Bulgarian Chemical Communications, Volume 49, Special Issue D (pp. 201 – 208) 2017

Synthesis and properties of several Betti bases as potential drugs

N. V. Georgieva[1]*, Z. L. Yaneva[1], S. D. Simova[2], G. D. Nikolova[3]

[1] *Chemistry Unit, Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Students Campus, Stara Zagora 6000, Bulgaria*

[2] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev street, bl. 9, 1113 Sofia, Bulgaria*

[3] *Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Trakia University, Armejska Str., 11, Stara Zagora 6000, Bulgaria*

Received April 18, 2017; Revised June 2, 2017

Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

The aim of the present study was to synthesize several Betti bases by a modified Betti reaction, to develop analytical methods for the quantitative determination and testing the microbiological and antioxidant activities. For the modification of the Betti reaction we used primary heterocyclic amine, aromatic aldehydes, 8-quinolinol and halogeno-substituted aromatic aldehydes. Betti bases were obtained at yield 80-98% and characterized by elemental analysis and NMR spectra. The applied analytical methods for quantitative determination of the studied Betti bases offered short analysis time, high precision, high linearity and satisfactory limit of detection (LOD) and limit of quantification (LOQ) values. The microbiological activity of studied Betti bases was tested against 23 test strains pathogenic microorganisms (different gram positive and gram negative). The microbiological screening proved their selective microbiological activity. According to the EPR study, the Betti bases possess radical structure and are likely to exhibit antioxidant activity. We assume that the potential of the presented Betti bases as a pharmacological compound is promising.

Key words: Betti bases; 8-quinolinol; UV-VIS spectroscopy, EPR spectroscopy; HPLC

INTRODUCTION

Quinolones are extensively investigated as broad spectrum anti-bacterial, anti-diabetic, anti-convulsant, anti-inflammatory, anti-viral, anti-fungal and anti-HIV agents [1–8]. In vitro studies show that the hydrochlorides of the Mannich bases of 8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline exhibit high cytotoxic activity against human cancer cells of leukemia and myeloma, as well as other tumors [6, 8]. The scientific literature presents reports demonstrating the antioxidant activity of quinoline-8-ol derivatives [9, 10]. The study of Betti bases chemistry started at the beginning of the 20th century. The reaction of primary aromatic amines with benzaldehyde and phenols or naphthols is called a Betti reaction although it represents a special case of the Mannich reaction and the products (aminonaphthols) obtained are known as Betti bases [11–15]. These bases have interesting biological applications, such as antibacterial, hypotensive, and bradycardic agents [3, 16–19]. The optically active Betti bases can be used as ligands to chelate with organometallic reagents in different reactions to provide highly efficient complex compounds [20–22]. However, various modifications have been made to prepare Betti base derivatives by using other naphthols or phenols, quilinols, and amines [23–25]. Many procedures for Betti reactions have also been successfully developed [26–31].

In our previous investigations we have found growth regulating activity of synthesized by us 7-aminobenzyl-8-hydroxyquinolines [32] and microbiological activity against a reference *Staphylococcus aureus* strain of the synthesized by us 2,5,7-substituted derivatives of 8-quinolinol [33].

The aim of the present study was to synthesize several Betti bases by a modified Betti reaction, to develop analytical methods for its quantitative determination and to test its microbiological and antioxidant activities.

EXPERIMENTAL

Reagents

The halogen-substituted aromatic aldehydes, quinolin-8-ol and 2-amino-6-methylpyridine used in the syntheses are commercially available (Fluka, Merck, Germany, > 99%) with analytical reagent

* To whom all correspondence should be sent:
E-mail: *nvgeorgieva@vmf.uni-sz.bg*

© 2017 Bulgarian Academy of Sciences, Union of Chemists in Bulgaria

grade. The amino benzylation of quinolin-8-ol was carried out by modified by us Betti reaction: three-component Mannich-type condensation of 8-hydroxyquinoline (0.01mol), chloro-, fluoro- and bromo-substituted benzaldehydes (0.01mol), 2-amino-6-methylpyridin-2-amino-6-methylpyridine (0.01mol) in absolute ethanol according to the scheme (Fig. 1):

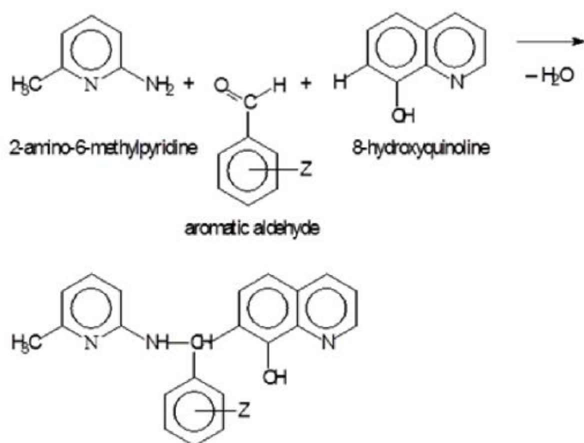

Fig. 1. Scheme of the reaction for the syntheses of 7-[(6-methylpyridin-2ylamino)(halogenophenyl)methyl)] quinolin-8ols, where Z is: 2-chloro; 3-chloro; 4-chloro; 2,4-dichloro; 2-bromo; 3-brom; 4-bromo and 2-fluoro.

The reaction mixtures were allowed to stand for 21 days at room temperature in a closed flask and the time (in hours) required for the precipitation of the products was reported. After completion of the process, the separated solid phase was filtered (no earlier than 21 days) and purified by recrystallization from an organic solvent (preferably a mixture of ethanol and acetone in the ratio 1:1).

NMR spectroscopy

^{1}H (600.1 and 250.1 MHz), ^{13}C (150.9 MHz) and ^{15}N (60.8 MHz) NMR spectra were acquired on Bruker AVANCE AV600 II+ and DRX 250 NMR spectrometers. All spectra were recorded in DMSO-d_6 at room temperature. Chemical shifts are referenced to the residual solvent ^{1}H (2.51 ppm) and ^{13}C (39.50 ppm) signals. Inverse detected ^{15}N NMR chemical shifts are referenced to external liquid NH_3. Unambiguous assignment of the NMR signals was made on the basis of the gradient enhanced versions of COSY, TOCSY, NOESY (ROESY), HSQC and HMBC experiments; Bruker TOPSPIN 3.5pl1 pulse library: cosygpmfqf, dipsi2etgpsi, noesygpphzs, hsqcedetgpsisp.3 and hmbcgp(lp)ndqf, 2015.

UV-VIS Spectrophotometry

Standard stock solutions (100 µg/ml) were prepared by dissolving the appropriate amount of 7-[(6-methylpyridin-2-ylamino)(2.4-dichlorophenyl) methyl]quinolin-8-ol (Bt4) in ethanol:HCl (pH 1.0). Working standard solutions (1–100 µg/mL) were prepared daily. The concentrations of Bt4 were measured with UV-VIS spectrophotometer DR 5000 Hach Lange (Germany), supplied with 10 mm quartz cells. All spectra were recorded in the UV region at λ 204 nm with 2 nm slit width, 900 nm min^{-1} scan speed and very high smoothing.

HPLC Study

RP-HPLC system comprising of a Hypersil BDS C18 (5 µM, 4.6 x 150 mm) column, Surveyor LC Pump Plus, PDA detector, and Surveyor Autosampler Plus (Thermo Fisher Scientific) was used. The used mobile phase consisted of a mixture of $MeOH:H_2O$ (40:60, v/v). The samples were monitored at 204 nm. The buffer pH was adjusted to 3.2 with H_3PO_4. The volume injected into the HPLC column was 20 µl.

All UV/VIS spectrophotometric and HPLC analyses were made in triplicate. The experimental data was analyzed by regression analyses and determination of the corresponding correlation coefficients (R^2) and relative standard deviation (RSD, %). The efficiency and accuracy of the developed UV/VIS and HPLC methods was estimated based on the calculated limit of detection (LOD) and limit of quantification (LOQ).

Microbiological activity

The obtained solid substances of the Betti bases were diluted in N,N'-dimethylformamide (DMF) and their microbiological activity was tested at concentrations of 4-500 µg/mL with Mueller-Hinton agar medium, against 23 strains of pathogenic microorganisms: *Listeria 215, Listeria 362, Listeria 1094, Swine erysipelas, Staphylococus 1, Staphyloc. 5, Staphyloc. 42, Staphyloc. 78, Staphyloc. 119, Bact. megat 10, Bact. megat 11, Bact. megat 16, Bact. megat 39, Bact. cereus 12, Past. mult. 10, Past. mult. SHp, Past. mult. R II, Salm. Dublin, Salm. Gollinarum 200, Salm. Enter 4767, E. coli 21, E. coli 56, E. coli 94.* The inoculation of the test strains was conducted in Stuart equipment with 23 nests. After the inoculation and the development of the bacterial culture in agar, the samples were incubated at 37ºC for several days.

The Betti base solutions were prepared for each petri dish separately with dissolved agar. Sterilized petri dishes and agar medium were used in the present work. The antibacterial activities of the compounds were evaluated by measuring the zone of inhibition on nutrient agar plate. The microbiological activities were tested for at least three times against all microorganisms and average values have been reported. Muller Hinton agar was used in the anti-bacterial study.

Electron paramagnetic resonance (EPR) study

EPR measurements were performed at room temperature on an X-band EMX_{micro}, spectrometer Bruker, Germany, equipped with standard Resonator. All EPR experiments were carried out in triplicate and repeated. Spectral processing was performed using Bruker WIN-EPR and SimFonia software. For the EPR study, direct EPR spectroscopy methods were used to investigate the antioxidant properties of 7-((6-methylpyridin-2-ylamino)(2,4-dichlorophenyl)methyl)quinolin-8-ol (Bt 4). By a direct EPR spectroscopy an EPR singlet signal was registered in the powdered form of Bt 4. Quartz capillaries were used as sample tubes. Sample tubes were filled with each powdered form and placed in the EPR microwave cavity. The EPR spectra of the Betti base in a powdered form were recorded at the following spectrometer settings: receiver gain 2×10^3, power 1.64 mW, center field 3513.50 G, sweep width 200 G, time constant 163.84 ms, sweep time 61.44 s, modulation amplitude 10 G, 5 scans per sample.

RESULTS AND DISCUSSION

Synthesis

As a result of the condensation between 2-amino-6-methylpyridin, 8-quinolinol and halogen-substituted aromatic aldehydes, eight 7-[(6-methylpyridin-2-ylamino)(halogenophenyl)methyl] quinolin-8-ols, with a common formula presented in Fig. 2, were obtained. The Betti bases, purified from the organic solvent are crystalline substances. The data for the common molecular formula, molecular mass, melting point, elemental analysis and yield of the newly synthesized derivatives of 8-hydroxyquinoline are presented in Table 1. It was established that the time required for extraction of the reaction products at room temperature is inversely proportional to their yield. The longer settling time could be explained by steric hindrance, as in the chemical structures of each of the Betti bases there is a substituent on the 2-position of the benzene ring.

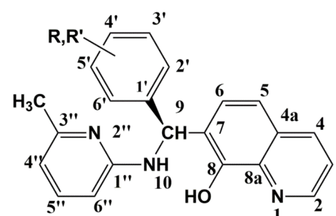

Fig. 2. Common formula of the Betti bases, where R is a substituent from 2-chloro (Bt1), 3-chloro (Bt2), 4-chloro (Bt3), 2-bromo (Bt5), 3-bromo (Bt6), 4-bromo (Bt7), 2-fluoro (Bt8) and R,R' is 2,4-dichloro (Bt4).

Confirmation of the Structure of the Betti Bases

The structures of the studied Betti bases were confirmed by their NMR spectra. They are also characterized by their melting point determined by Kofler microscope and by elemental analysis - nitrogen, carbon and hydrogen were determined (Table 1).

The NMR spectral assignment for the individual compounds is presented below:

R = 2'-Cl (Bt1): ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.93 (s, 1H, OH), 8.85 (dd, *J* = 4.2, 1.6 Hz, 1H, H-2), 8.30 (dd, *J* = 8.3, 1.6 Hz, 1H, H-4), 7.54 (dd, *J* = 8.3, 4.2 Hz, 1H, H-3), 7.48 – 7.23 (m, 8H), 7.10 (d, *J* = 7.8 Hz, 1H, NH-10), 6.98 (d, *J* = 7.8 Hz, 1H, H-9), 6.45 – 6.30 (m, 2H), 2.19 (s, 3H).

Table 1. Properties of the studied Betti bases with common formula shown in Fig. 2.

Betti bases	Bt1	Bt2	Bt3	Bt4	Bt5	Bt6	Bt7	Bt8
Empirical formula	$C_{22}H_{18}ClN_3O$	$C_{22}H_{18}ClN_3O$	$C_{22}H_{18}ClN_3O$	$C_{22}H_{17}Cl_2N_3O$	$C_{22}H_{18}BrN_3O$	$C_{22}H_{18}BrN_3O$	$C_{22}H_{18}BrN_3O$	$C_{22}H_{18}FN_3O$
MG	375.85	375.85	375.85	410.3	420.31	420.31	420.31	359.40
Time, h	96	17	12	33	39	22	19	15
M.P.,ºC	183-185	154-156	144-146	184-186	185-187	172-174	151-153	181-183
Yd, %	80	93	95	98	88	90	93	98
%,N Calc.	11.18	11.18	11.18	10.24	10.00	10.00	10.00	11.69
%,N Found	11.03	11.52	11.10	10.39	9.75	10.25	10.36	11.73
%,C Calc.	70.30	70.30	70.30	64.40	62.87	62.87	62.87	73.52
%,C Found	70.00	69.78	70.10	64.22	62.83	62.55	62.38	73.72
%,H Calc.	4.83	4.83	4.83	4.18	4.32	4.32	4.32	5.05
%,H Found	5.00	4.99	4.86	3.94	4.53	4.42	4.46	4.75

2nd isomer (25%) - 8.88 (dd, J = 4.1, 1.6 Hz), 8.46 (dd, J = 8.8, 1.6 Hz), 7.62 (dd, J = 8.8, 4.1 Hz), 6.98 (d, J = 8.0 Hz), 6.85 (d, J = 8.0 Hz), 2.24 (s).

R = 3'-Cl (Bt2): ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.03 (s, 1H, OH), 8.86 (dd, J = 4.2, 1.7 Hz, 1H, H-2), 8.29 (dd, J = 8.4, 1.6 Hz, 1H, H-4), 7.66 (d, J = 8.6 Hz, 1H, H-6), 7.54 (dd, J = 8.3, 4.2 Hz, 1H, H-3), 7.42 (d, J = 8.6 Hz, 1H, H-5), 7.42 (m, 1H, H-2'), 7.39 – 7.19 (m, 6H), 6.88 (d, J = 8.9 Hz, 1H, H-9), 6.48 (d, J = 8.4 Hz, 1H, H-6''), 6.38 (d, J = 7.2 Hz, 1H, H-4''), 2.23 (s, 3H).

R = 4'-Cl (Bt3): ^{1}H NMR (600.1 MHz, DMSO-d_6) δ 10.08 (s, 1H, OH), 8.86 (dd, J = 4.2, 1.6 Hz, 1H, H-2), 8.29 (dd, J = 8.3, 1.6 Hz, 1H, H-4), 7.64 (d, J = 8.6 Hz, 1H, H-6), 7.54 (dd, J = 8.3, 4.2 Hz, 1H, H-3), 7.41 (d, J = 8.6 1H, H-5), 7.38;7.35 (AA'XX', 4H, H-2',3',5',6'), 7.31 (d, J = 8.9 Hz, 1H, NH-10), 7.28 (dd, J = 8.3, 7.3 1H, H-5''), 6.85 (d, J = 8.7 Hz, 1H, H-9), 6.46 (d, J = 8.3 Hz, 1H, H-6''), 6.36 (d, J = 7.2 Hz, 1H, H-4''), 2.22 (s, 3H, CH_3-3''); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 157.43 (C-1''), 155.68 (C-3''), 149.60 (C-8), 148.31 (C-2), 142.78 (C-1'), 138.11 (C-8a), 137.24 (C-5''), 136.04 (C-4), 130.97 (C-4'), 128.92 (C-2',6'), 128.10 (C-3',5'), 127.52 (C-4a), 126.61 (C-6), 125.36 ((C-7), 121.72 (C-3), 117.46 (C-5), 111.31 (C-4''), 105.26 (C-6''), 51.06 (C-9), 24.24 (CH_3-3''); ^{15}N NMR (60.8 MHz, DMSO-d_6) δ 296 (N-1), 91 (d, J = 92, N-10), 266 (N-2'').

R, R' = 2',4'-Cl (Bt4): ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.00 (s, 1H, OH), 8.85 (dd, J = 4.2, 1.7 Hz, 1H, H-2), 8.30 (dd, J = 8.4, 1.7 Hz, 1H, H-4), 7.56 (d, J = 2.0 Hz, 1H, H-3'), 7.54 (dd, J = 8.3, 4.2 Hz, 1H, H-3), 7.45 (d, J = 8.4 Hz, 1H, H-6'), 7.40 (dd, J = 8.4, 2.0 Hz, 1H, H-5'), 7.38 (d, J = 8.5, 1H, H-5), 7.32 (d, J = 8.5, 1H, H-6), 7.27 (dd, J = 8.5, 7.5 Hz, 1H, H-5''), 7.15 (d, J = 7.6 Hz, 1H, NH-10), 6.95 (d, J = 7.6 Hz, 1H, H-9), 6.40 (d, J = 8.6 Hz, 1H, H-6''), 6.36 (d, J = 7.5 Hz, 1H, H-4''), 2.19 (s, 3H).

R = 2'-Br (Bt5): ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.85 (dd, J = 4.1, 1.6 Hz, 1H, H-2), 8.29 (dd, J = 8.4, 1.7 Hz, 1H, H-4), 7.61 (m, 1H, H-3'), 7.53 (dd, J = 8.4, 4.1 Hz, 1H, H-3), 7.45 – 7.16 (m, 7H), 7.10 (d, J = 7.7 Hz, 1H, NH-10), 6.91 (d, J = 7.7 Hz, 1H, H-9), 6.37 (m, 2H), 2.19 (s, 3H).

2nd isomer (21%) 8.88 (dd, J = 4.1, 1.5 Hz), 8.46 (dd, J = 8.7, 1.5 Hz), 7.62 (m), 6.98 (d, J = 8.1 Hz), 6.83 (d, J = 8.1 Hz), 2.25 (s).

R = 3'-Br (Bt6): ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.86 (dd, J = 4.2, 1.7 Hz, 1H, H-2), 8.29 (dd, J = 8.3, 1.6 Hz, 1H, H-4), 7.66 (d, J = 8.5 Hz, 1H, H-6), 7.57 (m, 1H, H-2'), 7.54 (dd, J = 8.3, 4.2 Hz, 1H, H-3), 7.42 (d, J = 8.5 Hz, 1H, H-5), 7.41 – 7.21 (m, 5H), 6.88 (d, J = 8.9 Hz, 1H, H-9), 6.47 (d, J = 8.2 Hz, 1H, H-6''), 6.38 (d, J = 7.1 Hz, 1H, H-4''), 2.23 (s, 3H).

R = 4'-Br (Bt7): ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.85 (dd, J = 4.2, 1.6 Hz, 1H, H-2), 8.28 (dd, J = 8.4, 1.7 Hz, 1H, H-4), 7.64 (d, J = 8.5 Hz, 1H, H-6), 7.53 (dd, J = 8.3, 4.2 Hz, 1H, H-3), 7.48 (d, J = 8.5 Hz, 1H, H-5), 7.50 – 7.25 (m, 5H), 6.84 (d, J = 8.7 Hz, 1H, H-9), 6.46 (d, J = 8.3 Hz, 1H, H-6''), 6.37 (d, J = 7.1 Hz, 1H, H-4''), 2.23 (s, 3H).

R = 2'-F (Bt8): ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.00 (s, 1H, OH), 8.84 (dd, J = 4.1, 1.6 Hz, 1H, H-2), 8.29 (dd, J = 8.3, 1.6 Hz, 1H, H-4), 7.54 (d, J = 8.5 Hz, 1H, H-6), 7.53 (dd, J = 8.5, 4.1 Hz, 1H, H-3), 7.40 (d, J = 8.9 Hz, 1H, H-5), 7.38 (m, 1H, H-6'), 7.27 (dd, J = 8.3, 7.2 Hz, 1H, H-5'', m, 1H, H-4'), 7.20 (d, J = 8.4 Hz, 1H, NH-10), 7.13 (m, 2H, H-3',5'), 7.04 (d, J = 8.4 Hz, 1H, C-9), 6.44 (d, J = 8.3 Hz, 1H, H-6''), 6.35 (dt, J = 7.2, 0.7 Hz, 1H, H-4''), 2.20 (s, 3H); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 160.12 (C-2' d, J = 245.4), 157.24 (C-1''), 155.62 (C-3''), 149.87 (C-8), 148.25 (C-2), 138.05 (C-8a), 137.11 (C-5''), 136.03 (C-4), 130.35 (C-1' d, J = 14.2), 128.94 (C-6' d, J = 4.3), 128.60 (C-4' d, J = 7.9) 127.58 (C-4a), 126.75 (C-6), 124.33 (C-7), 124.06 (C-5' d, J = 3.4), 121.69 (C-3), 116.99 (C-5), 115.20 (C-3' d, J = 21.5), 111.19 (C-4''), 105.28 (C-6''), 46.21 (C-9 d, J = 3.6), 24.28 (CH_3-2''); ^{15}N NMR (60.8 MHz, DMSO-d_6) δ 296 (N-1), 88 (d, J = 90, N-10), 266 (N-2'').

2nd isomer (12%) - 8.87 (dd, J = 4.1, 1.6 Hz), 8.48 (dd, J = 8.7, 1.6 Hz), 7.61 (dd, J = 8.7, 4.1 Hz), 6.42 (dt, J = 8.3, 0.7 Hz, 1H, H-4''), 6.38 (dt, J = 7.1, 0.7 Hz, 1H, H-4''), 2.24 (s).

For the ortho (2′) substituted derivatives two sets of signals are registered due to the hindered rotation around the C-9-C-1′ bond. The ratio of the two observed atropisomers depends on the nature of the substituents and on the temperature.

UV/VIS and HPLC methods for determination of 7-[(6-methylpyridin-2-ylamino) (2,4-dichlorophenyl)methyl]quinolin-8-ol (Bt4) in liquid phase

The UV/VIS spectra of Bt4 in acidic ethanol solutions (pH 1.0, Fig. 3 a,b) displayed maximum absorbance peaks in the UV region at λ=204 nm for the entire concentration range 1-100 μg/ml.

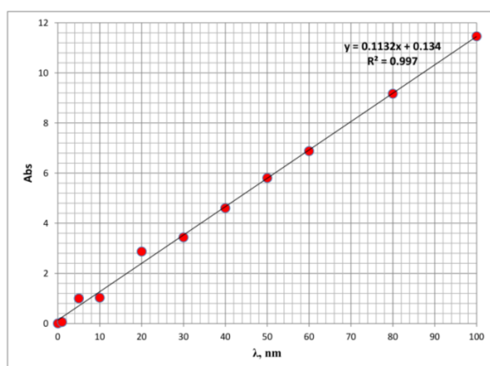

Fig. 3a. UV/VIS calibration curve.

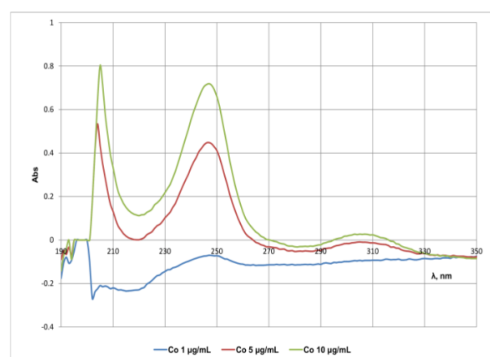

Fig. 3b. UV/VIS spectra of Bt4.

The obtained standard curve for Bt4 was linear over the tested range of initial concentrations C_o 1 - 100 µg/ml. The high value of the calculated correlation coefficient - R^2 0.9970, and the well-resolved UV absorption peaks of the heterocyclic compound proved the applicability of this method for determination of Bt4 concentrations in aqueous medium in the range C_o 1 - 100 µg/ml.

The HPLC experiments were conducted with ten Bt4 standard solutions in the concentration range 10 - 100 µg/ml. The used mobile phase consisted of methanol:H_2O (40:60, v/v). Optimal performance was obtained at a flow rate of 0.8 ml/min. The effect of temperature on the separation process was studied in the range 18 - 30°C. Satisfactory peak resolution and optimum analyses time were established at 30°C, at wavelength λ=204 nm. Bt4 was successfully detected within 1.5 min (Fig. 4 a,b). The standard calibration curve plotted, based on the spectral peak areas, characterized with a high correlation coefficient R^2 0.9825 and the following linear equation:

$$y = 3110.8x + 16864$$

The obtained HPLC chromatograms did not contain any interference peaks, which could influence the quantitative results. Besides, the base line is straight and stable with lower signal noise. The correlation regression coefficient of the obtained standard calibration curve at these HPLC conditions was sufficiently high, although lower than that of the UV/VIS method.

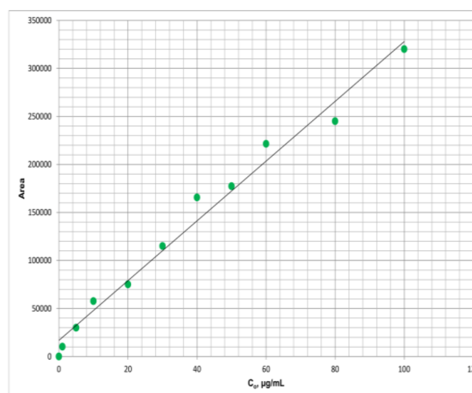

Fig. 4a. HPLC calibration curve.

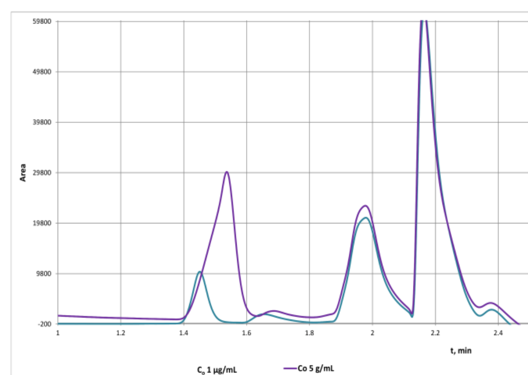

Fig. 4b. HPLC spectra of Bt4.

The values of LOD and LOQ were estimated according to the guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) based on the standard deviation of the response and the slope of the calibration curve of the analyte [34]. The values of these parameters are presented in Table 2.

The comparative analyses of the obtained experimental results revealed that the developed and applied UV/VIS spectrophotometric method characterized with the highest correlation coefficient and lowest SD value.

Table 2. Values of the characteristic parameters and error functions for the UV/VIS and HPLC methods.

Method	R^2	SD	S_x	S_y	LOQ, µg/mL	LOD, µg/mL
UV/VIS	0.9969	0.2201	0.0021	0.0999	2.9117	8.8233
HPLC	0.9825	14704	138.5093	6672.28	7.0782	21.4491

S_x – standard deviation of the slope; S_y – standard deviation of the intercept

Table 3. Microbiological activities of the studied Betti bases – MIC, μg/mL.

Test strains	Bt1, μg/mL	Bt2, μg/mL	Bt3, μg/mL	Bt4, μg/mL	Bt5, μg/mL	Bt6, μg/mL	Bt7, μg/mL	Bt8, μg/mL
Listeria 212	32	250	250	500	125	4	4/500	0
Listeria 362	32	500	500	+	250	500	500	250
Listeria 1094	32	250	500	500	250	250	250	250
Swine erysipelas	125	250	500	500	125	250	250	500
Staphylococus 1	64	250	250	500	125	+	500	250
Staphylococus 5	64	500	500	500	125	+	500	250
Staphylococus 42	64	500	500	500	500	+	+	250
Staphylococus 78	64	500	500	500	125	+	250	250
Staphylococus119	64	+	250	500	125	+	250	250
Bact. megat 10	64	500	500	500	250	500	500	250
Bact. megat 11	64	500	500	500		500	500	500
Bact. megat 39	64	500	500	500	250	500	500	250
Bact. megat 16	64	500	500	500	125	500	500	250
Bact. cereus 12	64	+	+	+	+	500	500	500
Past. mult. 10	0	4	4	+	4	4	4	4
Past. mult. SHp	0	4	4	+	8	8	8	4
Past. mult. R II	0	4	4	+	8	8	8	8
Salm. Dublin	250	+	+	+	+	+	+	+
S. Gollinarum200	250	500	+	+	+	+	+	+
Salm.enter4767	250	+	+	+	+	+	+	+
E. coli 21	250	+	+	+	500	500	+	64
E. coli 56	500	+	+	+	+	+	+	64
E. coli 94	500	+	+	+	+	+	+	64

"+" - registered growth of the tested pathogenic strains in the entire concentration range (4-500 μg/mL)
"0" – not registered growth of the tested pathogenic strains in the entire concentration range (4-500 μg/mL)

Besides, the determined LOD and LOQ values were the lowest. According to the spectra presented in Fig. 4b, however, solutions with Bt4 concentration lower than 5.0 μg/mL are characterized with not well resolved spectral peaks, i.e. the accuracy of the method below this limit is unsatisfactory. Among the basic advantages of the UV/VIS method is its cost-effectiveness and rapidity.

Regarding the developed HPLC methodology, and based on the data from Table 2, it could be concluded that it is characterized with lower accuracy and efficiency as the determined RSD, LOD and LOQ values were higher as compared to those of the UV/VIS method. The HPLC spectral data of Bt4 (Fig. 4b) revealed that its spectral peaks are characterized with great area. Besides, they were significantly more pronounced in the entire tested concentration range as compared to the UV/VIS peaks. The presence of two secondary peaks (at 2 and 2.2 min) could be attributed to the detection of methanol, as the area and height of these peaks remain constant at different initial Bt4 concentrations, which does not influence the analytical results. According to the spectra presented in Fig. 4b, the solutions with Bt4 concentration 1.0 μg/mL are characterized with a very well resolved spectral peak, i.e. the accuracy of the method is satisfactory. Another advantage of the HPLC method is the registered short retention time – 1.5 min.

Biological activities

Considering the wide range of biological activities of compounds containing phenolic hydroxyl group, including 8-hydroxyquinoline [1–8] and the physiological activity of the amino derivatives of the pyridine series [2–4, 11], we predicted high biological activity of the synthesized Betti bases. The results of the microbiological screening are presented in Table 3, where the compounds are denoted as in Table 1. The present study established that the microbiological activity depends on the number and position of the halogen-substitute in the benzene ring of the studied Betti bases. The presence of Cl-atom on position 2 of the benzene ring (Bt1) raised the bacteriostatic activity against all tested strains, as compared to the other Betti bases. All studied Betti bases, except Bt4, showed a bacteriostatic activity against test strains *Past. Mult.* in low concentrations (Table 3). Probably, the accumulation of Cl-atoms in the benzene ring of the Betti bases decreases the bacteriostatic activity. The presence of F-atom on position 2 of the benzene ring (Bt8) raised the bacteriostatic activity against *E. coli* strain (64

μg/mL, Table 3). The results of the microbiological screening of the studied Betti bases showed that they possess selective microbiological activity.

The established microbiological activity of the studied [(6-methylpyridine-2ylamino)(halogeno-phenyl)methyl]quinolin-8-ols was probably due, on the one hand to the presence of a quinoline ring in their molecular structure, and on the other - to the possibility of the –OH group on 8-position of the quinoline ring to form chelate complexes with traces of ions of some d-elements. Probably, the chelate complexes of 3d-elements and the derivatives of 8-hydroxyquinoline, which penetrated into the bacterial cell, lead to disturbance of the endo-cellular esterase within the cell, and caused violations of the metabolism of vital for the microorganisms enzymes. These observations of the prepared Betti bases correspond to those reported by other research teams [35]. The microbiological activity may also be due to the ability of 8-hydroxyquinoline derivatives to capture free radicals (spin - trap) [10], which allows us to continue our research in this direction and to investigate the antioxidant activity of Bt4.

EPR spectroscopy of Bt4

By a direct EPR spectroscopy, the EPR singlet signal, registered in the powdered forms of Bt4, exhibited almost a symmetrical single EPR signal (Fig. 5). According to it, the intensive central singlet in the EPR spectrum characterized with a g factor 1.9737 ± 0.0002 and width - 43 mT (Fig. 5).

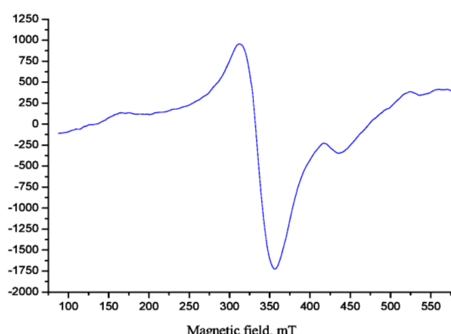

Fig. 5. EPR singlet signal in the powdered form of Bt4.

According to the registered EPR spectrum, Bt4 possesses radical structure. Considering the presence of a phenolic group in its structure (Fig. 2), we suppose that this group is involved in the formation of radical species and it could possibly capture reactive oxygen species (ROS), i.e. exhibit antioxidant activity. It is known that ROS such as superoxide radicals ($O_2^{-\bullet}$), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2) play a key role in human cancer development and has gained much support recently. They have been shown to possess several characteristics of carcinogens [36, 37].

Further, we hypothesized that the antioxidant action of the studied [(6-methylpyridin-2ylamino)(2,4-dichlorophenyl)methyl]quinolin-8-ol may be responsible for the beneficial effects of this compound, thus the other 6-methylpyridin-2ylamino)(halogenophenyl) methyl]quinolin-8-ols could be used as antioxidants. According to the result obtained in this study, the compounds tested can probably be used for the treatment of diseases associated with oxidative stress.

CONCLUSION

We investigated a three-component reaction of 8-hydroxyquinoline, halogeno-substituted benzaldehydes and 2-amino-6-methylpyridine, found a convenient methodology for the synthesis and efficient analytical methods for quantification in liquid phase of new type of Betti bases. The potential applications of this reaction in synthetic and medicinal chemistry might be significant. We assume that the potential of the studied Betti bases as pharmacological compounds is promising.

Acknowledgements: *The study was supported financially by Scientific Project №14-15, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria; The work was also supported by Projects UNA-17/2005 and DRNF-0213/2009 of Bulgarian National Science Fund, Sofia, Bulgaria.*

REFERENCES

1. V. Oliveri, G. Vecchio, *EJMECH.*, **120**, 252 (2016).
2. N. N. Chopde, *Int. J. Pharm. Pharm. Sci.,* **5**, 541 (2014).
3. C. Cardellicchio, M. A. Capozzi, F. Naso, *Tetrahedron: Asymmetry*, **21**, 507 (2010).
4. B. P. Mathew, A. Kumar, S. Sharma, P. K. Shukla, M. Nath, *EJMECH,* **45**, 1502 (2010).
5. R. Musiol, M. Serda M, S. Hensel-Bielowka, U. J. Polanski, *Curr. Med. Chem.*, **17**, 1960 (2010).
6. X. H. Ma, X. Y. Zhang, J. J. Tan, W. Z. Chen, C. X. Wang, *Acta Pharmacol. Sin.,* **25**, 950 (2004).
7. A. Y. Shen, S. N. Wu, C. T. Chiu, *J. Pharm. Pharmacol.*, **51**, 543 (1999).
8. I. M. Awad, A. A. Aly, A. M. Abdel-Alim, R. A. Ab, S. H. Ahmed, *J. Inorg. Biochem.,* **33**, 77 (1988).
9. S. Ariyasu, A. Sawa, A. Morita, K. Hanaya, M. Hoshi, I. Takahashi, B. Wang, S. Aoki, *Bioorg. Med. Chem.,* **22**, 3891 (2014).
10. O. Varagupta, P. Boonchoong, Y. Wongkrajang, *Bioorg. Med. Chem.*, **8**, 2617 (2000).

11. M. Betti, *Org. Synth. Coll.*, **1**, 381 (1944).
12. J. P. Phillips, R. W. Keown, Q. Fernando, *J. Org. Chem.*, **19**, 907 (1954).
13. J. P. Phillips, *Chem. Rev.*, **56**, 271 (1956).
14. J. P. Phillips, J. P. Barral, *J. Org. Chem.,* **21**, 692 (1956).
15. J. P. Phillips, J. T. Leach, *Trans. Ky. Acad. Sci.,* **24**, 95 (1964).
16. C. Cardellicchio, M. A. Capozzi, A. Faso, *Tetrahedron: Asymmetry,* **21**, 507 (2010).
17. M. Salamone, R. Amorati, S. Menichetti, C. Viglianisi, M. Bietti, *J. Org. Chem.*, **79**, 6196 (2014).
18. P. F. Kaiser, J. M. White, C. A. Hutton, *J. Am. Chem. Soc.,* **130**, 16450 (2008).
19. G. Cheng, X. Wang, R. Zhu, C. Shao, J. Xu, Y. Hu, *J. Org. Chem.*, **76**, 2694 (2011).
20. V. Prachayasittikul, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, *Drug Des. Devel. Ther.,* **7**, 1157 (2013).
21. H. Wang, Y. Dong, J. Sun, X. Xu, R. Li, J. Hu, *J. Org. Chem.,* **70**, 1897 (2005).
22. H. Wei, L. Yin, H. Luo, X. Li, A. S. Chan, *Chirality,* **23**, 222 (2011).
23. C. Cimarelli, D. Fratoni, A. Mazzanti, G. Palmieri, *Tetrahedron: Asymmetry*, **22**, 591 (2011).
24. I. Szatmári, F. Fülöp, *Tetrahedron,* **69**, 1255 (2013).
25. M. Marinova, K. Kostova, P. Tzvetkova, M. Tavlinova-Kirilova, A. Chimov, R. Nikolova, B. Shivachev, V. Dimitrov, *Tetrahedron:Asymmetry*, **24**, 1453 (2013).
26. A. Shahrisa, R. Teimuri-Mofrad, M. Gholamhosseini-Nazari, *Mol. Diversity,* **19**, 87 (2015).
27. R. Teimuri-Mofrad, A. Shahrisa, M. Gholamhosseini-Nazari, N. Arsalani, *Res. Chem. Intermed.,* **42**, 3425 (2016).
28. M. Shafiee, A. R. Khosropour, I. Mohammadpoor-Baltork, M. Moghadam, S. Tangestaninejad, V. Mirkhani, *Tetrahedron Lett.,* **53**, 3086 (2012).
29. A. Kumar, M. K. Gupta, M. Kumar, *Tetrahedron Lett.*, **51**, 1582 (2010).
30. J. P. Errico, B. Mugrage, I. Turchi, M. Sills, J. Ong, J. Allocco, P. Wines, *Patent* WO2011/85126 (2011).
31. R. A. Mekheimer, A. M. Asiri, A. M. Abdel Hameed, R. R. Awed, K. U. Sadek, *Green Processing and Synthesis*, **5**, 365 (2016).
32. I. Sergiev, N. Georgieva, A. Alexieva, E. Karanov, I. Petkov, *J. Ecol. Sci. - Ecology and Future*, **4**, 42 (2007).
33. A. Pavlov, N. Takuchev, N. Georgieva, *Biotechnol. Biotechnol. Equip.*, **26**(SE), 164 (2012).
34. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and methodology, Q2 (R1), 1 (2005).
35. G. L. Grunewald, V. H. Dahanukar, R. K. Jalluri, K. R. Criscione, *J. Med. Chem.*, **42**, 3315 (1999).
36. P. A. Cerutti, *Lancet,* **344**, 862 (1994).
37. R. H. Burdon, *Free Radic. Biol. Med.,* **18**, 775 (1995).

СИНТЕЗ И СВОЙСТВА НА НЯКОИ БЕТИ БАЗИ КАТО ПОТЕНЦИАЛНИ ЛЕКАРСТВЕНИ ПРЕПАРАТИ

Н. Георгиева[1]*, З. Янева[1], С. Симова[2], Г. Николова[3]

[1] *Ветеринарно-медицински факултет, Тракийски университет, Студентски град, 6000 Стара Загора, България*

[2] *Институт по органична химия с Център по фитохимия, Българска академия на науките, ул. Акад. Г. Бончев, бл. 9, 1113 София, България*

[3] *Медицински факултет, Тракийски университет, ул. „Армейска“11, 6000 Стара Загора, България*

Постъпила на 18 април 2017 г.; Коригирана на 02 юни 2017 г.

(Резюме)

Целта на настоящето изследване бе да се синтезират Бети бази чрез модифицирана реакция на Бети, да се разработят аналитични методи за количествено определяне и тестване на тяхната микробиологична и антиоксидантна активности. За модификацията на реакцията на Бети ние използвахме първичен хетероцикличен амин, 8-хидроксихинолин и халогено-заместени ароматни алдехиди. Приложените аналитични методи за количествено определяне на изследваните Бети бази са с висока точност, висока линейност и задоволителни стойности на граница на откриване (LOD) и граница на количествено определяне (LOQ). Бети базите бяха получени с добив 80-98% и охарактеризирани чрез елементен анализ и ЯМР спектри. Тяхната микробиологична активност бе тествана срещу 23 патогенни щамове микроорганизми (различни грам-положителни и грам-отрицателни). Микробиологичният скрининг доказа тяхната селективна микробиологична активност. Според ЕПР изследването Бети базите притежават радикалова структура и вероятно проявяват антиоксидантна активност. Ние предполагаме, че потенциалът на представените Бети бази като фармакологични препарати е голям.