

SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY EVALUATION OF NOVEL DERIVATIVES OF TROLOX WITH PHARMACOLOGICAL ALCOHOL AND AMINES

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ABSTRACT. In order to explore the novel anti-inflammatory agents, some novel Trolox derivatives were synthesized and characterized by IR, ¹H-NMR, ¹³C-NMR, mass and elemental analysis. The anti-inflammatory activities of the target compounds were evaluated via the croton oil-induced ear oedema test in Swiss mice. According to screened results, obtained compounds demonstrated considerable anti-inflammatory action.

KEY WORDS: Trolox derivatives, Anti-inflammatory agents, Oxidative stress, Croton oil-induced ear oedema test

INTRODUCTION

Inflammation is defined as a complex series of tissue changes that result in pain and fever [1]. It is a normal defensive response to a variety of noxious stimuli. However, uncontrolled inflammatory process results in extensive cell and tissue destruction, which is associated with various chronic inflammatory conditions. During the last three decades the development of non-steroidal anti-inflammatory drugs (NSAIDs) has shown to be one of the major advancements in chemotherapeutical research [2, 3]. These agents are among the most widely used drugs worldwide and represent a mainstay in the therapy of acute and chronic pain, fever and inflammation by blocking the formation of prostaglandins (PGs). PGs are well known as the mediators of inflammation, pain and swelling.

Trolox is a water-soluble analog of vitamin E. It is an antioxidant like vitamin E and it's used in biological or biochemical applications to reduce oxidative stress or damage. In view of the above, antioxidant compounds may be beneficial in the treatment of conditions involving oxidative stress. Multifunctional compounds, rationally designed to exhibit two or more pharmacological actions, are considered successful in the treatment of complex diseases, such as those affecting central nervous system, like Alzheimer diseases and Parkinsonism, immune system, such as arthritis and cancer, or cardiovascular system, like atherosclerosis [4-8]. Therefore, in this study, an antioxidant carboxylic acid has been esterified and amidated by cinnamyl alcohol, thiomorpholine and 3-pyridinepropanol (Scheme 1).

EXPERIMENTAL

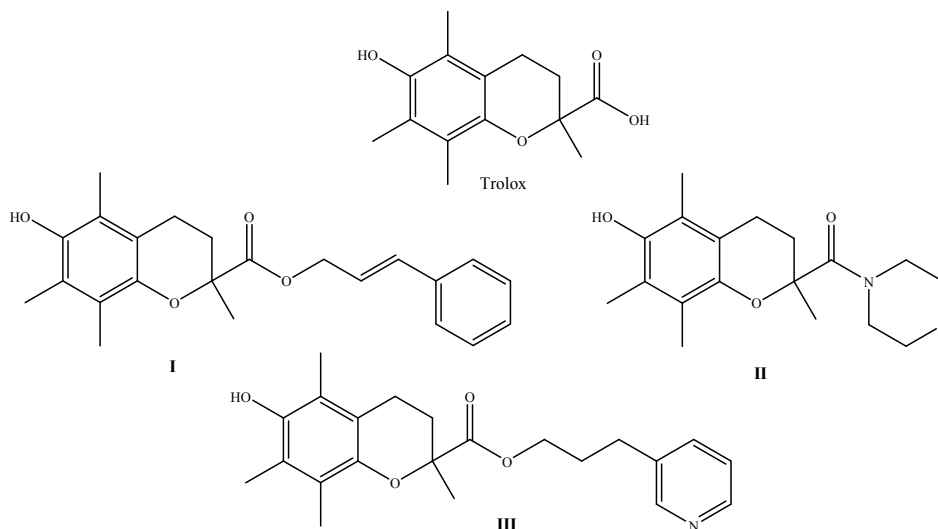
Material and equipments

All of chemicals and solvents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich chemical Co. (USA). Melting points (uncorrected) were determined with a digital Electrothermal melting point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). ¹H and ¹³C-NMR spectra were recorded with a Bruker 300 MHz (model AMX, Karlsruhe,

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Germany) spectrometer (Internal reference: TMS). IR spectra were recorded with a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, USA) spectrometer. Mass spectra were recorded with an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400.



Scheme 1. Structure formula of Trolox and their novel compounds (I-III).

Synthesis (Scheme 1 and 2)

Methods for the synthesis of the compounds I and III

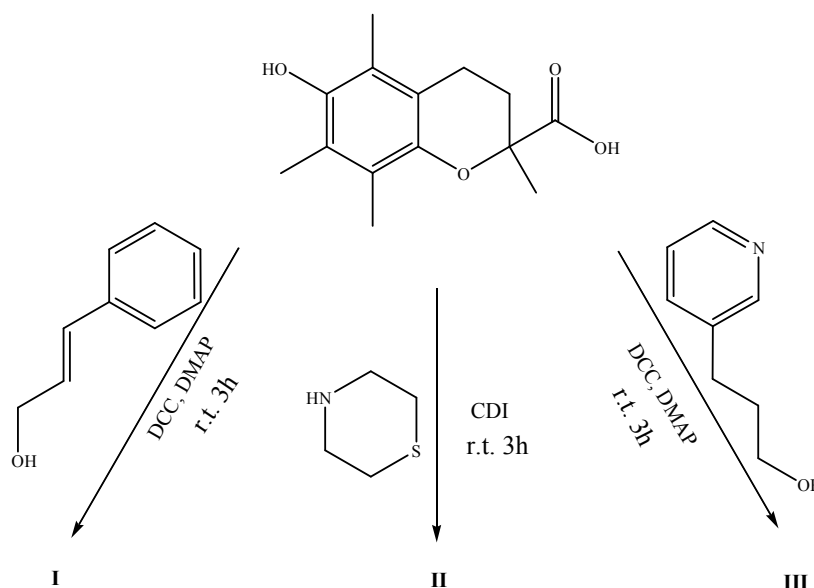
In a solution of Trolox (1 mmol) and cinnamyl alcohol/3-pyridinepropanol (1.1 mmol) in CH_2Cl_2 (10 mL), N,N' -dicyclohexylcarbodiimide (DCC, 1.3 mmol) and N,N -dimethylamino-pyridine (DMAP, 0.1 mmol) were added. The reaction mixture was stirred for about 3 h, and the reaction was checked by TLC to make sure that completion of reaction. The precipitated material was filtered off. The re-crystallization was carried out by adding petroleum ether (60-80 °C) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the crystals were collected to produce compound I and III.

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid 3-phenyl-allyl ester (I). White needle crystals; yield 65%, m.p. 104-106 °C; IR (KBr) (cm^{-1}): 3538 (O-H), 1760 (C=O ester); $^1\text{H-NMR}$ (CDCl_3), δ : 1.71 (s, 3H, 2- CH_3), 1.87 (m, 1H, C-3), 2.25, 2.27, 2.36 (3s, 9H, 5- CH_3 , 7- CH_3 , 8- CH_3), 2.65-2.72 (m, 1H, C-3 chroman equatorial), 2.45-2.65 (m, 2H, C-4 chroman), 4.75 (d, 2H, -O- CH_2 -CH=CH-), 6.25 (dt, 1H, -O- CH_2 -CH=CH-), 6.62 (d, 1H, -O- CH_2 -CH=CH-), 7.14-7.30 (m, 5H, ring cinnamyl); $^{13}\text{C-NMR}$ (CDCl_3) (ppm): 4.8, 16.3, 22.7, 41.3, 70.3, 75.5, 123.8, 124.1, 126.3, 128.7, 129.1, 132.8, 133.4, 145.1, 149.8, 152.1; Anal. calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_4$ (366.45): C 75.38, H 7.15; Found: C 75.08, H 6.96%; MS: m/z (regulatory intensity): 366 (100), 367 (26), 368 (4).

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid 3-pyridin-3-yl-propyl ester (III). White-light yellow crystals; yield 72%, m.p. 101-103 °C; IR (KBr) (cm^{-1}): 3337 (O-H), 1736 (C=O ester), 1577, 1601 (C-C aromatic); $^1\text{H-NMR}$ (CDCl_3), δ : 1.7 (s, 3H, 2- CH_3), 1.78-1.93 (m, 3H, C-3 chroman axial, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{Ar}$), 2.12, 2.17, 2.21 (3s, 9H, 5- CH_3 , 7- CH_3 , 8- CH_3 chroman), 2.51 (t, 2H, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 2.45-2.78 (m, 3H, C-3 chroman equatorial and C-4 chroman) 3.26-4.89 (m, 2H, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 7.41 (dd, 1H, C-5 pyridyl), 7.62 (d, 1H, C-4 pyridyl), 8.20 (s, 1H, C-2 pyridyl), 8.66 (d, 1H, C-6 pyridyl); $^{13}\text{C-NMR}$ (CDCl_3) (ppm): 4.9, 5.2, 5.8, 16.7, 22.8, 34.8, 35.9, 42.7, 81.7, 85.9, 124.8, 124.9, 126.7, 128.8, 129.1, 133.8, 134.4, 149.1, 155.1, 177.3; Anal. calcd. for $\text{C}_{22}\text{H}_{27}\text{NO}_4$ (369): C 71.52, H 7.37, N 3.79; Found: C 71.34, H 7.24, N 3.87%; MS: m/z (regulatory intensity): 369 (100), 370 (25), 371 (3.8).

Methods for the synthesis of the compounds II

Trolox (1 mmol) was dissolved in tetrahydrofuran (THF, 10 mL) and a solution of carbodiimidazole (CDI, 1.05 mmol), in the same solvent, was added. After 45 min, a solution of thiomorpholine (1.05 mmol) in THF (5 mL) was added and the mixture was left for 12 h with stirring at room temperature. Then, THF was evaporated, the resulting mixture was dissolved in CH_2Cl_2 (10 mL), successively washed with water, 5% aqueous HCl and NaHCO_3 solutions, dried and the re-crystallization was carried out by petroleum ether (60-80 °C). Then the mixture was filtered while it is cold and the crystals were collected to produce compound II.



Scheme 2. Schematic synthesis of novel compounds (I-III).

(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-thiomorpholin-4-yl-methanone (II). Faint white crystals; yield 78%; m.p. 177-179 °C; IR (KBr) (cm^{-1}): 3559 (O-H), 3350 (N-H), 1619 (C=O amide); $^1\text{H-NMR}$ (CDCl_3) δ : 1.69 (s, 3H, 2- CH_3), 1.75 (d, 1H, C-3 chroman axial), 2.22, 2.27, 2.32 (3s, 9H, 5- CH_3 , 7- CH_3 , 8- CH_3 chroman), 2.67 (bs, 4H, $-\text{CH}_2-\text{S}-\text{CH}_2-$), 2.56-2.88 (m, 3H, C-3 chroman equatorial and C-4 chroman), 4.12 (bs, 4H, $-\text{CH}_2-\text{N}-\text{CH}_2-$); $^{13}\text{C-NMR}$ (CDCl_3)

(ppm): 4.8, 16.4, 22.7, 34.2, 35.3, 42.3, 80.3, 85.5, 123.8, 126.3, 128.7, 129.1, 132.8, 133.4, 149.8, 152.1, 179.5; Anal. calcd. for C₁₈H₂₅NO₃S (335): C 64.45, H 7.51, N 4.18; Found: C 63.94, H 7.44, N 4.28%; MS: m/z (regulatory intensity): 335 (100), 336 (20.5), 337 (4.4).

Pharmacological methods

The topical anti-inflammatory activity was evaluated as inhibition of the croton oil-induced ear oedema in Swiss mice. Male Swiss mice (18-22 g), at the beginning of the experiment, were randomly housed in a temperature-controlled colony room under 12 h light/dark cycle. Rats were given free access to water and standard laboratory rat chow. All the experiments were conducted between 7 a.m. and 7 p.m., under a normal room light and at 25 °C. Groups (each group containing 10 mice) were used in all tests. The tested compounds and the reference drug were suspended in 0.5% sodium carboxymethyl cellulose (CMC), respectively. Inflammation was induced always in the late morning (10 a.m.-12 p.m.). Mice were anaesthetized with ketamine hydrochloride (145 mg/kg, intra-peritoneally) and inflammatory response was induced on the inner surface of the right ear (surface: about 1 cm²) by application of 20 µL of a 2% croton oil suspended in 42% aqueous ethanol. Control animals received only the irritant, whereas other animals received the irritant together with the tested substances. At the maximum of the oedematous response, 6 h later, mice were sacrificed and a plug (φ = 8 mm) was removed from both the treated (right) and the untreated (left) ears. Oedema was measured as the mass difference between the two plugs. The anti-inflammatory activity was expressed as the percentage of the oedema reduction in treated mice compared to that in the control mice. As reference, the non-steroidal anti-inflammatory drug ibuprofen was used. The results were expressed as mean±SD and statistical analysis was performed by means of student's t-test or by one-way analysis of variance followed by the Dunnett's test for multiple comparisons of unpaired data. Statistically, a p value of less than 0.05 was considered to be significant and a p value of less than 0.01 was considered to be very significant [9, 10].

RESULTS AND DISCUSSION

Chemistry

The designed compounds have been synthesized successfully as shown in Schemes 1 and 2. All target compounds were fully characterized by Mass Spectra, elemental analysis, infrared spectra, and ¹H, ¹³C-NMR spectra. For the synthesis of the novel compounds **I-III**, one known antioxidant acid was used: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). It was esterified with cinnamyl alcohol and 3-pyridinepropanol, and amidated with thiomorpholine, by classical methods with good yields. Preliminary pharmacological evaluation has been done for the designed compounds and it has been found that these compound exhibit anti-inflammatory effects compared to aspirin.

Biological evaluation

The anti-inflammatory effects of target compounds have been investigated by croton oil-induced ear oedema test, and the results show some target compounds induced oedema reductions and exhibit anti-inflammatory properties compared to aspirin (Table 1).

Table 1. Anti-inflammatory activities of target compounds on ear oedema induced by croton oil after topical administration at a dose of 200 mg/kg in mice.

Compound	Swelling (mg, X±SD)	Inhibition (%)	p
Control *	13.5±8.37		
Aspirin	7.90±3.15	46.9	<0.01
I	8.91±5.28	30.2	<0.05
II	9.16±5.88	27.9	<0.05
III	11.2±4.37	12.6	

*0.5% sodium carboxymethyl cellulose aqueous solution. In particular, compounds **I** and **II** showed the significant effects ($p < 0.05$) with 30.2% and 27.9% oedema inhibition, respectively, at the administered dose.

CONCLUSION

In conclusion, some novel Trolox derivatives were synthesized, characterized and evaluated for anti-inflammatory activities by the croton oil ear oedema test in mice as a model of acute inflammation. Two of them (**I** and **II**) showed significantly anti-inflammatory activities.

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