BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e18887

Article

Synthesis and Biological Activity of Trolox Amide Derivatives

Qian Xu[1,2,3], Luyun Zhang[2,3,4], Guangqing Xia[2,3,4], Dazhao Zhan[2,3], Junyi Zhu[2,3*], Hao Zang[1,2,3*]

[1]Yanbian University, College of Medicine, China, [2]Tonghua Normal University, School of Pharmacy and Medicine, China, [3]Tonghua Normal University, Green Medicinal Chemistry Laboratory, China, [4]Changchun University of Chinese Medicine, Jilin Ginseng Academy, China

A series of Trolox amide derivatives were synthesized by modifying the carboxyl groups of Trolox. Thirty target compounds were obtained and characterized through nuclear magnetic resonance and mass spectrometry. Trolox derivatives were employed to explore the potential structure-antioxidant activity relationships. The antioxidant activities of these compounds were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) and hydroxyl radical assays. DPPH scavenging activity test results illustrated that compounds exhibited scavenging activities similar to L-ascorbic acid and Trolox, with compounds **14a**, **18a**, **24a** and **26a** in particular exhibiting higher scavenging activities than L-ascorbic acid. The results demonstrated that compounds displayed ABTS scavenging activities similar to L-ascorbic acid and Trolox, with compounds **26a** and **29a** in particular having potency twofold higher. FRAP assay results indicated that compounds **11a**, **19a**, **25a**, **29a** and **30a** had activity similar to Trolox. The results revealed that compounds **6a** and **19a** had similarly high hydroxyl radical-scavenging activities as Trolox. The results of α-glucosidase experiments uncovered that compounds **10a**, **25a**, **28a** and **29a** had excellent inhibitory activity, which was similar to that of acarbose and different from Trolox. The results of acetylcholinesterase and butyrylcholinesterase experiments demonstrated that some compounds had weak anticholinesterase activities. **26a** and **29a** are important Trolox derivatives with better biological activity profiles and deserve further study.

Keywords: Trolox derivative. Antioxidant activity. Hypoglycemic activity. Anticholinesterase activity.

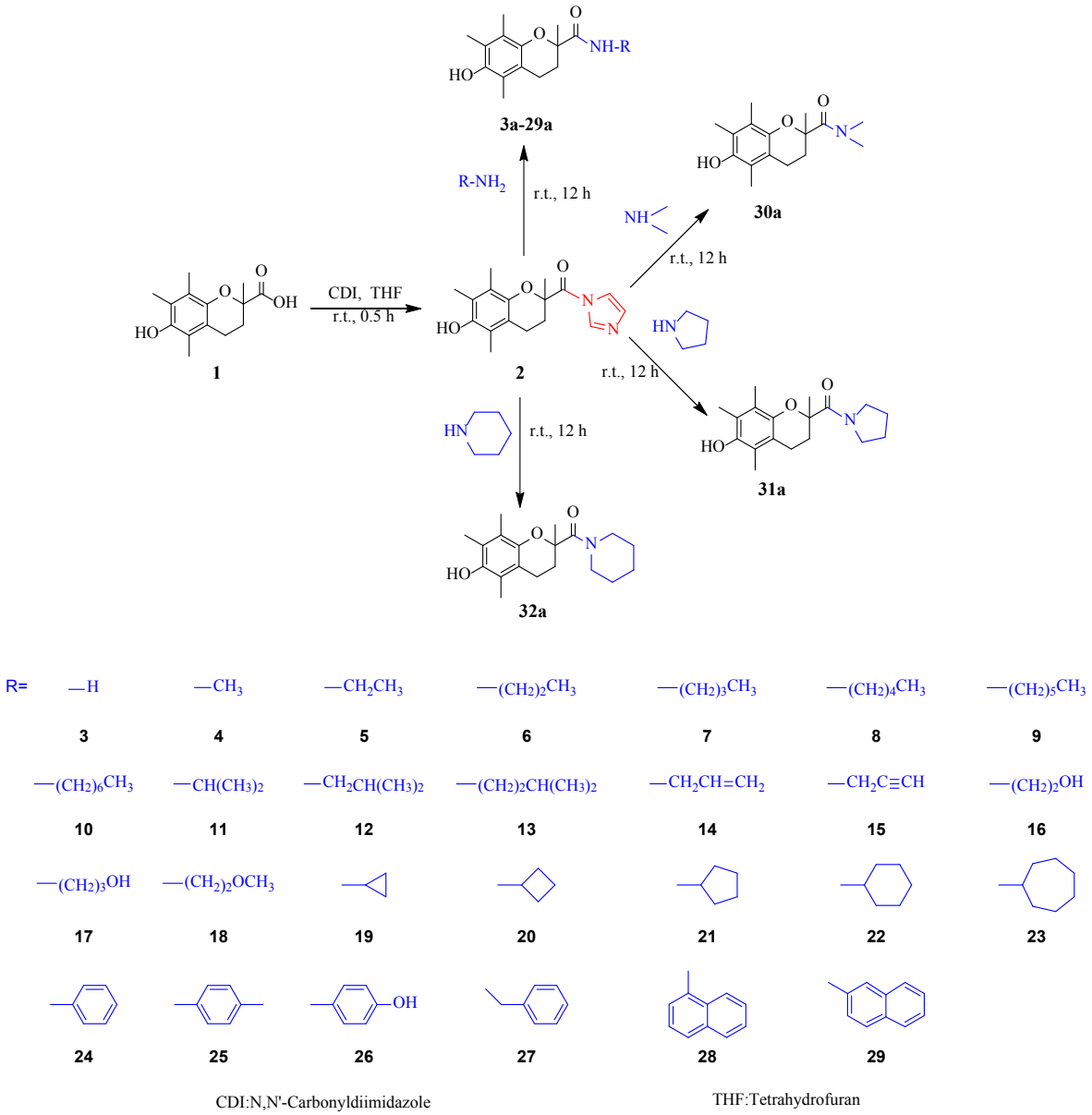
INTRODUCTION

α-Tocopherol (vitamin E) is one of the most effective antioxidants in living organisms. It is a chain-breaking radical scavenger and the first line of defense against lipid peroxidation in biofilms, (Brigelius-Flohe, Traber,1999) and it plays an important role in the treatment of oxidative stress-related diseases, as demonstrated in multiple clinical studies (Tucker, Townsend, 2005). Considering its powerful antioxidant effects, vitamin E is an ideal structure for developing excellent antioxidants. Trolox (compound **1**) is a representative vitamin E derivative in which the long alkyl chain has been removed. The antioxidant functional group is retained, and it has a certain water solubility because of the presence of carboxyl groups. Thus, it is used widely. In particular, **1** is often used as a positive control to measure the antioxidant capacity of other antioxidants (Zang *et al*., 2018). By reducing oxidative stress, **1** can increase wound healing (Vergauwen *et al*., 2015), reverse manganese-induced neurodevelopmental damage (Cordova *et al*., 2013), protect the hippocampal nerve after ischemia-reperfusion injury (Sarveazad *et al*., 2016), improve arthritis symptoms (Ponist *et al*., 2015), and prevent cigarette smoke-induced

***Correspondence:** Hao Zang. Yanbian University. College of Medicine. Yanji 133000, China. Phone: +86-435-320-9527. E-mail: zanghao_1984@163.com. ORCID: https://orcid.org/0000-0001-8357-2888. Junyi Zhu. Tonghua Normal University. School of Pharmacy and Medicine. Tonghua 134002, China. Phone: +86-435-320-9118. E-mail: swx0527@163.com.**.** Q. Xu: E-mail: 2017050591@ybu.edu.cn. H. Zang: E-mail: zanghao_1984@163.com. L. Zhang: E-mail: m18843581583@163.com. G. Xia: E-mail: qingguangx@163.com. D. Zhan: E-mail: 15043100585@163.com

lung damage (Messier *et al*., 2013). **1** can reduce the hemolysis of frozen red blood cells (Czubak *et al*., 2015), improve the quality of frozen semen (Varo-Ghiuru *et al*., 2015), and protect cold ovarian tissue (Brito *et al*., 2014). **1** can inhibit glioblastoma growth (Monticone *et al*., 2014). It is toxic to cancer cells such as breast and ovarian cancer cells (Miclea *et al*., 2015; Zakharova *et al*., 2016), and it inhibits breast cancer metastasis (Lee *et al*., 2014). In addition, **1** can also improve pig embryo development (Lee *et al*., 2015) and enhance follicular survival rates after monkey ovarian transplantation (Scalercio *et al*., 2015), regulate immunity (Slovak *et al*., 2016), and treat type 2 diabetes (Jin *et al*., 2014).

However, research on Trolox amide derivatives is scant. In this paper, **1** was used as a raw material to design and synthesize a series of Trolox amide derivatives. Considering the biological activities of **1**, we assayed the antioxidant (DPPH and ABTS, FRAP, and hydroxyl radical assays), α-glucosidase inhibition, and cholinesterase inhibition activities (acetylcholinesterase [AChE] and butyrylcholinesterase [BChE] inhibition assays) of the derivatives.



SCHEME 1 - Synthesis of compounds **3a**–**32a**

RESULTS AND DISCUSSION

Synthesis

The most common method for creating an amide bond is the use of carbodiimide condensing agents such as dicyclohexylcarbodiimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. The use of a condensing agent generally requires the addition of an acylation catalyst or an activator such as 4-dimethylaminopyridine or 1-hydroxybenzotriazole. One disadvantage of the reaction is the formation of urea, which is difficult to remove. N-Hydroxysuccinimide can also form an intermediate smoothly, but the intermediate requires purification. Therefore, N,N′-carbonyldiimidazole was used in the current study to react with carboxylic acid to obtain a highly active acyl imidazole. An amine was added directly, and the mixture was reacted for 12 h to obtain the target compound. The reaction was rapid, and the next reaction was conducted without purification.

Biological Activities

DPPH Assay

The radical scavenging activities of **3a**–**32a** in comparison with L-Ascorbic acid and **1** as determined using DPPH assays are shown in Table I. The compounds exhibited antioxidant capacity with IC_{50} values of 5.2–8.7 μM, versus 9.8 μM for L-ascorbic acid. In addition, **14a**, **18a**, **24a** and **26a** (IC_{50} = 5.2–6.6 μM) had better activity than L-ascorbic acid. The experimental results for **3a**–**13a** illustrated that alkyl substituents did not change antioxidant capacity. Compared with the activity of **5a**, the data for **16a** and **18a** demonstrated that hydroxyl and methoxyl substituents did not change antioxidant capacity. The experimental results for **19a**–**23a** indicated that cycloalkyl substituents did not change antioxidant capacity, and there was no difference in antioxidant capacity according to ring size. The experimental results for **24a** and **26a** revealed that phenyl and hydroxyphenyl substituents are beneficial.

ABTS Assay

The ABTS$^{\bullet+}$ assay is widely used to measure the radical-scavenging activity of antioxidants. The radical scavenging activities of **3a**–**32a** in comparison with L-ascorbic acid and **1** as determined using ABTS$^{\bullet+}$ assays are shown in Table I. The compounds exhibited antioxidant capacity with IC_{50} values of 11.4–27.2 μM, versus 28.6 μM for L-ascorbic acid. As shown in Table I, **26a** (IC_{50} = 11.4 μM) and **29a** (IC_{50} = 12.7 μM) were potent ABTS$^{\bullet+}$ scavengers. The experimental results for **3a**–**13a** illustrated that alkyl substituents did not increase antioxidant capacity. Compared with the results for **28a**, **29a** with a 2-substituted naphthalene ring had the higher antioxidant activity.

FRAP Assay

The results for the reducing power of **3a**–**32a** as evaluated using the FRAP assay (expressed as in millimoles of Fe (II) per gram) in comparison with L-ascorbic acid and **1** are summarized in Table I. The experimental results for **3a**–**13a** illustrated that alkyl substituents did not increase antioxidant capacity. Compared with the results for **28a**, **29a** with a 2-substituted naphthalene ring had the highest reducing power.

Hydroxyl Radical Assay

The radical scavenging activities of **3a**–**32a** in comparison with **1** as determined using hydroxyl radical assays are shown in Table I. **6a** and **19a** exhibited similarly potent hydroxyl radical-scavenging activity (IC_{50} = 716.0–733.3 μM) as **1** (IC_{50} = 670.1 μM).

TABLE I - Antioxidant activity of compounds **3a–32a**

Compd.	DPPH IC_{50}(μM)	ABTS IC_{50}(μM)	FRAP/(mmol·g^{-1})	·OH IC_{50}(μM)
3a	7.3±0.1	23.5±0.4	30.8±0.7	752.3±4.9
4a	7.6±0.1	21.4±0.2	30.2±0.9	851.1±14.9
5a	8.2±0.1	19.3±0.4	32.6±0.5	830.4±7.4
6a	7.9±0.2	20.5±0.6	31.7±0.2	733.3±2.0
7a	7.1±0.1	21.2±0.5	30.2±0.4	>1000
8a	7.7±0.1	18.3±0.3	33.6±0.4	955.1±13.8
9a	8.0±0.1	19.1±0.2	29.7±0.9	>1000
10a	8.6±0.2	24.0±0.5	30.1±0.9	891.3±4.2
11a	7.7±0.0	18.0±0.2	34.7±0.8	824.7±9.9
12a	8.1±0.1	18.2±0.4	31.7±0.6	>1000
13a	8.7±0.1	17.6±0.2	32.4±0.5	989.3±14.4
14a	5.2±0.1	19.7±0.4	29.5±0.2	>1000
15a	7.8±0.0	21.9±0.6	30.6±0.8	>1000
16a	7.2±0.1	16.8±0.4	31.5±0.5	878.0±13.9
17a	7.6±0.1	17.1±0.1	32.6±0.3	976.6±11.8
18a	6.6±0.2	23.0±0.3	32.1±0.7	985.6±16.3
19a	7.5±0.0	17.5±0.4	35.6±1.0	716.0±10.1
20a	8.0±0.1	25.6±0.2	26.9±0.3	905.5±24.6
21a	7.5±0.1	18.9±0.4	30.6±0.8	833.5±12.9
22a	7.5±0.1	24.7±0.3	27.4±0.1	983.3±18.7
23a	7.6±0.0	24.0±0.3	30.5±0.5	>1000
24a	5.2±0.1	27.2±0.1	30.8±0.4	>1000
25a	7.1±0.1	17.3±0.3	34.7±0.7	>1000
26a	6.1±0.1	11.4±0.3	30.6±0.2	>1000
27a	7.5±0.1	23.2±0.5	28.7±0.1	>1000
28a	7.7±0.2	24.2±0.1	27.9±0.8	>1000
29a	8.4±0.1	12.7±0.3	40.3±1.0	>1000
30a	8.1±0.1	17.0±0.1	34.6±0.7	779.3±11.6
31a	7.7±0.1	23.7±0.1	31.3±0.4	875.3±13.8
32a	7.7±0.1	19.4±0.3	30.7±0.4	>1000
L-Ascorbic acid	9.8±0.0	28.6±0.4	30.9±0.8	N.T.
1	7.0±0.0	21.8±0.5	34.5±0.9	670.1±6.4

N.T. indicates not test.
α-Glucosidase Inhibition Assay

The results for the α-glucosidase inhibition activities of **3a**–**32a** as evaluated using the α-glucosidase inhibition assay in comparison with **1** and acarbose are summarized in Table II. The experimental results for **24a**–**29a** indicated that aryl substituents are beneficial. The α-glycosidase inhibition activity of **29a** was closest to that of acarbose and significantly superior to that of **1**. **10a**, **25a–26a** and **28a** also had excellent inhibition activity.

AChE Inhibition Assay

The AChE activities of **3a**–**32a** in comparison with **1** and donepezil as determined using AChE Inhibition assays are shown in Table II. **3a**–**4a**, **9a**, **14a**, **26a**, **29a**–**32a** exhibited weaker AChE inhibition activities than donepezil.

BChE Inhibition Assay

The BChE activities of **3a**–**32a** in comparison with **1** and donepezil as determined using BChE inhibition assays are shown in Table II. **3a**–**4a**, **6a**, **8a**, **26a**, **29a**–**30a**, **32a** exhibited weaker BChE inhibition activities than donepezil.

TABLE II - Hypoglycemic activity, anticholinesterase activity of compounds **3a-32a**

Compd.	α-Glucosidase IC_{50}(μM)	AchE IC_{50}(μM)	BchE IC_{50}(μM)
3a	>800	876.5±13.9	723.2±8.6
4a	>800	690.2±7.6	796.5±18.9
5a	>800	>1000	>1000
6a	>800	>1000	980.2±19.3
7a	283.5±4.4	>1000	>1000
8a	645.2±7.2	>1000	845.2±17.1
9a	286.3±6.1	912.1±8.9	>1000
10a	199.3±0.8	>1000	>1000
11a	>800	>1000	>1000
12a	>800	>1000	>1000
13a	685.6±6.7	>1000	>1000
14a	>800	852.5±18.8	>1000
15a	>800	>1000	>1000
16a	>800	>1000	>1000
17a	>800	>1000	>1000
18a	>800	>1000	>1000
19a	>800	>1000	>1000
20a	>800	>1000	>1000
21a	704.8±7.5	>1000	>1000
22a	592.4±8.8	>1000	>1000
23a	338.5±6.5	>1000	>1000

TABLE II - Hypoglycemic activity, anticholinesterase activity of compounds **3a-32a**

Compd.	α-Glucosidase IC_{50}(μM)	AchE IC_{50}(μM)	BchE IC_{50}(μM)
24a	292.2±6.0	>1000	>1000
25a	190.4±3.8	>1000	>1000
26a	250.7±5.3	557.0±13.1	914.6±18.3
27a	526.7±9.6	>1000	>1000
28a	122.0±3.9	>1000	>1000
29a	79.7±0.6	794.5±11.7	579.4±11.5
30a	>800	498.0±10.2	534.9±15.7
31a	>800	535.0±12.8	>1000
32a	661.6±2.7	535.0±16.6	965.4±19.5
1	>800	>1000	>1000
Acarbose	60.9±1.0	N.T.	N.T.
Donepezil	N.T.	0.1±0.0	3.6±0.1

N.T. indicates not test.

EXPERIMENTAL

General Information

Yeast α-glucosidase (EC 3.2.1.20), porcine pancreatic α-amylase (EC 3.2.1.1), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8), S-Butyrylthiocholine chloride were purchased from the supplier (Sigma-Aldrich). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, 98%), N',N-carbonyldiimidazole (CDI, 98%), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ, 98%), acetylthiocholine iodide (ATCI, 98%), ferrous sulfate heptahydrate ($FeSO_4$•$7H_2O$, 99%), salicylic acid (99%), L-ascorbic acid (98%), anhydrous tetrahydrofuran (THF, 99.5%) were purchased from the supplier Energy Chemical. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 98%) was purchased from the supplier (TCI). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, 95%) 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB, 99%) were purchased from the supplier (Alfa Aesar). Acarbose 98% from Ark Pharm. p-Nitrophenyl-α-D-glucopyranoside (pNPG, 99%) from acros. Donepezil hydrochloride 98% from Adamas. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

Column chromatography was carried out on silica gel (200-300 mesh). Thin layer chromatography (TLC) was performed using silica gel 60 F_{254} plates. ^{1}H NMR and ^{13}C NMR spectra were measured on an AV-600 Spectrometer (Bruker, Germany) using tetramethylsilane as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) were performed on an Aglient 6520 Q-TOF (Agilent, USA) in positive ionization mode. Melting points were determined in open capillary tubes and the temperature was uncorrected.

Synthesis

A general experimental procedure for the synthesis of Trolox derivatives (**3a-32a**)

The target compounds were synthesised as outlined in our previously published work (Zang *et al*., 2014), **1** (0.4 mmol, 1.0 equiv), CDI (0.44 mmol, 1.1 equiv)

were placed in a dry standard Schlenk tube. Dry THF (1.0 mL) was added, the reaction mixture was stirred at room temperature for 0.5 h, followed by the addition of amine (0.4 mmol, 1.0 equiv). The reaction mixture was stirred at room temperature for 12 h, and the reaction was monitored with thin-layer chromatography. The crude reaction mixture was purified by flash silica gel column chromatography (petroleum ether: ethyl acetate 4:1) to obtain the corresponding product.

*6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**3a**) (Manzano et al., 2014).*

Light yellow solid, yield 54%; mp 210.3-212.1 °C (159 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.17(s, 1H), 6.72(s, 1H), 2.53(s, 1H), 2.44(d, J=8.5 Hz, 1H), 2.19(d, J=6.9 Hz, 1H), 2.08(s, 3H), 2.06(s, 3H), 1.99(s, 3H), 1.72-1.63(m, 1H), 1.38(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 175.7, 145.8, 144.0, 122.7, 121.1, 120.3, 117.0, 77.1, 29.2, 24.3, 20.2, 12.7, 12.0, 11.8. ESI-MS m/z 250.0759 $[M+H]^+$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-N,2,5,7,8-pentamethylchromane-2-carboxamide (**4a**)*

Light yellow solid, yield 82%; mp 174.0-174.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.47(s, 1H), 7.30(d, J=4.6 Hz, 1H), 2.59(d, J=4.7 Hz, 3H), 2.53(s, 1H), 2.40(d, J=7.7 Hz, 1H), 2.14(s, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.74-1.66(m, 1H), 1.35(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.8, 145.8, 143.9, 122.7, 121.3, 120.2, 117.0, 77.2, 29.4, 25.9, 24.1, 20.1, 12.8, 12.1, 11.8. ESI-MS m/z 264.0993 $[M+H]^+$.

*N-ethyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**5a**) (Jankowski et al., 2009).*

Yellow solid, yield 70%; mp 77.5-78.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.47(s, 1H), 7.31(s, 1H), 3.13-3.02(m, 2H), 2.52(s, 1H), 2.42(d, J=7.5 Hz, 1H), 2.16-2.11(m, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.72(s, 1H), 1.35(s, 3H), 0.94(t, J=7.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.1, 145.8, 143.9, 122.7, 121.2, 120.2, 117.1, 77.1, 33.4, 29.4, 23.9, 20.0, 14.7, 12.7, 12.1, 11.8. ESI-MS m/z 278.1117 $[M+H]^+$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-2,5,7,8-tetramethyl-N-propylchromane-2-carboxamide (**6a**) (Manzano et al., 2014).*

Light pink solid, yield 73%; mp 87.8-89.2 °C (94-95 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.21(t, J=5.9 Hz, 1H), 3.02(dd, J=11.8, 6.2 Hz, 2H), 2.53(s, 1H), 2.43(s,1H), 2.16(d, J=13.3 Hz, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.71(s, 1H), 1.36(s, 3H), 1.33(dd, J=7.2, 1.8 Hz, 2H), 0.70(t, J=7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.1, 145.8, 143.9, 122.7, 121.1, 120.2, 117.1, 77.2, 40.1, 29.5, 24.1, 22.3, 20.1, 12.7, 12.0, 11.8, 10.9. ESI-MS m/z 292.1248 $[M+H]^+$. Spectroscopic data were accordant with those previously reported.

*N-butyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**7a**) (Manzano et al., 2014).*

Light yellow solid, yield 72%; mp 94.3-95.9 °C (100-101 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.16(t, J=5.9 Hz, 1H), 3.08(s, 1H), 3.02(s, 1H), 2.53(s, 1H), 2.42(d, J=8.2 Hz, 1H), 2.20-2.15(m, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.71(d, J=5.6 Hz, 1H), 1.37(s, 3H), 1.30(d, J=2.9 Hz, 2H), 1.09(d, J=7.4 Hz, 2H), 0.78(t, J=7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.1, 145.8, 143.9, 122.6, 121.1, 120.2, 117.1, 77.2, 38.0, 31.1, 29.5, 24.2, 20.1, 19.1, 13.6, 12.7, 12.0, 11.7. ESI-MS m/z 306.1355 $[M+H]^+$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-2,5,7,8-tetramethyl-N-pentylchromane-2-carboxamide (**8a**)*

Light yellow solid, yield 69%; mp 88.0-88.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.14(t, J=5.9 Hz, 1H), 3.09(s, 1H), 2.99(s, 1H), 2.53(s, 1H), 2.42(d, J=8.5 Hz, 1H), 2.18(s, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.70(s, 1H), 1.37(s, 3H), 1.30(s, 2H), 1.16(d, J=7.4 Hz, 2H), 1.02(d, J=7.7 Hz, 2H), 0.78(t, J=7.3 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.0, 145.9, 143.9, 122.6, 121.1, 120.2, 117.1, 77.3, 38.2, 29.5, 28.6, 28.1, 24.3, 21.8, 20.1, 13.8, 12.7, 12.0, 11.7. ESI-MS m/z 320.1467 $[M+H]^+$.

*N-hexyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**9a**) (Jankowski et al., 2009)*

Light yellow solid, yield 71%; mp 83.5-85.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.47(s, 1H), 7.15(t, J=5.8 Hz, 1H), 3.14-3.04(m, 1H), 3.02-2.94(m, 1H), 2.52(s, 1H), 2.41(d, J=8.2 Hz, 1H), 2.21-2.15(m, 1H), 2.09 (s, 3H), 2.07(s, 3H), 1.98(s, 3H), 1.73-1.66(m, 1H), 1.37(s, 3H), 1.32-1.27(m, 2H), 1.19-1.09(m, 4H), 1.04(d, J=7.6 Hz, 2H), 0.81(t, J=7.1 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.0, 145.9, 143.8, 122.5, 121.0, 120.1, 117.1, 77.2, 38.2, 30.9, 29.5, 28.9, 25.6, 24.3, 22.0, 20.1, 13.8, 12.7, 12.0, 11.7. ESI-MS m/z 334.1579 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*N-heptyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**10a**)*

Light yellow solid, yield 66%; mp 51.6-53.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.47(s, 1H), 7.14(t, J=5.8 Hz, 1H), 3.09(s, 1H), 3.00(s, 1H), 2.52(d, J=6.0 Hz, 1H), 2.41(d, J=8.3 Hz, 1H), 2.23-2.16(m, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.72-1.67(m, 1H), 1.37(s, 3H), 1.34-1.18(m, 4H), 1.17-1.12(m, 4H), 1.03(s, 2H), 0.84(t, J=7.3 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.0, 145.9, 143.8, 122.5, 121.0, 120.1, 117.1, 77.2, 38.2, 31.2, 29.5, 28.9, 28.3, 25.9, 24.3, 22.0, 20.1, 13.9, 12.7, 12.0, 11.7. ESI-MS m/z 348.1695 $[M+H]^{+}$.

*6-hydroxy-N-isopropyl-2,5,7,8-tetramethylchromane-2-carboxamide (**11a**) (Manzano et al., 2014).*

Light yellow solid, yield 57%; mp 117.7-118.5 °C (121-124 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.49(s, 1H), 6.85(d, J=8.1 Hz, 1H), 3.81(d, J=7.7 Hz, 1H), 2.54(s, 1H), 2.45(s, 1H), 2.15-2.10(m, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.75(s, 1H), 1.35(s, 3H), 1.06(d, J=6.5 Hz, 3H), 0.93(d, J=6.6 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 172.3, 145.8, 143.8, 122.7, 121.0, 120.3, 117.1, 77.0, 40.4, 29.4, 23.7, 22.0, 22.0, 20.0, 12.7, 11.9, 11.8. ESI-MS m/z 292.1240 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-N-isobutyl-2,5,7,8-tetramethylchromane-2-carboxamide (**12a**) (Jankowski et al., 2009)*

Light yellow solid, yield 72%; mp 87.7-89.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.49(s, 1H), 7.11(t, J=6.1 Hz, 1H), 2.96(d, J=6.5 Hz, 1H), 2.82(d, J=6.5 Hz, 1H), 2.52(d, J=11.1 Hz, 1H), 2.42(d,J=8.8 Hz, 1H), 2.22-2.17(m, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.98(s, 3H), 1.71(d, J=1.5 Hz, 1H), 1.63-1.57(m, 1H), 1.38(s, 3H), 0.66(dd, J=6.7, 3.9 Hz, 6H). ^{13}C NMR (150 MHz, DMSO) δ 173.1, 145.8, 143.9, 122.7, 121.0, 120.3, 117.1, 77.3, 45.6, 29.5, 28.0, 24.3, 20.2, 19.5, 12.7, 12.0, 11.7. ESI-MS m/z 306.1354 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-N-isopentyl-2,5,7,8-tetramethylchromane-2-carboxamide (**13a**) (Jankowski et al., 2009)*

Light yellow solid, yield 62%; mp 96.3-97.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.11(t, J=5.9 Hz, 1H), 3.11(s, 1H), 3.02(s, 1H), 2.52(s, 1H), 2.42(d, J=8.2 Hz, 1H), 2.18(d, J=13.2 Hz, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.98(s, 3H), 1.70(s, 1H), 1.37(s, 3H), 1.23(d, J=6.4 Hz, 1H), 1.20(t, J=7.0 Hz, 2H), 0.75(dd, J=6.4, 3.0 Hz, 6H). ^{13}C NMR (150 MHz, DMSO) δ 173.0, 145.9, 143.9, 122.6, 121.1, 120.2, 117.1, 77.3, 37.9, 36.6, 29.5, 24.8, 24.2, 22.3, 22.2, 20.1, 12.7, 12.0, 11.7. ESI-MS m/z 320.1491 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*N-allyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**14a**)*

Light yellow solid, yield 64%; mp 100.5-101.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.49(s, 1H), 7.43(t, J=6.0 Hz, 1H), 5.79-5.68(m, 1H), 4.93(dd, J=10.4, 1.6 Hz, 1H), 4.84(dd, J=17.2, 1.7 Hz, 1H), 3.72(d, J=1.5 Hz, 1H), 3.67(s, 1H), 2.54 (s, 1H), 2.44(s, 1H), 2.19(s, 1H), 2.11(s, 3H), 2.08(s, 3H), 2.00(s, 3H), 1.72(s, 1H), 1.39(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.2, 145.8, 143.9, 135.3, 122.7, 121.2, 120.2, 117.1, 114.1, 77.3, 40.6, 29.5, 24.2, 20.1, 12.7, 12.1, 11.8. ESI-MS m/z 290.1636 $[M+H]^{+}$.

*6-hydroxy-2,5,7,8-tetramethyl-N-(prop-2-yn-1-yl) chromane-2-carboxamide (**15a**)*

Light yellow solid, yield 60%; mp 176.4-177.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.78(t, J=5.8 Hz, 1H), 7.48(s, 1H), 3.83(t, J=6.1 Hz, 2H), 3.01(t, J=2.2 Hz, 1H), 2.51(d, J=7.1 Hz, 1H), 2.42(s, 1H), 2.13(s, 1H), 2.10(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.78-1.69(m, 1H), 1.35(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.3, 145.8, 143.8, 122.7, 121.3, 120.2, 117.0, 81.4, 77.2, 72.4, 29.5, 28.3, 23.7, 19.9, 12.8, 12.1, 11.8. ESI-MS m/z 288.0836 $[M+H]^{+}$.

*6-hydroxy-N-(2-hydroxyethyl)-2,5,7,8-tetramethylchromane-2-carboxamide (**16a**) (Jankowski et al., 2009)*

Light yellow solid, yield 51%; mp 129.4-130.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.50(s, 1H), 7.24(t, J=5.6 Hz, 1H), 4.69(t, J=5.3 Hz, 1H), 3.36(s, 1H), 3.34(s, 1H), 3.19(s, 1H), 3.09(s, 1H), 2.54(s, 1H), 2.43(s, 1H), 2.11(s, 1H), 2.09(s, 3H), 2.07(s, 3H), 2.00(s, 3H), 1.75(s, 1H), 1.36(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.4, 145.9, 143.7, 122.7, 121.1, 120.3, 117.1, 77.2, 59.6, 41.1, 29.4, 23.9, 20.0, 12.8, 12.0, 11.8. ESI-MS m/z 294.0924 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-N-(3-hydroxypropyl)-2,5,7,8-tetramethylchromane-2-carboxamide (**17a**) (Jankowski et al., 2009)*

Light pink solid, yield 50%; mp 101.3-102.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.41(t, J=5.7 Hz, 1H), 4.49(t, J=5.1 Hz, 1H), 3.36(s, 1H), 3.33(s, 1H), 3.19-3.06(m, 2H), 2.54(s, 1H), 2.44-2.38(m, 1H), 2.12(s, 1H), 2.08 (s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.73(s, 1H), 1.49(s, 2H), 1.35(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.2, 145.8, 143.8, 122.7, 121.2, 120.2, 117.0, 77.1, 58.9, 36.5, 31.9, 29.4, 24.0, 20.0, 12.8, 12.0, 11.8. ESI-MS m/z 308.1015 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-N-(2-methoxyethyl)-2,5,7,8-tetramethylchromane-2-carboxamide (**18a**) (Jankowski et al., 2009)*

Yellow solid, yield 42%; mp 106.4-107.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.50(s, 1H), 7.20(t, J=5.0 Hz, 1H), 3.30(s, 1H), 3.21(s, 3H), 3.14(s, 3H), 2.54(s, 1H), 2.43(s, 1H), 2.14(s, 1H), 2.08(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.74(s, 1H), 1.36(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.4, 145.9, 143.7, 122.7, 121.0, 120.3, 117.1, 77.3, 70.3, 57.9, 38.2, 29.4, 24.1, 20.0, 12.7, 11.9, 11.8. ESI-MS m/z 308.1022 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*N-cyclopropyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**19a**) (Jankowski et al., 2009)*

Light yellow solid, yield 75%; mp 137.5-140.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.22(d, J=4.1 Hz, 1H), 2.59(d, J=3.9 Hz, 1H), 2.52(s, 1H), 2.45(s, 1H), 2.16-2.11(m, 1H), 2.06(s, 6H), 1.99(s, 3H), 1.76-1.67(m, 1H), 1.34(s, 3H), 0.63-0.53(m, 2H), 0.43-0.33(m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 174.5, 145.8, 143.9, 122.6, 121.2, 120.2, 117.1, 77.0, 29.4, 23.7, 22.4, 20.0, 12.7, 12.0, 11.8, 6.0, 5.8. ESI-MS m/z 290.1088 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*N-cyclobutyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**20a**)*

Light pink solid, yield 71%; mp 90.7-92.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 7.37(d, J=8.0 Hz, 1H), 4.16(d, J=8.2 Hz, 1H), 2.53(s, 1H), 2.44(s, 1H), 2.17-2.09(m, 5H), 2.08(s, 3H), 2.05(dd, J=7.5, 3.2 Hz, 1H), 1.99(s, 3H), 1.96(s, 1H), 1.84(s, 1H), 1.73(s, 1H), 1.58(d, J=6.4 Hz, 2H), 1.34(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 172.4, 145.8, 143.9, 122.7, 121.2, 120.3, 117.1, 76.9, 43.9, 30.1, 29.9, 29.4, 23.6, 20.0, 14.5, 12.7, 12.0, 11.8. ESI-MS m/z 304.1195 $[M+H]^{+}$.

*N-cyclopentyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**21a**)*

Yellow solid, yield 52%; mp 96.5-97.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.50(s, 1H), 6.82(d, J=7.6 Hz, 1H),

3.95(d, J=6.9 Hz, 1H), 2.53(s, 1H), 2.43(d, J=8.0 Hz, 1H), 2.18-2.12(m, 1H), 2.08(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.79-1.70(m, 2H), 1.68-1.61(m, 1H), 1.55-1.38(m, 5H), 1.37(s, 3H), 1.21-1.15(m, 1H). ^{13}C NMR (150 MHz, DMSO) δ 172.7, 145.9, 143.8, 122.7, 120.9, 120.4, 117.2, 77.1, 50.2, 32.2, 32.0, 29.4, 23.9, 23.2, 23.0, 20.1, 12.7, 11.9, 11.8. ESI-MS m/z 318.1310 $[M+H]^+$.

*N-cyclohexyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**22a**)*

Light yellow solid, yield 64%; mp 40.3-41.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.50(s, 1H), 6.77(d, J=8.2 Hz, 1H), 3.57-3.44(m, 1H), 2.54(s, 1H), 2.42(d, J=8.1 Hz, 1H), 2.18-2.12(m, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.74(s, 1H), 1.69(s, 1H), 1.54(s, 1H), 1.45(s, 2H), 1.37(s, 3H), 1.27-1.14(m, 4H), 1.11-1.03(m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 172.2, 145.8, 143.8, 122.7, 120.8, 120.4, 117.2, 77.2, 46.9, 31.8, 29.4, 25.0, 24.0, 24.0,24.0, 23.9, 20.0, 12.7, 11.9, 11.8. ESI-MS m/z 332.1436 $[M+H]^+$.

*N-cycloheptyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**23a**)*

Light yellow solid, yield 50%; mp 79.5-80.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.51(s, 1H), 6.77(d, J=8.2 Hz, 1H), 3.75-3.67(m, 1H), 2.55-2.50(m, 1H), 2.42(d, J=8.2 Hz, 1H), 2.15(s, 1H), 2.09(s, 3H), 2.07(s, 3H), 1.99(s, 3H), 1.77-1.67(m, 2H), 1.53-1.43(m, 5H), 1.42-1.28(m, 9H). ^{13}C NMR (150 MHz, DMSO) δ 171.9, 145.9, 143.8, 122.7, 120.8, 120.4, 117.2, 77.2, 49.1, 33.8, 33.7, 29.4, 27.3, 27.3, 24.1, 23.5, 23.2, 20.1, 12.7, 11.9, 11.8. ESI-MS m/z 346.1516 $[M+H]^+$.

*6-hydroxy-2,5,7,8-tetramethyl-N-phenylchromane-2-carboxamide (**24a**) (Moulin et al., 1998).*

Light yellow solid, yield 59%; mp 97.8-98.8 °C (93 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 9.14(s, 1H), 7.60(d, J=7.7 Hz, 2H), 7.54(s, 1H), 7.29(t, J=7.9 Hz, 2H), 7.06(t, J=7.4 Hz, 1H), 2.57(s, 2H), 2.33(s, 1H), 2.21(s, 3H), 2.11(s, 3H), 2.02(s, 3H), 1.83(s, 1H), 1.53(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 172.2, 146.1, 143.8, 138.2, 128.6, 128.6, 123.8, 122.8, 121.4, 120.4, 120.0, 120.0, 117.1, 77.5, 29.3, 23.8, 20.1, 12.8, 12.1, 11.8. ESI-MS m/z 326.0878 $[M+H]^+$.

*6-hydroxy-2,5,7,8-tetramethyl-N-(p-tolyl)chromane-2-carboxamide (**25a**)*

Yellow solid, yield 45%; mp 146.1-147.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.03(s, 1H), 7.52(s, 1H), 7.45(d, J=8.4 Hz, 2H), 7.09(d, J=8.3 Hz, 2H), 2.56(s, 2H), 2.30(s, 1H), 2.23(s, 3H), 2.18(s, 3H), 2.08(s, 3H), 1.99(s, 3H), 1.83-1.78(m, 1H), 1.50(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 171.9, 146.0, 143.8, 135.6, 132.7, 129.0, 129.0, 122.8, 121.4, 120.3, 120.0, 120.0, 117.1, 77.4, 29.3, 23.8, 20.4, 20.1, 12.8, 12.1, 11.8. ESI-MS m/z 340.0986 $[M+H]^+$.

*6-hydroxy-N-(4-hydroxyphenyl)-2,5,7,8-tetramethylchromane-2-carboxamide (**26a**)*

Light pink solid, yield 53%; mp 230.3-231.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.21(s, 1H), 8.87(s, 1H), 7.51(s, 1H), 7.32(d, J=8.8 Hz, 2H), 6.71-6.64(m, 2H), 2.63-2.51(m, 2H), 2.28(s, 1H), 2.16(s, 3H), 2.08(s, 3H), 1.99(s, 3H), 1.79(s, 1H), 1.48(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 171.5, 153.7, 146.0, 143.8, 129.7, 122.7, 121.9, 121.9, 121.3, 120.3, 117.1, 114.9, 114.9, 77.4, 29.3, 23.9, 20.1, 12.8, 12.1, 11.8. ESI-MS m/z 342.0782 $[M+H]^+$.

*N-benzyl-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxamide (**27a**) (Manzano et al., 2014).*

Light yellow solid, yield 50%; mp 92.7-94.6 °C (109-110 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.80(t, J=6.2 Hz, 1H), 7.53(s, 1H), 7.21-7.13(m, 3H), 6.95(d, J=6.9 Hz, 2H), 4.37(dd, J=15.4, 6.9 Hz, 1H), 4.18(dd, J=15.4, 5.7 Hz, 1H), 2.54(s, 1H), 2.43(s, 1H), 2.24(s, 1H), 2.10(s, 3H), 2.07(s, 3H), 2.01(s, 3H), 1.73(s, 1H), 1.43(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 173.4, 145.9, 144.1, 139.6, 128.1, 128.1, 126.4, 126.3, 126.3, 122.7, 121.3, 120.3, 117.2, 77.4, 41.8, 29.6, 24.5, 20.3, 12.8, 12.1, 11.8. ESI-MS m/z 340.1015 $[M+H]^+$. Spectroscopic data were accordant with those previously reported.

*6-hydroxy-2,5,7,8-tetramethyl-N-(naphthalen-1-yl) chromane-2-carboxamide (**28a**)*

Light yellow solid, yield 37%; mp 184.1-185.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.25(s, 1H), 7.91(d, J=8.2 Hz, 1H), 7.77(d, J=8.2 Hz, 1H), 7.65(d, J=7.3 Hz, 1H), 7.60(s, 1H), 7.49(s, 2H), 7.39-7.34(m, 1H), 7.31(d, J=8.4 Hz, 1H), 2.59(s, 2H), 2.44(s, 1H), 2.28(s, 3H), 2.15(s, 3H), 2.02(s, 3H), 1.86(s, 1H), 1.64(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 172.7, 146.2, 144.1, 133.6, 132.6, 128.2, 127.9, 126.0, 125.9, 125.6, 125.5, 123.0, 121.9, 121.5, 121.4, 120.7, 117.4, 78.1, 29.5, 24.7, 20.4, 12.8, 12.2, 11.8. ESI-MS m/z 376.0898 $[M+H]^{+}$.

*6-hydroxy-2,5,7,8-tetramethyl-N-(naphthalen-2-yl) chromane-2-carboxamide (**29a**)*

Red-brown solid, yield 43%; mp 91.8-92.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.38(s, 1H), 8.29(s, 1H), 7.87-7.79(m, 3H), 7.63(s, 1H), 7.53(s, 1H), 7.46(t, J=7.4 Hz, 1H), 7.40(s, 1H), 2.59(s, 2H), 2.37(s, 1H), 2.22(s, 3H), 2.09(s, 3H), 2.01(s, 3H), 1.86(s, 1H), 1.56(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 172.4, 146.1, 143.9, 135.8, 133.2, 130.0, 128.2, 127.4, 127.3, 126.4, 124.8, 122.8, 121.4, 120.7, 120.3, 117.1, 116.2, 77.5, 29.4, 23.8, 20.1, 12.8, 12.2, 11.8. ESI-MS m/z 376.0879 $[M+H]^{+}$.

*6-hydroxy-N,N,2,5,7,8-hexamethylchromane-2-carboxamide (**30a**) (Jankowski et al., 2009)*

Light yellow solid, yield 62%; mp 158.4-160.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.49(s, 1H), 3.19(s, 3H), 2.73(s, 3H), 2.57(s, 1H), 2.43(s, 2H), 2.07(s, 3H), 2.05(s, 3H), 1.97(s, 3H), 1.61-1.53(m, 1H), 1.50(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 171.8, 145.9, 143.7, 122.8, 120.6, 120.5, 117.2, 78.1, 37.5, 37.0, 31.2, 24.7, 20.7, 12.7, 12.0, 11.7. ESI-MS m/z 278.0991 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

*(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(pyrrolidin-1-yl)methanone (**31a**)*

White solid, yield 62%; mp 174.3-174.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 3.93-3.88(m, 1H), 3.29(s, 1H), 3.26-3.19(m, 2H), 2.52(s, 1H), 2.48-2.41(m, 2H), 2.07(s, 3H), 2.05(s, 3H), 1.98(s, 3H), 1.78(s, 2H), 1.70(s, 1H), 1.53(s, 2H), 1.45(s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 171.0, 145.8, 143.8, 122.9, 120.5, 120.4, 117.2, 78.0, 47.3, 47.0, 30.4, 26.5, 24.5, 22.5, 20.5, 12.7, 12.0, 11.8. ESI-MS m/z 304.1079 $[M+H]^{+}$.

*(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(piperidin-1-yl)methanone (**32a**) (Manzano et al., 2014).*

Yellow solid, yield 53%; mp 160.0-161.7 °C(160-161 °C in literature). ^{1}H NMR (600 MHz, DMSO) δ 7.48(s, 1H), 3.83(s, 2H), 2.60(s, 1H), 2.45(s, 1H), 2.41-2.37(m, 1H), 2.05(s, 6H), 2.01(s, 1H), 1.97(s, 3H), 1.59(s, 1H), 1.51(s, 2H), 1.48(s, 4H), 1.37(s, 4H). ^{13}C NMR (150 MHz, DMSO) δ 170.1, 145.8, 143.9, 122.7, 120.6, 120.5, 117.1, 78.2, 39.8, 39.8, 39.1, 31.3, 24.8, 24.8, 24.0, 20.6, 12.7, 11.9, 11.7. ESI-MS m/z 318.1224 $[M+H]^{+}$. Spectroscopic data were accordant with those previously reported.

Biological Activities Assay

DPPH Radical Assay

DPPH scavenging activity was assayed according to the method with slight modifications (Sharma, Bhat, 2009; Dong *et al*., 2017). Each sample (100 μL) in methanol at different concentrations (from 5 to 25 μM) was added to 100 μL of DPPH in methanol solution (50 μM). The solution was vortexed in 96-well plates for 10 s and then allowed to stand at room temperature for 20 min in the dark. The absorbance was recorded at 492 nm on a microplate spectrophotometer. L-Ascorbic acid and **1** were used as positive references. IC_{50} values (the concentrations required to scavenge 50% of the DPPH radicals present in the test solution) were calculated and expressed as the mean ± SD.

ABTS Radical Assay

ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity was assayed according to the method with slight modifications (Dong *et al*., 2017). Briefly, 1 mL of 2.6 mM of potassium persulfate was added to 1 mL of 7 mM of $ABTS^{\bullet+}$ solution,

and the mixture was incubated in the dark at room temperature for 12–16 h before use. The ABTS•$^{+}$ solution was diluted with methanol to provide an absorbance of 0.70 ± 0.02 at 734 nm. The diluted ABTS•$^{+}$ solution (190 µL) was added to sample fractions (10 µL) in DMSO at different concentrations (from 62.5 µM to 2 mM). A standard curve was constructed by measuring the reduction in absorbance of the ABTS•$^{+}$ solution at different concentrations of Trolox (0-4 mM). The plates were incubated at room temperature for 20 min in the dark. The absorbance was recorded at 734 nm on a microplate spectrophotometer. L-ascorbic acid and **1** were used as positive references. The scavenging rate was expressed as % scavenging and was calculated as follows: IC_{50} values were calculated and expressed as the mean ± SD.

$$\%scavenging = \left(1 - \frac{A_{sample} - A_{blank}}{A_{control}}\right) \times 100\%$$

FRAP Assay

Ferric reducing ability was assayed according to the method with slight modifications (Dong *et al.,* 2017). FRAP reagent was freshly made by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, and 20 mM aqueous ferric chloride ($FeCl_3$) solution at a 10:1:1 (v/v) ratio. The TPTZ solution was prepared on the same day. Each sample in DMSO solution (1 mM, 20 µL) was added to 180 µL of FRAP reagent, vortexed in 96-well plates for 10 s and then incubated at 37 ºC for 30 min in the dark. The absorbance was recorded at 595 nm on a microplate spectrophotometer. L-ascorbic acid and **1** were used as positive references. Ferrous sulfate ($FeSO_4$) at 10 different concentrations (from 0 to 8 mM) was used to construct a calibration curve. FRAP values were calculated and expressed as the mean ±SD.

Hydroxyl Radical (•OH) Assay

Hydroxyl radical scavenging activity was assayed according to the method with slight modifications (Guo *et al*., 2017). Each sample in DMSO solution (50 µL) (from 0.5 to 10 mM) was treated with 3 mM $FeSO_4$ solution (50 µL) and 3 mM H_2O_2 solution (50 µL), after which the mixture was vortexed in 96-well plates, incubated for 10 min, mixed with 6 mM salicylic acid solution (50 µL), and vortexed. The plates were incubated at room temperature for 30 min in the dark. The absorbance was recorded at 492 nm on a microplate spectrophotometer. **1** was used as a positive reference. IC_{50} values (the concentrations required to scavenge 50% of the hydroxyl radicals present in the test solution) were calculated and expressed as the mean ± SD.

α-Glucosidase Inhibition Assay

α-Glucosidase inhibition activity was assayed according to the method with slight modifications (Yuan *et al*., 2012). Each sample (20 µL) in DMSO solution (from 0.1 to 10 mM) was added to 100 µL of α-glucosidase solution (pH 6.9, 0.1 U/mL, in 0.1 M phosphate buffer). The mixture was vortexed in 96-well plates, incubated at 25 ºC for 10 min. Then, 50 µL pNPG solution (pH 6.9, 5 mM, in 0.1 M phosphate buffer) was added to each well, the mixture was incubated at 25 ºC for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer. Acarbose was used as a positive reference. The α-glucosidase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

AChE Inhibition Assay

AChE inhibition activity was assayed according to the method with slight modifications (Ozturk *et al*., 2011). Each sample in 10 % DMSO solution (20 µL) (from 1 to 10 mM) was added to 120 µL of phosphate buffer (pH 8.0, 0.1 M) and 20 µL of AChE solution (pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The mixture was incubated at 25 ºC for 15 min. Then, 20 µL of ATCI solution (pH 8.0, 1.78 mM, in 0.1 M phosphate buffer) and 20 µL of DTNB solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the

mixture was incubated at 25 ºC for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer. Donepezil was used as a positive reference. The AChE inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

BChE Inhibition Assay

BChE inhibition activity was assayed according to the method with slight modifications (Ozturk *et al*., 2011). Each sample in 10 % DMSO solution (20 µL) (from 1 to 10 mM) was added to 120 µL of phosphate buffer (pH 8.0, 0.1 M) and 20 µL of BChE solution (pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The mixture was incubated at 25 ºC for 15 min. Then, 20 µL of butyrylthiocholine chloride solution (pH 8.0, 0.4 mM, in 0.1 M phosphate buffer) and 20 µL of DTNB solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the mixture was incubated at 25 ºC for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer. Donepezil was used as a positive reference. The BChE inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

Statistical Analysis

All the experiments were carried out in triplicate and the data were analyzed using SPSS software (Version 22.0) and Origin software (Version 8.0).

CONCLUSIONS

A series of Trolox amide derivatives all exhibited good antioxidant activity. **28a**–**29a** displayed similar α-glucosidase inhibition activity as acarbose, whereas some compounds displayed weaker inhibition activities for both cholinesterases. Both **26a** and **29a** had good performance in various biological assays, and further research is underway. This study revealed that Trolox derivatives with antioxidant activity are potentially beneficial for human health and worthy of further investigation.

ACKNOWLEDGEMENTS

This work was supported by the Science and Technology Development Funds of Jilin Province (YDZJ202201ZYTS186, 20160520044JH). We thank Joe Barber Jr., PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

REFERENCES

Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. FASEB J. 1999;13(10):1145-1155.

Brito DC, Brito AB, Scalercio SR, Percario S, Miranda MS, Rocha RM, et al. Vitamin E-analog Trolox prevents endoplasmic reticulum stress in frozen-thawed ovarian tissue of capuchin monkey (Sapajus apella). Cell Tissue Res. 2014; 355(2):471-480.

Cordova FM, Aguiar AS Jr, Peres TV, Lopes MW, Goncalves FM, Pedro DZ, et al. Manganese-exposed developing rats display motor deficits and striatal oxidative stress that are reversed by Trolox. Arch Toxicol. 2013; 87(7):1231-1244.

Czubak K, Antosik A, Cichon N, Zbikowska HM. Vitamin C and Trolox decrease oxidative stress and hemolysis in cold-stored human red blood cells. Redox Rep. 2017;22(6):445-450.

Dong LM, Jia XC, Luo QW, Zhang Q, Luo B, Liu WB, et al. Phenolics from Mikania micrantha and their antioxidant activity. Molecules. 2017;22(7):e1140-1- e1140-10.

Guo Z, Lin D, Guo J, Zhang Y, Zheng B. In vitro antioxidant activity and in vivo anti-fatigue effect of sea horse (Hippocampus) peptides. Molecules. 2017;22(3):e482-1-e482-11.

Jankowski OD, Wesson KE, Mollard P, Shrader WD. 4-(p-quinonyl)-2-hydroxybutanamide derivatives for treatment of mitochondrial diseases. US Patent No.20090118257. 2009.

Jin L, Tu J, Jia J, An W, Tan H, Cui Q, et al. Drug-repurposing identified the combination of Trolox C and Cytisine for the treatment of type 2 diabetes. J Transl Med. 2014;12:153-1-153-7.

Lee JH, Kim B, Jin WJ, Kim JW, Kim HH, Ha H, et al. Trolox inhibits osteolytic bone metastasis of breast cancer through both PGE2-dependent and independent mechanisms. Biochem Pharmacol. 2014; 91(1):51-60.

Lee S, Park EJ, Moon JH, Kim SJ, Song K, Lee BC. Sequential treatment with resveratrol and/or trolox improves development of porcine embryos derived from parthenogenetic activation and somatic cell nuclear transfer. Theriogenology. 2015;84(1):145-154.

Manzano JI, Lecerf-Schmidt F, Lespinasse MA, Di Pietro A, Castanys S, Boumendjel A, et al. Identification of specific reversal agents for Leishmania ABCI4-mediated antimony resistance by flavonoid and trolox derivative screening. J Antimicrob Chemother. 2014;69(3):664-672.

Messier EM, Bahmed K, Tuder RM, Chu HW, Bowler RP, Kosmider B. Trolox contributes to Nrf2-mediated protection of human and murine primary alveolar type II cells from injury by cigarette smoke. Cell Death Dis. 2013;4:e573-1-e573-11.

Miclea I, Fuss V, Zahan M, Orlovschi D, Miclea V. Influence of Trolox and Quercetin combinations on human ovarian cancer cell line a2780 and human breast cancer cell line T47D-KBluc. Bull Univ Agric Sci Vet Med Cluj-Napoca, Anim Sci Biotechnol. 2015;72(2):182-187.

Monticone M, Taherian R, Stigliani S, Carra E, Monteghirfo S, Longo L, et al. NAC, tiron and trolox impair survival of cell cultures containing glioblastoma tumorigenic initiating cells by inhibition of cell cycle progression. PLoS One. 2014;9(2):e90085-1-e90085-14.

Moulin C, Duflos M, Le Baut G, Grimaud N, Renard P, Caignard D. Synthesis and anti-inflammatory activity of N-(aza)arylcarboxamides derived from Trolox. Eur J Med Chem. 1998;33(4):321-329.

Ozturk M, Kolak U, Topcu G, Oksuz S, Choudhary MI. Antioxidant and anticholinesterase active constituents from Micromeria cilicica by radical-scavenging activity-guided fractionation. Food Chem. 2011;126(1):31-38.

Ponist S, Slovak L, Kuncirova V, Fedorova T, Logvinenko A, Muzychuk O, et al. Inhibition of oxidative stress in brain during rat adjuvant arthritis by carnosine, trolox and novel trolox-carnosine. Physiol Res. 2015;64(S4):S489-S496.

Sarveazad A, Babahajian A, Yari A, Goudarzi F, Soleimani M, Nourani M. Neuroprotective role of trolox in hippocampus after ischemia reperfusion injury in mouse. Int J Vitam Nutr Res. 2016;86(3-4):228-234.

Scalercio SR, Amorim CA, Brito DC, Percario S, Oskam IC, Domingues SF, et al. Trolox enhances follicular survival after ovarian tissue autograft in squirrel monkey (Saimiri collinsi). Reprod Fertil Dev. 2015; 28(11):1854-1864.

Sharma OP, Bhat TK. DPPH Antioxidant Assay Revisited. Food Chem. 2009;113(4):1202-1205.

Slovak L, Ponist S, Kuncirova V, Mihalova D, Fedorova T, Bauerova K. Evaluation of the effect of carnosine, its novel derivative trolox-carnosine and trolox in a pre-clinical study focussing on the regulation of immunity. Eur Pharm J. 2016;63(1):16-19.

Tucker JM, Townsend DM. Alpha-tocopherol: roles in prevention and therapy of human disease. Biomed Pharmacother. 2005;59(7):380-387.

Varo-Ghiuru F, Miclea I, Hettig A, Ladosi I, Miclea V, Egerszegi I, et al. Lutein, Trolox, ascorbic acid and combination of Trolox with ascorbic acid can improve boar semen quality during cryopreservation. CryoLetters. 2015;36(1):1-7.

Vergauwen H, Tambuyzer B, Jennes K, Degroote J, Wang W, De Smet S, et al. Trolox and ascorbic acid reduce direct and indirect oxidative stress in the IPEC-J2 cells, an in vitro model for the porcine gastrointestinal tract. PLoS One. 2015;10(3):e0120485-1-e0120485-18.

Yuan T, Wan C, Liu K, Seeram NP. New maplexins F-I and phenolic glycosides from red maple (Acer rubrum) bark. Tetrahedron. 2012;68(4):959-964.

Zang H, Sun JM, Huang XG, Ji Y, Dai TT, Gao XC, et al. Synthesis and biological activity of N-substituted carnosine amide derivatives. Chem J Chin Univ. 2014;35(12):2567-2573.

Zang H, Shen P, Wang EP, Xu Q, Zhang LY, Xia GQ, et al. Synthesis and biological activity of tyrosol ester derivatives. Chem J Chin Univ. 2018;39(1):64-70.

Zakharova OD, Frolova TS, Yushkova YV, Chernyak EI, Pokrovsky AG, Pokrovsky MA, et al. Antioxidant and antitumor activity of trolox, trolox succinate, and α-tocopheryl succinate conjugates with nitroxides. Eur J Med Chem. 2016;122:127-137.

Received for publication on 22nd October 2018
Accepted for publication on 29th September 2019