Bulgarian Chemical Communications, Volume 42, Number 1 (pp. 40–45) 2010

Synthesis and evaluation of novel carbazole derivatives as free radical scavengers

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Received July 29, 2009

A series of carbazole conjugated with different aminophenols and substituted aminophenols were synthesized by base catalyzed condensation reaction. The key intermediate 1-(9H-carbazol-9-yl)-2-chloroethanone**,** was obtained by N-acylation of carbazole with chloroacetyl chloride. The newly synthesized compounds were characterized by spectral and elemental analysis data and studied for their radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Butylated hydroxy anisole (BHA) was used as a reference antioxidant compound and the comparative study with newly synthesized compounds was also done. Among the analogues, 1-(9H-carbazole-9-yl)-2-(4-hydroxy-3-methoxy-phenylamino)ethanone, bearing electron donating methoxy substituent in the phenolic moiety, showed predominant activity.

Key words: carbazole, 1-(9H-carbazol-9-yl)-2-chloroethanone and radical scavenging activity.

INTRODUCTION

Free radicals, which are generated in many bioorganic redox processes, may induce oxidative damage in various components of the body (e.g. lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations, were recently reported [1]. Reactive oxygen species have been recognized to play an important role in the initiation and or progression of various diseases such as ischemia-reperfusion injury, atherosclerosis, and inflammatory injury [2]. There is a growing interest on natural and synthetic antioxidants as a protective strategy against these diseases by block or removal of oxidative stress [3]. Free radical formation is associated with the normal natural metabolism of aerobic cells. The oxygen consumption inherent in cell growth leads to the generation of a series of oxygen free radicals. The interaction of these species with lipid molecules produces new radicals: hydroperoxides and different peroxides [4–5]. This group of radicals (superoxide, hydroxyl and lipid peroxides) may interact the biological systems in a cytotoxic manner. Free radicals and their uncontrolled production, in fat, are responsible for several pathological processes, such as certain tumors (prostate and Colon cancers) and coronary heat diseases [6]. The reducing properties of diarylamines make them very important as antioxidants, especially as radical scavengers [7]. In fact most representative examples of antioxidants are hindered phenols and diphenylamine derivatives [8]. The reaction of $RO_2^{\bullet}$ radicals with secondary amine seems to proceed according to the mechanism proposed by Thomas [9], the H-transfer reaction from the N–H bond to peroxyl occurring in a first steps leads to aminyl radical ($RR'N^{\bullet}$), which react again with $RO_2^{\bullet}$ radical giving nitroxide radicals ($RR_1NO^{\bullet}$) in a second step.

Antioxidants are now forged as the drug candidate to combat several diseases. In the literature some tricyclic amines and their chemical structure showed antioxidant neuroprotective activity *in vitro* [10]. Recently, radical scavenging activity of amino acid analogues of 10-methoxy-5H-dibenz[b,f]azepine, a tricyclic amine has been reported [11]. Herein, carbazole belonging to the same class of compound is taken as model compound. Carbazole is one of the aromatic heterocyclic organic compound and its derivatives are known as alkaloids from plants, and many of these show antioxidative and biological activities, such as antitumor, psychotropic, anti-inflammatory, antihistaminic, and antibiotic activities [12–15]. Owing to the widespread applications, synthetic and biological activity evaluation of carbazole and their derivatives has been subject of intense investigations. In the course of the development of new antioxidants, we have interested in novel carbazole derivatives based on the preliminary findings that carbazole has an antioxidant properties.

CHEMISTRY

Carbazole was synthesized by applying known method [15]. The active sites for the coupling of different aminophenols and substituted aminophenols to the basic molecule was very less thus, we select the N-acylation reaction in order to obtain the

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key intermediate in which the coupling of different aminophenols and substituted aminophenols can be done very easily with simple experiment protocol with good yield. The selection of aminophenols and substituted aminophenols was done on the basis of its chemical feasibility. The synthesis of carbazole analogues conjugated with different aminophenols and substituted aminophenols was realized in two steps. In the first step, the key intermediate 1-(9H-carbazol-9-yl)-2-chloroethanone was prepared in good yield by N-acylation of carbazole with chloroacetyl chloride in the presence of triethylamine as base (Scheme 1). In the second step, further coupling of respective aminophenols and substituted aminophenols to the intermediate was done by base condensation reaction to obtain the novel carbazole analogues (Scheme 2).

EXPERIMENTAL

Materials and Methods

DPPH was purchased from Sigma Aldrich, chloroacetyl chloride, triethylamine, benzene, diethyl ether, ethyl acetate, *n*-hexane, tetrahydrofuran, anhydrous potassium carbonate, methanol, chloroform, sodium bicarbonate, anhydrous sodium sulphate and aminophenols like 2-aminophenol, 3-aminophenol, 4-aminophenol, substituted aminophenols like 4-nitro-2-aminophenol and 4-methoxy-2-aminophenol were all of analytical grade and procured from Merck. TLC aluminium sheets - Silica gel 60 F_{254} was also purchased from Merck. All the reported melting points were taken in open capillaries and are reported uncorrected. The IR spectra were recorded on a FT-IR021 model in KBr disc. The ^{1}H NMR spectra were recorded on Jeol GSX 400 MHz spectrophotometer using $CDCl_3$ as a solvent and the chemical shift (δ) are in ppm relative to internal standard. The Mass spectra were recorded on Waters-Q-TOF Ultima spectrometer.

Synthesis of 1-(9H-carbazol-9-yl)-2-chloroethanone (**2**)

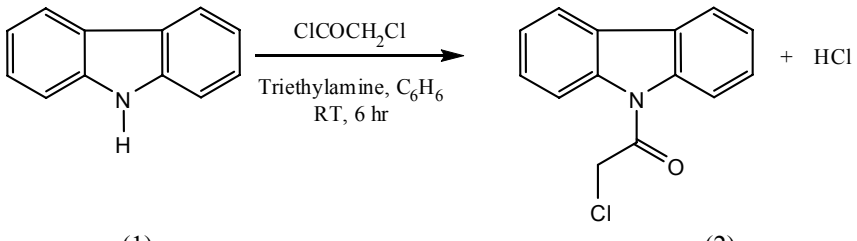
To the well stirred solution of carbazole (2 mM) and triethylamine (2.2 mM) in 50 ml benzene, chloroacetyl chloride (2.2 mM) in 25 ml benzene was added drop by drop for about 30 min. Then the reaction mixture is stirred at room temperature for about 6 hr. Progress of the reaction is monitored by TLC 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% $NaHCO_3$ solution followed by distilled water. Finally the ether layer is dried over anhydrous Na_2SO_4. The pale yellow solid product was obtained by desolventation through rotary evaporator at 35ºC.

Carbazole (**1**). Light yellow solid, yield 73%, melting point 218–220ºC, IR (KBr) ν_{max} (cm^{-1}): 3418.21 (N–H), 2360.4–2922.59 (Ar–H); ^{1}H NMR ($CDCl_3$) δ: 10.2 (s, N–H, 2H), 7.2–8.33 (m, Ar–H, 8H). Mass (m/z, %): M^+ 167.8; Anal. calcd. for $C_{12}H_9N$: C, 86.20; H, 5.43; N, 8.38%; Found: C, 86.21; H, 5.42; N, 8.37%.

1-(9H-*carbazol*-9-*yl*)-2-*chloroethanone* (**2**): Light yellow solid, yield 85%, melting point 209–212ºC, IR (KBr) ν_{max} (cm^{-1}): 1600.8 (C=O), 2378.4–2872.9 (Ar–H); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.36 (d, CH_2–C=O, 2H), 7.78–8.33 (m, Ar–H, 8H). Mass (m/z, %): M^+ 243; Anal. calcd. for $C_{14}H_{10}ClNO$: C, 69.00; H, 4.14; N, 5.75%; Found: C, 69.01; H, 4.16; N, 5.73%.

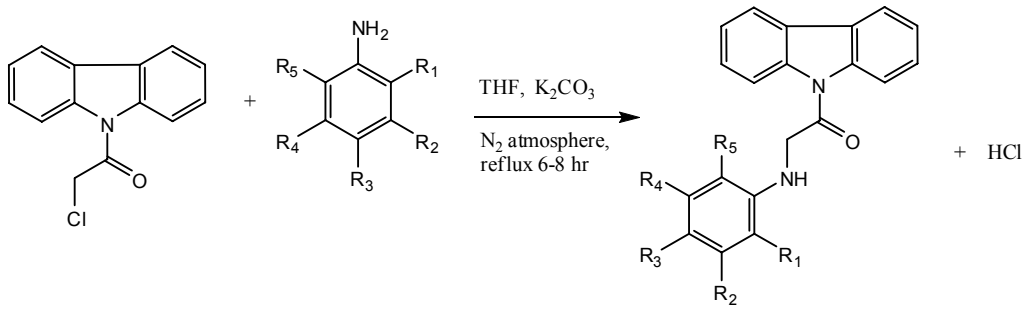
*General procedure for the synthesis of 1-(9H-carbazol-9-yl)-2-chloroethanone conjugated with different aminophenols and substituted aminophenols (**2a**–**e**).*

2-aminophenol (1.2 mM) in THF (25 mL) was treated with K_2CO_3 (600 mg) under N_2 atmosphere. Later the solution of 1-(9H-carbazol-9-yl)-2-chloroethanone (1 mM) in THF (25 mL) was added drop by drop for 30 min. The reaction mixture was refluxed for 6–8 hr. The progress of the reaction mixture was monitored by TLC. The reaction mixture was then desolventized in rotary evaporator and the compound is extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. The yellow semisolid was obtained by further desolventation in rotary evaporator at 50ºC.



(1) (2)

Scheme 1.



(**2**) (**2a**–**e**)

Compound	R_1	R_2	R_3	R_4	R_5
2a	H	H	OH	H	H
2b	OH	H	H	H	H
2c	H	OH	H	H	H
2d	H	H	OH	NO_2	H
2e	H	H	OH	OCH_3	H

Scheme 2.

1-(9H-carbazol-9-yl)-2-chloroethanone derivatives conjugated with 3-aminophenol, 4-aminophenol, substituted aminophenols like 4-nitro-2-aminophenol and 4-methoxy-2-aminophenol were obtained by following same procedure. The analogues were separated and purified by column chromatography by using mixture of chloroform/methanol = 85:15. The products were characterized by IR, mass, ^{1}H NMR and elemental analysis.

1-(9H-Carbazole-9-yl)-2-(4-hydroxy phenylamino)ethanone (**2a**). Light yellow solid, yield 74%, melting point 222–224ºC, IR (KBr) ν_{max} (cm^{-1}): 3413.21 (N–H), 1600.8 (C=O), 2364.5– 2922.59 (Ar–H), 3201.3–3412.6 (Ph–OH); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.34 (d, CH_2–C=O, 2H), 7.71–8.32 (m, Ar–H, 8H), 6.2 (s, NH, 1H), 6.7–6.8 (m, Ph–Ar–H, 4H), 10 (s, Ph–OH, 1H). Mass (m/z, %): M^+ 316; Anal. calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86%; Found: C, 75.91; H, 5.12; N, 8.88%.

1-(9H-Carbazole-9-yl)-2-(2-hydroxy phenylamino)ethanone (**2b**). Light yellow solid, yield 71%, melting point 178–182ºC, IR (KBr) ν_{max} (cm^{-1}): 3401.21 (N–H), 1601.8 (C=O), 2360.4–2921.5 (Ar–H), 3378.2–3446.6 (Ph–OH); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.35 (d, CH_2–C=O, 2H), 7.76–8.33 (m, Ar–H, 8H), 6.3 (s, NH, 1H), 6.5–7.2 (m, Ph–Ar–H, 4H), 10.1 (s, Ph–OH, 1H); Mass (m/z, %): M^+ 316; Anal. calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86%; Found: C, 75.91; H, 5.12; N, 8.88%.

1-(9H-Carbazole-9-yl)-2-(3-hydroxy phenylamino) ethanone (**2c**). Light yellow solid, yield 66%, melting point 200–202ºC, IR (KBr) ν_{max} (cm^{-1}): 3412.21 (N–H), 1600.6 (C=O), 2362.4–2920.9 (Ar–H), 3377.3–3456.4 (Ph–OH); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.33 (d, CH_2–C=O, 2H), 7.73–8.36 (m, Ar–H, 8H), 6.1 (s, NH, 1H), 6.0–7.2 (m, Ph–Ar–H, 4H), 9.8 (s, Ph–OH, 1H). Mass (m/z, %): M^+ 316; Anal. calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86%; Found: C, 75.91; H, 5.12; N, 8.88%.

1-(9H-Carbazole-9-yl)-2-(4-hydroxy-3-nitro phenylamino)ethanone (**2d**). Light brown solid, yield 77.6%, melting point 220–224ºC, IR (KBr) ν_{max} (cm^{-1}): 3411.21 (N–H), 1600.5 (C=O), 2368.4–2922.5 (Ar–H), 3360.3–3455.3 (Ph–OH); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.31 (d, CH_2–C=O, 2H), 7.77–8.39 (m, Ar–H, 8H), 6.2 (s, NH, 1H), 7.1–7.9 (m, Ph–Ar–H, 3H), 10.3 (s, Ph–OH, 1H);). Mass (m/z, %): M^+ 361; Anal. calcd. for $C_{20}H_{15}N_3O_4$: C, 68.48; H, 4.18; N, 11.63%; Found: C, 68.46; H, 4.19; N, 11.64%.

1-(9H-Carbazole-9-yl)-2-(4-hydroxy-3-methoxy phenylamino)ethanone (**2e**). White solid, yield 83.4%, melting point 189–193ºC, IR (KBr) ν_{max} (cm^{-1}): 3412.21 (N–H), 1613.9 (C=O), 2361.4–2932.5 (Ar–H), 3377.3–3455.6 (Ph–OH); ^{1}H NMR ($CDCl_3$) δ (ppm): 4.30 (d, CH_2–C=O, 2H), 7.71–8.31 (m, Ar–H, 8H), 6.4 (s, NH, 1H), 7.0–7.7 (m, Ph–Ar–H, 3H), 9.8 (s, Ph–OH, 1H), 3.5 (s, OCH_3, 3H). Mass (m/z, %): M^+ 346; Anal. calcd. for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09%; Found: C, 72.83; H, 5.21; N, 8.07%.

RADICAL SCAVENGING ACTIVITY

The newly synthesized compounds were screened for their radical scavenging activity using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The compounds under studies were dissolved in distilled ethanol (50 mL) to prepare 1000 μM solution. Solutions of different concentrations (10, 25, 50, 100, 200 and 500 μM) were prepared by serial dilution and the free radical scavenging activity was studied.

DPPH radical scavenging activity

The DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois [16]. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 500 μM) 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 the dark room at RT for 20 min. A DPPH blank was prepared without 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 DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

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

Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The radical scavenging activity of BHA and ascorbic acid was also measured and compared with that of the newly synthesized compound.

RESULTS AND DISCUSSION

N-Acylation of carbazole was affected initially by using Na_2CO_3 as a base and benzene as solvent .Only poor yields was achieved. Instead, when triethylamine was used as base the yield of the product improved significantly (i.e, about 85%) in stirring mode at about 30–35ºC.

Scheme 1 shows the reaction pathway of the N-acylation of carbazole (**1**) in the presence of triethylamine as base affords key intermediate 1-(9H-carbazol-9-yl)-2-chloroethanone (**2**), Further base condensation with different aminophenols and substituted aminophenols were carried out to get the target analogues (**2a**–**e**) (Scheme 2).

The obtained analogues were characterized by various spectroscopic techniques like IR, Mass, ^{1}H NMR and elemental analysis.

The IR spectra of key intermediate showed the absence of N–H stretching at 3418 cm^{-1} and addition of C=O stretching at 1600 cm^{-1} respectively. 1H NMR reveals the absence of N–H proton at 11.2 ppm and the presence of –CH_2 protons as doublet at 4.36 ppm. All the respective aromatic protons were signaled at 7.78–8.33 ppm. These data reveals the N-acylation of carbazole was successful under our experimental protocol.

Further the coupling of various aminophenols and substituted aminophenols was done by base condensation reaction in the presence of K_2CO_3 as base. The IR spectra of all the target analogues showed broad stretching at a region at 3201–3456 cm^{-1} for phenolic –OH, all the conjugated analogues showed N–H stretching at 3401–3413 cm^{-1}. ^{1}H NMR spectra of all 1-(9H-carbazol-9-yl)-2-chloroethanone derivatives (**2a**–**2e**) showed multiplet for Ar–H proton at δ 6.0–8.39 ppm. All the conjugated analogues showed sharp singlet peak at δ 9.8–10.3 ppm corresponding to phenolic –OH. Compound (**2e**) showed sharp singlet peak at δ 3.5 ppm corresponding to –OCH_3 group.

All the analogues showed mass according to their M^+ ions.

The radical scavenging effects of newly synthesized compounds were examined in the present study using radicals generated by DPPH.

Radical scavengers reacts with DPPH, which is a stable free radical and convert it to 2,2-diphenyl-1-picrylhydrazine. The degree of discoloration indicates the scavenging potentials of the compounds. The percentage DPPH activities of all the newly synthesized compounds are showed in the Figure 1.

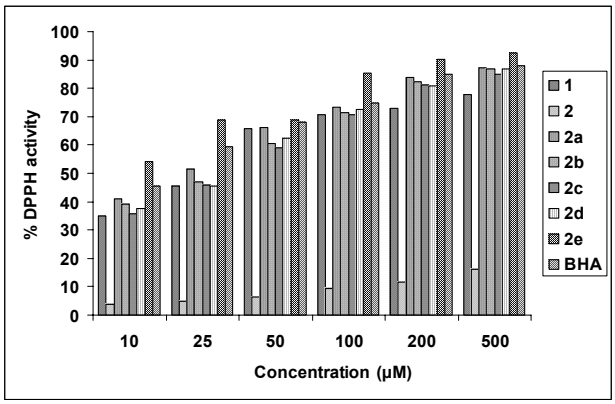


Fig. 1. % DPPH radical scavenging activity of carbazole and newly synthesized analogues. Each value represents the mean ± SD (n = 3).

From the figure, all the compound showed DPPH activity in concentration dependent manner. On the other hand, the half inhibition concentration (IC_{50}) for all the newly synthesized analogues including the reference antioxidant BHA was calculated graphically using a linear regression algorithm and showed in Table 1.

Initially, the key intermediate (**2**) showed negligible activity, but coupling of different aminophenols and substituted aminophenols (**2a**–**2e**) increases the activity. All the analogues demonstrated signi-

ficant radical scavenging effect. Among them, the methoxy substituted analogues (**2e**) was found to be more potent followed by *p*-aminophenol analogue (**2a**).

Table 1. 50% Inhibition of DPPH radical by the carbazole and its analogues. where – corresponds to no 50% inhibition.

Compound	IC50 (μM)
1	60.34 ± 1.94
2	–
2a	18.32 ± 0.56
2b	21.67 ± 0.86
2c	25.55 ± 1.11
2d	20.87 ± 0.94
2e	15.12 ± 0.43
BHA	16.23 ± 1.23

The presence of electron donating methoxy substitutent in the phenolic compounds is known to increase the stability of the radical and hence, the antioxidant activity [17]. Thus the introduction of a methoxy group to aminophenol increases the hydrogen donation ability and therefore increases the radical scavenging capacity. But the introducing of electron withdrawing NO_2 group slightly decreases the scavenging capacity.

The radical scavenging activity of all the newly synthesized analogues was compared with the standard antioxidant i.e., BHA. 1-(9H-carbazole-9-yl)-2-chloro ethanone conjugated with methoxy substituted aminophenol (**2e**) showed dominant activity than the BHA, whereas all the analogues showed less activity than the BHA.

The increased DPPH radical scavenging activity of all the newly synthesized compounds is as follows **2e** > BHA> **2a** > **2d** > **2b** > **2c** > **1** > **2**

CONCLUSION

We have synthesized a ray of carbazole analogues conjugated with different aminophenols and substituted aminophenols. The synthetic protocol proposed by us, reproduces the convenient way for the target compounds. The synthesized compounds were evaluated for their DPPH radical scavenging activity. Initially, the key intermediate 1-(9H-carbazole-9-yl)-2-chloro ethanone (**2**) showed negligible activity, whereas coupling of different aminophnols and substituted aminophenols enhance the radical scavenging activity. Among the analogues carbazole conjugated with 4-methoxy-2-aminophenol exhibited more potent inhibition of DPPH radical scavenging activity and also more potent than the standard BHA.

Our study provides evidence that carbazole derivative bearing different aminophenols and substituted aminophenols exhibits interesting DPPH radical scavenging activity. These analogues may be useful in the treatment of pathologies, in which free radical oxidation plays a fundamental role. This may warrant further in depth biological evolutions. Work is in progress to design, synthesize and evaluate addition compound in this and related systems.

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СИНТЕЗ И ПРЕЦЕНКА НА НОВИ КАРБАЗОЛОВИ ПРОИЗВОДНИ КАТО АНТИОКСИДАНТИ

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Постъпила на 29 юли 2009 г.

(Резюме)

Синтезирани е серия съединения от карбазол спрегнат с различни аминофеноли и заместени аминофеноли чрез алкална каталитична реакция на кондензация. Ключовото междинно съединение 1-(9H-карбазол-9-ил)-2-хлоретанон е получено чрез N-ацилиране на карбазол с хлорацетилхлорид. Новосинтезираните съединения са охарактеризирани чрез спектрални и елементен анализи и е изследвана тяхната активност като антиоксиданти с 2,2-дифенил-1-пикрилхидразил (DPPH). Направено е сравнително изследване на новосинтезираните съединения като антиоксиданти в сравнение с бутилхидроксианизол (BHA). От аналозите по-висока активност показа 1-(9H-карбазол-9-ил)-2-(4-хидрокси-3-метоксифениламино)етанона, който има електронодорен метокси заместител във фенолната част.