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Synthesis and *in vitro* study of redox properties of pyrrole and halogenated pyrrole derivatives

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Abstract: The redox balance plays a crucial role in maintaining biological processes under normal conditions. Antioxidants inhibit and reduce harmful oxidation processes, while pro-oxidants can act as anti-cancer agents by promoting ROS-mediated cell death. The aim of this study is to compare the redox properties of seven newly synthesised tribromopyrrole derivatives with three novel and four previously synthesized non-halogenated analogues in an *in vitro* model (in human serum) and with exogenously induced oxidative stress. The obtained values of their oxy scores (OS) were compared and the result showed that four non-halogenated pyrrole derivatives with secondary amide group **M2**, **M10**, **M11** and **M12** have lower OS values than Trolox, a water-soluble analogue of vitamin E with proven antioxidant properties. All four compounds show strong resistance to oxidative stress, which is reflected in the maintenance of negative OS values when exposed to exogenous oxidative stress using TBH in the reaction mixture. This capability to resist invading ROS should be expected also in an endogenous environment, where constant prooxidant production takes place at a low, homeostatic level, but even more so in pathological conditions. The tribrominated derivative **M15** showed prooxidant activity with a significantly higher OS value than all other compounds tested. The comparison of the dose-response of Trolox and the five compounds with the lowest OS also shows that compounds **M2**, **M7** and **M10** have better antioxidant activity than Trolox.

Keywords: oxidative stress; antioxidant; prooxidant; synthesis; pyrroles.

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INTRODUCTION

In the complex web of cell biology, oxidative processes play a crucial role in maintaining homeostasis and regulating various physiological functions. A central role in this complicated network is played by reactive oxygen species (ROS), a class of highly reactive species that serve both as important signaling molecules and potential indicators of cell damage. While ROS are essential components of various cellular processes, an imbalance in their production and elimination can lead to oxidative stress, a disorder that plays a role in a variety of pathological conditions, including cancer. Reactive oxygen species include a number of molecules, *e.g*., superoxide anions, hydrogen peroxide and hydroxyl radicals (•OH), which are produced during normal cellular metabolism. These species are involved in important cellular signaling pathways such as redox signaling, but also pose a threat as they can cause oxidative damage to biomolecules such as proteins, lipids and nucleic acids.[1,2]

Oxidative stress occurs when the cellular antioxidant defense mechanisms are overwhelmed, leading to an accumulation of ROS that exceeds the cell's ability to neutralise them. This imbalance can have various causes, including metabolic disorders. Importantly, the consequences of oxidative stress extend beyond the immediate cellular environment. Persistent oxidative stress can trigger genomic instability and promote mutagenesis, creating a favorable environment for tumour development and progression. Cancer cells develop mechanisms to keep high oxidative stress under control. This enables the involvement of ROS in the angiogenesis, invasiveness and metastatic capacity of cancers. At each stage of cancer development, cancer cells are adapted to high levels of ROS, which enables their survival in conditions unbearable for normal cells.[3,4] Furthermore, the intricate interplay between prooxidant activities and cellular antioxidant defense mechanisms contributes to an even more complex understanding of redox dynamics in cancer biology.[5,6]

Pyrrole is a heterocyclic compound whose derivatives show diverse pharmacological properties. The flat, electron-rich ring of pyrrole is highly susceptible to electrophilic attack and can bind to numerous biomolecules through hydrogen bonding and π–π stacking interactions.[7] This essential structural element is found in various naturally occurring structures such as chlorophyll, haemoglobin, myoglobin, cytochromes, vitamin B12 and bile pigments such as bilirubin and biliverdin.[8] The pyrrole subunit can be considered as privileged structure in medicinal chemistry extensively used as a key structural element in antifungals,[9] antimicrobials,[10] anti-inflammatory agents,[11] HMG-CoA reductase inhibitors[12] and antitumour agents.[13] There are some commercially available drugs such as ketorolac (**1**), tolmetin (**2**) and zomepirac (**3**), which are trisubstituted pyrrole derivatives used as non-steroidal anti-inflammatory drugs (Fig. 1).

Due to the considerable reactivity of pyrroles with electrophiles, halogenated derivatives of pyrroles are widespread in nature. The enzymatic incorporation of halogens into the biosynthesis of natural compounds allows fine-tuning of electronic and steric properties, which influence the affinity and selectivity of a molecule's interactions with its biological target. The antibiotic properties of certain natural halogenated pyrroles, such as pentabromopseudilin (**4**), pyoluteorin (**5**) and pyrrolnitrin (**6**, Fig. 1), have long been recognized.[14] This class of compounds also has some anticancer and antifungal activity and inhibits cholesterol biosynthesis. Pyrrolomycines (**7**), polyhalogenated pyrrole metabolites isolated from the fermentation broth of Actinosporangium and *Streptomyces* species, also show antibiotic activity.

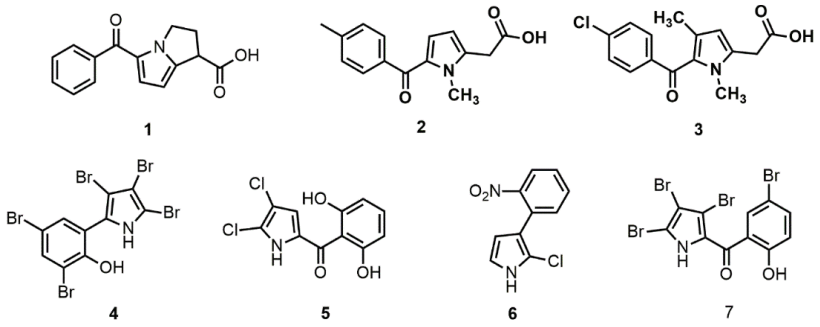


Fig. 1. Structures of some important pyrrole derivatives.

Typical antioxidants such as polyphenols neutralize oxygen radicals by a process that usually involves the transfer of hydrogen atoms (HAT) and results in a stabilized phenolic radical that does not continue the oxidative chain process. It has been shown that pyrroles, which have an N–H bond, also act as hydrogen atom donors and have antioxidant activity.[15] The antioxidant activity of various pyrrole derivatives, such as pyrrole-2-carbaldehydes,[16] pyrrole-2,5-diones,[17] pyrrole-based dihydropyrimidines,[18] hydrazides[19] and formazans,[20] has been demonstrated. Also, our previous study of substituted coumarins and the related isocoumarins and phthalides also showed the positive effect of azolyl substituents on the antioxidant/prooxidant balance of these compounds.[21]

Exploration of the antioxidant properties of pyrazole derivatives, revealed that the introduction of a chlorine atom into the aromatic ring increases the antioxidant capacity of these derivatives compared to non-halogenated ones.[22] Bearing in mind this fact, we envisioned to explore antioxidant/prooxidant potential of a series of newly synthesized tribromopyrrole derivatives in comparison with non-halogenated analogues in biological medium (serum pool of healthy volunteers). The unique pyrrole derivatives used in this study are easily accessible via chemistry that we have recently reported and further developed.[23]

EXPERIMENTAL

General

All chemicals used for the synthesis were obtained from commercial sources and were of reagent grade purity or better (Merck, Sigma Aldrich, Fluka, Fisher Scientific, *etc*.). ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded at 400 and 101 MHz, respectively, using a Bruker Ascend 400 (400 MHz) spectrometer. Deuterochloroform was used as the solvent and chemical shifts are reported in ppm (δ) downfield from tetramethylsilane as the internal standard. Mass spectral data were recorded using an Orbitrap XL. Flash chromategraphy used a silica gel 60 (230–400 mesh), while thin-layer chromatography (TLC) was carried out using alumina plates with a 0.25 mm silica layer (Kieselgel 60 F254 Merck). The compounds were visualized by staining with potassium permanganate solution. Synthetic procedures are listed in the Supplementary data.

Sample collection

A serum pool was formed from samples of healthy individuals remaining after routine laboratory procedures. The use of patient data is excluded in this study. The selected samples included individuals whose key biochemical parameters were within the reference ranges for metabolites, which served as confirmation of their overall good health. The aliquots of the serum pool were frozen at −80 °C and used several months after the first collection. The substances to be analyzed, dissolved in DMSO at an initial concentration of 10 mmol/L, were mixed with the aliquots of the serum pool at a ratio of 1:9, thereby restricting sample dilution to 10 % and preserving the biomatrix. The final concentration for all tested substances was maintained at 1 mmol/L. This was followed by a two-hour incubation at 37 °C and the analyses were performed in duplicate, both alone and in combination with the exogenously added prooxidant *tert*-butyl hydroperoxide (TBH) at a concentration of 0.25 mmol/L, in an equivolume ratio.

Evaluation of biochemical parameters

The study involved the analysis of four redox status parameters using already published spectrophotometric methods. Two parameters defined prooxidant properties, total oxidative status (TOS) and prooxidant–antioxidant balance (PAB), while the other two, total antioxidant status (TAS) and total sulfhydryl groups (SHG), described antioxidant potential.

Serum TOS, a sum of lipid hydroperoxides and H_2O_2 concentrations, was determined by Erel's method modified in our laboratory. This involves the oxidation of ferrous ion in the *o*-dianisidine complex to ferric ion by oxidants present in the sample. The intensity of the color is proportional to the total amount of oxidant molecules in the sample. Calibration was performed with an aqueous solution of hydrogen peroxide (2–200 μmol/L) and the results are expressed as μmol H_2O_2 equivalent/L.[26,27]

Serum PAB is a measure of H_2O_2 concentration in an antioxidant environment measured by a previously published method.[28] The method involves the simultaneous reaction of 3,3'--5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide and antioxidants such as uric acid. The reaction of hydrogen peroxide and chromogen is enzymatically catalyzed by peroxidase, whereas the reaction of serum antioxidants and chromogen is a non-enzymatic chemical process. The standard solutions were prepared by combining different proportions (0–100 %) of 1 mmol/L H_2O_2 with 6 mmol/L uric acid. The absorbance was measured at 450 nm and the PAB values are expressed in arbitrary units corresponding to the percentage of H_2O_2 in the standard solution. All measurements were performed using the micro-plate reader SPECTROstar Nano Microplate Reader (BMG Labtech, Ortenberg, Germany).

TAS represents the total concentration of all reductive substances in the blood and was measured using Erel's method, which was further optimized in the laboratory.[27] The assay involves the oxidation of reduced 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) with hydrogen peroxide in an acidic medium. The antioxidants in the serum cause a discoloration of the reagent and the extent of the discolouration is proportional to their concentration.[29] Calibration was performed with Trolox, a water-soluble analogue of vitamin E, in the measuring range of 200–2000 μmol/L and the absorbance measured at 660 nm. The assay results are given in micromole Trolox equivalents/L.

SHG values were measured using a modification of the Ellman method by Kotur-Stevuljevic *et al*. The method used 10 mM 5,5'-dithiobis(2-nitrodithiobenzoic acid) (DTNB) as a reagent.[27] In a basic environment (pH 9.0), DTNB reacts with aliphatic thiol compounds to produce 1 mole of *p*-nitrophenol anion per mole of thiol. The absorbance was measured at 412 nm and the calibration used reduced glutathione as a standard in the concentration range of 0.01–4.0 mM.

Prooxidant score, antioxidant score and oxy score

The oxy score (OS) is calculated as the difference between the prooxidant score (average *Z*-scores of the measured prooxidants and their products, including TOS and PAB) and the antioxidant score (average *Z*-scores of the measured antioxidants, such as TAS and SHG). The *Z*-score is calculated as the difference between the original value and the control value divided by the standard deviation (*SD*) of the control samples' value. A higher oxy score indicates a poorer redox status (weaker antioxidant protection and a higher content of prooxidants).

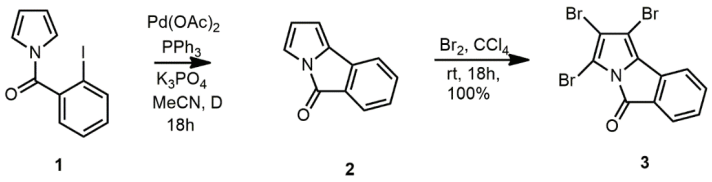
Statistical analysis

Data are presented as median values (25^{th}–75^{th} percentile values). For the inter-groups comparison Kruskal–Wallis ANOVA and post-hoc Mann–Whitney U test were used. The *P* value below 0.05 was considered as statistically significant.

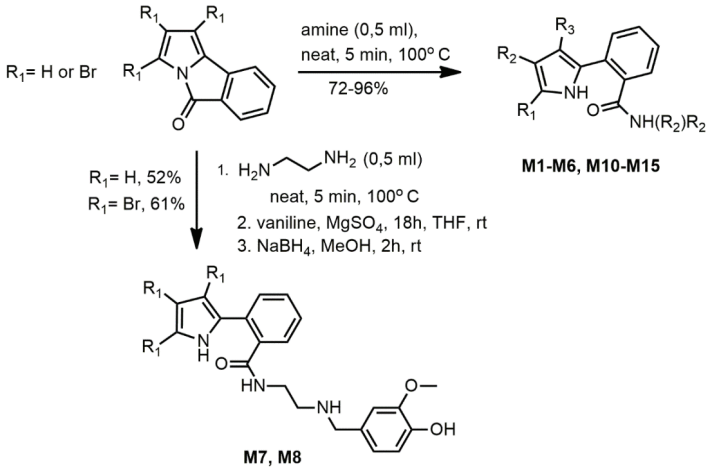
RESULTS AND DISCUSSION

Recently, we reported a novel method for the selective arylation of pyrrole derivatives in which the arylation agent fulfils a dual function: protection of the NH moiety and C(2) arylation.[23] During our mechanistic insight into this reaction, we found that treatment of acylpyrrole **1** with $Pd(OAc)_2/PPh_3$ and K_3PO_4 as a base in refluxing acetonitrile gives a tricyclic compound **2** (Scheme 1). The electrophilic aromatic substitution of the pyrrole ring with Br_2 in CCl_4 led to the formation of a tribrominated pyrrole derivative **3**, a structural motif found in several biologically active compounds. The subsequent ring opening of **2** or **3** with primary and secondary amine nucleophiles produces pyrrole derivatives **M1**–**M6**, **M10**–**M15** which are subjected to examination in antioxidant assays (Scheme 2). If an amine is used as a solvent, the reaction proceeds very quickly. After heating to 100 °C for 5 min, amides are formed in 72–96 % yields. An alternative with 3 equivalents of amine in acetonitrile as solvent gave comparable yield of product, but in this case overnight heating in boiling solvents is required. Furthermore, ring opening of compounds **2** and **3** with 1,2-diaminoethane, followed by reductive amination of the primary amino group with vanillin (3-hydroxy-4-methoxybenzaldehyde), led to compounds **M7** and **M8**, in which the structures of pyrrole

are combined with a phenolic ring with proven antioxidant activity. In order to investigate the significance of the pyrrole ring for antioxidant activity, a compound without the pyrrole ring, **M9**, was also synthesized, which was achieved by reductive amination of benzylamine and vanillin.[25]



Scheme 1. Synthesis of tricyclic pyrrole derivatives.



Scheme 2. Synthesis of pyrrole derivatives for the investigation of redox properties.

In order to estimate antioxidant potential of newly synthesized bromopyrrole derivatives and their non-halogenated analogues, we measured two prooxidant (TOS and PAB) and two antioxidant (TAs and SHG) parameters (data presented in the Supplementary material to this paper). The experiments were performed without or with externally added *t*-butyl hydroperoxide to mimic the conditions prevailing during the development of pathological processes. The main objective of this analysis is the calculation of three redox scores (without and with the addition of TBH), as their value indicates the level of prooxidant/antioxidant activity in the biological medium (serum pool). Typically, a low oxy score is associated with significant antioxidant capabilities of the compounds tested. Oxy scores, when considering the addition of TBH, reflect the capacity of the system to resist the effects of exogenous prooxidant.

The structures of all tested compounds are outlined in Fig. 2 and their redox scores are listed in Table I.

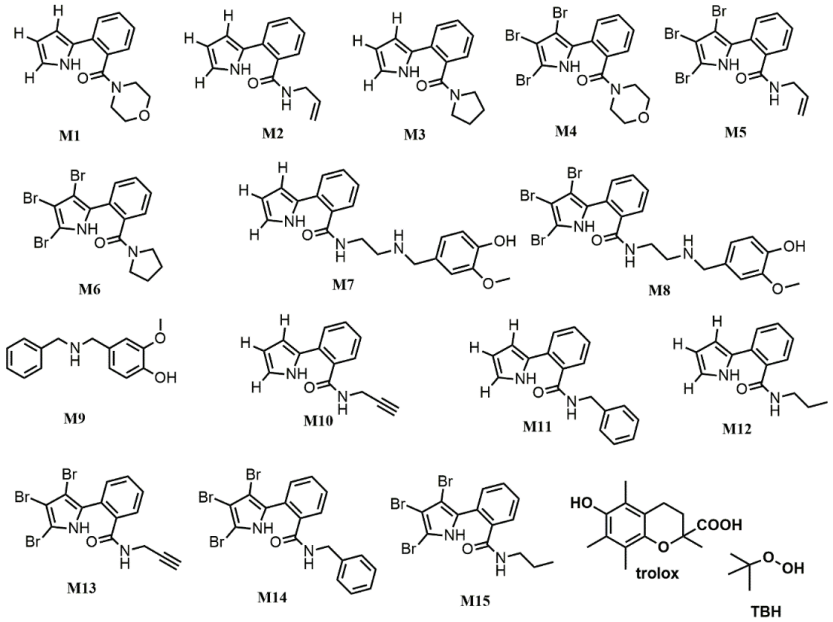


Fig. 2. Structures of tested compounds, Trolox and TBH.

TABLE I. Calculated values of prooxy, antioxy and oxy score of tested compounds; data presented as medians and 25th–75th percentile values in brackets; entries **a**–**o**: samples without TBH; entries **a′–o′**: samples with TBH

Entry	Compound	Prooxy score	Antioxy score	Oxy score
–	blank (0)	–3.3 (–5.9–0.0)	–0.4 (–2.2–0.6)	–1.1 (–4.2–0.0)
a	M1	–8.7 (–9.2–(–)8.1)	7.0 (6.9–7.1)	–15.7 (–16.1–(–)15.3)
b	M2	–18.3 (–19.3–(–)17.3)	8.6 (8.0–9.2)	–26.9 (–27.3–(–)26.5)
c	M3	–11.1 (–11.4–(–)10.9)	6.1 (6.0–6.2)	–17.2 (–17.5–(–)16.9)
d	M4	–7.9 (–8.5–(–)7.3)	7.2 (4.9–9.6)	–15.1 (–18.1–(–)12.2)
e	M5	–1.5 (–2.5–(–)0.5)	3.6 (3.6–3.6)	–5.1 (–6.1–(–)4.1)
f	M6	–1.3 (–1.4–(–)1.2)	4.8 (4.7–4.9)	–6.1 (–6.3–(–)6.0)
g	M7	–14.7 (–15.4–(–)13.9)	11.8 (11.2–12.4)	–26.4 (–26.6–(–)26.3)
h	M8	–2.6 (–3.2–(–)2.0)	8.0 (8.0–8.0)	–10.6 (–11.2–(–)10.0)
i	M9	–3.0 (–3.4–(–)2.5)	8.4 (8.3–8.5)	–11.4 (–12.0–(–)10.8)
j	M10	–18.5 (–21.3–(–)15.6)	4.9 (0.3–9.5)	–23.4 (–25.2–(–)21.6)
k	M11	–26.4 (–27.5–(–)25.4)	–2.4 (–3.6–(–)1.2)	–24.1 (–24.2–(–)23.9)
l	M12	–34.2 (–36.4–(–)32.0)	–6.1 (–6.8–(–)5.3)	–28.1 (–29.6–(–)26.7)
m	M13	–12.7 (–13.9–(–)11.6)	–11.6 (–12.7–(–)10.5)	–1.1 (–3.3–1.1)
n	M14	–12.4 (–14.7–(–)10.1)	5.9 (2.8–9.1)	–18.4 (–19.3–(–)17.5)
o	M15	14.3 (11.7–16.9)	2.7 (0.9–4.5)	11.6 (7.2–16.0)
a′	M1+TBH	6.0 (5.6–6.4)	2.8 (2.5–3.2)	3.2 (2.5–3.9)
b′	M2+TBH	–2.5 (–2.6–(–)2.5)	3.5 (3.2–3.8)	–6.1 (–6.4–(–)5.7)
c′	M3+TBH	4.0 (–0.6–8.7)	1.6 (0.2–3.0)	2.5 (–3.6–8.5)
d′	M4+TBH	6.6 (6.5–6.7)	1.7 (1.4–1.9)	4.9 (4.6–5.2)
e′	M5+TBH	9.5 (8.5–10.4)	1.2 (0.8–1.5)	8.3 (7.0–9.6)

TABLE I. Continued

Entry	Compound	Prooxy score	Antioxy score	Oxy score
f′	M6+TBH	12.6 (12.5–12.7)	–0.6 (–1.0–(–)0.2)	13.1 (12.8–13.4)
g′	M7+TBH	1.7 (1.0–2.5)	–1.2 (–9.6–7.1)	3.0 (–6.2–12.1)
h′	M8+TBH	10.3 (9.5–11.0)	3.5 (2.7–4.3)	6.8 (5.2–8.3)
i′	M9+TBH	10.2 (8.3–12.1)	1.8 (0.5–3.1)	8.3 (5.1–11.5)
j′	M10+TBH	–26.5 (–27.8–(–)25.2)	–4.6 (–5.8–(–)3.5)	–21.9 (–24.3–(–)19.5)
k′	M11+TBH	–20.3 (–21.6–(–)19.0)	–7.5 (–9.6–(–)5.4)	–12.8 (–16.2–(–)9.3)
l′	M12+TBH	–13.7 (–19.0–(–)8.4)	4.6 (1.1–8.0)	–18.3 (–27.1–(–)9.5)
m′	M13+TBH	2.5 (0.7–4.2)	–3.9 (–5.5–(–)2.3)	6.3 (3.0–9.7)
n′	M14+TBH	4.8 (–0.4–9.9)	–11.7 (–12.8–(–)10.7)	16.5 (10.3–22.7)
o′	M15+TBH	3.2 (1.6–4.8)	–11.0 (–11.2–(–)10.9)	14.2 (12.8–15.6)
–	Trolox+serum	–7.2 (–20.5–6.1)	13.1 (13.0–13.3)	–20.3 (–33.8–(–)6.8)
–	DMSO+serum	–5.4 (–10.7–0.5)	0.0 (–5.5–4.2)	–3.7 (–5.4–(–)3.4)
–	TBH+serum	10.2 (0.7–21.5)	–4.4 (–10.3–0.1)	(11.0–21.4)

Most of the substances had a negative oxy score, lower then blank serum, which indicates their antioxidant potential. Compounds **M2** (*OS* –26.9, entry **b**, Table I), **M7** (*OS* –26.4, entry **g**), **M10** (*OS* –23.4, entry **j**), **M11** (*OS* –24.1, entry **k**) and **M12** (*OS* –28.1, entry **j**) had lower oxy score than Trolox (*OS* –20.3), water-soluble analogue of vitamin E with proven antioxidant activity. As an exception, the brominated pyrrole derivative **M15** (*OS* 11.6, entry **o**) stands out with a positive oxy score and may exhibit prooxidant properties, which may be a useful protective function like cytostatic, bacteriostatic and antivirotic.[30] When comparing non-halogenated and halogenated pyrrole derivatives, the non-halogenated analogues showed lower OS values for all matching pairs. The investigation of the antioxidant activity mechanism of N–H pyrroles revealed that the pyrrolyl radical adopts the 5π electron system after hydrogen atom transfer (HAT), the stability of which largely depends on the electronic properties of the substituents on the aromatic ring. It was shown that the electron-donating groups on the benzene ring of 2-arylpyrroles stabilise the generated radical and increase the antioxidant activity of the compound.[15] From this it can be concluded that the electron-accepting properties of the three bromine atoms could destabilise the pyrrolyl radical and negatively influence the antioxidant activity. Further SAR analysis demonstrated some additional facts. Non-halogenated pyrrole derivatives with tertiary amide group **M1** (*OS* –15.7, entry **a**) and **M3** (*OS* –15.7, entry **c**), obtained in the reaction with secondary amines, had less negative *OS* values than derivatives **M2** (*OS* –26.9, entry **b**), **M10** (*OS* –23.4, entry **j**), **M11** (*OS* –24.1, entry **k**) and **M12** (*OS* –28.1, entry **l**) obtained in reactions with primary amines. This is not surprising considering that in a study on the antioxidant activity of carboxamide it was shown that the N–H bond of the amide can also be a site for the abstraction of the H atom.[31] It was also interesting to compare the OS values for amides with propyl, propenyl and propynil groups. For the non-halogenated

pyrrole derivatives **M12** (*OS* –28.1, entry **l**), **M2** (*OS* –26.9, entry **b**) and **M10** (*OS* –23.4, entry **j**), the values were comparable, while for the brominated derivatives, the compound with saturated propyl group **M15** (*OS* 11.6, entry **o**) showed prooxidant activity, with a significantly higher positive OS value compared to the propenyl and propynyl derivatives **M5** (*OS* –5.1, entry **e**) and **M13** (*OS* –1.1, entry **m**). Clear evidence for the essential role of the pyrrole ring is provided by the comparison of two compounds obtained by reductive amination with vanillin, an aldehyde with a phenolic ring with proven antioxidant activity.[32] The vanillin derivative **M7** (*OS* –26.4, entry **g**), which has a pyrrole ring, showed better antioxidant properties than the benzyl derivative **M9** (*OS* –11.4, entry **i**).

OS was also determined in the presence of *t*-butyl hydroperoxide, indicating the potential of the compounds to resist oxidative stress. Preserved negativity of the *OS* in samples containing compounds **M2** (*OS* –6.1, entry **b′**), **M10** (*OS* –21.9, entry **i′**), **M11** (*OS* –12.8, entry **j′**) and **M12** (*OS* –18.3, entry **k′**) is a sign of its antioxidant strength, even in the presence of prooxidants. The change in *OS* towards positive values in other samples speaks in favor of its lower ability to respond to oxidative stress.

Oxy scores (*OS*) for all compounds are also outlined in Fig. 3 which summaries the results from both experiments (without and with TBH) after 2 h incubation at 37 °C in comparison with Trolox used as standard.

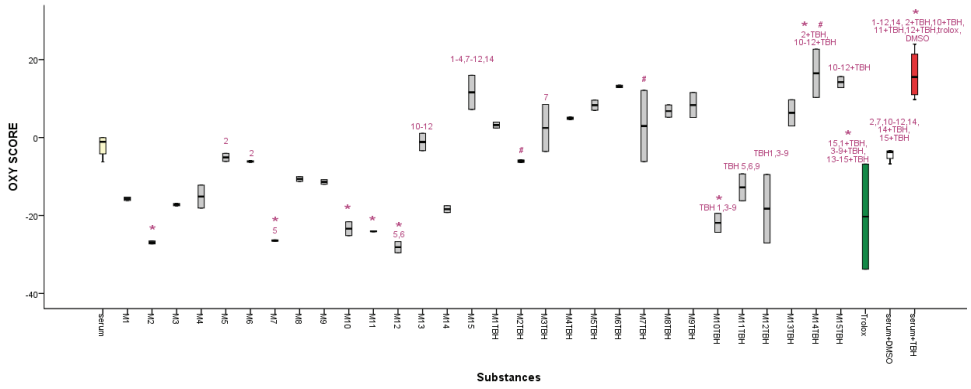


Fig. 3. Oxy score (OS) in tested substances with and without TBH, along with native serum, serum with Trolox (2000 μmol/L) and serum with TBH (0.25 mM); * – $P < 0.05$, *vs*. native serum; # – $P < 0.05$, *vs.* the same substance sample without TBH; numbers: statistically significant difference *vs*. distinct substance without or with TBH.

The lowest *OS*, observed in samples **M2**, **M7** and **M10**–**12** was the reason why we continued the dose-response analyses with these 5 samples. A water-soluble analogue of vitamin E (Trolox) was prepared in 5 concentrations: 2000,

1000, 500, 250 and 125 μmol/L in water. In addition, 3 dilutions of compounds **M2**, **M7** and **M10**–**M12** (0.5, 0.25 and 0.125 mmol/L from starting 1 mmol/L) were prepared in DMSO. The oxy scores were calculated and a graphical representation is shown in Fig. 4. A concentration dependence can be observed for all tested substances (the compounds show lower antioxidant activity, which is indicated by higher *OS* in samples with lower concentrations). A comparison of the *OS* shows that compound **M2** is a better antioxidant than compound **M7**, although both compounds have excellent antioxidant properties.

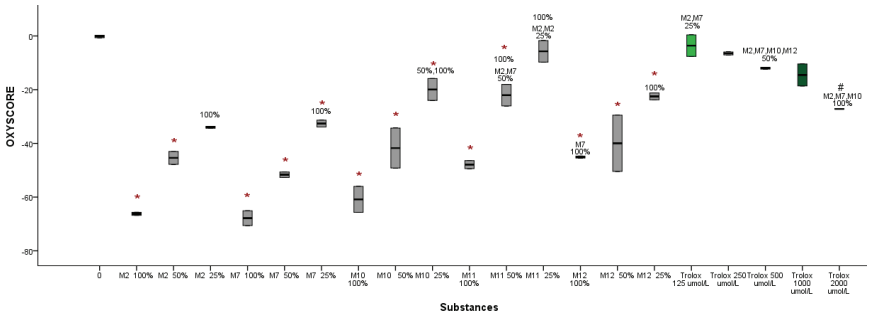


Fig. 4. Oxy score in three different concentrations of M2, M7, M10, M11 and M12 samples, so as in samples with Trolox (125–2000 μmol/L); *– $P < 0.05$ *vs.* native serum (blank); 100 %, 50 %: $P < 0.05$ *vs*. sample of the same substance with different concentration; Mx, My…Mi %: $P < 0.05$ *vs*. indicated sample of a specific dilution; # – $P < 0.05$ *vs*. Trolox (125 μmol/L).

In the case of Trolox, a concentration dependence is also observed, *i.e*., the *OS* is significantly lower in serum samples with higher concentrations of Trolox. However, compared to samples containing compounds **M2**, **M7** and **M10**, its antioxidant activity is less pronounced (higher *OS* values, although still negative).

CONCLUSION

In this work, we have described an efficient and simple method for the preparation of tribrominated and non-halogenated analogue pyrrole derivatives. Our investigation of the redox properties of the synthesized compounds shows that the tribrominated derivative **M15** exhibits strong prooxidant activity. The four non-halogenated compounds with a secondary amide group, namely **M2**, **M10**, M**11** and **M12**, have lower oxy score (*OS*) values than Trolox, an analogue of vitamin E with proven antioxidant activity. In addition, all four compounds show strong resistance to oxidative stress. A comparison of the dose-response of compounds **M2**, **M7** and **M10** with Trolox shows that the synthesized compounds exhibit better antioxidant activity than Trolox. Therefore, they can be further developed as effective antioxidants.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12931, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И *IN VITRO* СТУДИЈА РЕДОКС ОСОБИНА ХАЛОГЕНОВАНИХ И НЕХАЛОГЕНОВАНИХ ДЕРИВАТА ПИРОЛА

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Редокс равнотежа игра кључну улогу у одржавању биолошких процеса у нормалним условима. Антиоксиданси инхибирају и смањују штетне процесе оксидације, док прооксиданси могу деловати као антиканцерски агенси промовишући ћелијску смрт посредовану реактивним кисеоничним врстама. Циљ овог рада је да се упореде редокс својства седам новосинтетисаних деривата трибромопирола и нехалогенованих аналога (три новосинтетисана и четири претходно синтетисана) у *in vitro* моделу (у хуманом серуму) и са егзогено индукованим оксидативним стресом. Упоређене су добијене вредности њихових окси скорова (*OS*) и резултат је показао да четири нехалогенована деривата пирола са секундарном амидном групом **M2**, **M10**, **M11** и **M12** имају ниже вредности *OS* од Тролокса, аналога витамина Е растворљивог у води са доказаним антиоксидативним својствима. Сва четири једињења показују јаку отпорност на оксидативни стрес, што се огледа у одржавању негативних вредности *OS* када су изложене егзогеном оксидативном стресу коришћењем ТВН у реакционој смеши. Ову способност да се одупиру ROS треба очекивати и у ендогеном окружењу, где се константна производња прооксиданата одвија на ниском, хомеостатском нивоу, али још више у патолошким стањима. Трибромовани дериват **M15** је показао прооксидативну активност са значајно вишом *OS* вредношћу од свих осталих тестираних једињења. Такође, упоредне анализе доза-одговор тестираних пет једињења са најнижим *OS* и Тролокса показују да Тролокс има слабију антиоксидативну активност од једињења **M2**, **M7** и **M10**.

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