Bulgarian Chemical Communications, Volume 41, Number 1 (pp. 72–79) 2009

Synthesis of amino acid analogues of 10-methoxy dibenz[b,f]azepine and evaluation of their radical scavenging activity

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Received May 19, 2008; Revised October 6, 2008

A method for the synthesis of L-aminoacid (L-tyrosine, L-phenylalanine, L-hydroxyproline and L-threonine) analogues of 10-methoxy-dibenz[b,f]azepine is proposed. 10-Methoxy-dibenz[b,f]azepine, as a basic molecule was prepared by a known method. The key intermediate 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one**,** was obtained by N-acylation of 10-methoxy-dibenz[b,f]azepine with 3-chloro-propionylchloride. Further coupling of the respective L-aminoacids was accomplished to produce 3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz[b,f] azepin-5-yl)-3-oxopropylamino)propanoic acid, 2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)-3-phenyl-propanoic acid, 3-hydroxy-1-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropyl)pyrrolidine-2-carboxylic acid, and 3-hydroxy-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)butanoic acid**,** respectively. The synthesized compounds were evaluated in regard to their potential over the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. Butylhydroxy anisole (BHA) and ascorbic acid (AA) were used as reference antioxidant compounds and also a comparative study with the newly synthesized compounds was done. Under the present experimental conditions, the analogues containing L-tyrosine, L-hydroxyproline and L-threonine**,** possess a direct scavenging effect by trapping the stable DPPH free radical. L-Hydroxyproline analogues showed a significant radical scavenging activity among the synthesized analogues. DPPH activity of the pure L-aminoacids was also determined and a comparative study with the newly synthesized products was done. The DPPH activity of products was found to be greater than that of the L-aminoacids.

Key words: 10-methoxy-5H-dibenz[b,f]azepine, 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one, L-aminoacids, radical scavenging activity.

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INTRODUCTION

Free radicals and active oxygen species have been related to cardiovascular and inflammatory diseases, and even have a role in cancer and ageing [1–2]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicines is experiencing an upsurge of interest in medically useful antioxidants [3–4].

A series of compounds that can scavenge radicals by trapping, initiating and/or propagating radicals, are called 'antioxidants' [5]. In biological systems, the definition for antioxidants has been extended to any substance that when it is present in low concentrations, compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate [6]. There is an expanding quest for using antioxidative molecules because they have the capacity to quench free radicals, thereby protecting cells and tissues from oxidative damage. Numerous natural or synthetic antioxidant compounds have been tested with success in various disease models as well as in clinics [7]. Antioxidants are now expected as the drug candidate to combat these diseases. In the literature some tricyclic amines and their chemical structures shows antioxidant neuroprotective activity *in vitro* [8]. Nowadays, the free-radical scavenging mechanism of aromatic amines (Ar_2NHs) has been discussed from the view point of chemical kinetics [9].

10-Methoxy-5H-dibenz[b,f]azepine **1**, is common basic fused tricyclic amine, which belongs to the family of 5H-dibenz[b,f]azepine i.e., iminostilbene. It is used as an intermediate for the synthesis of the registered anticonvulsant drug oxcarbazepine [10], the structure of which has recently been reported [11]. Dibenz[b,f]azepine and its derivatives have been variously reported as having antiallergic activity, specifically antihistaminic activity, spasmolytic, serotonin antagonistic, anticonvulsive, antiemetic, antiepileptic, anti-inflammatory, sedative and fungicidal action [12]. In our earlier studies, the DPPH activity of the basic molecule i.e., 10-methoxy-5H-dibenz[b,f]azepine, was determined and reported [13]. From the studies, the basic molecule possesses significant 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity, so further we plan to synthesize its

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new analogues and try to explore the radical scavenging activity by coupling different L-aminoacids.

The research work on free radicals provides theoretical information for the medicinal development, and supplies some *in vitro* methods for quick-optimizing drugs, it attracts more scientific attention of bioorganic and medicinal chemists. In addition to the traditional O–H bond type of antioxidant, tricyclic amines having N–H bond function as antioxidant have attracted much research attention because Ar_2NHs have always been the central structure in many currently used drugs [14]. Usually phenolic compounds were found to have antioxidant and radical scavenging activity; they also inhibit LDL oxidation [15–16]. In the literature aminoacids and some of their derivatives were also found to have antioxidant activity [17].

In the present study we have used a model compound 3-chloro-1-(10-methoxy-5H-dibenz[b,f] azepine-5-yl)propan-1-one to verify the possibility of obtaining the L-aminoacid analogues of 10-methoxy-dibenz[b,f]azepine. Before coupling of different L-aminoacids to the key intermediate, their DPPH activity was evaluated. Since their structure may justify a possible intervention in free radical process we have selected some of the free L-aminoacids to explore better the chemistry and the biological activities. The L-aminoacid analogues of 10-methoxy dibenz[b,f]azepine were synthesized and their structure was established by chemical and spectral analyses. The newly synthesized compounds were investigated in regard to their *in vitro* DPPH free radical scavenging potential and compared to commercially available synthetic antioxidants namely butylated hydroxyanisole (BHA) and ascorbic acid (AA) and also with the L-aminoacids (L-tyrosine, L-phenylalanine, L-hydroxyproline and L-threonine). These studies reflect the possibility for therapeutic uses and application as a source of synthetic antioxidants.

CHEMISTRY

10-Methoxy-5H-dibenz[b,f]azepine **1**, was synthesized applying a known method [10]. The active sites for the coupling of different L-aminoacids to the basic molecule were less and also the methoxy group in the basic molecule is an important group, which can play an important role for the DPPH activity. Hence we select the N-acylation reaction in order to obtain the key intermediate in which the coupling of different L-aminoacids can be done very easily with simple experimental procedure with good yield. Here in the key intermediate $ClCH_2–CH_2–CO–$ plays an important role for the coupling of L-aminoacids. In the present study aminoacids having L-configuration were used for the coupling. The synthesis of L-aminoacid analogues of 10-methoxy-5H-dibenz[b,f]azepine was realized in two steps. In the first step, the key intermediate 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one **a**, was prepared in good yield by N-acylation of 10-methoxy-dibenz[b,f]azepine with 3-chloro-propionylchloride in the presence of triethyl amine as base (Scheme 1). In the second step, further coupling of respective L-aminoacid to the intermediate were done to obtain the L-aminoacid analogues of 10-methoxy-5H-dibenz[b,f]azepine **b–e**, (Scheme 2).

EXPERIMENTAL

Materials and methods

The following compounds and materials supplied by Sigma Aldrich, were used: L-tyrosine, L-phenylalanine, L-hydroxyproline, L-threonine and 5H-dibenz[b,f]azepine. 3-Chloro-propionylchloride, triethyl amine, benzene, methanol, chloroform, diethyl ether, acetic acid, ethyl acetate, sodium bicarbonate, anhydrous sodium sulphate were all of analytical grade of purity and procured from Merck. TLC aluminium sheets-silica gel 60 F_{254} were also purchased from Merck.

The IR spectra were recorded on a FT-IR021 model in KBr disc. The ^1H NMR spectra were recorded on Jeol GSX 400 MHz spectrophotometer using $CDCl_3$ as a solvent and the chemical shifts (δ) are in ppm relative to the internal standard. The mass spectra were recorded on Waters-Q-TOF Ultima spectrophotometer.

Synthesis of 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one

To the well stirred solution of 10-methoxy-dibenz[b,f]azepine (2 mM) and triethyl amine (2.2 mM) in 50 ml benzene, 3-chloro-propionyl chloride (2.2 mM) in 25 ml benzene was added drop by drop for about 30 min. Then the reaction mixture was stirred at room temperature for about 6 h. The progress of the reaction was monitored by TLC using 9:1 hexane:ethyl acetate mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water and extracted in diethyl ether. The ether layer was washed twice with 5% $NaHCO_3$ and once with distilled water. Finally the ether layer was dried with anhydrous Na_2SO_4. The pale-brown semi solid product was obtained by desolventation through rotary evaporator at 50°C.

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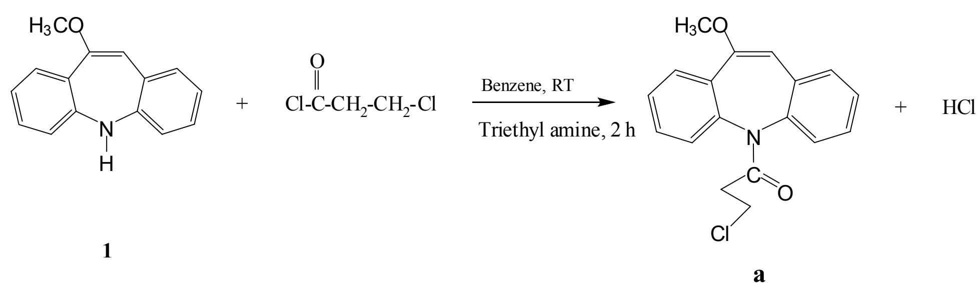

Scheme 1.

a + tyrosine: Methanol, K_2CO_3, Reflux, 8h → b + HCl

a + phenylalanine: Methanol, K_2CO_3, Reflux, 6h → c + HCl

a + 3-hydroxyproline: Methanol, K_2CO_3, Reflux, 6h → d + HCl

a + threonine: Methanol, K_2CO_3, Reflux, 6h → e + HCl

Scheme 2

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3-Chloro-1-(10-Methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one, ***a***. Pale brown semi solid, yield 86%. IRS (KBr) 2467.7–3321.6 (OH– carboxylic acid), 1687.1 (C=O), 2835.7 and 2958.4 (CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.86 (t, 2, α C=O, 2H), 3.90 (t, 2, β C=O, 2H), 5.92 (s, 1, CH, 1H), 3.71 (s, 3, OCH_3, 3H), 7.19–7.81 (m, 7, Ar–H, 7H), 8.23 (d, 1, Ar–H, 1H). MS (m/z, % abundance): 314 (M^+ 96), 312 (8), 310 (20), 308 (4), 316 (41). Anal. calc. for $C_{18}H_{16}ClNO_2$: C, 68.90; H, 5.14; N, 4.46; O, 10.20; Found: C, 68.12; H, 5.19; N, 4.30; O, 10.36%.

Synthesis of 3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino) propanoic acid

L-Tyrosine (1.2 mM) in methanol (25 mL) was neutralized with triethyl amine (1.2 mM). To this solution K_2CO_3 (600 mg) was added. Later the solution of 3-chloro-1-(10-methoxy-5H-dibenz[b,f] azepine-5-yl)propan-1-one (1 mM) in methanol (50 mL) was added drop by drop for 30 min. The reaction mixture was refluxed for 6–8 h. The progress of the reaction was monitored by TLC. The reaction mixture was then desolventized in a rotary evaporator and the compound was extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. The brown semi-solid was obtained by further desolventation in a rotary evaporator at 50°C.

L-Phenylalanine, L-hydroxyproline and L-threonine aminoacid analogues of 10-methoxy-dibenz [b,f]azepine were obtained by the same procedure. The analogues were separated and purified by column chromatography using mixture of chloroform/methanol/acetic acid 85:15:3. The products were characterized by IRS, Mass spectroscopy, ^1H NMR and elemental analysis.

3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz [b,f]azepin-5-yl)-3-oxopropylamino)propanoic acid, ***b***. Brownish semi-solid, Yield 73%; IRS (KBr): 3321.4 (N–H), 2339.4–3062.8 (OH– carboxylic acid), 1671.8 (C=O), 1599.3 and 1618.5 (CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.36 (d, 2, α C=O, 2H), 2.98 (d, 2, β C=O, 2H), 5.92 (s, 1, CH, 1H), 3.7 (s, 3, OCH_3, 3H), 6.78–8.33 (m, 12, Ar–H, 12H), 9.40 (s, 1, Ar–OH, 1H), 11.5 (s, 1, COOH, 1H), 2.40 (s, 1, NH, 1H), 4.1 (s, 1, CH, 1H), 3.11–3.40 (t, 2, CH_2, 2H). MS (m/z, % abundance): 458.31 (M^+ 97), 468 (11), 457 (47), 456 (41), 455 (40). Anal. calcd. for $C_{27}H_{26}N_2O_5$: C, 70.73; H, 5.72; N, 6.11; O, 17.45: Found: C, 70.92; H, 5.12; N, 6.71; O, 17.25%.

2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)-3-phenylpropanoic acid, ***c***. Brownish semi-solid , Yield 69%; IRS (KBr): 3318.0 (N–H), 2071.0–30628 (OH– carboxylic acid), 1675.9 (C=O), 1566.1 and 1620.3 (CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.36 (d, 2, α C=O, 2H), 2.98 (d, 2, β C=O, 2H), 5.92 (s, 1, CH, 1H), 3.7 (s, 3, OCH_3, 3H), 7.30–8.3 (m, 13, Ar–H, 13H), 11.5 (s, 1, COOH, 1H), 2.40 (s, 1, NH), 4.1 (t, 1, CH, 1H), 3.11–3.40 (t, 2, CH_2, 2H). MS (m/z, % abundance): 458.31 (M^+ 29), 441 (25), 443 (20), 440 (62). Anal. calcd. for $C_{27}H_{26}N_2O_4$ C, 73.28; H, 5.92; N, 6.33; O, 14.46; Found: C, 73.01; H, 5.87; N, 6.78; O, 14.94%.

3-hydroxy-1-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropyl)pyrrolidine-2-carboxylic acid, ***d***. Brownish semi-solid, yield 68%; IRS (KBr): 2311.4 and 3418.8 (OH– carboxylic acid), 1670.6 (C=O), 1566.3 and 1619.1 (CH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.36 (d, 2, α C=O, 2H), 2.98 (d, 2, β C=O, 2H), 5.92 (s, 1, CH, 1H), 3.7 (s, 3, OCH_3, 3H), 7.19–7.81 (m, 7, Ar–H, 7H), 11.5 (s, 1, COOH, 1H), 1.72–1.95 (m, 2, CH_2, 2H), 2.25–2.35 (q, 2, CH_2, 2H), 3.80 (q, 1, CH, 1H), 3.31 (d, 1, CH, 1H). MS (m/z, % abundance): 408 (M^+ 61), 410 (45), 406 (2), 404 (2), 410 (10). Anal. calcd. for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33; O, 14.46; Found: C, 73.56; H, 5.84; N, 6.28; O, 14.11%.

3-hydroxy-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)butanoic acid, ***e***. Brownish semi-solid, yield 71%. IRS (KBr): 3308.8 (N–H), 2748.1–3066.4 (OH-carboxylic acid), 1676.8 (C=O), 1566.1and 1619.4 (CH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.36 (d, 2, α C=O, 2H), 2.98 (d, 2, β C=O, 2H), 5.92 (s, 1, CH, 1H), 3.7 (s, 3, OCH_3, 3H), 7.19–7.81 (m, 7, Ar–H, 7H), 11.5 (s, 1, COOH, 1H), 5.1 (s, 1, OH, 1H), 4.1 (t, 1, CH, 1H), 1.22 (d, 3, CH_3, 3H), 3.65 (d, 1, CH, 1H). MS (m/z, % abundance): 396 (M^+ 15), 397 (17), 399 (12), 400 (15). Anal. calcd. for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86; O, 19.59%; Found: C, 67.79: H, 5.89; N, 6.74; O, 19.01%.

Radical scavenging activity

In the present study, the newly synthesized compounds were screened in regard to their DPPH free radical scavenging activity. The DPPH evaluation of different L-aminoacids (L-tyrosine, L-phenylalanine, L-hydroxyproline and L-threonine) was also carried out and a comparative study towards newly synthesized products was also done. The compounds under studies were dissolved in distilled ethanol (50 mL) to prepare 1000 μM solution. Solutions of different concentrations (10, 50, 100, 200 and 500 μM) were prepared by serial dilution and the free radical scavenging activity was studied.

The DPPH radical scavenging effect was evaluated according to the method first employed by Blois [18]. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each

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compound solution having different concentrations (10, 50, 100, 200 and 500 μM). These were taken in different test tubes, 4 mL of a 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in a dark room at room temperature for 20 min. A DPPH blank was prepared without any compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining concentration of DPPH was calculated. The percentage decrease in the absorbance was recorded for each concentration, and the percentage quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

$$\textit{Radical scavenging activity} = [(A_0 - A_1)/A_0]\times 100 ,$$

where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The radical scavenging activities of BHA and ascorbic acid were also measured and compared with that of the newly synthesized compound. The % DPPH activities for all the pure L-aminoacids and newly synthesized compounds were determined and showed in Figures 1 and 2. On the other hand, the half inhibition concentration (IC_{50}) for all the newly synthesized compounds and L-aminoacids including the reference antioxidants was calculated graphically using a linear regression algorithm and showed in the Table 1.

RESULTS AND DISCUSSIONS

The key intermediate 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one was prepared with a good yield by N-acylation reaction. In practice, 10-methoxy 5H-dibenz[b,f]azepine and 3-chloro-propionyl chloride were mixed at 1:1.2 ratio. Triethylamine was used to maintain basic conditions. The reaction was carried out for 6 h at room temperature. The final product was separated from the reaction mixture by washing the HCl salt (precipitate) with distilled water and the compound was extracted with diethyl ether. Further, it was washed with 5% $NaHCO_3$ to remove the remaining quantity of acids. Then the organic phase was separated and desolventized by vacuum-distillation and finally the product was isolated through column chromatography by using 9:1 hexane:ethyl acetate mixture as mobile phase.

The direct inclusion of L-aminoacids to 10-methoxy-5H-dibenz[b,f]azepine was unfavourable, because here the active sites for the direct inclusion are less and the experimental procedure was difficult, which leads to the major disadvantages for direct coupling of L-aminoacid. So, to overcome these problems, a key intermediate was needed to couple L-aminoacid. Thus we synthesized 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one, where the inclusion of L-aminoacids can be done very easily with simple experimental procedure resulting good yield.

Aminoacid analogues of 10-methoxy-5H-dibenz [b,f]azepine were obtained by reacting respective L-aminoacid:3-chloro-1-(10-methoxy-5H-dibenz[b,f] azepine-5-yl)propan-1-one = 1.2:1 mM. The respective L-aminoacids were obtained dissolved in methanol. 600 mg of anhydrous K_2CO_3 was added and stirred for 30 min. 3-Chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one in methanol was added drop by drop and refluxed for 6 h. The reaction was monitored by TLC. The methanol containing the final product was desolventized by vacuum-distillation and the product was extracted with ethyl acetate. Further, the ethyl acetate layer was washed with water to remove K_2CO_3 and dried over anhydrous Na_2SO_4. Desolventation by vacuum-distillation was done and the final product was isolated by column chromatography by using chloroform/methanol/acetic acid = 85: 15: 3 as mobile phase.

The newly synthesized compounds were screened in regard to their DPPH free radical scavenging activity. The DPPH test provided information about the reactivity of the tested compounds with a stable free radical. Because of its odd electron, the DPPH radical showed a strong absorption band at 517 nm in visible light region (a deep purple color). As this electron is paired off in the presence of a free radical scavenger, the absorption band vanishes and the resulting discolouration is stochiometric with respect to the number of electrons taken up. This bleaching of DPPH colouring, which occurs in the odd electron of the radical is paired, thus it is representative of the capacity of the compounds to scavenge free radicals independently. Initially, before coupling the L-aminoacids to the key intermediate the DPPH activity for the respective L-aminoacids was evaluated. Some comparative studies including standard antioxidants and the newly synthesized products were also done. Our study reveals that the L-aminoacids have potential with respect to DPPH activity showing slightly lower activity than the products and standard antioxidants.

Figure 1 illustrates the DPPH activity – L-aminoacids like L-tyrosine and L-hydroxyproline exhibited good activity, L-threonine showed average activity and L-phenylalanine showed negligible

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activity. All the L-aminoacids showed lower activity than the standards like ascorbic acid and BHA. Increase in activity was observed after coupling these L-aminoacids to the intermediate. This may be due to the presence of OCH_3, in the basic molecule. Keeping standard antioxidants in mind, comparative studies with the standard antioxidants (Ascorbic acid and **BHA**) and the newly synthesized compound were done. The DPPH free radical scavenging ability of the newly synthesized compounds is showed in Figure 2.

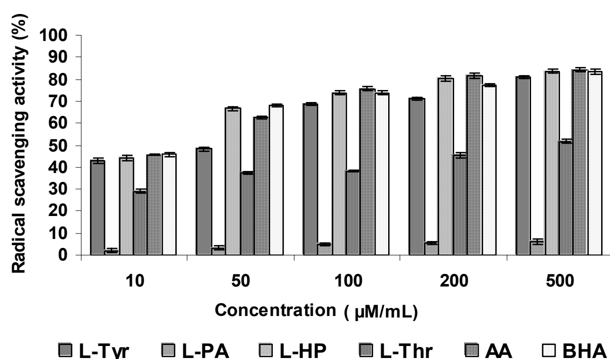

Fig. 1. DPPH free radical scavenging ability of respective L-aminoacids. Values represent arithmetic means ± SD (n = 3). Where: L-Tyr = L-tyrosine, L-PA = L-phenylalanine, L-HP = L-hydroxyproline, L-Thr = L-threonine, AA = Ascorbic acid and BHA = Butylated hydroxyanisole.

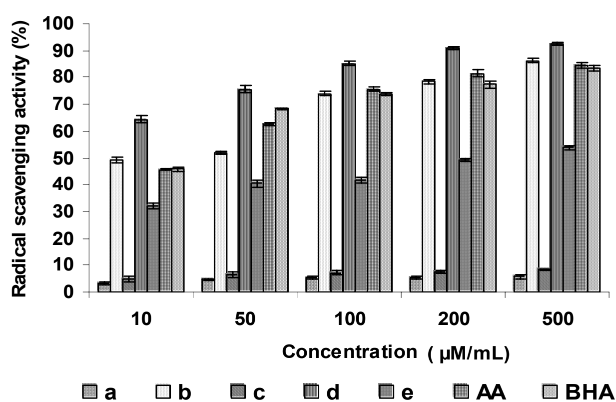

Fig. 2. DPPH free radical scavenging ability of 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one and its L-aminoacid analogues.
Values represent arithmetic means ± SD (n = 3).
The notations are: **a**. 3-chloro-1-(10-methoxy-5H-dibenz [b,f]azepine-5-yl) propan-1-one; **b**. 3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino) propanoic acid; **c**. 2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)-3-phenyl-propanoic acid; **d**. 3-hydroxyl-1-(3-10-methoxy-5H-dibenz[b,f]azepine-5yl)-3-oxopropyl)pyrrolidine-2-carboxylic acid; **e**. 3-hydroxy-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropyl amino)butanoic acid. AA - ascorbic acid; BHA - butylated hydroxyanisole.

The half inhibition concentration (IC_{50}) for all the L-aminoacids and newly synthesized compounds including standard antioxidants is summarized in the Table 1 and Table 2.

Table 1. 50% Inhibition of DPPH radical by L-aminoacid including standard antioxidants. The sign (–) corresponds to negligible activity. Values represent arithmetic means ± SE (n = 3). AA - ascorbic acid; BHA - butylated hydroxyanisole.

Compound	IC_{50}, µM/mL
L-Tyrosine	47±0.90
L-Phenylalanine	–
L-Hydroxyproline	4.1 ±0.87
L-Threonine	176.0± 1.2
AA	4.94 ± 0.63
BHA	5.26 ± 0.87

Table 2. 50% inhibition of DPPH radical by 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one and its L-aminoacid analogues. The sign (–) corresponds to negligible activity. AA - ascorbic acid; BHA - butylated hydroxyanisole.

Compound	IC_{50}, µM/mL
a	–
b	40 ± 1.02
c	–
d	2.9 ± 0.92
e	160.0 ± 1.21
AA	4.94 ± 0.63
BHA	5.26 ± 0.87

IC_{50} - concentration required for 50% reduction of 0.1 mM DPPH radical. Values represent arithmetic means ± SE (n=3). The notations are: **a** - 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one; **b** - 3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)propanoic acid; **c** - 2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)-3-phenylpropanoic acid; **d** - 3-hydroxyl-1-(3-10-methoxy-5H-dibenz[b,f]azepine-5yl)-3-oxopropyl)pyrrolidine-2-carboxylic acid; **e** - 3-hydroxy-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3oxopropylamino)butanoic acid. AA – Ascorbic acid; BHA - Butylated hydroxyanisole.

Initially the DPPH free radical scavenging capacity of 3-chloro-1-(10-methoxy-5H-dibenz[b,f] azepine-5-yl)propan-1-one **a**, was assessed and found not to be effective. Consequently, molecules with L-aminoacid groups were coupled to enhance the radical scavenging activity effect. Analogues of L-tyrosine **b**, L-hydroxyproline **d**, and L-threonine **e**, aminoacids showed promising radical scavenging activity with a major activity for L-hydroxyproline analogues **d**. When L-tyrosine molecule is coupled to 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one **a,** the activity will be enhanced due to the presence of phenolic group. The activity disappears due to the absence of –OH group in L-phenylalanine. When L-hydroxyproline is coupled to the intermediate, the RSA increases with a major activity due to the presence of –OH group, attached to the five member heterocyclic ring. May be the presence of free –OH group in L-threonine showed

the average activity over all the analogues. These results showed the major importance of the L-aminoacid substituent in DPPH free radical effects. An increasing order of DPPH activity for the synthesized compounds can be given as follows:

3-hydroxyl-1-(3-10-methoxy-5H-dibenz[b,f]azepine-5yl)-3-oxopropyl)pyrrolidine-2-carboxylic acid, **d** > 3-(4-hydroxyphenyl)-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)propanoic acid, **b** > 3-hydroxy-2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3oxopropylamino)butanoic acid, **e** > 2-(3-(10-methoxy-5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino)-3-phenyl propanoic acid, **c** > 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl) propan-1-one, **a**. The synthesized compounds were compared to internal standards (AA and BHA). Among the synthesized compounds L-tyrosine analogues **b**, showed almost equal activity compared to standards and L-hydroxyproline analogues **d**, showed more effective DPPH activity than the standards.

CONCLUSION

The method, proposed by us, reproduces the synthesis of L-aminoacid analogues of 10-methoxy-5H-dibenz[b,f]azepine. The synthesized compounds were characterized with the help of spectroscopic technique and were screened in regard to their ability to DPPH radical scavenging activity. Based on the biological assay it was found that the key intermediate 3-chloro-1-(10-methoxy-5H-dibenz[b,f] azepine-5-yl)propan-1-one **a**, showed no effect towards DPPH activity, but the moiety of 3-chloro-1-(10-methoxy-5H-dibenz[b,f]azepine-5-yl)propan-1-one containing L-tyrosine **b**, L-hydroxyproline **d,** and L-threonine **e,** showed promising DPPH radical scavenging activity. However, L-hydroxyproline analogue **d**, was found to have most effective radical scavenging activity compared to the other analogues and also internal standards (AA and BHA). The comparative studies on DPPH activity of L-aminoacids with the newly synthesized products reveal that L-aminoacids possess lower DPPH activity compared to the respective products and the standard antioxidants. Our study provides evidence that several L-aminoacid analogues of 10-methoxy-5H-dibenz[b,f]azepine exhibit interesting direct DPPH free radical activity. These effects may be useful in the treatment of pathologies, in which free radical oxidation plays a fundamental role.

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СИНТЕЗ НА АМИНОКИСЕЛИННИ АНАЛОЗИ НА 10-МЕТОКСИ-ДИБЕНЗ[b,f]АЗЕПИН И ОЦЕНКА НА ТЯХНАТА АКТИВНОСТ КАТО АНТИОКСИДАНТИ

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Постъпила на 19 май 2008 г.; Преработена на 6 октомври 2008 г.

(Резюме)

Предложен е метод за синтез на L-аминокиселинни (L-тирозинов, L-фенилаланинов, L-хидроксипролинов и L-треонинов) аналози на 10-метокси-дибенз[b,f]азепин. 10-Метокси-дибенз[b,f]азепин като основна молекула е синтезиран по известен метод. Ключовото междинно съединение 3-хлоро-1-(10-метокси-5H-дибенз[b,f]азепин-5-ил)пропан-1-он е получено чрез N-ацилиране на 10-метокси-дибенз[b,f]азепин с 3-хлоро-пропионилхлорид. Проведено е последващо сдвояване на съответните L-аминокиселини до получаване съответно на 3-(4-хидроксифенил)-2-(3-(10-метокси-5H-дибенз[b,f]азепин-5-ил)-3-оксопропиламино)пропанова киселина, 2-(3-(10-метокси-5H-дибенз[b,f]азепин-5-ил)-3-оксопропиламино)-3-фенилпропанова киселина, 3-хидрокси-1-(3-(10-метокси-5H-дибенз[b,f]азепин-5-ил)-3-оксопропил)пиролидин-2-карбоксилна киселина и 3-хидрокси-2-(3-(10-метокси-5H-дибенз[b,f]азепин-5-ил)-3-оксопропиламино)бутанова киселина. Оценена е активността на получените съединения като антиоксиданти по отношение на 1,1-дифенил-2-пикрилхидразил (ДФПХ) свободни радикали. Бутилхидроксианизол (БХА) и аскорбинова киселина са използвани като стандартни антиоксиданти и е направено сравнение с новополучените съединения. При използваните експериментални условия, аналозите съдържащи L-тирозин, L-хидроксипролин и L-треонин имат пряко действие като анти-оксиданти чрез улавяне на стабилния ДФПХ радикал. L-хидроксипролиновите аналози показват значителна активност като анти-оксиданти сред синтезираните аналози. Активността на чистите L-аминокиселини спрямо ДФПХ също е оценена и е сравнена с тази на синтезираните продукти. Установено е, че продуктите проявяват по-висока антиоксидантна активност спрямо тази на L-аминокиселините.

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