(\mathrm{A}) + (1 - f)\,\mathrm{B}$$

ED50 of VAL

fraction of the corresponding ED50 in the drug mixture (f = 0.5)

ED50 of each antidepressant alone

$$\mathrm{ED50\ add} = f(\mathrm{A}) + (1 - f)\,\mathrm{B}$$

ED50 of VAL

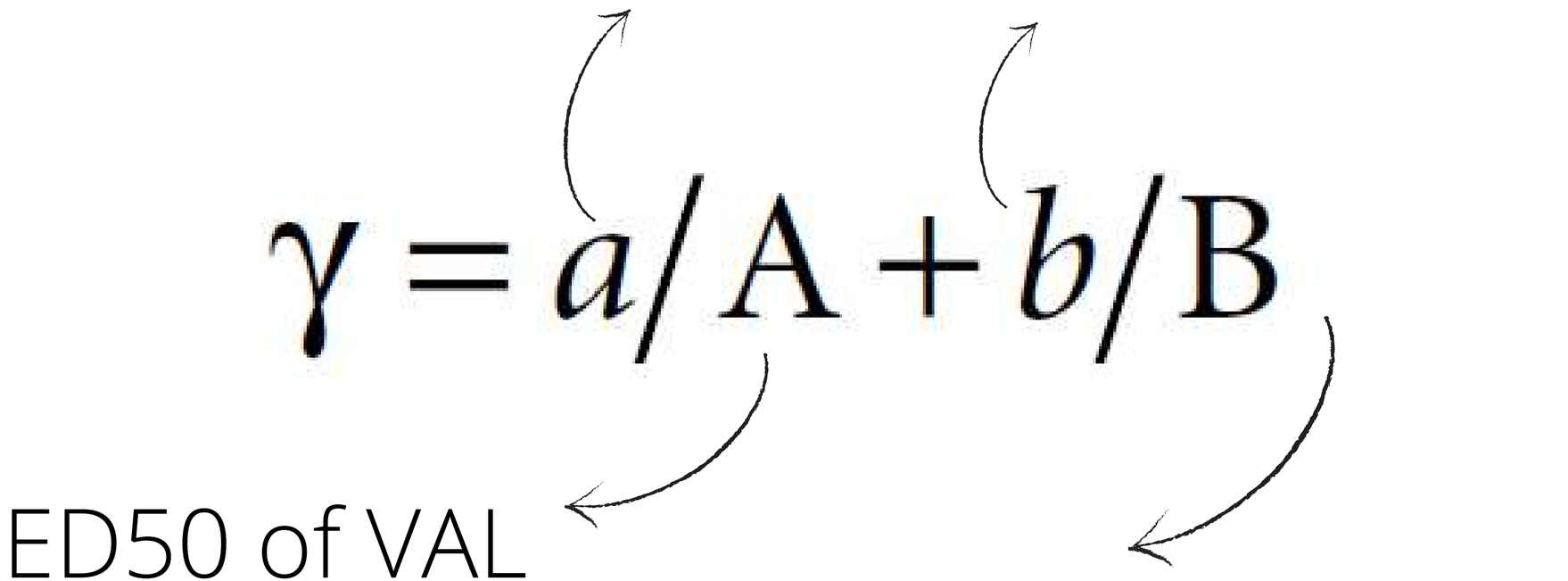
fraction of the corresponding ED50 in the drug mixture (f = 0.5)

ED50 of each antidepressant alone

Drug/drug combination	ED50 (CI 95%)	
VAL	2.34 (0.45–12.2)	
Bupropion	9.12 (5.19–16.04)	
Imipramine	25.34 (7.9–31.1)	
Desipramine	16.47 (7.92–34.21)	
	ED50 mix (CI 95%)	ED50 add (CI 95%)
VAL + bupropion	0.8 (0.38–1.7)	5.73 (3.38–9.71)*
VAL + imipramine	2.91 (2.04–4.15)	13.84 (6.91–27.7)*
VAL + desipramine	3.83 (2.29–6.41)	9.41 (5.32–16.64)*

*P < 0.05: significantly different from the corresponding ED50 mix (Student's t-test for independent means), indicating a synergic interaction.

amounts of each drug (a: VAL; b: antidepressants)
in the combination (obtained from the ED50 mix)



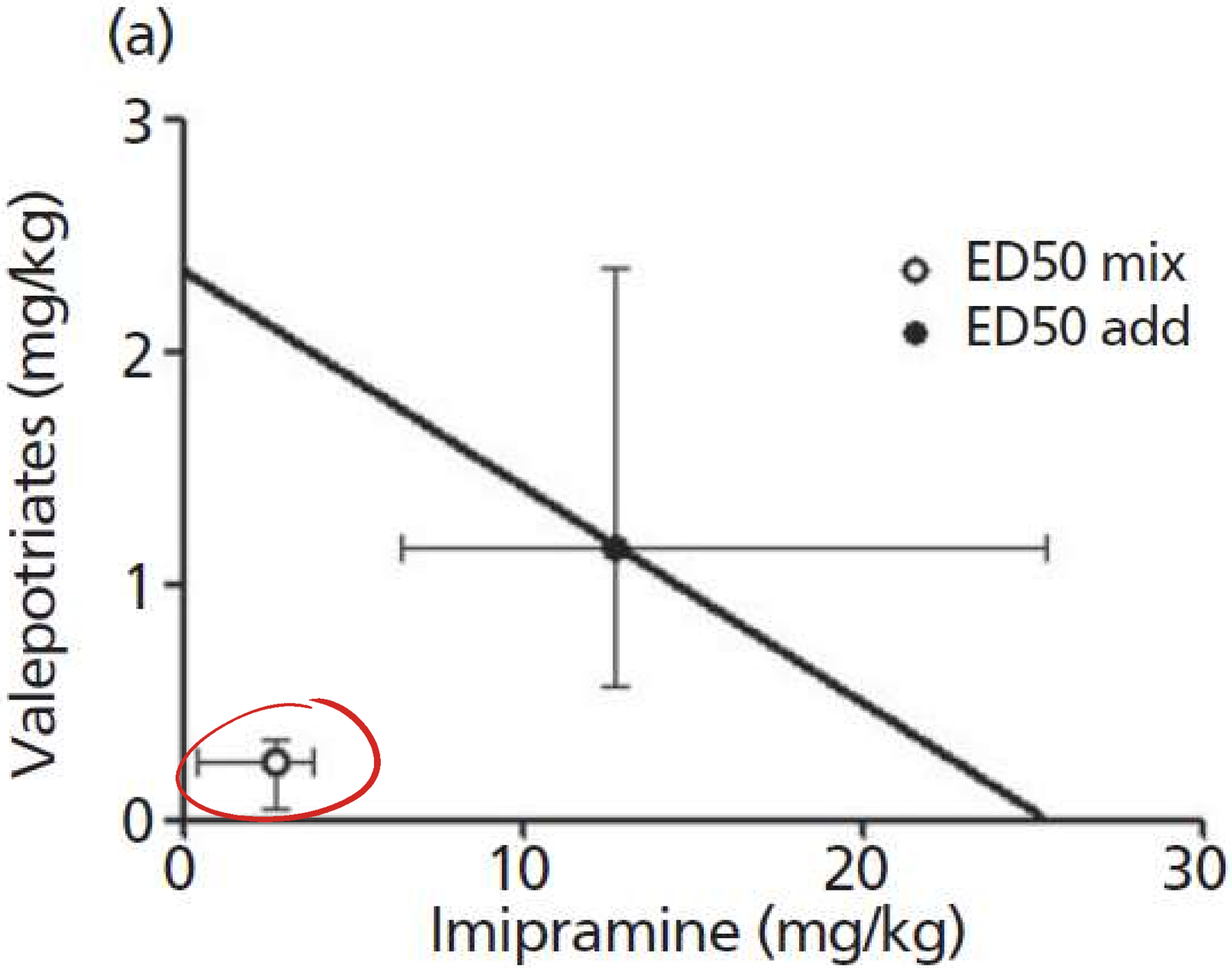
Drug/drug combination	ED50 (CI 95%)		
VAL	2.34 (0.45–12.2)		
Bupropion	9.12 (5.19–16.04)		
Imipramine	25.34 (7.9–31.1)		
Desipramine	16.47 (7.92–34.21)		
	ED50 mix (CI 95%)	ED50 add (CI 95%)	γ
VAL + bupropion	0.8 (0.38–1.7)	5.73 (3.38–9.71)*	0.14
VAL + imipramine	2.91 (2.04–4.15)	13.84 (6.91–27.7)*	0.21
VAL + desipramine	3.83 (2.29–6.41)	9.41 (5.32–16.64)*	0.41

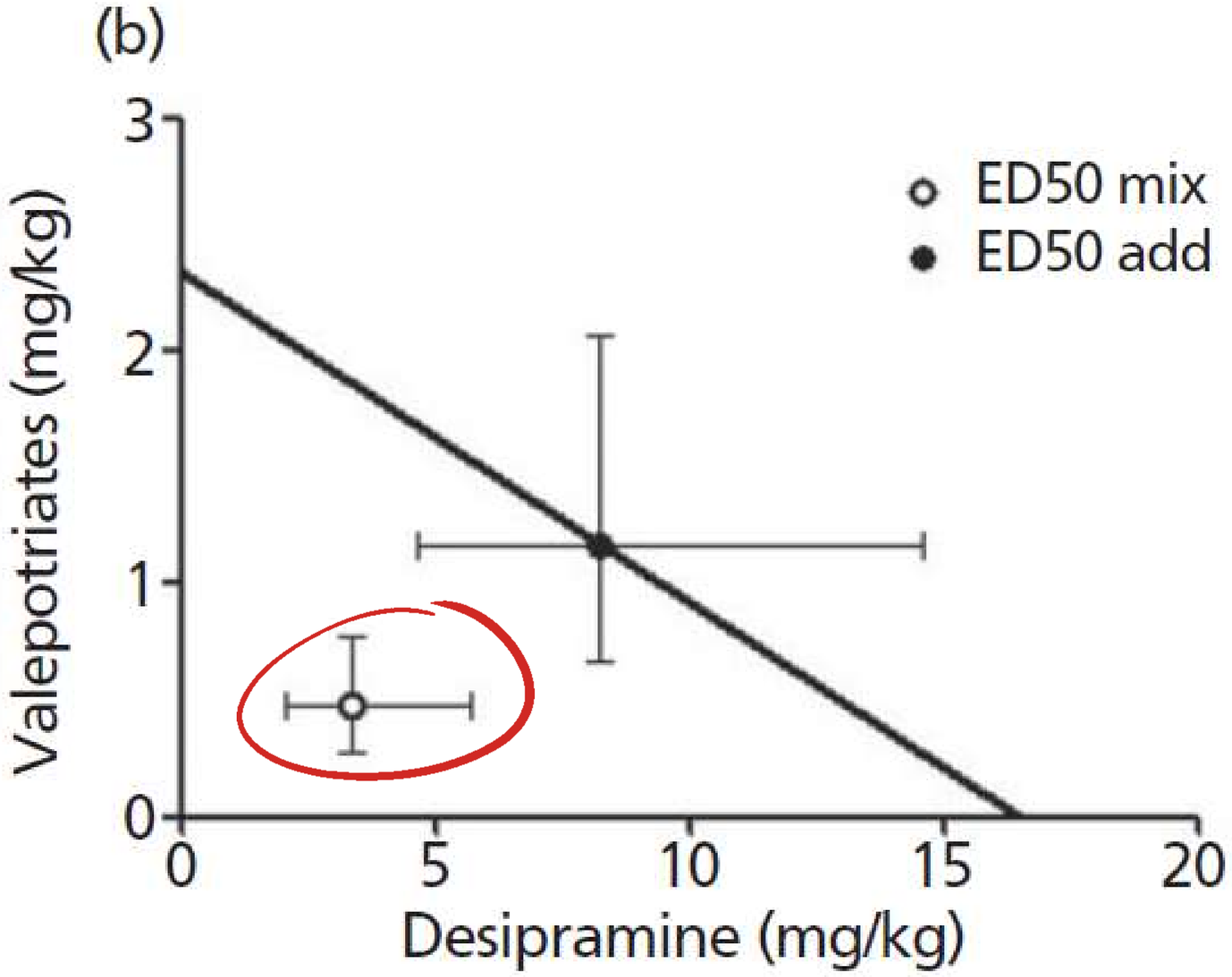
*P < 0.05: significantly different from the corresponding ED50 mix (Student's t-test for independent means), indicating a synergic interaction.

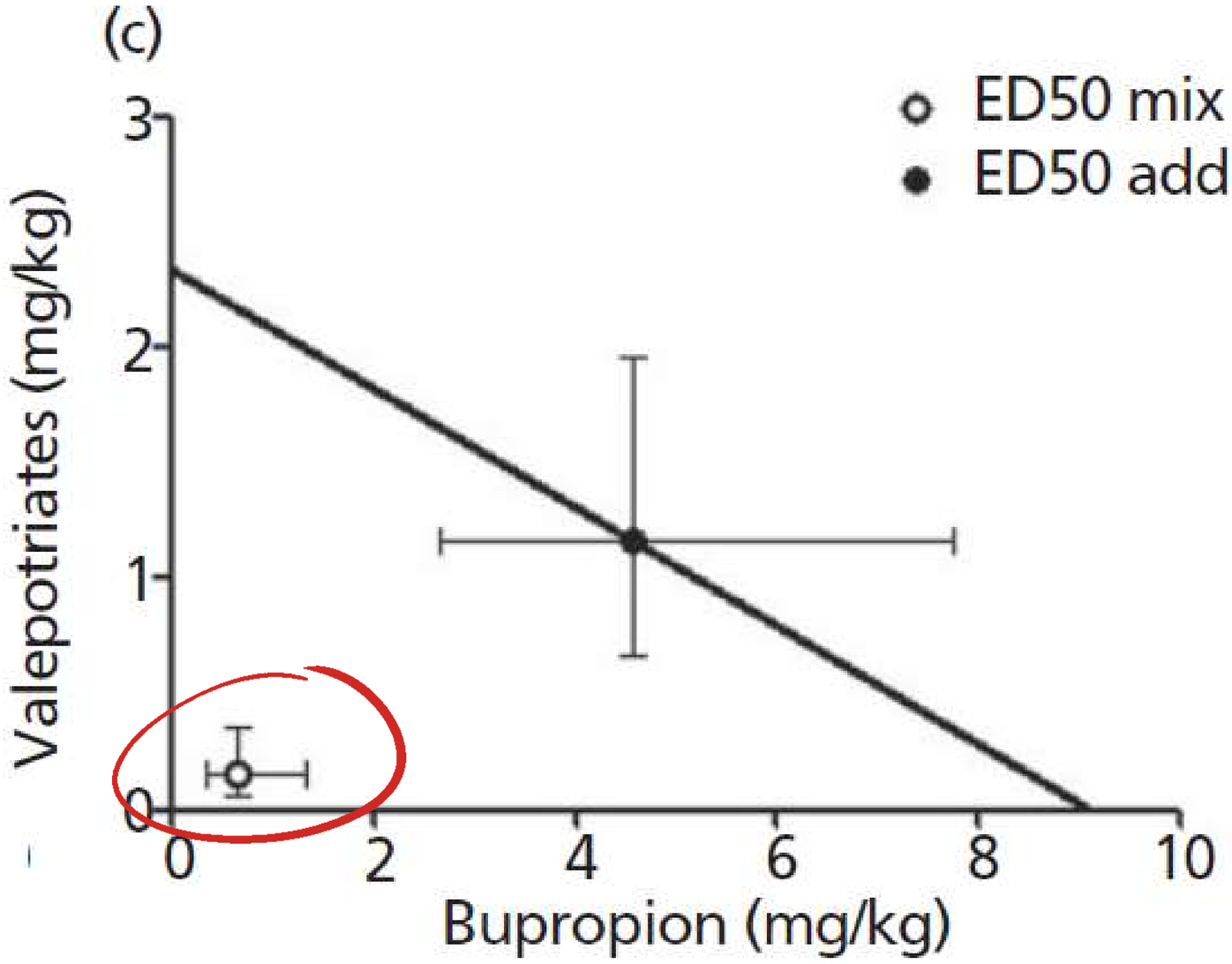
$\gamma \approx 1$ = additive interaction;

$\gamma > 1$ = antagonistic interaction;

$\gamma < 1$ = synergistic interaction.


(a)
3
2
1
0
Valepotriates (mg/kg)
ED50 mix
ED50 add
0
10
20
30
Imipramine (mg/kg)


(b)
3
2
1
0
Valepotriates (mg/kg)
ED50 mix
ED50 add
0
5
10
15
20
Desipramine (mg/kg)


(c)
ED50 mix
ED50 add
Valepotriates (mg/kg)
3
2
1
0
0
2
4
6
8
10
Bupropion (mg/kg)

Isobolographic analysis of the dual-site synergism in the antinociceptive response of tramadol in the formalin test in rats

Amaury J. Pozos-Guillén [a], Patricia Aguirre-Bañuelos [b], Abraham Arellano-Guerrero [b], Gilberto Castañeda-Hernández [c], Carlos Hoyo-Vadillo [c], José Pérez-Urizar [b,*]

[a] Facultad de Estomatología, Universidad Autónoma de San Luís Potosí, San Luís Potosí, México
[b] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, San Luís Potosí, México
[c] Sección Externa de Farmacología, CINVESTAV, México D.F., México

0022-3565/11/3371-312–320$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2011 by The American Society for Pharmacology and Experimental Therapeutics
JPET 337:312–320, 2011

Synergistic Interaction between the Two Mechanisms of Tapentadol in Analgesia

W. Schröder, T. M. Tzschentke, R. Terlinden, J. De Vry, U. Jahnel, T. Christoph, and R. J. Tallarida

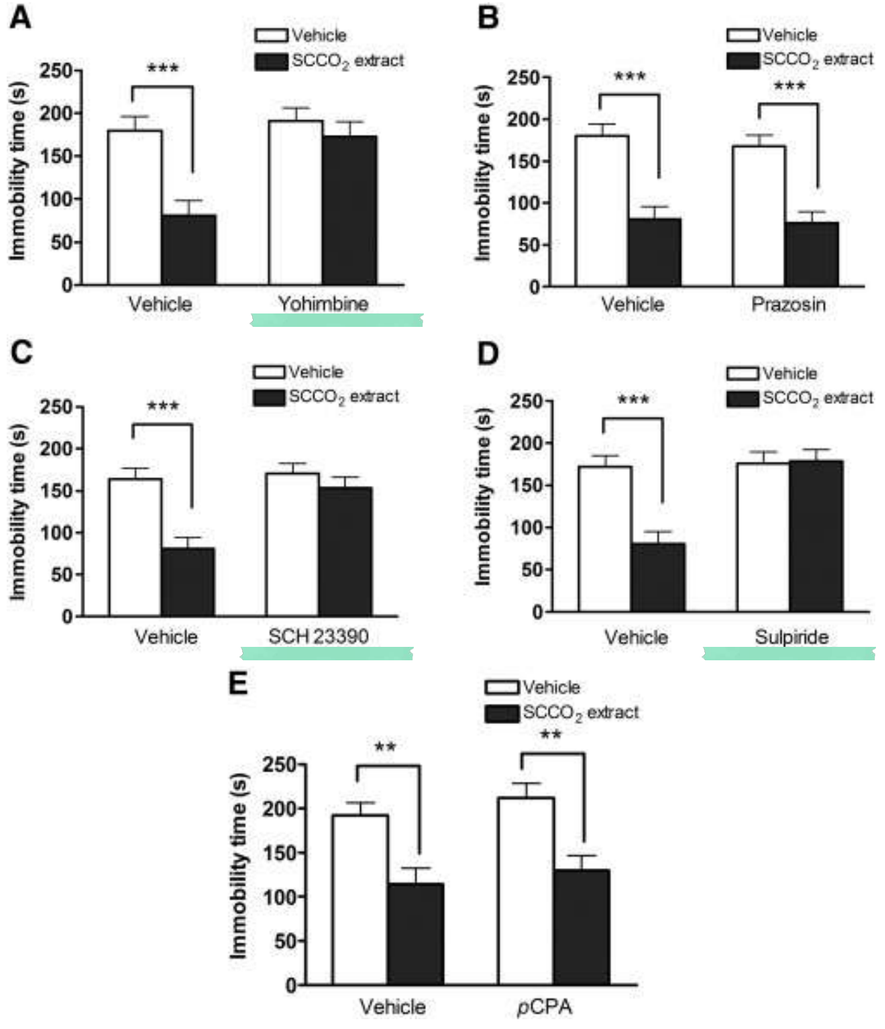
Departments of Pharmacology (W.S., T.M.T., J.D.V., T.C
Development (U.J.), Grünenthal GmbH, Aachen, Germa
Medicine and Center for Substance Abuse Research, F

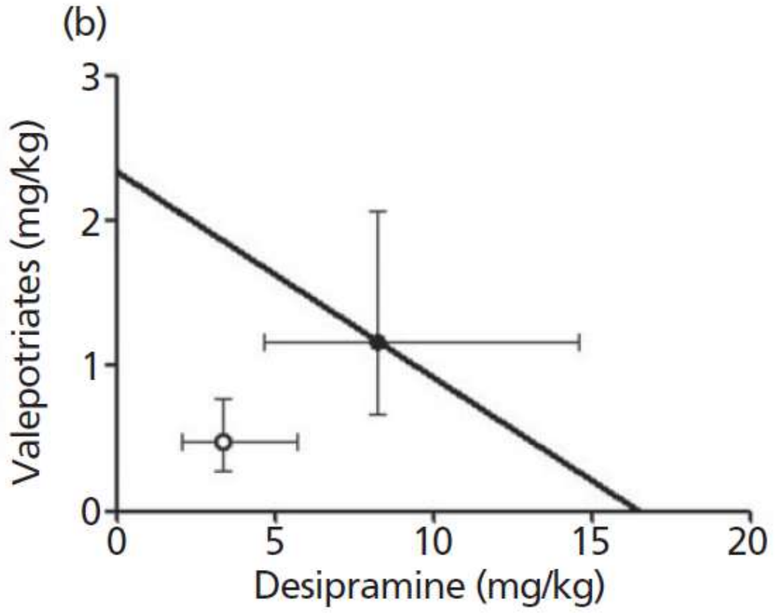
macokinetics (R.T.) and Global Preclinical Research and
f Pharmacology, Temple University School of

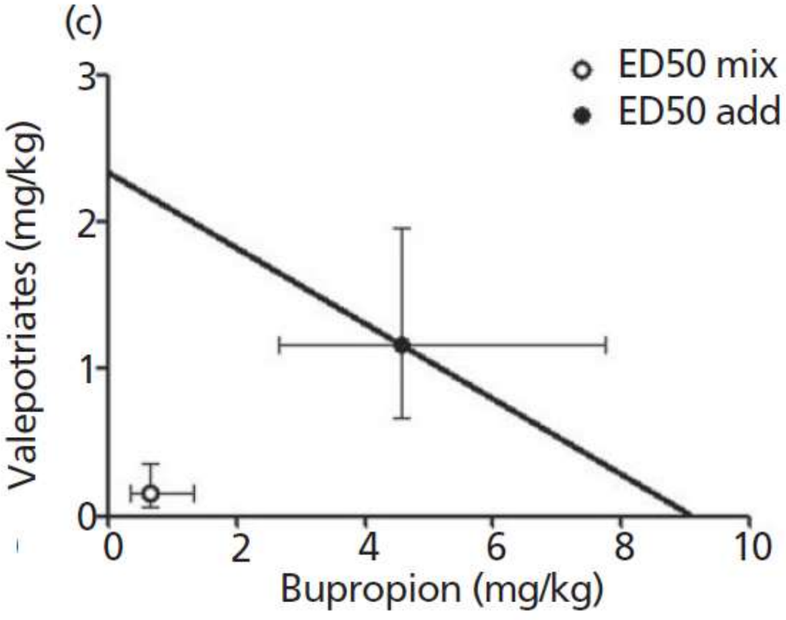
Tramadol and several anticonvulsants synergize in attenuating nerve injury-induced allodynia

Ellen E. Codd [a,*], Rebecca P. Martinez [a], Lory Molino [a], Kathryn E. Rogers [a], Dennis J. Stone [a], Ronald J. Tallarida [b]

In addition, isobolographic analyses can be used to provide clues as to the mechanism of action of substances and to suggest potential lines of investigation.[21] For instance, the occurrence of synergism between two substances may indicate that the effect of each drug is mediated by distinct pathways or that the substances act at two distinct sites.[22–24]


A
Vehicle
SCCO2 extract
Immobility time (s)
***
Vehicle
Yohimbine
B
Vehicle
SCCO2 extract
Immobility time (s)
***
***
Vehicle
Prazosin
C
Vehicle
SCCO2 extract
Immobility time (s)
***
Vehicle
SCH 23390
D
Vehicle
SCCO2 extract
Immobility time (s)
***
Vehicle
Sulpiride
E
Vehicle
SCCO2 extract
Immobility time (s)
**
**
Vehicle
pCPA


(b)
Valepotriates (mg/kg)
Desipramine (mg/kg)


(c)
ED50 mix
ED50 add
Valepotriates (mg/kg)
Bupropion (mg/kg)

A valepotriate-enriched fraction from **Valeriana glechomifolia** decreases DNA methylation and up-regulate TrkB receptors in the hippocampus of mice.

Müller LG, Biojone C, Sales AJ, Betti AH, Herzfeldt V, Joca SRL, Rates SMK.

Behav Pharmacol. 2020 Jun;31(4):333-342. doi: 10.1097/FBP.0000000000000534.

PMID: 31860564

Effects of diene valepotriates from **Valeriana glechomifolia** on Na+/K+-ATPase activity in the cortex and hippocampus of mice.

Müller LG, Salles L, Lins HA, Feijó PR, Cassel E, Vargas R, von Poser GL, Noël F, Quintas LE, Rates SM.

Planta Med. 2015 Feb;81(3):200-7. doi: 10.1055/s-0034-1396200. Epub 2015 Jan 23.

PMID: 25615276

Diene Valepotriates from **Valeriana glechomifolia** Prevent Lipopolysaccharide-Induced Sickness and Depressive-Like Behavior in Mice.

Müller LG, Borsoi M, Stolz ED, Herzfeldt V, Viana AF, Ravazzolo AP, Rates SM.

Evid Based Complement Alternat Med. 2015;2015:145914. doi: 10.1155/2015/145914. Epub 2015 Jun 11.

PMID: 26170871 **Free PMC article.**

A valepotriate-enriched fraction from **Valeriana glechomifolia** decreases DNA methylation and up-regulate TrkB receptors in the hippocampus of mice.

Müller LG, Biojone C, Sales AJ, Betti AH, Herzfeldt V, Joca SRL, Rates SMK.

Behav Pharmacol. 2020 Jun;31(4):333-342. doi: 10.1097/FBP.0000000000000[illegible]

PMID: 31860564

Effects of diene valepotriates from **Valeriana glechomifolia** on Na+/K+-ATPase activity in the cortex and hippocampus of mice.

Müller LG, Salles L, Lins HA, Feijó PR, Cassel E, Vargas R, [illegible]oser GL, Noël F, Quintas LE, Rates SM.

Planta Med. 2015 Feb;81(3):200-7. doi: 10.1055/s-[illegible]. Epub 2015 Jan 23.

PMID: 25615276

Diene Valepotriates from **Valeriana glechomifolia** Prevent Lipopolysaccharide-Induced Sickness and Depressive-Like Behavior in Mice.

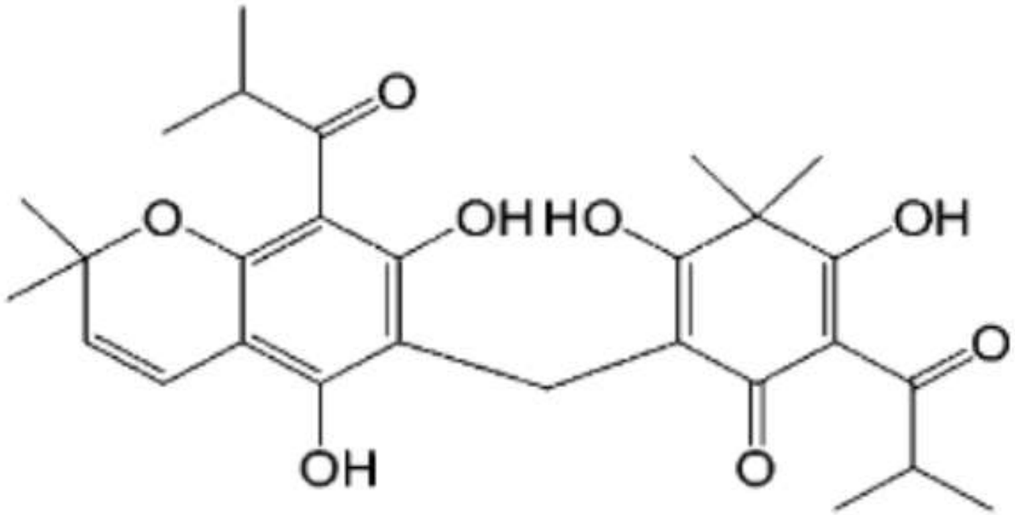
Müller LG, Borsoi M, Stolz ED, Herzfeldt V, Viana AF, Ravazzolo AP, Rates SM.

Evid Based Complement Alternat Med. 2015;2015:145914. doi: 10.1155/2015/145914. Epub 2015 Jun 11.

PMID: 26170871 Free PMC article.

Multitarget molecules

Uliginosin B



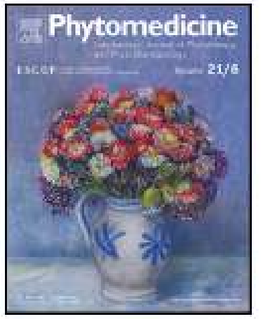
Hypericum polyanthemum Klotzsch ex Reichardt
(Hypericaceae)

ELSEVIER

Contents lists available at ScienceDirect

Phytomedicine

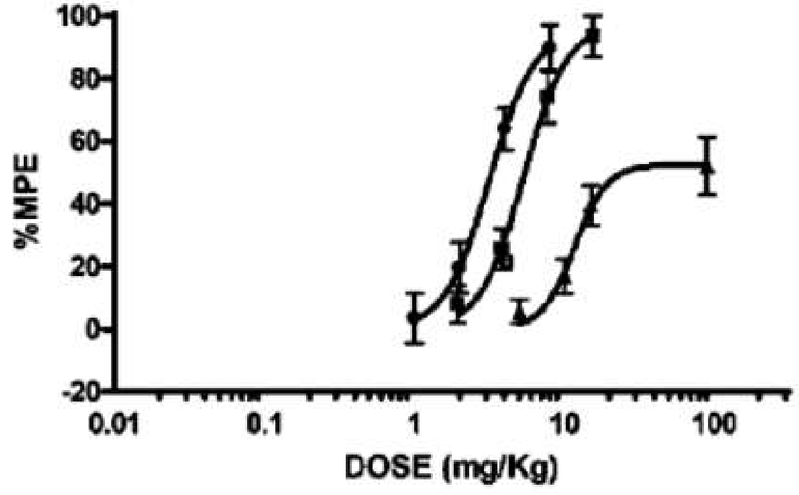
journal homepage: www.elsevier.de/phymed



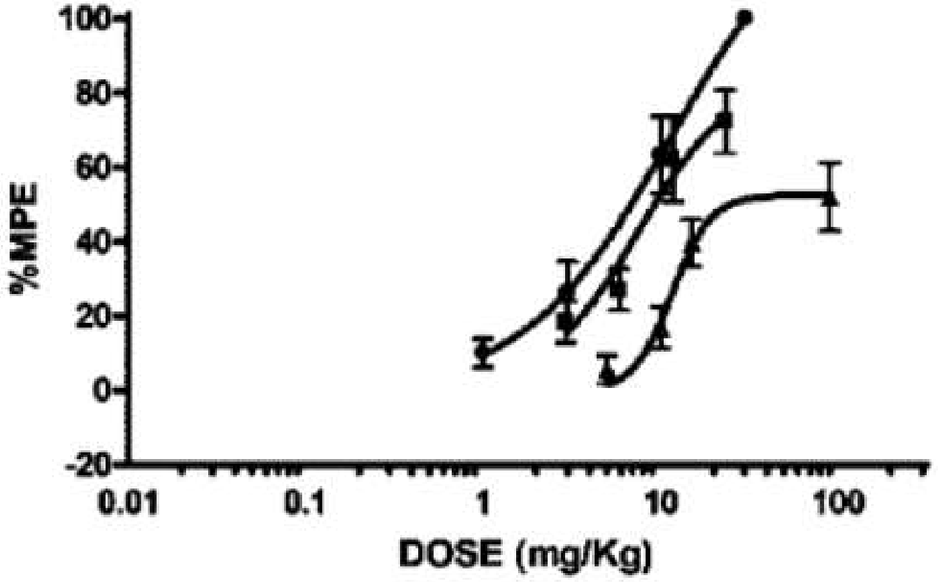
Determination of pharmacological interactions of uliginosin B, a natural phloroglucinol derivative, with amitriptyline, clonidine and morphine by isobolographic analysis

Eveline D. Stolz [a], Liz G. Müller [a], Camila B. Antonio [a], Paola F. da Costa [a], Gilsane L. von Poser [a], François Noël [b], Stela M.K. Rates [a,*]

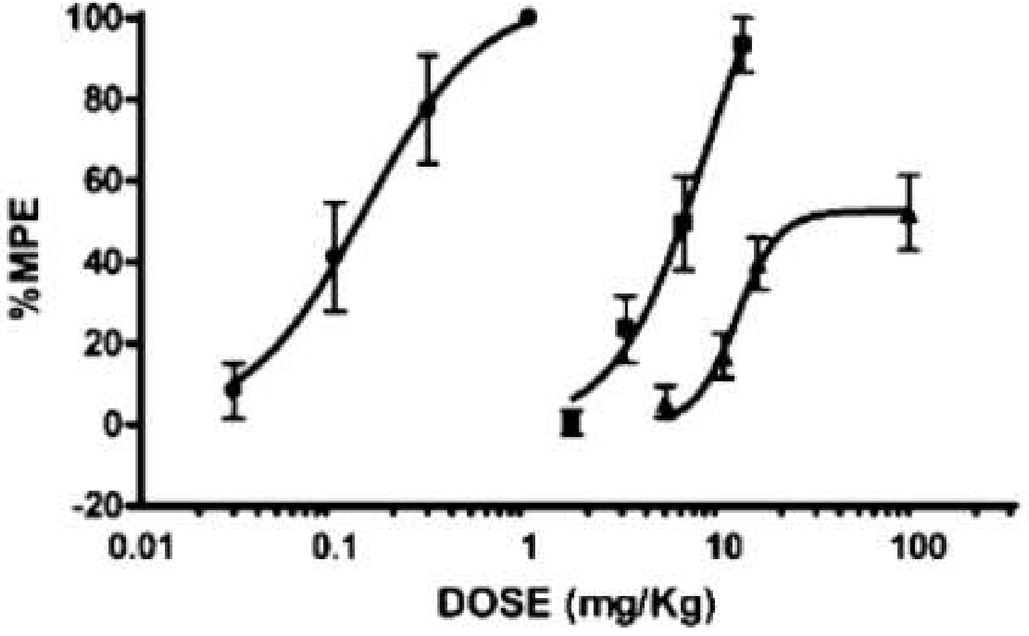
[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[b] *Laboratório de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*



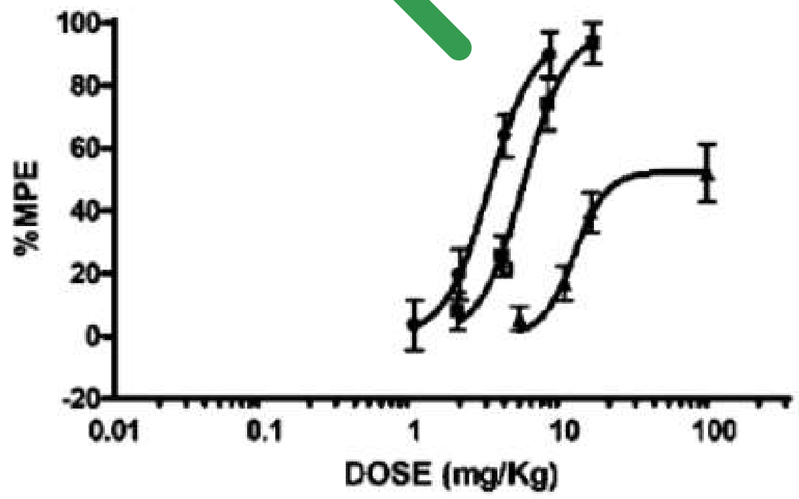
■ ULI+MOR
▲ ULI
● MOR



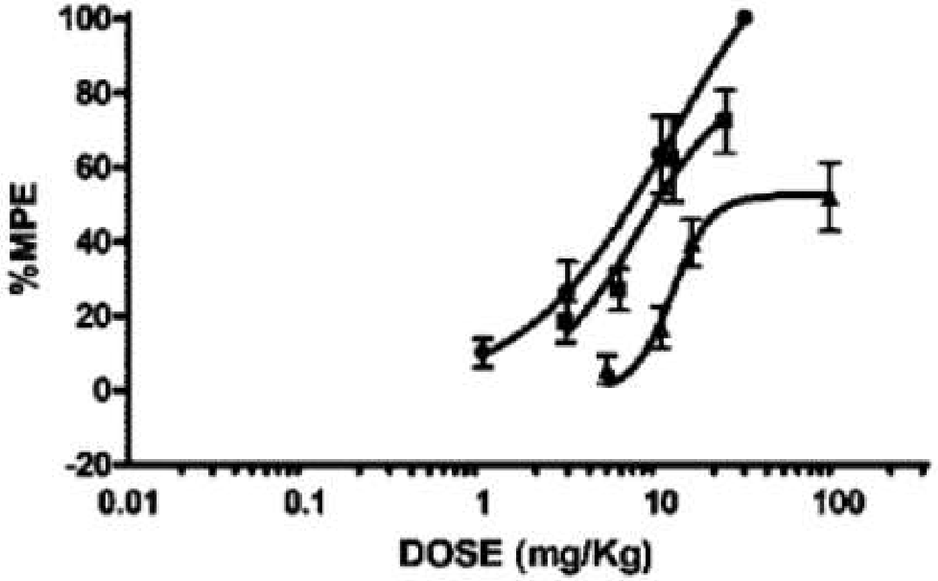
■ ULI+AMI
▲ ULI
● AMI



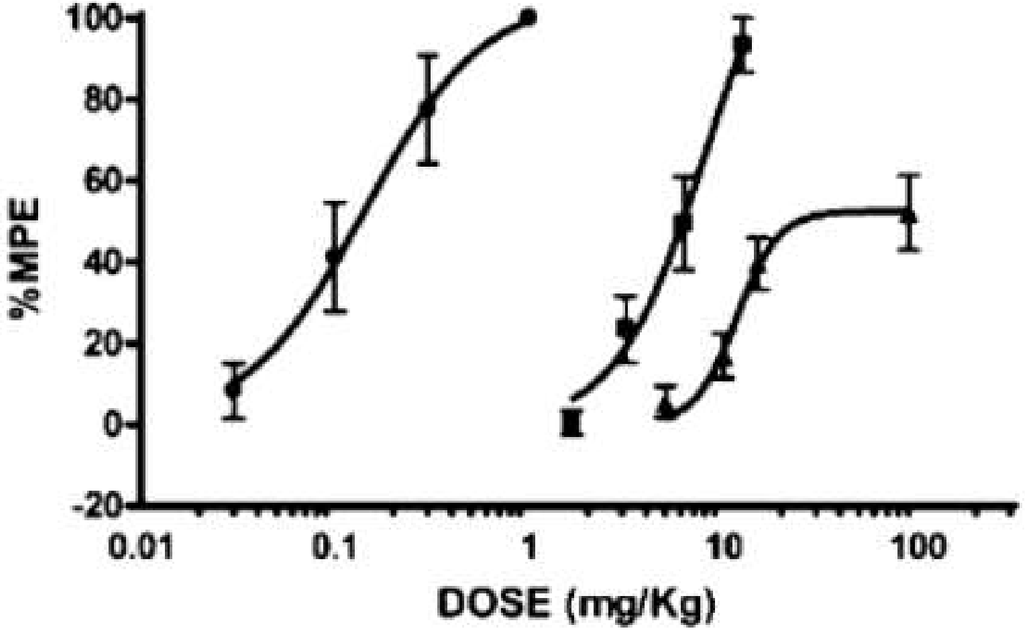
■ ULI+CLO
▲ ULI
● CLO



ULI+MOR
ULI
MOR



ULI+AMI
ULI
AMI



ULI+CLO
ULI
CLO

$$E_i = (E_{\max} D^h)/(D^h + ED_{50}^h)$$

where: Ei is the selected effect level (50% MPE), D is the equieffective dose for this effect level and *h* is the Hill coefficient.

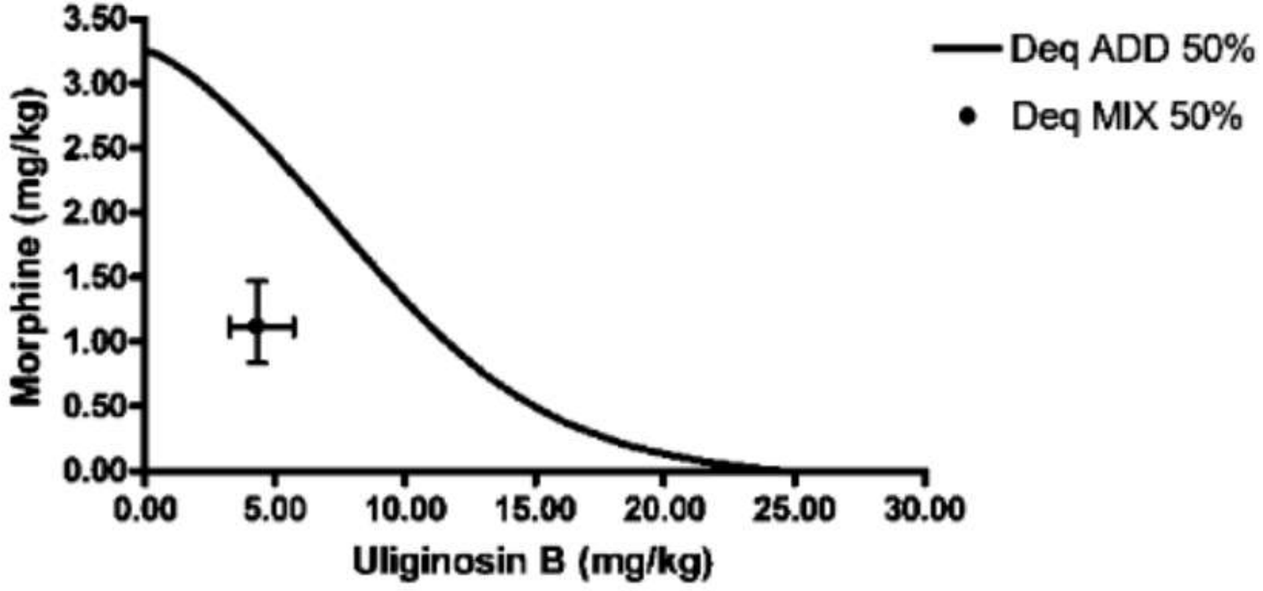
Table 1
Effective doses for the antinociceptive effect of uliginosin B, morphine, amitriptyline and clonidine alone and in combination in the hot-plate test. The ED_{50} and the doses producing 50% of the maximum possible analgesic effect ($D_{50\%}$) with their respective maximum effect (E_{max}) and Hill coefficient (h) were determined by non-linear regression. Doses results are expressed as mean with their 95% confidence interval (CI).

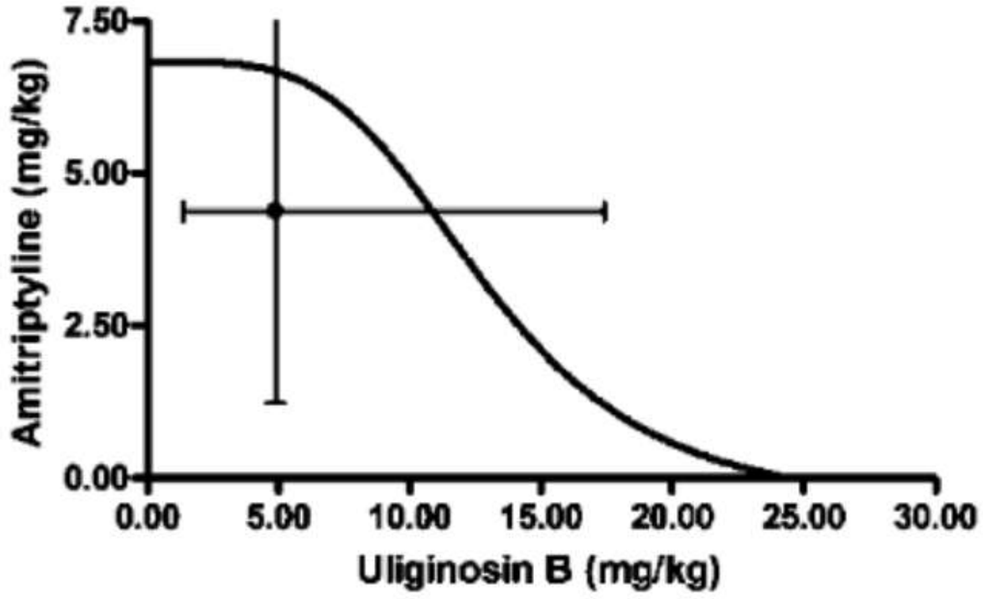
Treatments	ED_{50} (95% CI)	$D_{50\%}$ (95% CI)	$E_{max} \pm$ S.E.M.	h
Uliginosin B	11.67 (9.03 – 15.07)	24.51 (6.62 – 32.14)	52.52 ± 6.45	4.025
Morphine	3.15 (2.28 – 4.35)	3.26 (2.33 – 4.55)	95.38 ± 12.68	2.950
Amitriptyline	11.51 (2.22 – 59.70)	6.84 (1.86 – 25.06)	136.50 ± 48.06	1.054
Clonidine	0.14 (0.06 – 0.32)	0.13 (0.06 – 0.28)	105.60 ± 18.50	1.430
Morphine/Uliginosin B	5.39 (4.08 – 7.11)	5.44 (4.10 – 7.21)	97.80 ± 10.44	2.993
Amitriptyline/Uliginosin B	7.50 (2.57 – 21.88)	9.29 (2.61 – 32.89)	85.47 ± 35.71	1.615
Clonidine/Uliginosin B	8.03 (2.19 – 29.44)	6.16 (2.12 – 17.86)	133.10 ± 68.15	1.907

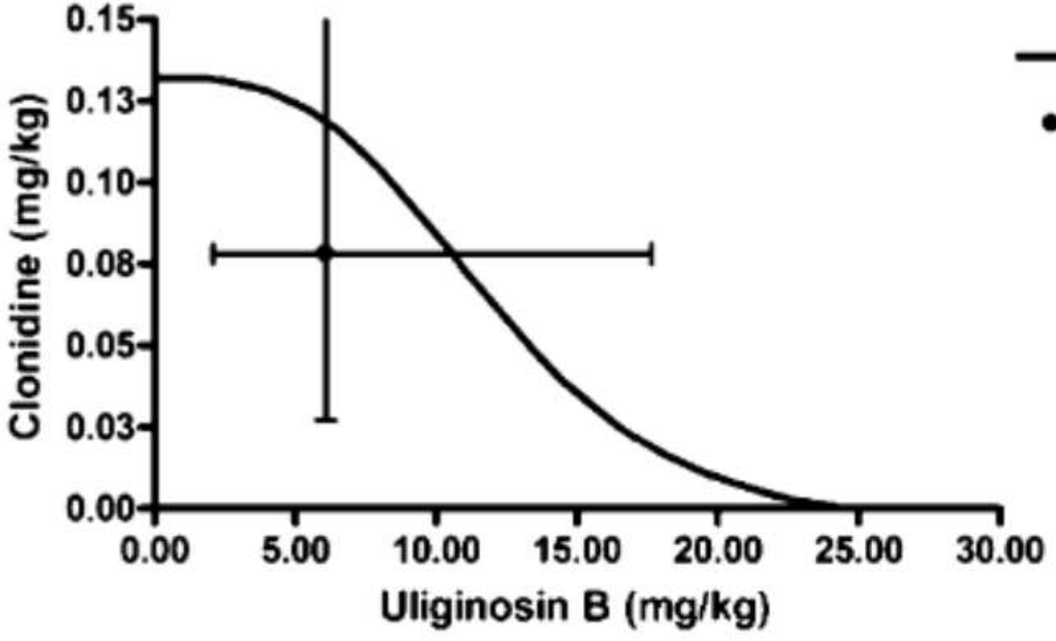
The additive line was calculated using the following equation:

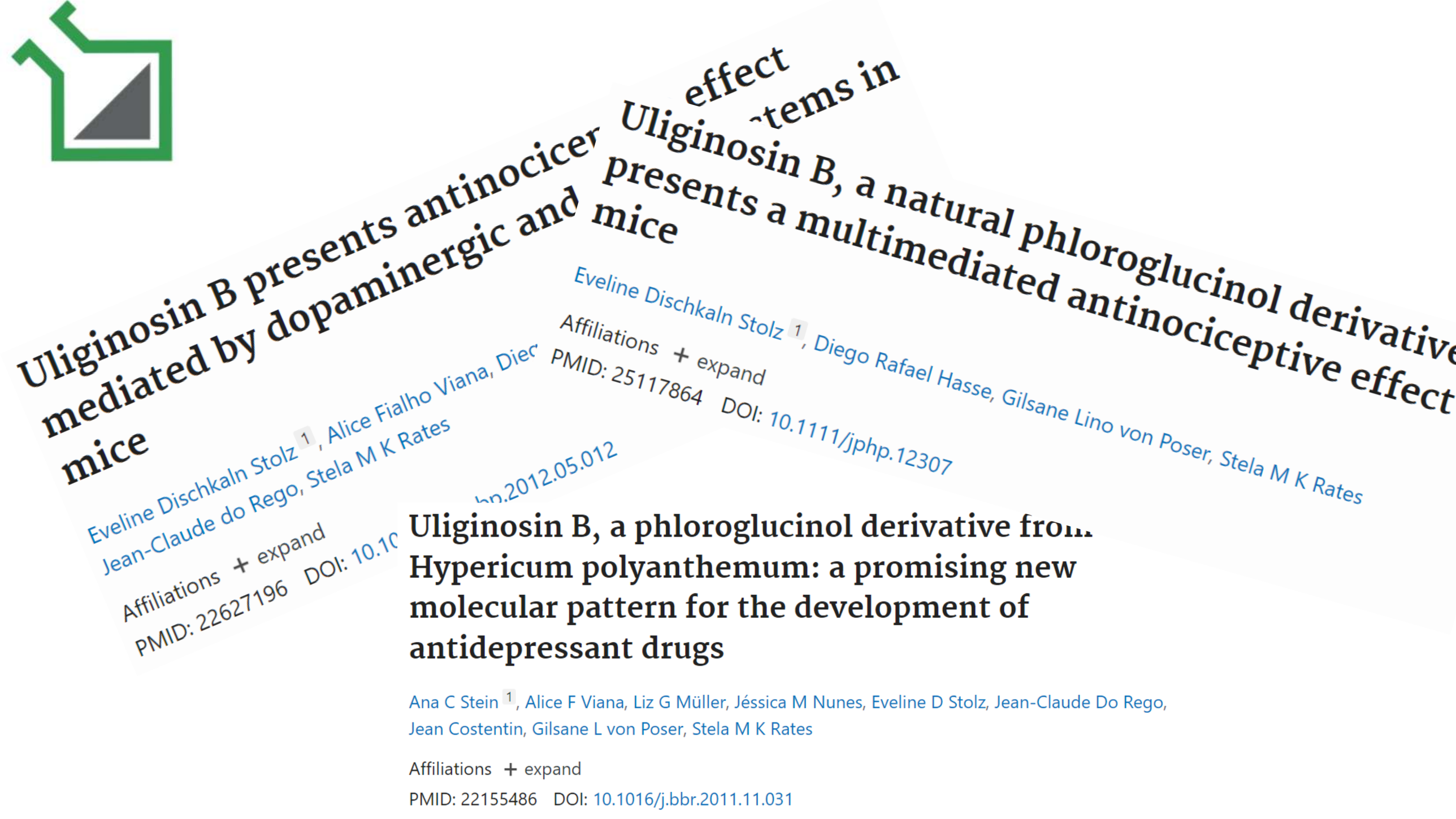
$$B_i = b + D_B/[(E_B/E_A)(1 + D_A^{h_A}/a^{h_A}) - 1]^{1/h_B}$$

where: Bi is the equi-effective dose for the drug B alone (morphine, amitriptyline or clonidine) at the level effect (50% MPE); (*a*, *b*) pairs are the doses of drug A and B defining the isobole of additivity; DA and DB are the ED50 values; EA and EB are the Emax values; hA and hB are the Hill coefficients for drug A and B, respectively


Morphine (mg/kg)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Uliginosin B (mg/kg)
Deq ADD 50%
Deq MIX 50%


Amitriptyline (mg/kg)
7.50
5.00
2.50
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Uliginosin B (mg/kg)


Clonidine (mg/kg)
0.15
0.13
0.10
0.08
0.05
0.03
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Uliginosin B (mg/kg)
Deq ADD 50%
Deq MIX 50%


Uliginosin B presents antinocicer
mediated by dopaminergic and
mice
Eveline Dischkaln Stolz 1, Alice Fialho Viana, Die
Jean-Claude do Rego, Stela M K Rates
Affiliations + expand
PMID: 22627196 DOI: 10.1
effect
-tems in
Uliginosin B, a natural phloroglucinol derivative
presents a multimediated antinociceptive effect
mice
Eveline Dischkaln Stolz 1, Diego Rafael Hasse, Gilsane Lino von Poser, Stela M K Rates
Affiliations + expand
PMID: 25117864 DOI: 10.1111/jphp.12307
Uliginosin B, a phloroglucinol derivative from
Hypericum polyanthemum: a promising new
molecular pattern for the development of
antidepressant drugs
Ana C Stein 1, Alice F Viana, Liz G Müller, Jéssica M Nunes, Eveline D Stolz, Jean-Claude Do Rego,
Jean Costentin, Gilsane L von Poser, Stela M K Rates
Affiliations + expand
PMID: 22155486 DOI: 10.1016/j.bbr.2011.11.031

THANK YOU!

UFRJ

UFRGS
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

CAPES

CNPq

UNOCHAPECÓ

NEUROPSICOFARMACOLOGIA

Pré-clínica

UNOCHAPECÓ

A COMUNIDADE DO CONHECIMENTO

CIÊNCIAS AMBIENTAIS

mestrado e doutorado

unochapeco.edu.br/cienciasambientais

Thank you!

III SIMPÓSIO INTERNACIONAL EM INVESTIGAÇÕES QUÍMICO-FARMACÊUTICAS

I ENCONTRO IBERO-AMERICANO DE PLANTAS MEDICINAIS Dr. Mahabir Gupta

I CONGRESSO LUSO-BRASILEIRO DE CIÊNCIAS E TECNOLOGIAS EM SAÚDE

28 a 30 de setembro de 2022, Itajaí-SC-Brasil

UNIVALI
UNIVERSIDADE DO VALE DO ITAJAÍ

UNOCHAPECÓ
A COMUNIDADE DO CONHECIMENTO

Isobolographic analysis of the interaction between synthetic drugs and natural products: synergistic, additive or antagonistic effects?

Liz Girardi Müller
lizmuller@unochapeco.edu.br

Itajaí
September 29th 2022