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Original article

Design, Prins-cyclization reaction promoting diastereoselective synthesis of 10 new tetrahydropyran derivatives and *in vivo* antinociceptive evaluations

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ABSTRACT

We described in this article the very efficient 2,6-*cis* ou 2,4,6-*cis* diastereoselective synthesis (2 or 3 steps, 62–65% global yields) from Prins-cyclization reaction as synthetic key-step to tetrahydropyran rings construction of 10 new congeners compounds (**3**–**12**) designed from Naproxen structure. These tetrahydropyran derivatives were *in vivo* bioevaluated on antinociceptive effect in the acetic acid-induced abdominal writhing test, the tail-flick test, the rota-rod performance and open field tests. All new compounds showed greater antinociceptive activity compared to compound **1a**, an analgesic tetrahydropyran derivative previously described by us. We can detach the high activity of tetrahydropyran derivative **10** which presented 87.5% inhibition (14% inhibition was presented by **1a**) in the acetic acid-induced abdominal writhing test. Besides that the tail-flick tests indicate compounds **7** and **10** as the most actives. All these new compounds showed no toxicity in mice in all biologically studied models.

1. Introduction

Substituted tetrahydropyranyl moieties are extensively distributed in the natural product structures that present a large pharmacological profile [1,2]. Many methodologies are being developed aiming to prepare these structures, *e.g.* the hetero-Diels–Alder, intramolecular Michael, the cyclization of diols and δ-hydroxyketones, the iodolactonization, the unsaturated alcohols undergo seleno-etherification, the epoxide opening, the Prins-cyclization reaction and others [3]. The Prins-cyclization reaction is a relatively new and very efficient reaction that has significantly advanced in the last years demonstrated by a number of applications described in the literature [4].

In the previous articles we described the first diastereoselective synthesis of (±)-*cis*-(6-ethyl-tetrahydropyran-2-yl) formic acid (**1a**) [5], and for the (-)–(S,S)–**1b** acid [6] (Fig. 1) both of them using Prins-cyclization reactions as key-step on synthetic strategy to construct the tetrahydropyran skeletons. Compound **1a** presented important antinociceptive (analgesic and anti-inflammatory) properties. This compound was proposed as responsible for the bioactivity of the isolated *Vitex cymosa* sp extract [7]. However, when spectroscopic data of synthetic **1a** were compared with the natural product we have found that lactone **2** [8] was the actual natural product responsible for the analgesic activity of *V. cymosa* sp (Fig. 1). Fortunately, this mistake [7] was very convenient for us, considering that unpublished **1a** and **1b** compounds showed higher antinociceptive activities than *V. cymosa* sp extract, and so compound **1a**–**b** could be presented as a new promising non-steroidal prototype to antinociceptive class of drugs.

The antinociceptive activity of **1a** was evaluated in mice on acetic acid-induced abdominal writhing, on *tail-flick* test, on hot-plate test, on formalin test, on reduction of spontaneous activity [5,9]. It described that the opioid receptor antagonist Naloxone totally reverted effects on **1a** in all models. In fact, the pharmacological profile described for **1a** indicating that it substance can mediate antinociception at peripheral and central sites even when orally administered through activation of opioid receptors [9]. Although, **1a** induced less tolerance when compared to morphine. Recently, our research group described pharmacological profile to pure enantiomer **1b** [10] indicating that **1b** also develops significant antinociceptive activity and, at least part of its effects seems to be mediated by the opioid system.

It is well established that there are multiple pain states and that these states differ not only in the reported sensation of pain, but

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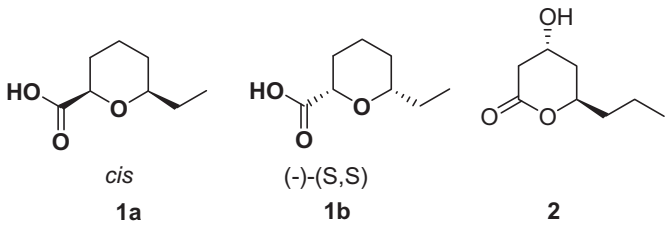


Fig. 1. (±)-Cis-(6-ethyl-tetrahydropyran-2-yl) formic acid (**1a**), (−)–(S,S)-**1b** acid and (±)-lactone **2** structures.

also in the pharmacology of therapeutic agents and in the sites of the nervous system where the pain is generated and can be altered [11]. Current pharmacological therapy for pain comes primarily from two classes of compounds: non-steroidal anti-inflammatory drugs and opioids. Up to now there is a continuous search for new pharmacologically active analgesic agents with minor adverse effects.

In our continuing search for bioactive substances [12–18] we present in this article the designed, diastereoselective synthesis, *in vivo* antinociceptive and toxicological evaluation to a new congener series of tetrahydropyranyl derivatives: the acids **3** and **4**, its synthetic precursors **5**–**8** and also the derivatives **9**–**12** (Fig. 2). Even compound **1a** has already been extensively investigated in the previous work, it was also bioevaluated *in vivo* in this article as reference compound.

We realized the design these new compounds inspirited on (±)-naproxen structure **13** (Fig. 3), a PGHS1/PGHS2 enzymes inhibitor [19] which present the carboxylic acid and the naphthyl moieties. In our design we included a spacer group [20] from (±)-Naproxen structure (**13**), producing the virtual structure **14** (Scheme 1), followed by the conformational restriction strategy [21], producing the virtual structure **15**. The use of the bio-isosterism concept [22] led to the α and β naphthyl tetrahydropyranyl acids **3** and **4**. These new compounds can be prepared at diastereoselective form from the Prins-cyclization as strategy to construction of its tetrahydropyran skeletons like the **1a** and **1b** prototype [3].

Even knowledge that (±)-Naproxen (**13**) does not present an opioid-type action [19] like our prototypes **1a** and **1b** we also choose **13** as starting structure for *design* of the tetrahydropyranyl acids **3** and **4** (Fig. 2) due to the presence of the pharmacophoric and auxoforic groups like **1a** and **1b** and also because **13** present a strong antinociceptive activity, being an PGHS1 and PGHS2 enzyme inhibitor [19]. In addition, compounds **3** and **4** and these derivatives presented, respectively, a good *in silico* calculated enzyme inhibitor and GPCR ligand scores from the on line

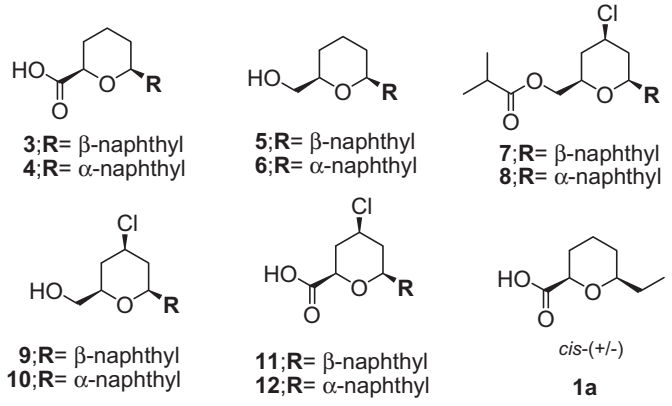


Fig. 2. Designed, synthesized and bioevaluated new compounds (**3**–**12**) and **1a** as biological reference compound.

Molinspiration cheminformatics® program (Table 1) [22,23]. It is important to detach that compounds **1a**, **3**–**12** (Fig. 2 and Table 1) are in agreements to the Lipinski's Rule of Five [24], also calculated by the Molinspiration cheminformatics® program. Recently, several publications in Medicinal Chemistry have been described using Molinspiration cheminformatic® program assisting the *design* of new drugs [25].

2. Results and discussion

2.1. Chemistry

2.1.1. Improved synthesis of (±)-cis-(6-ethyl-tetrahydropyran-2-yl) formic acid (*1a*)

We began our experimental work optimizing of the previous diastereoselective synthesis of compound **1a** [5].

The 2,2-dimethoxyethyl isobutyrate (**19**) is a strategic compound that has been used in several nucleosides synthesis and other applications [26]. For example **19** was used for the synthesis of mescarine [27], oxetanocin [28], kallolide A [29], (±)-kumausallene and (+)-epi-kumausalene [30]. The synthesis of 2,2-dimethoxyethyl isobutyrate (**19**) could be performed in high yield by reaction between potassium isobutyrate (**16**) and 2-chloro-1,1dimethoxyethane (**17**) in *N*,*N*-dimethyl formamide (DMF), through a nucleophilic substitution leading to an 2-chloro-1,1-dimethoxyethane (**18**) in high yield [31], followed by hydrolysis with aqueous formic acid resulting 2,2-dimethoxyethyl isobutyrate (**19**) [32]. Using these protocols we could easily prepare **19** on scales of 60 g in two steps on high yield (Scheme 1).

The synthesis of (±)-homoallylic alcohol (**20**), was performed through a Barbier reaction between allyl bromide and aldehyde **19** (1.0 Equiv.) in water in the presence of tin chloride (dihydrate) (1.5 Equiv.), potassium iodide (3.0 Equiv.) and solution of saturated ammonium chloride at room temperature for 2 h, resulting in **20** on 80% of yield (Scheme 2) [33].

The (±)-homoallylic alcohol **20** was employed as the substrate for the Prins cyclization reaction with propanal, mediated by $AlCl_3$ as the Lewis acid [34], dissolved in dried CH_2Cl_2 for 6 h, resulting tetrahydropyran derivative **21** in 85–90 % yield (Scheme 2). An only diastereoisomer was obtained by CG-MS analysis (see experimental section).

Based on the most commonly accepted reaction mechanism explaining the 2,4,6-*cis* preferential geometry for this reaction, proposed by Li and Yang et al. [35], we propose the mechanism shown in Fig. 4. The mechanism begins with the nucleophilic attack of alcohol **20** on the complexed aldehyde with $AlCl_3$ (step **a**) leading to formation of intermediate **22**. Step b is a proton exchange followed by elimination (step **c**) with the formation of intermediate **24**. The step d (slow step) subsequently occurs by synchronously nucleophilic attack of chloride ion through the transition state **25**, which produces **21**. It should be noted that the attack of chloride ion occurs preferentially on equatorial position which is a more stable transition state (Fig. 4).

Another proposal explaining the stereoselectivity on C_4 (Chlorine) of tetrahydropyran derivatives was based on the theoretical studies by Alder et al. [36]. They proposed that the favoring equatorial C_4 selectivity obtained on this reaction originates from the geometry of the cationic intermediate (*e.g.* **26**, Fig. 4) involved in the reaction.

According to the Alder interpretation, the interaction between one of the *n* electrons pair of oxygen with two *σ* electrons pairs (one in each of the C–C bonds of the ring cyclic σ) and the empty p-orbital of carbocation makes particular stability on the system. This particular geometry favors the nucleophilic attack of chloride ion on *exo*-face of **26** (Fig. 5) leading the C_4-equatorial product **21**.

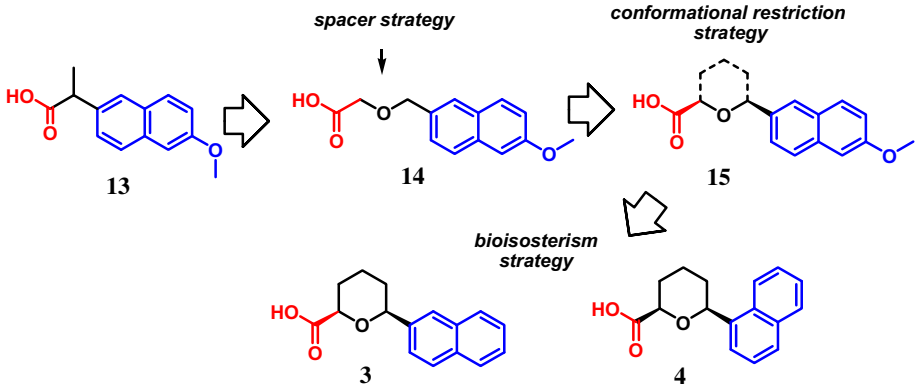


Fig. 3. Designed to acids **3** and **4** from (±)-Naproxen structure (**13**).

Comparison of **21** prepared here with spectroscopic data of this same compound previously described by us [9] corroborated its relative geometry.

The reduction of esters was carried out by reaction with sodium borohydride in anhydrous ethanol under reflux where it was successful resulting in yields of 90–100% [37]. The subsequent chlorine atom removing could be performed *one pot* from reduction of ester **21** producing the alcohol **22** in quantitative yield (step iii, Scheme 2) by using lithium aluminum hydride on refluxing dried THF for 12 h [38]. The alcohol **22** were efficiently oxidized by nitric acid, furnishing the carboxylic acids **1a** in 100% yield [39]. In our previous article [5] this synthetic step was performed by Jones reagent on 91% yield. This protocol presented here is cheaper and avoids the utilization of chromium (VI) compounds which are highly toxic for both acute and chronic exposures and can cause cancer and care must be taken when handling chromium (VI) reagents [40]. It should be noted that in previous article [9] we prepare **1a** in four steps from **19** in 33% yield and here **1a** is prepared in four steps from **19** in 72%.

2.1.2. Synthesis of new tetrahydropyranyl derivatives

The preparation of the new acids **3** and **4** (Scheme 3) was obtained in accordance with the experimental procedures developed to acid **1a** in this article. Curiously, the Prins reaction between alcohol **18** and β-naphtaldehyde to prepare **7** and α-naphtaldehyde to prepare **8** was more efficient on chloroform as solvent than dichloromethane (steps i/ii, Scheme 3). Moreover, when these reactions were made in different reaction times we obtained increases in yields (85% of **7** and 100% of **8**). The subsequent reduction of compounds **7** and **8** may be chemoselectively performed depending on chosen reduction reagent. Only the ester group in **7** and **8** was reduced using sodium borohydride preparing compounds **9** and **10** in quantitative yields (steps iii/iv, Scheme 3). Differently using lithium aluminum hydride both, dechlorination reaction of C_4 of **7** and **8** and transformations of esters moieties on corresponding alcohols could be made in *one pot* reactions (steps vii/viii) producing alcohols **5** and **6** respectively. Finally, oxidations of **9** and **10** to **11** and **12** (steps v/vi) and oxidation of **5** and **6** to **3** and **4,** respectively (steps ix/x) were quantitatively performed using HNO_3 (Scheme 3).

2.1.3. Geometries determinations of new tetrahydropyranyl derivatives

In accordance of the results described here to preparation of **1a** an only diastereoisomer was obtained for new compounds (**3**–**12**) evaluated by GC–MS analysis. As previously discussed here the geometries 2,6-*cis* (to compounds **3**–**6**) and 2,4,6-*cis* (to compounds **7**–**12**) are expected based on the Prins-cyclization reaction proposed mechanism [3,4].

The scalar constant coupling values obtained on **3**–**12** were not sufficient to establish accurately the relative 2,4,6 *cis* geometries to these new compounds. However, the 2D-NOESY spectrum, *e.g.* on compound **10** (Fig. 6) presented significant signals of correlation between H_a–H_b, H_a–H_c and H_b–H_c that determine unequivocally the 2,4,6-*cis* geometry, confirming the mechanistic prediction. Besides that, computational studies on **10** and **10a** (**10a** is a C_4-epimer of **10,** Fig. 6) at M06-2X/6-311++G(d,p) as level of calculations, were able to determinate the theoretical distances between H_a, H_b and H_c (see data in Table 2).

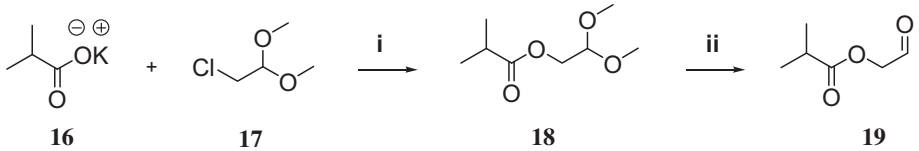
These theoretical results corroborate the 2D-NOESY spectrum. If the structure **10a** was a correct structure would not be expecting correlation signals between H_a–H_b and H_b–H_c due to the large distance between these atoms (see Table 2). We have shown in Fig. 7 two different visions for each of the calculated structures **10** and **10a**. So, 2D-NOESY and theoretical results corroborated the correct relative geometry for this compound as **10**.

It should be noted that it was possible to observe one intramolecular hydrogen bond (IHB) between the alcoholic hydrogen and oxygen in the tetrahydropyran ring in **10** (O–H···O = 2.36 Å) (Fig. 7). The presence of this IHB on **10** must have an important role in the conformational stability as well as the interaction with the biological receptor.

2.2. Biology

2.2.1. Effect of tetrahydropyranyl derivatives on acetic acid-induced writhing

Intraperitoneal injection of acetic acid (1.2%) induced a total of 51 ± 8.1 writhes in a period of 30 min. The mice were pre-treated orally with tetrahydropyranyl derivatives at a dose of 30 mg/kg, control and vehicle (Table 3).



Scheme 1. (i) 1.5 Equiv. **16**, 1 Equiv. **17**, DMF, 80 °C, 4 h, 90–95%. (ii) Dimethylacetal **18**, HCO_2H/H_2O (8:2), r.t., 12 h, 80–85%.

Table 1
Enzyme inhibitor, GPCR ligand scores and number of Lipinski's rule violations calculated by Molinspiration cheminformatic® program.

Compound	Enzyme inhibitor	GPCR ligand	LRV[a]
1a	−0.06	−0.55	0
3	+0.54	+0.48	0
4	+0.47	+0.51	0
5	+0.48	+0.38	0
6	+0.40	+0.42	0
7	+0.16	+0.21	1[b]
8	+0.10	+0.24	1[c]
9	+0.37	+0.31	0
10	+0.30	+0.35	0
11	+0.43	+0.40	0
12	+0.36	+0.44	0

[a] Number of Lipinski's rule violations.
[b] log $P = 5.029$.
[c] log $P = 5.005$. A molecule contradicts Lipinski's rule when two or more violations of this rule are calculated.

The acetic acid-induced writhing method is able to determine antinociceptive effects of compounds and dose levels that might seem to be inactive in other methods [41]. The results showed that all the new tetrahydropyranyl derivatives had a significant reduction in the number of abdominal writhes compared to the control group, showing an antinociceptive effect. The compounds **5**, **10** and **12** were the most effective (55.7%, 87.5% and 54.4%, respectively) (Table 3).

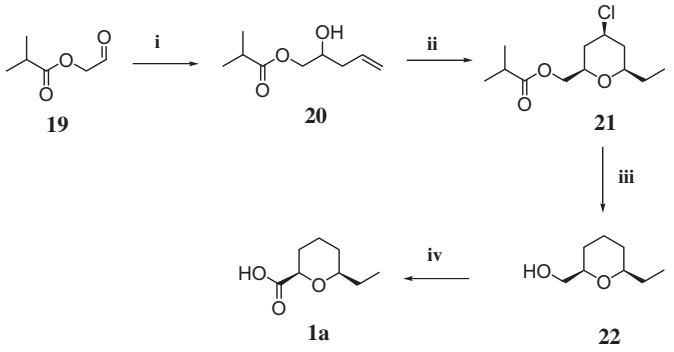
According to previous results [9], the compound **1a** showed antinociceptive activity in a dose of 50 mg/kg (approximately 15% inhibition in the number of abdominal writhes), from there we decided to evaluate and compare this compound with the new tetrahydropyranyl derivatives in a lower dosage (30 mg/kg). Table 3 shows that all new compounds showed greater antinociceptive activity than compound **1a**.

2.2.2. Effect of tetrahydropyran derivatives in the tail-flick test

The tail-flick test was used to assess the central activity of the compounds, since this test is predominantly a spinal reflex, and is considered to be selective for centrally acting analgesic substances, whereas peripherally acting analgesics are known to be inactive against thermal stimuli [42,43].

Fig. 8(A–D) shows that all the compounds presented a result significantly greater than the control group, except compound **12**, in the tail-flick test throughout the experiment, confirming the central antinociceptive effect of these compounds, however compounds **4** and **7** (Fig. 8A and B), and **8**, **10** and **11** (Fig. 8C and D) produced greater effect than the compound **1a**.

In Fig. 8A and C, graphs represent time–effect curve. In Fig. 8B and D, graphs represent the area under the curve (AUC) calculated for each time–effect curve. The dose of compounds was 30 mg/kg (p.o.). The results are presented as percentage increase over the baseline or area under the curve (AUC); $n = 6$ per group. Statistical significance was calculated by the analysis of variance followed by Bonferroni's test. $^{*}P < 0.05$ relative to the control group. Where no error bars are shown, it is because they are smaller than the symbol.



Scheme 2. Improved synthesis of **1a**. (i) Aldehyde **19** (1 Equiv.), H_2O, $SnCl_2$ (1.5 Equiv.), KI (3.0 Equiv.), allyl bromide, NH_4Cl, r.t., 2 h, 80 %; (ii) **20** (1 Equiv.), propanal, $AlCl_3$, CH_2Cl_2, 0 °C, 6 h, 85–90%; (iii) **21**, $LiAlH_4$, THF, 100 °C, 12 h, 100%; and (iv) **22**,concentrated nitric acid, r.t, 4 h, 100%.

2.2.3. Effect of tetrahydropyran derivatives in the rota-rod performance and open field tests

The rota-rod performance (forced motor activity) and open-field (spontaneous motor activity) tests were used to exclude the possibility that the antinociceptive action of tetrahydropyran derivatives could be related to nonspecific disturbances in the locomotors activity of the animals. We observed that at dose that has antinociceptive action (30 mg/kg, p.o.), tetrahydropyran derivatives did not alter the motor performance of mice in both tests (Fig. 9).

2.2.4. Toxicological evaluation in vivo of tetrahydropyran derivatives

All tetrahydropyran derivatives described in this paper were evaluated for their acute toxicity in mice. No symptom of intoxication was observed in animals (disorientation, hyperactivity, piloerection and hyperventilation). Investigated compounds were not toxic after oral administration (LD_{50} >2000 mg/kg).

3. Conclusion

In this article we presented firstly a high improvement on the analgesic tetrahydropyran **1a** preparation (from 33% in previous article to 72% presented here). After, we described the *design* and total synthesis for 10 novel tetrahydropyranyl derivatives (compounds **3**–**12**), using the Prins-cyclization reactions as key-step to construction on diastereoselective form the 2,4-*cis* and 2,4,6-*cis* tetrahydropyranyl rings. This non-toxic congener series of compounds presented high analgesic activity observed in animal models (mice). All new compounds showed greater antinociceptive effect in the acetic acid-induced abdominal writhing test, compared to compound **1a** previously described by us. We can detach the high activity of tetrahydropyranyl derivative **10** which presented 87.5% inhibition (**1a** presented only 14% inhibition). The tail-flick test demonstrated compounds **7** and **10** as the most actives. The new compounds showed central antinociceptive activity (except compound **12**) without producing motor impairment. Compound **7** could be prepared in 65% yield (2 steps from aldehyde **19**) and compound **10** could be prepared in 65% yield (3 steps from aldehyde **19**). Finally, considering a significant increase between the antinociceptive values of **1a** and compounds **3**–**12** we concluded that the strategy shown in Fig. 3 was very satisfactory. We also can notice that **1a** score values is negative (Table 1) and differently all new compounds **3**–**12** showed positive values of scores for both enzymatic inhibition and ligands GPCR. These results are in according of obtained antinociceptive values. We believe that these new class of compounds discovered here are candidates for analgesics. Our complementary studies on related new compounds are now in progress.

4. Experimental procedures

4.1. Chemistry

4.1.1. General methods

All commercially available reagents and solvent were obtained from commercial providers and used without further purification. Reactions were monitored by TLC using Silica gel 60 UV254

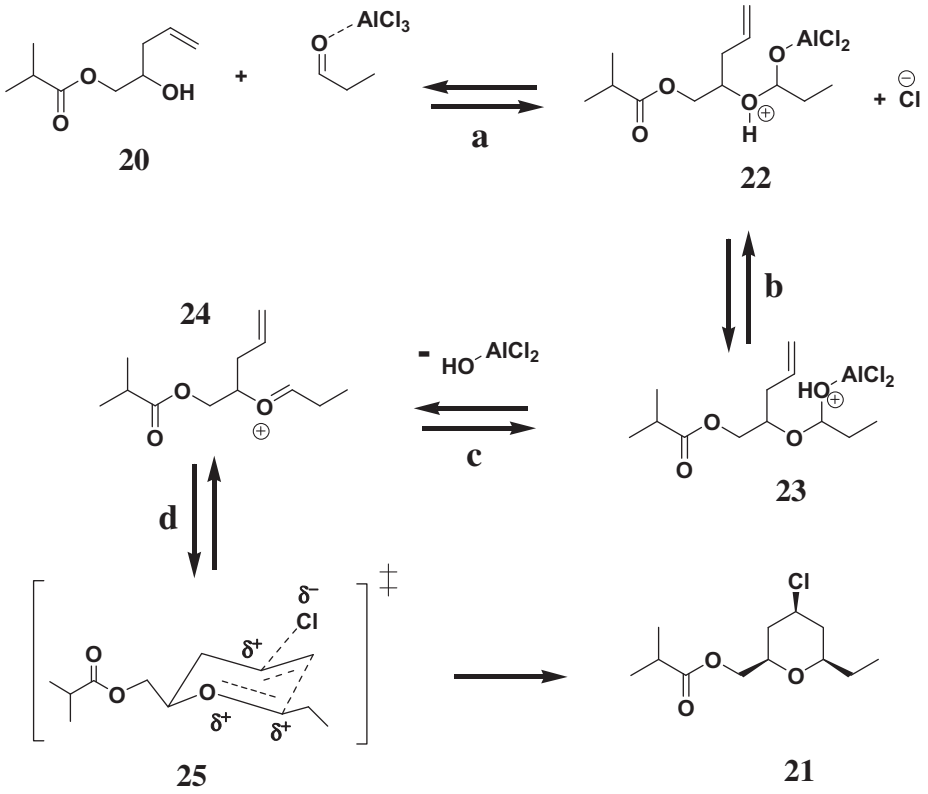


Fig. 4. Proposal mechanism to the **21** preparation based on the Li and Yang studies.

Macherey–Nagel pre-coated silica gel plates; detection was by means of a UV lamp and revelation to vanillin. Flash column chromatography was performed on 300–400 mesh silica gel. Organic layers were dried over anhydrous $MgSO_4$ or Na_2SO_4 prior to evaporation on a rotary evaporator. ^1H NMR and ^{13}C NMR spectra were recorded using Varian Mercury Spectra AC 20 spectrometer (400 MHz and 200 MHz for ^1H, 101 MHz and 50 MHz for ^{13}C) in $CDCl_3$. Chemical shifts were reported relative to internal tetramethylsilane (δ 0.00 ppm) for ^1H, and $CDCl_3$ (δ 77.0 ppm) for ^{13}C. FTIR spectra were recorded on a Shimadzu spectrophotometer model IRPrestige-21 in KBr pellets. MS data were measured with a Shimadzu GCMS – QP2010 mass spectrometer. High-resolution mass spectra were determined using a Shimadzu spectrophotometer LC-MS-IT-TOF.

*4.1.2. Synthesis of 2,2-dimethoxyethyl isobutyrate (**18**) [31]*

The reaction was performed using a solution of chloroacetaldehyde dimethyl acetal (**17**, 201 mmol, 24.92 g, 22.70 mL) in DMF (300 mL) was added potassium isobutyrate (**16**, 110.6 mmol, 13.95 g) and the mixture was refluxed at 150 °C for 2 h. Additional **16** (110.6 mmol, 13.95 g) was charged to the reactions mixture portion wise while refluxing. The reflux was continued for 13 h and mixture was allowed to room temperature. Water (300 mL) was added, and the resulting mixture was extracted with EtOAc (3 × 100 mL). The organic phase was dried with sodium sulfate anhydrous and concentrated under reduced pressure where **18** was obtained a 80% yield; IR (KBr, cm^{-1}): 1130, 1195 (C–O), 1739 (CO_2), 2974 (C–H sp^3); ^1H NMR ($CDCl_3$, 200 MHz): δ 1.19 (d, 6H, *J* 6.84 Hz), 2.67 (m, 1H), 3.41 (s, 6H), 4.12 (d, 2H, *J* 5.22 Hz), 4.54 (m, 1H); ^{13}C

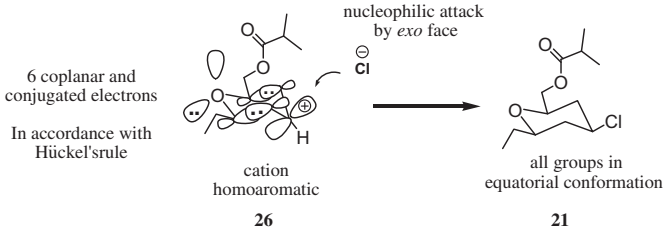
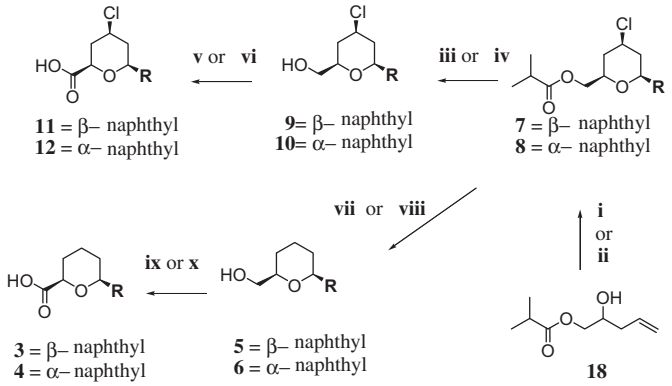


Fig. 5. The Alder's model to the equatorial diastereoselectivity on C_4 of the Prins cyclization reactions.



Scheme 3. Synthesis of new tetrahydropyranyl derivatives. (i) **18** (1 Equiv.), β-naphtaldehyde, $AlCl_3$ (1.3 Equiv.), $CHCl_3$, 0 °C, 6 h–85%/6days–85%; (ii) **18** (1 Equiv.), α-naphtaldehyde, $AlCl_3$ (1.3 Equiv.), $CHCl_3$, 0 °C, 6 h–85%/6days-100%; (iii) **7**, $NaBH_4$ (1 Equiv.), EtOH, 100 °C, 3 h, 85–90 %; (iv) **8**, $NaBH_4$ (1 Equiv.), EtOH, 100 °C, 3 h, 85–90 %; (v) **9**, concentrated nitric acid, r.t, 4 h, 100%; (vi) **10**, concentrated nitric acid, r.t, 4 h, 100%; (vii) **7**, $LiAlH_4$, THF, 100 °C, 12 h, 100%; (viii) **8**, $LiAlH_4$, THF, 100 °C, 12 h, 100%; (ix) **5**, concentrated nitric acid, r.t, 4 h, 100%; and (x) **6**, concentrated nitric acid, r.t, 4 h, 100%.

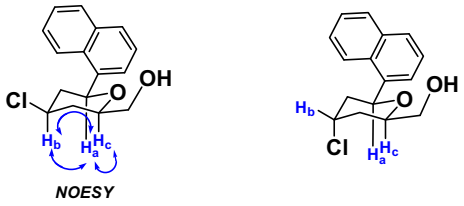


Fig. 6. Compounds **10** and **10a** representation (see data in Table 2).

NMR ($CDCl_3$, 50 MHz): δ 18.7, 34.1, 53.5, 53.7, 62.6, 101.4, 171.8; GC: RT = 6.94 min (100%); Mass calculated: 176.1, $C_8H_{16}O_4$.

*4.1.3. Synthesis of 2-oxoethyl isobutyrate (**19**) [32]*

A solution of 2,2-dimethoxyethyl isobutyrate (**18**, 12.4 mmol, 2.19 g) in formic acid aqueous solution (HCO_2H/H_2O = 8:2 v/v, 30 mL) was stirred at room temperature for 12 h. After the end of reaction (verified by TLC), the mixture where extracted (3 × 50 mL of CH_2Cl_2) and the organic phase was washed with a saturated solution of $NaHCO_3$. The organic phase was dried with sodium sulfate anhydrous and concentrated under reduced pressure producing 80% yield; IR (KBr, cm^{-1}): 1654, 1724 (CO_2), 2704 (-CHO), 2931(C–H sp^3); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.19 (d, 3H, J = 4 Hz), 1.23 (d, 3H, J = 8 Hz), 2.63 (m, 1H), 4.21 (s, 1H), 4.65 (d, 1H), 9.61 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 18.87, 18.70, 33.64, 68.44, 176.52, 195.85; GC: RT = 13.73 min (100%); Mass calculated: 130.06, $C_6H_{10}O_3$.

*4.1.4. Synthesis of 2-hydroxypent-4-enyl isobutyrate (**20**) [33]*

The 2-oxoethyl isobutyrate aldehyde (**19**, 25 mmol, 3.25 g) was added to water (120 mL) containing potassium iodide (75 mmol, 12.45 g), stannous chloride dehydrate (37.55 mmol, 8.45 g) and allyl bromide (3 mmol). The orange solution turns white by addition of saturated ammonium chloride (80 mL). The stirring was continued for 2 h at room temperature. After the end of reaction, the reaction mixture was extracted with CH_2Cl_2 (3 × 50 ml) gave an organic phase, washed with water, dried with sodium sulfate anhydrous, concentrated under reduced pressure and purified by flash chromatography on silica gel (AcOEt:hexane 3:7 as eluent.) The products were concentrated under reduced pressure yielding a pale oil in 75% yield; IR (KBr, cm^{-1}): 1157, 1199 (C–O), 1392 (C–O–H), 1732 (C=O), 2939, 2978 (C–H sp^3), 3456 (O–H) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.17 (d, 6H, J = 8 Hz), 2.31(m, 2H), 2.6 (sp, 1H, J = 8 Hz), 3.68 (m, 1H), 3.96 (m, 1H), 4.12 (m, 1H), 4.93–5.17 (m, 2H), 5.78 (m, 1H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 18.74, 18.94, 33.92, 38.01, 67.69, 69.17, 118.47, 133.46, 177.33; GC: RT = 7.92 min (100%); Found: $C_9H_{16}O_3$ 172.1.

4.1.5. Procedure general for Prins-cyclization: a typical procedure for synthesis of derivates tetrahydropyrans

A typical procedure for the Prins cyclization reaction [5], aluminum chloride ($AlCl_3$, 1.5 mmol, 0.2 g) was place in a dried flask under magnetic stirrer followed by addition of 3 mL dichloromethane (dried under calcium hydride). After, a solution of homoallylic alcohol (**20**, 1.0 mmol, 0.172 g), the corresponding aldehyde (1.0 mmol), in 3 mL of dichloromethane was slowly place into this flask at 0 °C. The mixture reaction is stirring at this temperature for 6 h. After this time, 5 mL of a saturated sodium bicarbonate solution $NaHCO_3$ is added. The organic phase into reaction mixture was separated and washed with brine, dried with Na_2SO_4 and the evaporated under reduced pressure. This crude product is then submitted to flash column chromatography, yielding a colorless oil in 85% yield. We have also observed that the addition of $CHCl_3$ in place of CH_2Cl_2 occurs an improvement in the yield of the reaction between 85 and 100%.

Table 2
Calculated distances (Å) between $H_a/H_b/H_c$ in **10** and **10a** at M06-2X/6-311++G(d,p) as level of calculations.

Compound **10**		Compound **10a**	
H_a–H_b	2.56609	H_a–H_b	3.78258
H_a–H_c	2.35396	H_a–H_c	2.32529
H_b–H_c	2.64134	H_b–H_c	3.84439

*4.1.5.1. (4-Chloro-6-(naphthalen-2-yl)-tetrahydro-2H-pyran-2-yl) methyl isobutyrate (**7**).* This product was obtained using (1.0 mmol, 0.156 g) of β-naphthaldehyde, resulting in 85% yield in both CH_2Cl_2 or $CHCl_3$ solvents. The product was purified by silica gel column chromatography using EtOAc/hexane (1:10, v/v) as eluent. IR (KBr, cm^{-1}): 821 and 748 (C–H aromatic), 1249 and 1157 (C–O ester), 1627 and 1462 (C=C aromatic), 1735 (C=O ester), 2970 (C–H sp^3), 3055 (=C–H aromatic) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 1.21 (dd, 6H, J = 4 Hz), 1.86 (m, 2H), 2.5 (m, 1H), 2.62 (m, 1H), 3.85 (m, 1H), 4.3 (m, 3H), 4.57 (d, J = 12 Hz), 7.48 (m, 3H), 7.83 (m, 4H); ^{13}C NMR ($CDCl_3$ 50 MHz): 18.96, 33.88, 38.54, 43.96, 55.05, 65.99, 74.83, 78.62, 123.85, 124.50, 125.93, 126.12, 127.61, 127.97, 128.17, 132.98, 133.19, 138.25, 176.86; GC: RT = 27.17 min (100%); Mass calculated: 346.1, $C_{20}H_{23}ClO_3$; HRMS – mass calculated for $C_{20}H_{23}ClO_3[(M + Na)^+]$ 369,0979; Found 369,0979.

*4.1.5.2. (4-Chloro-6-(naphthalen-1-yl)-tetrahydro-2H-pyran-2-yl) methyl isobutyrate (**8**).* This product was obtained using (1.0 mmol, 0.156 g) of α-naphthaldehyde, resulting in 85% yield when CH_2Cl_2 was used and 100% yield when $CHCl_3$ was used. The product was purified by silica gel column chromatography using EtOAc/hexane (1:10, v/v) as eluent. IR (KBr, cm^{-1}): 779 and 732 (C–H aromatic), 1246 and 1157 (C–O ester), 1597 and 1465 (C=C aromatic), 1735 (C=O ester), 2873 (C–H sp^3), 3051 (=C–H aromatic) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 1.19 (dd, 6H, J = 8 Hz), 1.83 (q, 1H, J = 12 Hz), 2.07 (q, 1H, J = 12 Hz), 2.31 (m, 1H), 2.60 (m, 2H), 3.95 (m, 1H), 4.27 (m, 1H), 5.09 (d, 1H, J = 12 Hz), 7.7 (m, 7H); ^{13}C NMR ($CDCl_3$ 50 MHz): 18.97, 33.89, 38.60, 42.72, 55.20, 66.08, 75.16, 75.81, 123.02, 123.29, 125.42, 125.52, 126.09, 128.49, 128.90, 130.31, 133.77, 136.06, 176.85; GC: RT = 24.42 min (100%); Mass calculated: 346.1, $C_{20}H_{23}ClO_3$; HRMS – mass calculated for $C_{20}H_{23}ClO_3[(M + Na)^+]$ 369,0979; Found 369,0979.

*4.1.5.3. (4-Chloro-6-ethyl-tetrahydro-2H-pyran-2-yl)methyl isobutyrate (**21**).* This product was obtained using (1.0 mmol, 0.156 g) of propionaldehyde, resulting in 88% yield when used in CH_2Cl_2 and 90% yield when used in $CHCl_3$. The product was purified by silica gel column chromatography using EtOAc/hexane (1:10, v/v) as eluent. IR (KBr, cm^{-1}): 757 (C-Cl), 1150 and 1193 (C–O ester), 1737 (C=O ester), 2972 and 2878 (C–H sp^3), cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.93 (t, 3H, J = 4 Hz), 1.18 (d, 6H, J = 7 Hz), 1.55 (m, 5H), 2.10 (m, 1H), 2.60 (sp, 1H, J = 7.1 Hz), 3.20 (m, 1H), 3.60 (m, 1H), 3.90–4.10 (m, 3H); ^{13}C NMR ($CDCl_3$ 50 MHz): δ 9.76, 18.93, 28.61, 33.87, 38.76, 41.87, 55.32, 66.08, 74.32, 78.16, 176,83; GC: RT = 10.25 min (100%); Mass calculated: 248.1, $C_{12}H_{21}ClO_3$.

4.1.6. General procedure for the reduction of esters in alcohols using sodium borohydride [37]

In a dried Argon atmosphere flask under magnetic stirrer was placed $NaBH_4$ (6 mmol) dissolved in 10 mL of anhydrous ethanol following by addition of the product corresponding of Prins-cyclization dissolved in 10 mL of anhydrous ethanol to this flask at 0 °C. The reaction mixture was refluxed for 3 h at temperature 100 °C. After the end of reaction, a saturated aqueous ammonium chloride (10 mL) was placed at 0 °C and the reaction mixture was

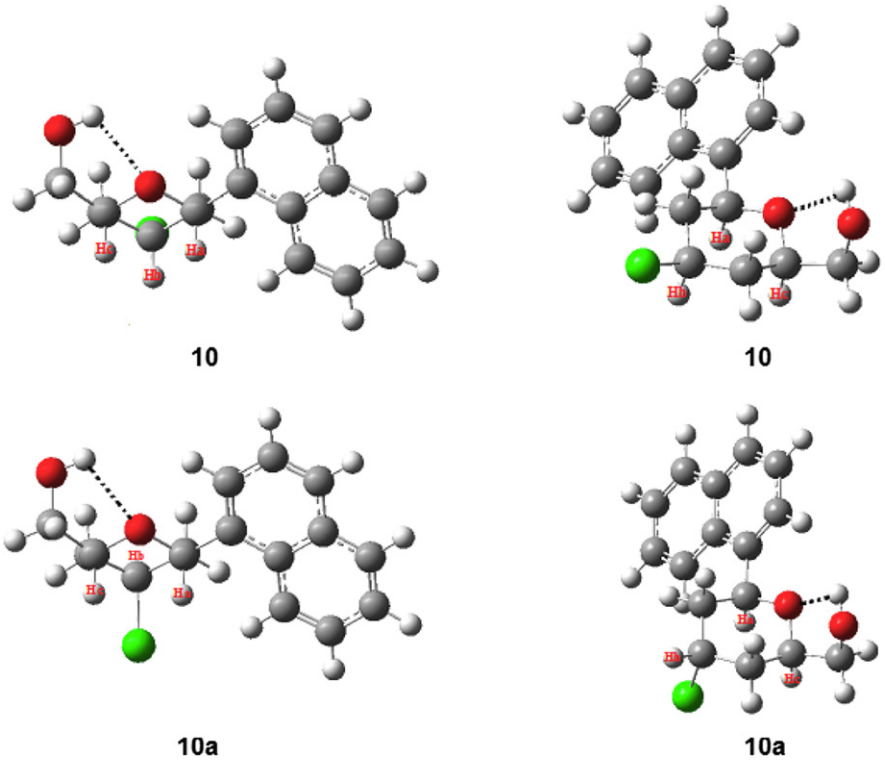


Fig. 7. Two different visions for each of the calculated structures **10** and **10a** at M06-2X/6-311++G(d,p) as level of calculation. See distances data between Ha–Hb–Hc in Table 2.

extracted with CH_2Cl_2 (3 × 20 ml) gave an organic phase, dried with sodium sulfate anhydrous, concentrated under reduced pressure providing the tetrahydropirans derivatives in high yields.

*4.1.6.1. (4-Chloro-6-(naphthalen-2-yl)-tetrahydro-2H-pyran-2-yl) methanol (**9**).* Reaction of 1.0 mmol (0.346 g) of **7** produces 100% yield of **9** as a yellow oil IR (KBr, cm^{-1}): 817 and 748 (C–H aromatic), 1053 (C–O), 1600 and 1442 (C=C aromatic), 2862 (C–H sp^3), 3383 (O–H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.77 (m, 1H), 1.95 (q, 1H $J = 12$), 2.10 (s, 1H), 2.16 (dd, 1H), 2.45 (dt, 1H), 3.69 (m, 2H), 4.21 (m, 1H), 4.56 (dd, 1H), 4.83 (s. 1H), 7.47 (m, 3H), 7.83 (m, 4H), RMN ^{13}C (101 MHz; $CDCl_3$) δ: 38,06; 42,80; 55,45; 65,62; 75,80; 78,01; 123,71; 124,45; 125,85; 125,71; 126,29; 128,63; 128,97; 130,45; 133,85; 136,29; GC: RT = 25.30 min (100%); Mass calculated $C_{16}H_{17}ClO_2$ 346.1; HRMS – Mass calculated for $C_{16}H_{17}ClO_2$[(M + Na)] 299.0612; Found 299.0612.

*4.1.6.2. (4-Chloro-6-(naphthalen-1-yl)-tetrahydro-2H-pyran-2-yl) methanol (**10**).* Reaction of 1.0 mmol (0.346 g) of **8** produces 90% yield of **10** as a yellow oil. IR (KBr, cm^{-1}): 779 and 860 (C–H aromatic), 1068 (C–O), 1396 and 1597 (C=C aromatic), 2862 (C–H sp^3), 3390 (O–H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.24 (m, 1H),1.78 (q, 1H, $J = 12$ Hz), 2.10 (dd, 1H, $J = 12$ Hz), 2.19 (m, 2H), 2.57 (dt, 1H), 3.68 (m, 1H), 3.77 (m, 1H), 4.28 (m, 1H), 5.10 (t, 1H), 7.76 (m, 7H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: 38.01, 42.74, 55.32, 65.55, 75.60, 77.93, 122.88, 123.24, 125.34, 125.61, 126.25, 128.57, 128.93, 130.32, 133.72, 136.09; GC: RT = 22.50 min (100%); Mass calculated: $C_{16}H_{17}ClO_2$ 346.1; HRMS – mass calculated for $C_{16}H_{17}ClO_2$[(M + Na)] 299.0616; Found 299.0616.

4.1.7. General procedure for the reduction of esters in alcohols using lithium aluminum hydride [38]

In dried Argon atmosphere flask under magnetic stirrer was place lithium aluminum hydride ($LiAlH_4$, 2.5 mmol) and 5 mL THF (dried under sodium/benzophenone), was slowly added at 0 °C the product corresponding of Prins cyclization dissolved in 5 mL dried THF. The mixture was refluxed for 12 h at temperature 100 °C. After the end of reaction, a saturated aqueous ammonium chloride (10 mL) and excess of THF was removed in vacuo. The crude reaction mixture was extracted with CH_2Cl_2 (3 × 20 ml) gave an organic phase, dried with sodium sulfate anhydrous, concentrated under reduced pressure providing the tetrahydropirans derivatives in high yields (90–100%).

*4.1.7.1. (6-(Naphthalen-2-yl)-tetrahydro-2H-pyran-2-yl) methanol (**5**).* Reaction of 1.0 mmol (0.346 g) of **7** produced 100% yield of **5** as a yellow liquid. IR (KBr, cm^{-1}): 867 and 879 (C–H aromatic), 1041 (C–O), 1458 (C=C aromatic), 2862 (C–H sp^3), 3417 (O–H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.58 (m, 6H), 3.58 (m, 1H), 4.73 (t, 1H, $J = 8$ Hz); 7.55 (m, 7H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: 23.50, 26.86, 33.63, 66.34, 78.74, 79.86, 124.34, 125.11, 126.12, 127.60, 127.66, 127.83, 127.92, 128.26, 132.89, 133.33; RT = 23.20 min (100%); Mass

Table 3
Antinociceptive activity of tetrahydropyranyl derivatives on acetic acid-induced abdominal writhes in mice (30 mg/kg, p.o.).

Compound	Number of writhes ± SD	Inhibition (%)
Control	51 ± 8.1	–
Vehicle	47 ± 7.1	7.8
1a	43.8 ± 6.2	14.1
3	29 ± 3.5*	43.1
4	26 ± 3.4*	49
5	22.6 ± 3.6*	55.7
6	31 ± 6.0*	39.2
7	28.3 ± 2.5*	44.4
8	31.4 ± 5.2*	38.4
9	27.3 ± 4.1*	46.6
10	6.4 ± 1.3*	87.5
11	36.7 ± 4.5*	28.1
12	23.3 ± 4.8*	54.4

SD – standard deviation; *$P < 0,05$ against control group.

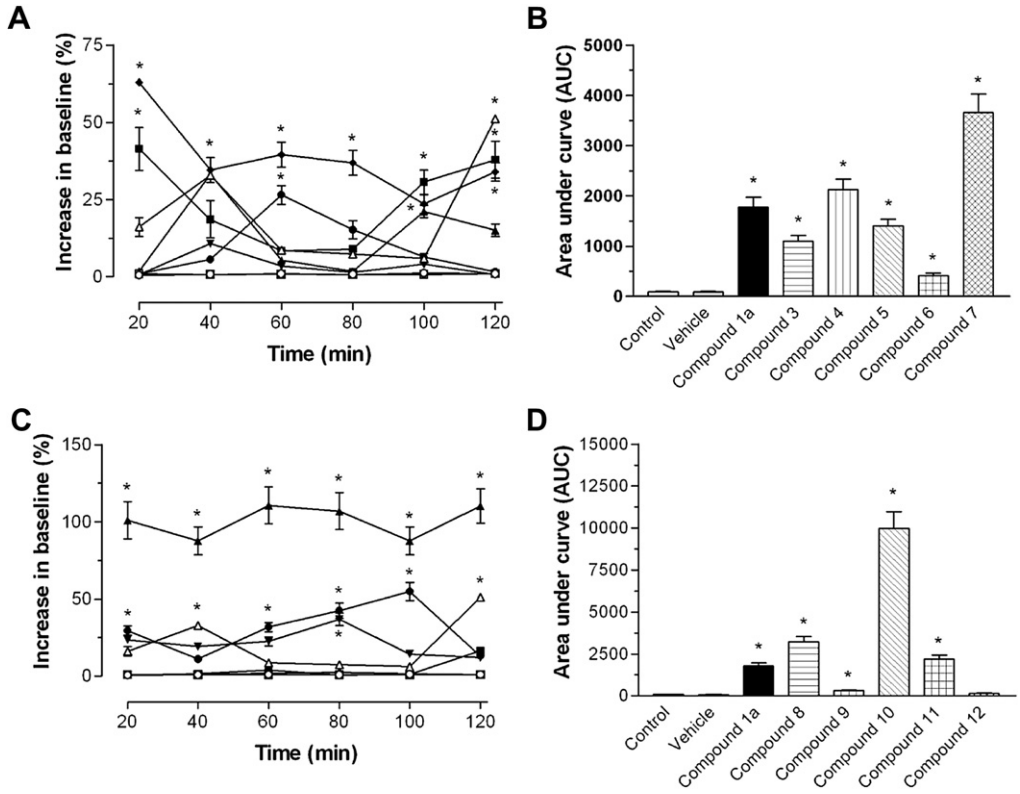


Fig. 8. Effects of tetrahydropyran derivatives in the tail-flick. In (A), (-○-) Control, (-□-) Vehicle, (-△-) Compound 1a, (-●-)Compound 3, (-■-)Compound 4, (-▲-) Compound 5, (-▼-) Compound 6, (-♦-) Compound 7. In (C), (-○-) Control, (-□-) Vehicle, (-△-) Compound 1a, (-●-) Compound 8, (-■-) Compound 9, (-▲-) Compound 10, (-▼-) Compound 11, and (-♦-) Compound 12.

calculated $C_{16}H_{18}O_2$ 242.1; HRMS − mass calculated for $C_{16}H_{17}ClO_2[(M+Na)]$ 265.0610; Found 265.0610.

*4.1.7.2. (6-(Naphthalen-1-yl)-tetrahydro-2H-pyran-2-yl) methanol (**6**).* Reaction of 1.0 mmol (0.346 g) of **8** produces 100% yield of **6** as a pale oil IR (KBr, cm^{-1}): 867 and 879 (C−H aromatic), 1041 (C−O), 1458 (C═C aromatic), 2862 (C−H sp^3), 3417 (O−H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 0.91 (m, 1H), 1.20 (m, 2H), 1.99 (m, 2H), 2.51 (m, 1H), 2.69 (m, 1H), 4.19 (m, 2H), 4.68 (t, 1H, $J = 12$ Hz), 8.03 (m, 7H); RMN ^{13}C (400 MHz; $CDCl_3$) δ: 23.65, 27.00, 32.20, 66.34, 76.67, 79.16, 122.88, 123.58, 125.79, 125.93, 126.25, 128.01, 128.46, 128.59, 128.79, 133.72; RT = 23.20 min (100%); Mass calculated $C_{16}H_{18}O_2$ 242.1; HRMS − mass calculated for $C_{16}H_{17}ClO_2[(M+Na)]$ 265.1029; Found 265.1029.

*4.1.7.3. (6-Ethyl-tetrahydro-2H-pyran-2-yl) methanol (**22**) [5].* Reaction of 1.0 mmol (0.144 g) of **21** produce 100% yield of **22** as a pale oil IR (KBr, cm^{-1}): 1178, 1227 (C−O), 1375 (C−OH), 2877, 2931 and 2962 (C−H sp^3), 3383 (O−H) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.94 (t, 3H, $J = 8$ Hz), 1.15−1.25 (m, 1H), 1.48−1.64 (m, 4H), 1.95 (s, 1H), 2.04 (m, 1H), 2.15 (m, 1H), 3.28 (m, 1H), 3.44−3.64 (m, 3H), 4.04 (m, 1H); ^{13}C NMR ($CDCl_3$ 50 MHz): δ 9.75, 28.66, 38.21, 41.92, 55.50, 65.60, 76.88, 78.04; GC: RT = 8.81 min (100%); Mass calculated: 144.1, $C_8H_{16}O_2$.

4.1.8. General procedure for the synthesis oxidation of alcohols [39]

The appropriate alcohol (1.0 mmol) was added in a stirring flask following by slow addition of HNO_3 at 80% of concentration (5.0 mmol, 0,315 g). The reaction occurred with evolution of heat and nitrogen oxides that caused the brown color of the reaction mixture. After the end of heat evolution the reaction mixture was maintained for 3−4 h at 30 °C until discoloration, then it was poured into 50 ml of cold water, extracted with chloroform (3 × 50 mL), the extract was dried with Na_2SO_4. The chloroform was distilled off and the carboxylic acid obtained on 100% yield.

*4.1.8.1. 6-Ethyl-tetrahydro-2H-pyran-2-carboxylic acid (**1a**).* Reaction of 1.0 mmol (0.144 g) of **21** produces 100% yield of **1a** as a yellow solid. IR (KBr, cm^{-1}): 1280(C−O), 1730 (C═O), 2877, 2931 and 2966 (C−H sp^3), 3140 (O−H) cm^{-1}; ^1H NMR ($CDCl_3$, 40): δ 0.97 (t, 3H, $J = 8$ Hz), 1.68 (m, 5H), 2.19 (m, 1H), 2.56 (m, 1H), 3.36 (m, 1H), 4.05 (t, 2H, $J = 12$ Hz), 8.81 (s, 1H); 0 MHz ^{13}C NMR ($CDCl_3$ 101 MHz): δ 9.67, 28.32, 38.56, 41.07, 54.21, 74.67, 78.62, 173.53; GC: RT = 10.20 min (100%); Mass calculated: 158.10, $C_8H_{14}O_3$.

*4.1.8.2. 6-(naphthalen-2-yl)-tetrahydro-2H-pyran-2-carboxylic acid (**3**).* reaction of 1.0 mmol (0.242 g) of **5** producing 100% yield of **3** after purifications by silica gel flash chromatography (EtOAc/hexane ,1:10 as eluent).as a dark colored solid. IR (KBr, cm^{-1}): 763, 825 (C−H aromatic), 1280, 1338 (C−O), 1523 (C═C aromatic), 1720 (C═O), 2927 (C−H sp^3), 3429 (O−H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.62 (m, 4H), 3.49(m, 2H), 4.17 (m, 1H), 4.73 (m, 1H), 8.08 (m, 7H), 10.20 (s, 1H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: 19.15, 24.29, 33.21, 76.25, 76.42, 125.30, 126.42, 126.76, 127.78, 128.29, 128.47, 128.67, 129.52, 132.36, 135.91, 172.37; HRMS - Mass calculated for $C_{16}H_{16}O_3$: 256.1099; Found 256.7039.

*4.1.8.3. 6-(Naphthalen-1-yl)-tetrahydro-2H-pyran-2-carboxylic acid (**4**).* Reaction of 1.0 mmol (0.242 g) of **6** producing 100% yield of **4** after purifications by silica gel flash chromatography (EtOAc/hexane, 1:10 as eluent).as a dark colored solid. IR (KBr, cm^{-1}): 763,

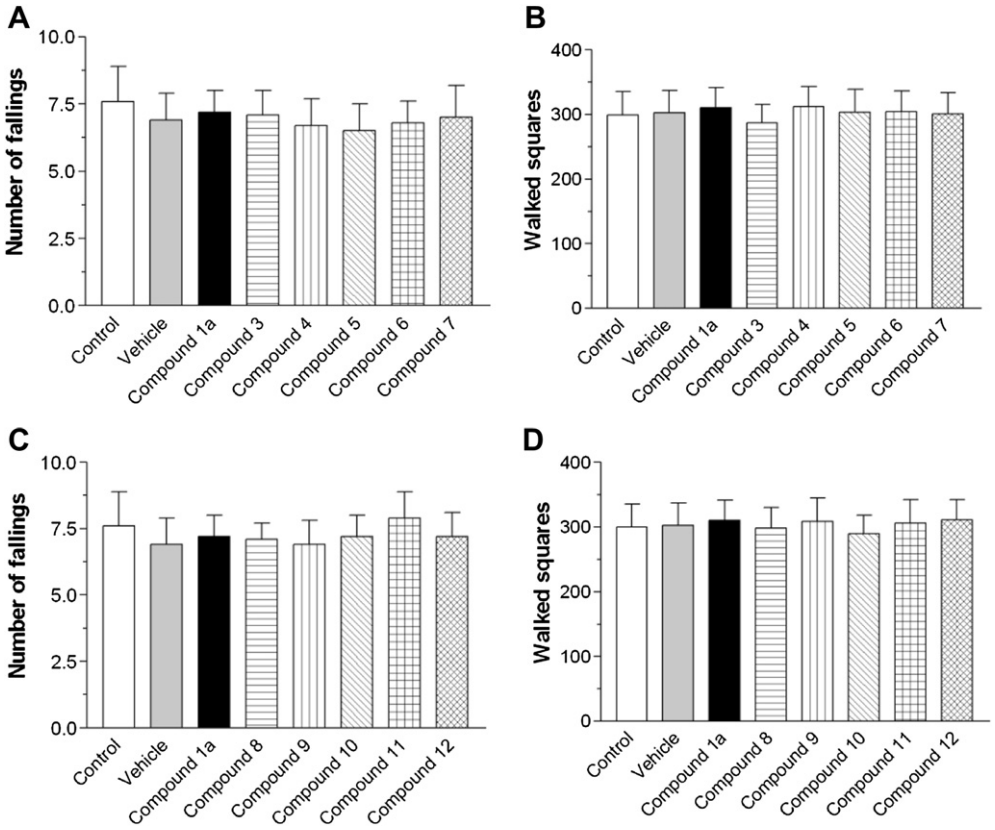


Fig. 9. Effects of tetrahydropyran derivatives in the Rota-rod Performance and open-field tests. In (A) and (C), graphs represent number of fallings of mice. In (B) and (D), graphs represent the number of walked squares by mice. The dose of compounds was 30 mg/kg (p.o.); $n = 6$ per group. Statistical significance was calculated by the analysis of variance followed by Bonferroni's test. $^{*}P < 0.05$ relative to the control group.

825 (C—H aromatic), 1280, 1338 (C—O), 1523 (C═C aromatic), 1720 (C═O), 2927 (C—H sp^3), 3429 (O—H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.97 (m, 2H), 2.45(m, 2H), 3.05 (m, 2H), 3.98 (m, 1H), 4.89 (m, 1H), 7.83 (m, 7H), 10.39 (s, 1H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: RMN 13C (101 MHz; CDCl3) δ: 29,70; 38,44; 42,48; 75,23; 75,87; 122,62; 123,59; 125,55; 125,68; 126,40; 128,75; 129,02; 130,00; 133,66; 135,28; 173,66; HRMS – mass calculated for $C_{16}H_{16}O_3$: 256.1099; Found 255.0735.

*4.1.8.4. 4-Chloro-6-(naphthalen-2-yl)-tetrahydro-2H-pyran-2-carboxylic acid (**11**).* Reaction of 1.0 mmol (0.276 g) of **9** producing 100% yield of **11** after purifications by silica gel flash chromatography (EtOAc/hexane, 1:10 as eluent).as a dark colored solid. IR (KBr, cm^{-1}): 794, 837 (C—H aromatic), 1257, 1338 (C—O), 1523 (C═C aromatic), 1724 (C═O), 2924 (C—H sp^3), 3429 (O—H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 2.00(m, 3H), 2.67(m, 1H), 4.12 (m, 1H), 4.36 (m, 1H), 5.20 (m, 1H), 8.07 (m, 7H), 10.12 (s, 1H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: 38.19, 43.33, 54.14, 74.91, 75.14, 125.31, 126.79, 127.80, 128.33, 129.54, 132.20, 132.37, 133.15, 135.95, 137.08, 172.34; HRMS – mass calculated for $C_{16}H_{15}ClO_3$: 290.7415; Found 293.1571.

*4.1.8.5. 4-Chloro-6-(naphthalen-1-yl)-tetrahydro-2H-pyran-2-carboxylic acid (**12**).* Reaction of 1.0 mmol (0.276 g) of **10** producing 100% yield of **12** after purifications by silica gel flash chromatography (EtOAc/hexane, 1:10 as eluent) as a dark colored solid. IR (KBr, cm^{-1}): 794, 837 (C—H aromatic), 1257, 1338 (C—O), 1523 (C═C aromatic), 1724 (C═C═O), 2924 (C—H sp^3), 3429 (O—H) cm^{-1}; RMN ^1H (400 MHz; $CDCl_3$) δ: 1.94 (m, 2H), 2.53(m, 2H), 4.13 (m, 2H), 4.65 (m, 1H), 8.12 (m, 7H), 10.14 (s, 1H); RMN ^{13}C (101 MHz; $CDCl_3$) δ: 21.72, 29.70, 38.44, 75.23, 75.87, 122.62, 123.59, 125.55, 125.68, 126.40, 128.75, 129.02, 130.00, 133.66, 135.28, 173.66; HRMS – mass calculated for $C_{16}H_{15}ClO_3$: 290.7415; Found 289.0301.

4.2. Computational details

Initially, Relaxed Potential Energy Surface Scan (RPESS) was performed using GAUSSIAN 09W® package [44], at semi-empirical AM1 level, considering important rotational degrees of freedoms for MBHA's (sigma bonds). For this purpose, dihedral angles were frozen in steps of 10° and the remainder portion of molecule was optimized. From potential energy curves for each molecule, the lowest energy conformation was selected and submitted to a full optimization at M06-2X/6-311++g(d,p) as level of calculations. All real frequencies have confirmed the presence of the local minimum. No imaginary frequency was observed. The structures were visualized by GaussView 5® program.

4.3. Pharmacology

4.3.1. Animals

The experiments were carried out on male Albino-Swiss mice (body weight 20–24 g). The animals were housed in wire mesh cages in a room at 20 ± 2 °C and exposed to a 12 h light:12 h dark cycle. The animals had free access to standard pellet diet, tap water was given *ad libitum*. The protocol for this study was approved by the ethics committee for Animal Research of the Federal Rural University of Rio de Janeiro (COMEP – UFRRJ) under number 002/2009.

Control and experimental groups consisted of 6 animals each. The investigated compounds were administered orally (p.o.) as the

suspension in 5% ethyl acetate (vehicle) in constant volume of 5 mL/kg.

4.3.2. Statistical analysis

All experimental groups were composed of 6 animals. The results are presented as the mean ± SD in the acetic acid-induced writhing, rota-rod and open field tests, and percentage increase over the baseline or area under the curve (AUC) in the tail-flick test. Statistical significance between groups was performed by the application of one way analyses of variance (ANOVA) followed by Bonferroni's test. $P < 0.05$ was considered as statistically significant. The estimated LD50 was obtained by fitting the data points representing the percentage of deaths with increasing doses of the compounds up to 2000 mg/kg calculated by nonlinear regression method using the Graph Pad Prism software version 3.0 (San Diego, CA, USA).

4.3.3. Antinociceptive evaluations

4.3.3.1. Acetic acid-induced abdominal writhing. Mice were used as described previously [45]. In brief, the total number of writhes after the i.p. administration of 1.2% (v/v) acetic acid was recorded over a period of 30 min, starting immediately after acetic acid injection. The pattern of abdominal writhes is the appearance of strong abdominal contractions, stretching the body of the animal, followed by elongation of hind limbs and abdomen contact with the floor of the counting chamber.

4.3.3.2. Tail-flick test. The test was performed as previously described [46]. The mice were kept in an acrylic tube and then placed on equipment to perform tail-flick test. A light beam is focused to approximately 4 cm from the tip of the tail and the tail withdrawal latency is automatically registered. The light intensity was adjusted for baseline values between 4 and 6 s;this intensity has not changed and the animals that had baseline values outside these limits were excluded from the experiment. Measures of latency time were made at intervals of 20 min between each one. The first two measures were made before drug administration. The average of these measures is called "baseline". After drug administration six measures of the latency time was performed. Antinociception was quantified as either the (IBL) percentage increase over the baseline at each measurement time, or the area under the curve (AUC) of responses from 20 to 120 min after drug administration, calculated according to the following formula based on the trapezoid rule: [AUC = 20 × IBL [(20 min) + (40 min) - + … + (120 min)/2].

4.3.4. Locomotor activity

4.3.4.1. Rota-rod performance test. The rota-rod performance test is an established method for evaluating motor impairment and ataxia [47]. The day before the test the animals were trained twice to maintain the equilibrium for 5 min on a roller apparatus ('Rotarod for mice', U. Basile, Italy). The speed selector was set to 10 rev/min. Twenty-four hours later, mice were treated orally with tetrahydropyran derivatives (30 mg/kg, p.o.) and vehicle; and 60 min after administration were placed on the roller for 5 min. Neurological deficit was evaluated by the inability of the animal to remain on the roller for the test period and reported as number of falling of animals.

4.3.4.2. Open-field test. The procedure was similar to the method described by [48]. Mice received tetrahydropyran derivatives (30 mg/kg, p.o.) and vehicle by oral administration and were placed individually in an observation chamber (60 min after oral administration) whose floor was divided into 50 squares (5 cm × 5 cm). Total numbers of squares by which mouse walked during 5 min were counted. The spontaneous activity was quantified as either number of squares walked within 5 min after compound administration.

4.3.5. In vivo toxicological evaluation of tetrahydropyran derivatives

Acute toxicity test was performed according to the World Health Organization (WHO) guideline [49] and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals [50]. The investigated compounds were administered orally in increasing doses up to 2000 mg/kg. The animal behavior was observed from 5 h after a single administration of the compounds and subsequently monitored daily until the 14th day. Acute toxicity was expressed by the required dose in g/kg body weight to cause death in 50% of animals tested (LD 50).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.046.

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