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ANTIOXIDANT ACTIVITY OF XANTHONE DERIVATIVES

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Abstract: Certain xanthone derivatives, such as these present in mangosteen fruits, show strong antioxidant activity. On the other hand, evidences accumulated that oxidative stress is involved in epileptogenesis. Therefore, the aim of the present study was to estimate total antioxidant capacity (expressed as a ferric reducing antioxidant power - FRAP) and evaluate ability to scavenge free radicals (DPPH methods) by xanthone derivatives showing antiepileptic activity. Selected 2-(aminomethyl)-9*H*-xanthen-9-one derivatives shared structural features, such as chlorine substituent in xanthone ring and different chiral (or not) alkanol groups at the nitrogen atom. The results of antioxidant activities among racemates revealed the highest activity for compound (***R*/*S*)-3** (31.7% in diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and (0.184 ± 0.003 mM Fe^{2+}/L) in FRAP assay. Among tested pair of enantiomers we observed that (***R*)-1** and (***R*)-2** showed higher reduction capacity ((***R*)**–1: 0.096 ± 0.007 mM Fe^{2+}/L; (***R*)-2**: 0.048 ± 0.005 mM Fe^{2+}/L, respectively) and stronger DPPH scavenging activity ((***R*)-1**: 31 ± 3.0%; (***R*)-2**: 29 ± 2.5%, respectively) comparing to their (*S*)-enantiomers and racemates.

Keywords: xanthone derivatives, oxidative stress, DPPH test, FRAP

Reactive oxygen species (ROS) are formed in oxidation of various cell constituents as DNA, lipids and proteins and consequently cause oxidative damage of cellular substance leading to cell death (1). The oxidative damage of DNA induced by ROS lead to certain cancers, and ROS may also play a role in cell cycle progression. ROS are implicated in numerous pathological events including metabolic disorders, cellular aging, reperfusion damage of DNA, inflammation and atherosclerosis (2). In that light, searching for new, antioxidant agents seems to be well grounded (3). It was demonstrated that oxidative stress and reactive oxygen species production are involved also in epilepsy pathogenesis. The xanthone nucleus comprises an important class of oxygenated heterocycles. They are found in natural products, especially in higher plants, lichens and fungi. Plants belonging to the *Guttiferae* family are the richest in xanthone derivatives. The most popular is *Garcinia mangostana*, a tropical evergreen tree. Its origin is in Southeast Asia. It can now be found in Northern Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Malaysia, Thailand, and other tropical countries. The edible fruit is deep reddish purple when ripe. In Asia, it is known as the "Queen of Fruits" due to its pleasant flavor (4). The fruit's hull of α-, β- and γ-mangosteen, 3-isomangosteen, gartanin, 8-desoxygartanin has been used for hundreds of years in Southeast Asia as a medicine for skin infections, wounds, dysentery and diarrhoea, but those biological benefits were not related with the xanthone structure (5). Nowadays, mangosteen is used as an ingredient in several popular commercially available nutritional supplements with antioxidant, cardiovascular, immunestimulating activity, including Vemma (Vemma Co.) and Xango (Xango Co.) (6). It is known from the literature that xanthone derivatives are characterized with diverse biological activities including tuberculostatic (7), antimicrobial (8), cardiovascular (9), antiinflamatory (10) and antioxidant (11, 12). Taking into account antioxidative properties, compounds can act as metal chelators, free radical scavengers, as well as inhibitors of lipid peroxidation (13). There are evidences that oxidative stress can be involved in seizure generation (14, 15). What is more, beneficial effects of antioxidants in animal models of epileptic seizures was observed (16), therefore, antioxidant properties could be recommended for newly synthesized antiepileptic com-

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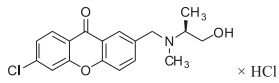
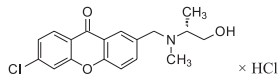
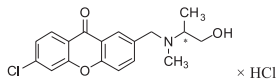
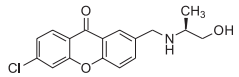
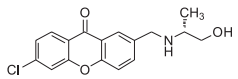
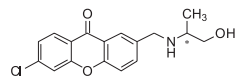
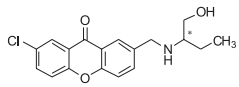
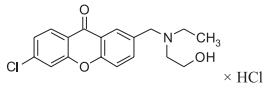
pounds. New derivatives of xanthone are the subject of the research carried out in our Department of Bioorganic Chemistry. Anticonvulsant activity and neurotoxicity were evaluated under the Anticonvulsant Screening Program (ASP) at the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA.

Most of the structures mentioned below proved their beneficial properties and were previously described (17-20). Compounds **2** ***R***,***S***; **2** ***R*** and **2** ***S*** displayed significant anti-MES (maximal electroshock) activity in mice with protective index (TD_{50}/ED_{50}) of 5.84, 6.23 and 6.85, corresponding to that of phenytoin, carbamazepine and valproate. Moreover, compound **2** ***S*** revealed low micromolar affinity to the voltage-dependent Ca^{2+} channels, comparable to that of carbamazepine (17, 18). Xanthone derivatives from the first series (**1** ***S***, **1** ***R***, **1** ***R***,***S***) were not so effective and acted in higher doses than second series (**2** ***R***,***S***; **2** ***R*** and **2** ***S***) (18, 19).

Compound **3** ***R***,***S*** with chlorine atom at position 7 in the xanthone scaffold was active in MES

Table 1. Xanthone derivatives containing chlorine in the aromatic ring and different alkyl substituent at the nitrogen atom.

Compound	Structure and IUPAC name
(*S*)-1	 (*S*)-6-chloro-2-{[(1-hydroxypropan-2-yl)(methyl)amino]methyl}-9H-xanthen-9-one hydrochloride
(*R*)-1	 (*R*)-6-chloro-2-{[(1-hydroxypropan-2-yl)(methyl)amino]methyl}-9H-xanthen-9-one hydrochloride
(*R*,*S*)-1	 (*R*,*S*)-6-chloro-2-{[(1-hydroxypropan-2-yl)(methyl)amino]methyl}-9H-xanthen-9-one hydrochloride
(*S*)-2	 (*S*)-6-chloro-2-{[(1-hydroxypropan-2-yl)amino]methyl}-9H-xanthen-9-one
(*R*)-2	 (*R*)-6-chloro-2-{[(1-hydroxypropan-2-yl)amino]methyl}-9H-xanthen-9-one
(*R*,*S*)-2	 (*R*,*S*)-6-chloro-2-{[(1-hydroxypropan-2-yl)amino]methyl}-9H-xanthen-9-one
(*R*,*S*)-3	 (*R*,*S*)-2-chloro-7-{[(1-hydroxybutan-2-yl)amino]methyl}-9H-xanthen-9-one
4	 6-Chloro-2-{[ethyl(2-hydroxyethyl)amino]methyl}-9H-xanthen-9-one hydrochloride

test in the dose of 30 mg/kg in 87% of the examined mice (20).

Herein we report on radical scavenging and antioxidant activities of the synthesized xanthone derivatives using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

MATERIALS AND METHODS

The reagents such as vitamin C, quercetin, trolox, 2,2-diphenyl-1-picrylhydrazyl and 2,4,6-tri-(2-pyridyl)-s-triazine were of analytical grade and were obtained from Sigma-Aldrich Chemical Company (Steinheim, Germany). All other reagents ($FeSO_4$, $FeCl_3$ and methanol) were purchased from Avantor Performance Materials Poland S.A.

Xanthone derivatives: **(*R*/*S*)-1**; **(*S*)-1**; **(*R*)-1**; **(*R*/*S*)-2**; **(*S*)-2**; **(*R*)-2**; **(*R*/*S*)-3** and **4** (Table 1) were synthesized at the Department of Bioorganic Chemistry, UJ CM, Kraków, Poland. Their structures were confirmed by ^{1}H NMR and IR spectra (17-20). Enantiomers were checked for their purity by measuring their specific rotation (19, 20). For the studies, compounds were dissolved in water using ultrasounds and tested in the following concentrations: 0.3, 0.6, 1, 50 and 200 μM. Reference antioxidants were used in the same concentrations as tested compounds: *L*-ascorbic acid (vit. C, dissolved in water), trolox (dissolved in methanol) and quercetin (polyphenol dissolved in methanol). Spectrophotometer Cecil CE 7200 BioAquarius (Cecil Instruments Limited, Cambridge, UK) was applied for the measurement of absorbance. Assays were carried out in triplicate. Results are given as the means with standard deviation.

DPPH free radical scavenging activity assay

Antioxidant properties of the investigated group of compounds were measured by DPPH test based on the method reported by Blois (21). In general, DPPH in its stable radical form absorbs at 517 nm, but upon reduction by an antioxidant present in a sample its absorption decreases. Solution (0.6 mM) of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was prepared in methanol. Fixed volume (25 μL) of solutions of each compound or reference substance (0.3–200 μM) were added to 1 mL of DPPH. Solutions were mixed and incubated in dark at room temperature for 30 min. After that time, the absorbance of samples were measured at 517 nm (As) against blank sample (methanol) (Ab). As a control, absorbance of DPPH solution with 25 μL of distilled water was measured (Ac). The capability of tested compounds to scavenge the DPPH radical (antioxidant activity) was calculated using the following equation:

$$DPPH\ [\%] = \frac{Ac - As}{Ac - Ab} \times 100$$

where *Ac* was the absorbance of the control, *As* of the sample and *Ab* of the blank (methanol).

Measurement of the total antioxidant capacity

The FRAP assay, which is the modification of Benzie and Strein's method (22), was applied to measure the ability of xanthone derivatives to reduce Fe^{3+} to Fe^{2+} ions in acidic environment (pH 3.6). Fe^{2+} ions in the presence of 2,4,6-tripyridyl-*S*-triazine (TPTZ) forms Fe^{2+}-TPTZ intensive blue complexes, with maximum absorbance at 593 nm. Reaction mixture consisted of acetate buffer 0.3 M, (pH 3.6); TPTZ 0.01 M and iron(III) chloride 0.02 M. Samples were prepared by adding 50 μL of tested compound or standard in different concentrations (0.3–200 μM) to 1 mL of reaction mixture. After mixing, samples were incubated at 37°C for 30 min. After that time, the absorbance was measured at a wavelength of λ = 593 nm. Blank test was performed similarly, but to a reaction mixture 50 μL of distilled water was added. The antioxidant activity was expressed in micromoles of ferrous ions per liter produced by tested compound or standard,

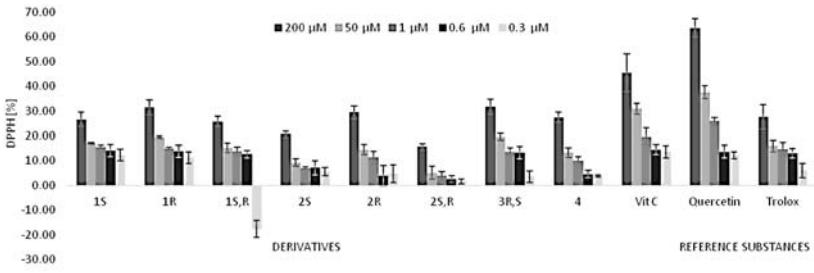


Figure 1. DPPH [%] concentration values for the standard and xanthone derivatives of the series **1**, **2** and substances **3*R*,*S*** and **4**

which was calculated from the calibration curve of iron(II) sulfate (100 to 1000 μM/L of $FeSO_4$).

RESULTS AND CONCLUSIONS

Number of reports concerning studies on plant extracts from *Garcinia rigida, Garcinia mangostana, Cudrania tricuspidata* or *Cratoxylum cochinchinense* demonstrated their various biological activities, such as anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxic and anti-HIV (23-26). Plants mentioned above are source of xanthone derivatives, that were isolated and identified as (poly)phenols, possessing one or more hydroxyl groups in the aromatic scaffold (27, 28). Among synthetic xanthone derivatives, we reported some compouds with promising anticonvulsant properties being derivatives of 6- or 7-chloro-2-(aminomethyl)-9*H*-xanthen-9-one. For this study, we selected derivatives with defined anticonvulsant activity possessing hydroxyl group in the side alkyl chain (Table 1). Their ability to "sweep off" free radicals was measured and expressed as a percentage of activity (DPPH test - Fig. 1). In case of the FRAP method, production of Fe^{2+} in the presence of xanthone derivatives was calculated (FRAP test - Fig. 2).

Figure 1 illustrates a decrease in the concentration of DPPH due to the scavenging ability of xanthone derivatives and standards. The scavenging effect decreased in the order of quercetin > vitamin C > **(*R*/*S*)-3** > **(*R*)-1** > **(*R*)-2** > trolox > **4** > **(*S*)-1** > **(*R*/*S*)-1** > **(*S*)-2** > **(*R*/*S*)-2** yielding 63.5, 45.4, 31.7, 31.4, 27.6, 27.3, 26.6, 25.6, 20.6 and 15.6%, at the concentration of 200 μM, respectively. Changing concentrations of tested compounds from 200 to 50 μM resulted in greatest difference in outcomes for all substances. In case of **(*R*/*S*)-2**, a decrease in scavenging activity was 67%, while for vit. C was only 32%. At the lowest tested concentration (0.3 μM) the calculated scavenging activity for racemic **(*R*/*S*)-2** was of negative value, what can suggest prooxidative properties of racemic compound. Such effect was not observed in case of both of its enantiomers **(*S*)-2** and **(*R*)-2**. Comparing scavenging effect of pairs of enantiomers and racemates (comp. **1** and **2**) it can be readily observed that *R*-enantiomers are more potent free radicals scavengers (Fig. 1). We have previously reported some differences in free radical scavenging activity between stereoisomers of β-carboline derivatives (29).

In case of FRAP assay, the highest Fe^{2+} content in the tested samples was observed for compounds **(*R*)-1** and **(*R*/*S*)-3** (Fig. 2). The reducing power of these compounds was higher than all three reference substances tested. Only at the highest concentration (200 μM), vitamin C showed stronger antioxidative activity than compound **(*R*)-1**, while quercetin at concentration 1 μM more effectively reduced Fe^{3+} than **(*R*/*S*)-3**. Concerning chirality of xanthone derivatives, both *R*-enantiomers ((**(*R*)-1** and **(*R*)-2**) showed the tendency to increase value of FRAP with the decrease of concentration. In case of standards, only vit. C decreased FRAP value when diluting samples, thus showing reducing properties. For compound **(*S*)-1**, FRAP values did not significantly differ in all of the tested concentrations. Compound **(*R*/*S*)-2** was the least active in the studied group of xanthone derivatives. Generally (*R*)-enantiomers showed stronger ferric reducing antioxidant power than their (*S*)-enantiomers and racemates. Additionaly, **(*R*/*S*)-1** showed prooxidative properties at the lowest tested concentration (0.3 μM), what overlapped with the observation in DPPH assay.

When comparing antioxidant activity of tested compounds with their antiepileptic properties and structure, most active in central nervous system compound **(*S*)-2** (17, 18) with chlorine atom in the position of 6 and propanol as alkanol substituent,

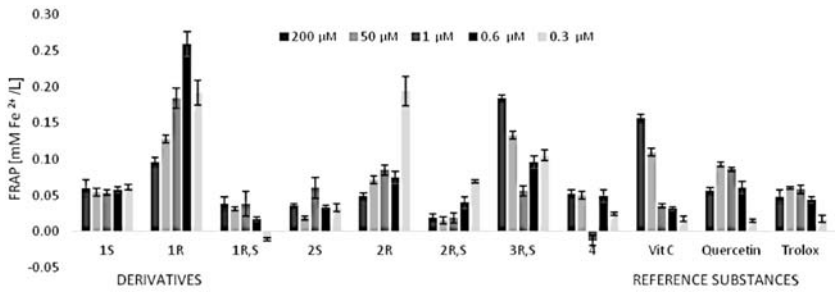


Figure 2. FRAP [mM Fe^{2+}/L] concentration values for the standard and investigated substances of the series **1**, **2** and substances ***3R,S*** and **4**

possessed only very mild antioxidant properties. Racemic **(*R*/*S*)-3**, bearing chlorine in position 7 of xanthone scaffold and butanol substituent at nitrogen atom, showed moderate anticonvulsant protection (19) and revealed the highest antioxidant effect in tested series of compounds. On the other hand, **(*R*)-1**, with chlorine substituent in position 6 of xanthone structure being tertiary amine with propanol substituent and methyl group, exhibited the highest ferric reduction power but in preliminary anticonvulsant screening was less effective than other compounds (17, 18).

In conclusion, the obtained results of antioxidant studies for compounds affecting CNS functions in the group of 2-(aminomethyl)-9*H*-xanthen-9-one derivatives with alkanol substituent at the nitrogen entitled to lead further studies to search for new antiepileptic structure with good antioxidant properties. It requires of course more advanced methodology.

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