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# Synthesis and evaluation of antibacterial and antioxidative activities of carbazole derivatives

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Seven compounds were synthesized by known methods, and their antibacterial activity was evaluated against *Bacillus subtilis* and *Escherichia coli* using a disk diffusion method. Antioxidative activity was evaluated using free 1,1-diphenyl-2-picryl-hydrazyl radical scavenging assay and ferric reducing antioxidant power methods. The disk diffusion method revealed that 6 out of 7 tested compounds showed antibacterial activity against tested strains, they inhibited the growth of bacteria at various concentrations, from 31.25 to 250 μg/ml. 3-Cyano-9*H*-carbazole, 3-iodo-9*H*-carbazole and 3,6-diiodo-9*H*-carbazole showed a stronger antibacterial activity against *Bacillus subtilis* compared to the reference drug amoxicillin. 1,3,6-Tribromo-9*H*-carbazole showed a stronger activity against *Escherichia coli.* All tested compounds showed a weak antioxidative activity by the 1,1-diphenyl-2-picryl-hydrazyl radical scavenging assay and ferric reducing antioxidant power assay methods.

**Keywords:** antibacterial agents, antioxidative activity, carbazole, disk diffusion method, *Bacillus subtilis* and *Escherichia coli*

## INTRODUCTION

Even though there are many antibacterial agents, multidrug resistant bacteria pose a huge threat to public health and have become one of the biggest health problems in the last decade. Therefore, there is an urgent need to develop and provide novel and more potent antibacterial agents to overcome drug resistance [1].

Carbazole and its derivatives are an important type of nitrogen-containing aromatic heterocyclic compounds that can be found in the nature or synthesized by various methods. In nature carbazole is found as an alkaloid that is isolated from various parts of the plant [2]. Such carbazole ring containing alkaloids are carbomycins that

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were first isolated from *Streptoverticillium ehimense* and showed a good activity against various organisms, which made carbazoles a desirable target for further biological research [3]. Carbazole and its derivatives are extensively used in various chemistry fields such as photoelectrical materials, dyes and supramolecular recognition [4–6]. Also, it is known that natural and synthesized carbazole derivatives have shown good biological activities.

It has been observed that carbazole compounds exhibit multiple mechanisms of antibacterial activity action. One is that carbazole compounds increase membrane permeability by inhibiting specific enzymatic processes. Increased penetration of free radicals violates integrity of bacterial cells [7]. Second, the mechanism of action is that carbazole compounds can interact with bacterial DNA by forming non-covalent interactions with DNA gyrase [8]. Therefore, various N and C substituted carbazoles are attractive targets to develop and produce new antibacterial agents with two possible antibacterial mechanisms of action, that could help resolve the drug resistance problem.

Even though scientific and clinical communities made a big step towards understanding how to fight with various bacterial infections, the emergence of multidrug resistant bacteria such as *Escherichia coli* [9], which remains a frequent cause of various urinary tract, enteric and systemic infections in humans [10], continues to present challenges to human health.

In this work, we have chosen known carbazole compounds with appropriate halogens, cyano and alkyl groups at various carbazole positions to evaluate their antibacterial activities against Gram-negative, *Escherichia coli*, and compare it to Gram-positive, *Bacillus subtilis*, as possible lead structures for further development and research of antibacterial agents; as well as to evaluate and compare the influence of different substitutes and different carbazole ring positions on antioxidant properties, seeing that there was no published information about antioxidant activities of selected compounds.

## EXPERIMENTAL

### Materials and methods

9*H*-Carbazole (**I**), 4,4'-dibromobiphenyl (**III**), 9-ethyl-9*H*-carbazole (**5**) and 1,6-dibromohexane were purchased from Sigma Aldrich.

$^{1}$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded on a Varian Unity Inova 300 apparatus.

### Synthesis

**3-Bromo-9*H*-carbazole (II)**. A solution of *N*-bromosuccinimide (NBS) (4.48 g, 23.92 mmol) in 10 mL DMF was added slowly to a solution of 9*H*-carbazole (**I**) (4 g, 0.02392 mol) in dichloromethane (75 mL) in a two-necked round bottom flask. The reaction mixture was stirred for 3 h at room temperature. The mixture was extracted with $H_2O$ and dried over anhydrous $MgSO_4$ for 12 h and filtered. The excess of the solvent was evaporated and dried under vacuum. The final compound was obtained in 98% (5.76 g) as a white solid. M. p. 188–189°C. $^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.25 (m, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.43 (m, 1H), 7.5 (d, *J* = 6.6 Hz, 1H), 8.0 (d, *J* = 7.8 Hz, 1H), 8.08 (s, 1H), 8.1 (s, 1H), 8.18 (d, *J* = 4 Hz, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 139.9, 138.1, 128.6, 126.7, 125.2, 123.4, 122.5, 120.6, 120.0, 112.3, 112.1, 110.9.

**3-Cyano-9*H*-carbazole (1a)** [11]. A solution of 3-bromo-9*H*-carbazole (**II**) (23.08 g, 94 mmol) and cuprous cyanide (9.33 g, 0.103 mol) in *N*-methylpyrrolidone (300 ml) was heated at 200°C for 5 h. The cooled reaction mixture was poured on to water (600 ml) and the precipitate was filtered off and washed with ethyl acetate (3 × 50 ml). The filtrate was extracted with ethyl acetate (3 × 250 ml) and the combined ethyl acetate extracts were washed with water (150 ml) and brine (150 ml), dried using $MgSO_4$ and concentrated in vacuum. The residue was crystallized from heptane and recrystallized from acetonitrile (70 ml) acquiring 7.16 g, 40% of **1a** as a white solid. M. p. 180–181°C. $^{1}$H NMR (300 MHz, DMSO) δ 11.91 (s, 1H), 8.85 (s, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.78 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H). $^{13}$CNMR (75 MHz, DMSO): δ 142.6, 141.2, 129.5, 127.9, 126.5, 123.6, 122.5, 121.9, 121.5, 120.8, 113.0, 112.5, 101.1.

**4,4'-Dibromo-2-nitrobiphenyl (IV).** 4,4'-Dibromobiphenyl (III) (10 g, 32 mmol) was dissolved in glacial acetic acid (120 mL), and the mixture was stirred and heated to 100°C for 24 h. Then, fuming concentrated nitric acid (95%, 40 mL) was added and the resulting mixture was allowed to react for another 30 min. After the reaction, the solution was cooled to room temperature, the crude product

was filtered. After recrystallization from ethanol, the title compound was obtained in 91% yield as white crystals. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, $J$ = 1.8 Hz, 1H), 7.76 (dd, $J_1$ = 8.1 Hz, $J_2$ = 1.8 Hz, 1H), 7.54–7.59 (m, 2H), 7.31 (s, 1H), 7.14–7.18 (m, 2H).

**2,7-Dibromo-9*H*-carbazole (V).** 7.8 g (22 mmol) of 4,4'-dibromo-2-nitrobiphenyl (IV) was dissolved in phosphorous acid triethyl ester (30 mL) and the mixture was heated to 150°C under the protection of argon. The system was allowed to react for 24 h and a brown solution was obtained. The volatile solvents were then removed by vacuum distillation. The solution left was purified by column chromatography with ethyl acetate/petroleum ether (10:1, v/v) as the eluent. Finally, a white solid was obtained for 2,7-dibromocarbazole (**V**) in 48% yield. $^1$H NMR (300 MHz, acetone-$d_6$): δ 10.64 (s, 1H), 8.09 (d, $J$ = 8.4 Hz, 2H), 7.75 (d, $J$ = 1.8 Hz, 2H), 7.37 (dd, $J$ = 8.4, 1.8 Hz, 2H).

**2,7-Dicyano-9*H*-carbazole (1b)** [12]. Copper(I) cyanide (CuCN) (14.7 g, 164.3 mmol) was added to a stirred solution of 2,7-dibromo-9*H*-carbazole (IV) (12.28 g, 37.75 mmol) in DMF (150 mL). The solution was maintained at reflux under nitrogen for 9 h. In the reaction mixture 40 mL of ethylenediamine and 20 g of CuCN were used to give a white powder (7.92 g, 96.6%). M. p. 279–281°C. $^1$H NMR (300 MHz, acetone-$d_6$): δ 11.3 (br, 1H), 8.46–8.43 (d, 2H), 8.06 (s, 2H), 7.62–7.59 (d, 2H). $^{13}$C NMR (75 MHz, acetone-$d_6$): δ 141.0, 126.1, 123.4, 123.1, 120.1, 116.8, 110.7.

**3-Iodo-9*H*-carbazole (2a)** [13]. 9*H*-carbazole (**I**) (16.7 g, 101 mmol) was dissolved in boiling glacial acetic acid (250 mL) and KI (11.73 g, 135 mmol) was added. The solution was cooled, ground potassium iodate (23.42 g, 150 mmol) was added, and the mixture was boiled until it acquired a clear straw-coloured tint (10 min). The hot solution was decanted from the undissolved potassium iodate, and it was cooled to 45°C. The faintly brown plates were rapidly filtered off and recrystallized from alcohol, and the solution was allowed to cool to 45°C. The faintly brown plates were rapidly filtered off and recrystallized from ethanol; the solution was allowed to cool to 45°C and filtered, yielding 9.73 g, 47% of **2a** as a brown solid. M. p. 202°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.11 (s, 1H), 8.04 (d, $J$ = 1.7 Hz, 1H), 7.68 (dd, $J$ = 1.7 Hz, 1H), 7.42–7.49 (m, 2H), 7.22–7.28 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 139.5, 138.6, 134.1, 129.2, 126.6, 125.9, 122.1, 120.5, 119.9, 112.6, 110.7.

**3,6-Diiodo-9*H*-carbazole (2b)** [13]. To a solution of 9*H*-carbazole (I) (5.00 g, 30.43 mmol) in acetic acid (85 mL) potassium iodide (6.67 g, 40.17 mmol) was added. With stirring, potassium iodate (9.77 g, 45.65 mmol) was slowly added into the mixture and refluxed for 10 min. The reaction mixture was cooled to room temperature, filtered and washed with acetic acid (50 mL) to yield in 96% (11.94 g) as pale-yellow powder. M. p. 208–211°C. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, $J$ = 1.6 Hz, 2H), 8.07 (s, 1H), 7.67 (d, $J$ = 8.8 Hz, 1.6 Hz, 2H), 7.20 (d, $J$ = 8.4 Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 138.4, 134.7, 129.2, 123.4, 112.6, 82.4.

**1,3,6-Tribromo-9*H*-carbazole (3)** [14]. To a stirred solution of 9*H*-carbazole (I) (1 mmol) in dichloromethane (50 mL), containing silica (10 g), a solution of NBS (0.890 g, 5.0 mmol) in dichloromethane (75 mL) was added dropwise. The reaction mixture was stirred for appropriate time in the absence of light at ambient temperature until TLC indicated that it was complete. The reaction mixture was then filtered, and the silica washed with dichloromethane (3 × 15 mL). The combined extracts were washed with water (100 mL) and the organic layer was dried and evaporated to yield **3** as a white solid in 96%. M. p. 172–173°C. $^1$H NMR (300 MHz, acetone-$d_6$): δ 7.50 (d, $J$ = 8.0 Hz, 1H), 7.54 (d, $J$ = 8.0 Hz, lH), 7.67 (s, 1H); 8.26 (s, 2H); 10.70 (s, 1H). $^{13}$C NMR (75 MHz, acetone-$d_6$): δ 105.19, 112.1, 113.1, 114.2, 123.3, 124.4, 124.8, 125.5, 130.5, 131.1, 138.5, 139.8.

**1,6-Di(3-iodo-9*H*-carbazolyl)hexane (4)** [15]. A mixture containing (12 g, 41 mmol) of 3-iodo-9*H*-carbazole (**2a**), (3.9 g, 16 mmol) of 1,6-dibromohexane, and (0.4 g, 1.2 mmol) of KOH were heated to reflux in 70 mL of acetone, and then (1.8 g, 32 mmol) of powdered potassium hydroxide was added. After refluxing for 12 h, during which time a white precipitate formed, acetone was removed, and the reaction product was dissolved in chloroform. The obtained suspension was filtered, and the solution was washed with water. After removal of the solvent, the product was purified by two crystallizations from acetone to yield 4.7 g, 44% of white crystals. $^1$H NMR (300 MHz, $CDCl_3$): δ 1.24–1.31 (m, 4H), 1.67–1.82 (m, 4H), 4.15 (t, $J$ = 6.9 Hz, 4H), 7.04 (d, $J$ = 8.8 Hz, 2H), 7.16–7.64 (m, 8H), 7.99 (d, $J$ = 7.8 Hz, 2H), 8.35 (s, 2H).

**Evaluation of antibacterial activity**

Antibacterial activity of the compounds was tested using the disk diffusion method [16]. In this study inhibition of bacterial growth was investigated against Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli*. The solution (1 mg/ml) of the compounds was prepared in DMSO and then diluted to various concentrations (31.25–1000 μg/ml) in DMSO. Bacterial cultures were cultivated in Petri dishes at 37°C for 24 h on the Luria-Bertani (LB) agar medium [17]. 50 μL inoculum containing bacterial cells were spread across the LB agar medium. Sterile filter paper disks were soaked in 25 μL of each compound solution, and then the disks were put on the LB agar medium. Amoxicillin and ciprofloxacin were used as positive control, and DMSO was used as the negative control. Petri dishes were incubated aerobically at 37°C and examined for zones of inhibition after 24 h. The inhibition zones were measured using a ruler (Fig. 1).

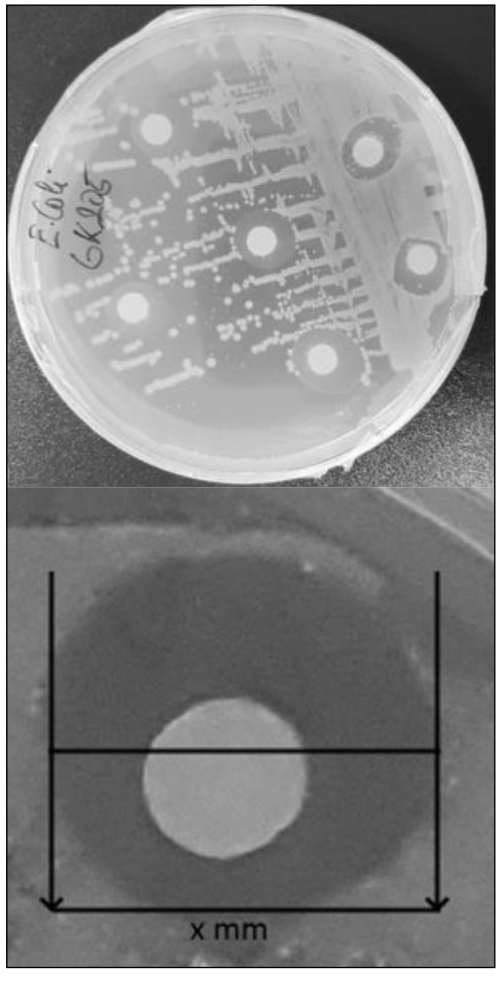



**Fig. 1.** Top: disk placement onto a Petri dish. Bottom: bacterial growth inhibition zone evaluation

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay**

The free radical scavenging activity of the compounds was measured by the DPPH method [18]. At first, a solution (5 mM) of the compounds was prepared in DMSO. Then, a 1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was added to the solutions of the selected compounds. The mixture was vigorously stirred and allowed to stand at room temperature. After 20 min, the absorbance of the reaction mixture was measured at 517 nm with a UV-1280 spectrophotometer (Shmidazu). The activity of DPPH radical scavenging was calculated according to the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_b - A_a)}{A_b} \cdot 100.$$

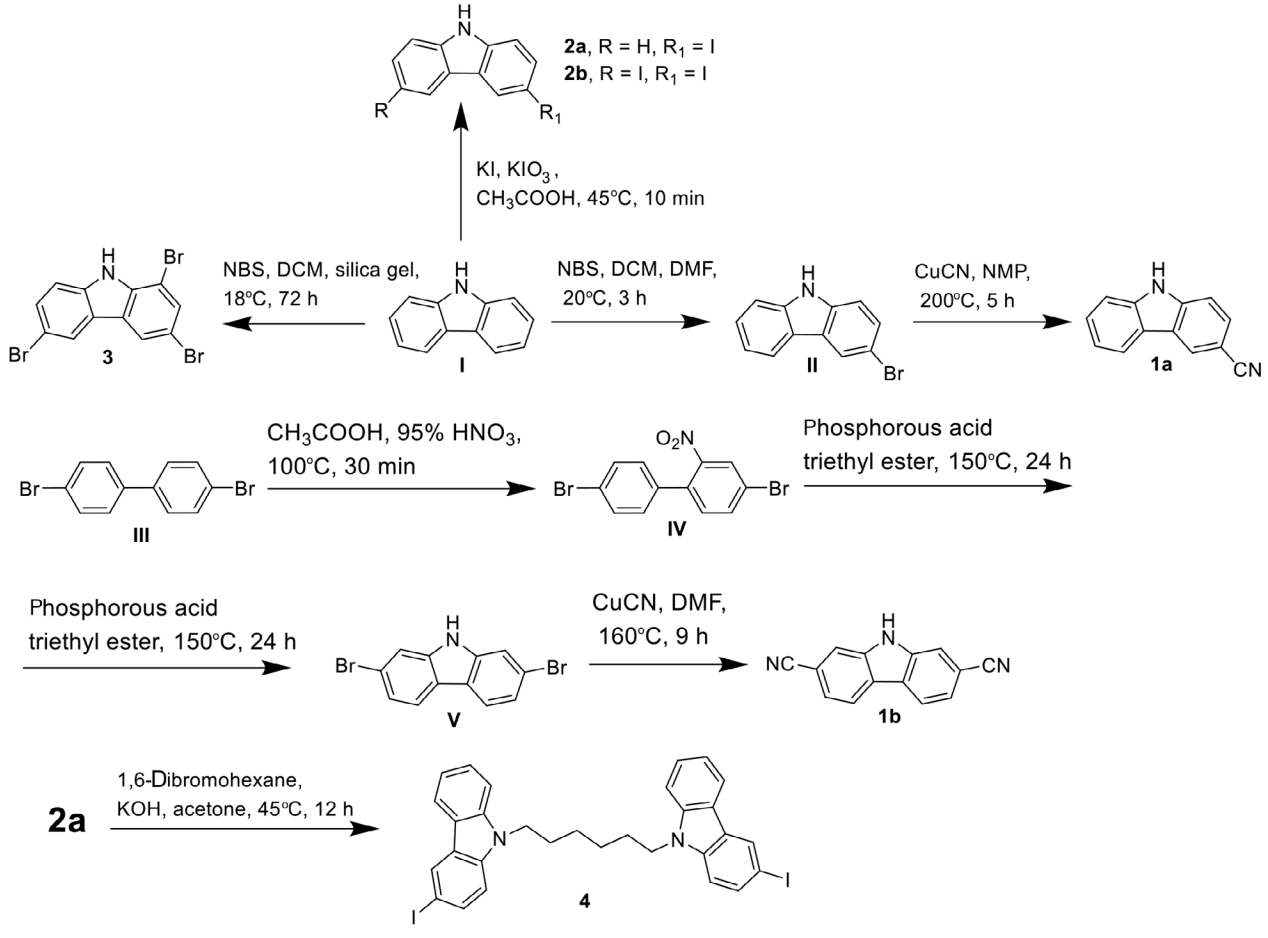
Here $A_b$ is the absorbance of the control reaction, and $A_a$ is the absorbance in the presence of the compounds. Each experiment was repeated three times.

**Ferric reducing antioxidant power assay (FRAP)**

Reducing properties were investigated using the FRAP method, which is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous coloured form in the presence of antioxidants [19]. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, also 2.5 ml of $FeCl_3$ (20 mM) and 25 ml of acetate buffer (0.3 M, pH = 3.6). 100 μL of the tested compounds (5 mM) were mixed with 3 mL of the FRAP reagent. Afterwards, the reaction mixture was incubated at 37°C. After 24 h, the absorbance of the reaction mixture at 593 nm was measured spectrophotometrically. For comprising of the calibration curve, five concentrations of $FeSO_4 \cdot 7H_2O$ (5, 10, 15, 20, 25 μM) were used and the absorbances were measured as a sample solution [20]. Each experiment was repeated three times.

## RESULTS AND DISCUSSION

A series of carbazole-based derivatives have been designed and synthesized (Scheme, Table 1), and their antibacterial activity was evaluated against *Bacillus subtilis* and *Escherichia coli*. Various functional




**Scheme.** Synthetic routes to compounds **1a–4**

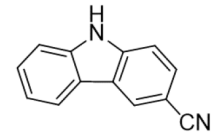
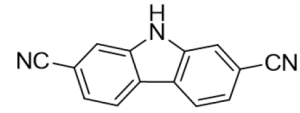
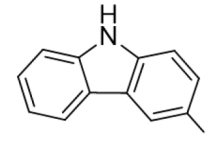
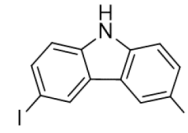
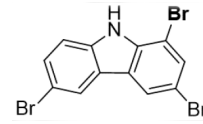
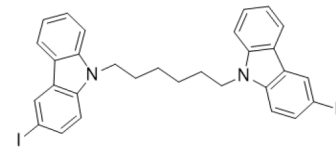
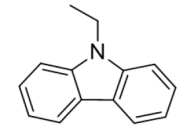
groups were introduced into carbazole-based compounds in order to investigate their biological activity.

Under the standard reaction conditions, when the mixture of **II**, CuCN in NMP was stirred in air at 155°C for 22 h, 3-cyano-9*H*-carbazole (**1a**) was isolated in 30% yield. The reaction of dibromide **V** with CuCN in DMF gave 2,7-dicyano-9*H*-carbazole (**1b**) within 9 h. Other materials 3-iodo-9*H*-carbazole (**2a**) and 3,6-diiodo-9*H*-carbazole (**2b**) were synthesized from commercially available 9*H*-carbazole (**I**) by Tucker iodination with $KI/KIO_3$ in acetic acid. Production of 1,3,6-tribromo-9*H*-carbazole (**3**) provided a simple and clean synthesis of 9*H*-carbazole (**I**) by use of three equivalents of NBS/silica. 9,9'-(Hexane-1,6-diyl)bis(3-iodo-9*H*-carbazole) (**4**) was synthesized by the alkylation reaction with an excess amount of 1,6-dibromohexane in acetone. All derivatives (**1a, 1b, 2a, 2b, 3** and **4**) were characterized by using $^{1}H$ NMR, $^{13}C$ NMR spectroscopies and mass spectrometry.

Compounds **1a–5** were evaluated for their antibacterial activity against the strains of *Bacillus subtilis* and *Escherchia coli* by the disk diffusion method. The antibacterial activity of the tested compounds was compared with the activity of the known antibiotics – amoxicillin, 62.50 μg/ml (171.1 μM) and ciprofloxacin, 3.90 μg/ml (11.8 μM).

Carbazole-based compounds **1a**, **2a** and **2b** were the most active against Gram-positive *Bacillus subtilis*, they showed inhibitory activity at 31.25 μg/ml (162.6, 106.6, 74.6 μM) (Table 2). These compounds showed a greater activity than that of amoxicillin, which was active only at 62.5 μg/ml (171.1 μM). Brominated carbazole compound **3** was slightly less active, 62.5 μg/ml (154.8 μM), but still comparable to amoxicillin. Against Gram-negative *Escherichia coli* strains the selected compounds were slightly less active, but still showed good results. Three -Br groups containing carbazole compound **3** were the most active at 31.25 μg/ml (77.4 μM), it showed a greater activity than that of

Table 1. **Structures of the carbazolyl fragment containing compounds 1a–5**

| Abbreviation | Name | Structure |
|---|---|---|
| **1a** | 3-Cyano-9*H*-carbazole |  |
| **1b** | 2,7-Dicyano-9*H*-carbazole |  |
| **2a** | 3-Iodo-9*H*-carbazole |  |
| **2b** | 3,6-Diiodo-9*H*-carbazole |  |
| **3** | 1,3,6-Tribromo-9*H*-carbazole |  |
| **4** | 9,9′-(Hexane-1,6-diyl)bis(3-iodo-9*H*-carbazole) |  |
| **5** | 9-Ethyl-9*H*-carbazole |  |

amoxicillin 62.5 μg/ml (171.1 μM). Compounds **2a** and **2b** were not as active as against *Bacillus subtilis*, but still comparable to amoxicillin and showed activity at 62.5 μg/ml (213.2, 149.2 μM). Ciprofloxacin suppressed the growth of tested bacteria at 3.9 μg/ml (11.8 μM).

Evaluation of the antibacterial activity revealed that -Br, -CN and -I groups introduced into various carbazole ring positions determined its antibacterial activity. We can see that -Br carbazole compound **3** had a greater activity against Gram-negative, *Escherichia coli,* and -I groups containing carbazole compounds **2a** and **2b** showed better results against Gram-positive, *Bacillus subtilis,* strains. Interestingly, compound **1a** containing only one -CN group at the C-3 position showed a significant inhibitory activity against both tested bacteria, whereas compound **1b** containing two -CN groups at C-2 and C-7 positions, showed a very weak activity. This suggests that the substitutes introduced into C-3 and C-6 positions have a greater influence on antibacterial properties than the substitutes introduced into C-2 and C-7 positions. This tendency has also been observed among isopropanol-conjugated carbazole azole derivatives [6]. They noted that the -Br substitute at the carbazole C-3 position had a greater antibacterial activity than that of the -Br substitute at the C-2 position containing carbazole [6].

Table 2. **Minimum inhibitory concentration (MIC) values of the synthesized compounds against *Bacillus subtilis* and *Escherichia coli* strains**

| Compound | *Bacillus subtilis* | | *Escherichia coli* | |
|---|---|---|---|---|
| | MIC, μg/ml (μM) | Zone of inhibition at MIC value, mm | MIC, μg/ml (μM) | Zone of inhibition at MIC value, mm |
| **1a** | 31.25 (162.6) | 5.3 | 125 (650.3) | 6.30 |
| **1b** | 1000 (4603.6) | 9.50 | 1000 (4603.6) | 9.67 |
| **2a** | 31.25 (106.6) | 5.47 | 62.5 (213.2) | 6.17 |
| **2b** | 31.25 (74.6) | 6.33 | 62.5 (149.2) | 6.00 |
| **3** | 62.5 (154.8) | 7.40 | 31.25 (77.4) | 5.95 |
| **4** | 125 (181.6) | 7.15 | 1000 (1496.3) | 7.75 |
| **5** | NA | NA | NA | NA |
| **Amoxicillin** | 62.5 (171.1) | 6.67 | 62.5 (171.1) | 6.80 |
| **Ciprofloxacin** | 3.90 (11.8) | 7.90 | 3.90 (11.8) | 7.40 |

DMSO for negative control, NA for no inhibitory activity.

It should also be noted that compound **4** containing two 3-iodo-9*H*-carbazolyl fragments showed a weaker activity than that of compound **2a**, made of only a 3-iodo-9*H*-carbazolyl fragment. Also, compound **5**, consisting of an ethyl group at the C-9 position, did not show any antibacterial activity against the tested bacteria. This could suggest that introducing alkyl or other closed form structures has a negative effect on antibacterial properties.

Antioxidant activities of compounds **1a–5** were evaluated using the (1,1-diphenyl-2-picrylhy-drazyl) (DPPH) radical scavenging method (Fig. 2) and the ferric reducing antioxidant power (FRAP) assay (Fig. 3).

The DPPH assay method is based on the reduction of DPPH. When a free radical DPPH meets with a hydrogen donor, it is reduced to the DPPHH therefore the free radical DPPH which has purple colour is decolourized to yellow. Measuring this change absorption at 517 nm allows the calculations of antioxidant activity [21].

As seen from the results presented in Fig. 2, 5 out of 7 compounds showed weak to moderate DPPH radical scavenging properties. The best

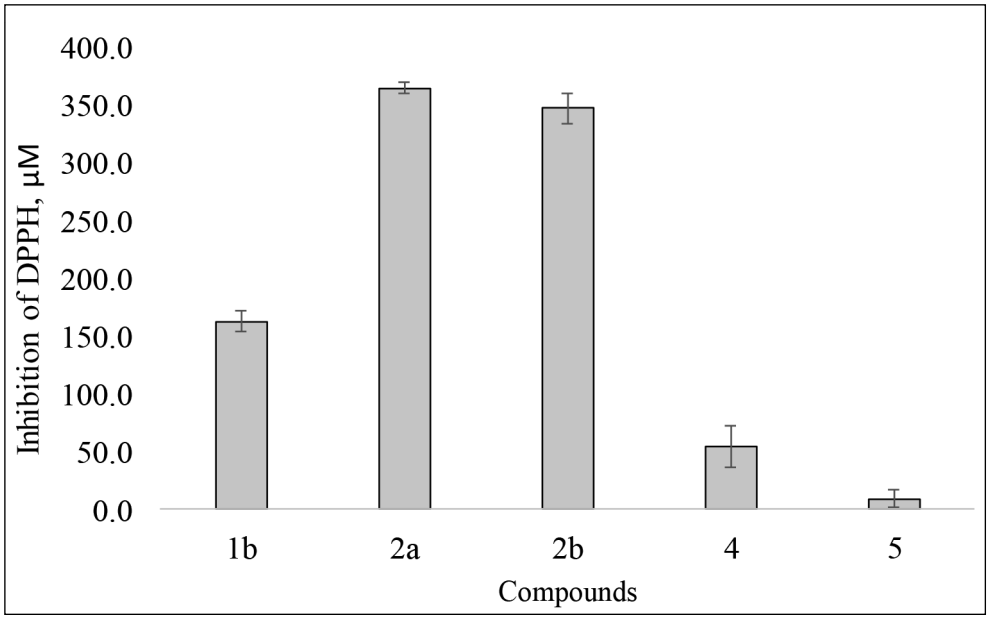



**Fig. 2.** DPPH radical scavenging properties of compounds **1b–2b, 4–5**

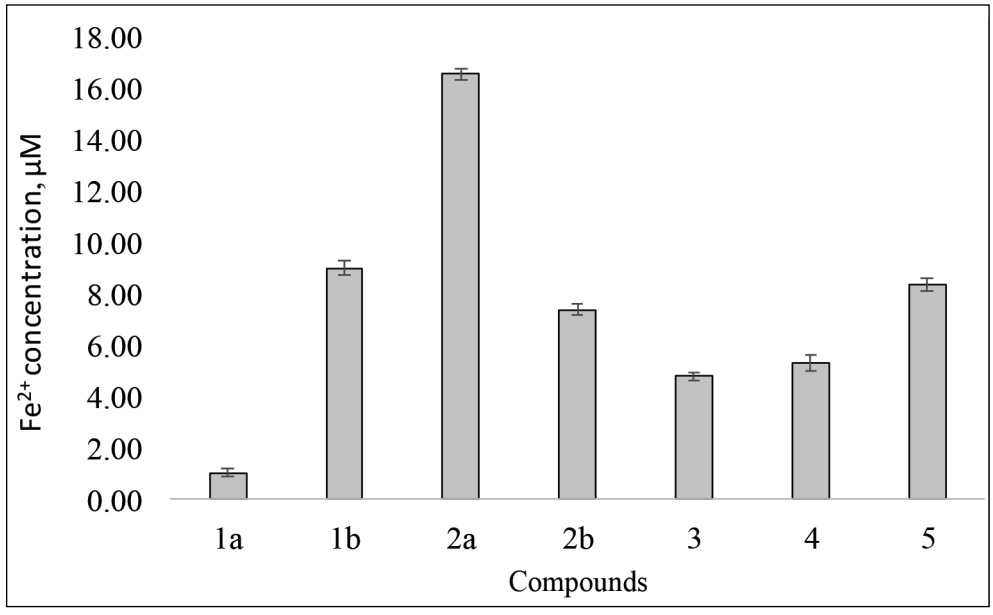



**Fig. 3.** Antioxidant activity of compounds **1a–5** according to the FRAP method

antioxidative activity was shown by ascorbic acid (797.57 µM), which was used as standard. Standard moderate radical scavenging abilities were shown by compounds **2a** (365.64 µM) and **2b** (348.02 µM), containing one and two -I atoms at C3 and C6 carbazole positions. Two -CN groups containing compound **1b** showed two times weaker activity, at 163.00 µM, whereas compound **1a** with only one -CN group showed no radical scavenging activity at the tested concentration. Compound **5**, with an introduced ethyl substitute at the C9 position, and two 3-iodo-9*H*-carbazolyl fragments containing compound **4** showed weak radical scavenging abilities, 9.53 µM and 54.33 µM, respectively. Compound **3**, containing three -Br atoms, showed no antioxidative activity.

The radical scavenging activities of compounds **1a–5** may be attributed to the electronic effects of substituent groups on the carbazole scaffold and the free -NH group at the C9 carbazole position. The importance of the free -NH group can be seen between compound **2a**, containing one –I atom at the C3 position, which showed the best radical scavenging ability, and compound **4**, containing two 3-iodo-9*H*-carbazolyl fragments and no free -NH group, which showed a lot weaker radical scavenging activity.

The FRAP method is based on electron transfer rather than hydrogen atom transfer [17]. The method is based on the ability of an antioxidant to reduce the $Fe^{3+}$ complex with 2,4,6-trypyridyl-s-triazine ($Fe(TPTZ)^{3+}$) to the $Fe^{2+}$ complex ($Fe(TPTZ)^{2+}$) [22]. The increase in absorbance of the intensely blue coloured $Fe^{2+}$ complex is measured at 593 nm and results can be expressed as $Fe^{2+}$ concentration (µM).

The results, shown in Fig. 3, revealed that compared to ascorbic acid (115.67 µM), which was used as standard, all tested compounds showed a weak activity to reduce $Fe(TPTZ)^{3+}$ to $Fe(TPTZ)^{2+}$. Out of the tested compounds, compound **2a**, containing one -I atom, at the C3 carbazole ring position, showed a notably higher ability (16.60 µM) to reduce $Fe(TPTZ)^{3+}$. Compounds **1b**, **2b**, **3**, **4** and **5** showed similar results (4.80–9.02 µM). Lastly, only at 1.05 µM, compound **1a** showed the weakest antioxidant activity according to the FRAP assay.

Even though the literature review revealed that good reductive properties were shown by carbazoles containing -OH [23], $-NO_2$ and $OCH_3$ [24], the ability to reduce $Fe(TPTZ)^{3+}$ to $Fe(TPTZ)^{2+}$ of the tested compounds **1a–5** can also be related to the electronic effects of substituent groups and the redox properties of these compounds.

## CONCLUSIONS

In summary, various mono-, di- and tri-substituted carbazole derivatives, containing appropriate halogens, cyano and alkyl groups, were synthesized. Screening of their antibacterial activity has displayed that against *Bacillus subtilis* the most

active antibacterial agents were 3-cyano-9*H*-carbazole, 3-iodo-9*H*-carbazole and 3,6-diiodo-9*H*-carbazole, they suppressed the growth of bacteria at a concentration of 31.25 μg/ml (162.6, 106.6, 74.6 μM). *Escherichia coli* bacteria were the most sensitive to 1,3,6-tribromo-9*H*-carbazole, which inhibited their growth at a concentration of 31.25 μg/ml (77.4 μM). The antibacterial activity assay also revealed that the carbazole derivatives substituted at C-3 and C-6 positions displayed better antibacterial activities than the carbazoles substituted at C-2 and C-7 positions. Also, it should be noted that the carbazoles containing -I substitutes better suppressed the growth of Gram-positive bacteria and the -Br containing carbazole better inhibited the growth of Gram-negative bacteria. The antioxidant activity assay revealed that the tested compounds displayed weak to moderate radical scavenging properties according to the 1,1-diphenyl-2-picryl-hydrazyl method and weak reductive properties according to the ferric reducing antioxidant power method, resulting in that CN, -Br and -I substitutes introduced into carbazole did not determine good radical scavenging and reducing properties.

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## KARBAZOLO DARINIŲ SINTEZĖ BEI JŲ ANTIBAKTERINIŲ IR ANTIOKSIDACINIŲ SAVYBIŲ VERTINIMAS

*S a n t r a u k a*

Pagal žinomus metodus buvo susintetinti septyni junginiai ir, taikant diskelių difuzijos metodą, ištirtos jų antibakterinės savybės dviems bakterijoms (*Bacillus subtilis* ir *Escherichia coli*). Antioksidacinės savybės buvo nustatytos remiantis laisvųjų 1,1-difenil-2-pikrilhidrazilo radikalų sugaudymo ir geležies jonų redukcijos antioksidacinio aktyvumo nustatymo metodais. Diskelių difuzijos metodu buvo nustatyta, kad šeši iš septynių junginių turėjo antibakterinį poveikį tirtoms bakterijoms ir jų augimą slopino esant įvairioms junginių koncentracijoms – nuo 31,25 iki 250 μg/ml. *Bacillus subtilis* bakterijas stipriau veikė 3-ciano-9*H*-karbazolas, 3-jod-9*H*-karbazolas ir 3,6-dijod-9*H*-karbazolas, o *Echerichia coli* bakterijas – 1,3,6-tribrom-9*H*-karbazolas, palyginti su amoksicilinu. Tirti junginiai pasižymėjo silpnomis vidutinėmis radikalų sugaudymo savybėmis, kai buvo taikytas 1-difenil-2-pikrilhidrazilo laisvųjų radikalų sugaudymo metodas, ir silpnomis redukcinėmis savybėmis taikant geležies jonų redukcijos antioksidacinio aktyvumo nustatymo metodą.