Synthesis and Investigation of Antimicrobial and Antioxidant Activity of 3-Nitro-*N*-(3-chloro-2-oxo-substituted-phenyl-azetidin-1-yl)naphtho [2,1-*b*]furan-2-carboxamides

K. SHASHIKALA DEVI[#], M.RAMAIHAH[§], D.L. ROOPA[§] and V.P VAIDYA^{§*}

[#]Department of Chemistry, Maharani's Science College for Women
Bangalore, Karnataka, India

[§]Department of Chemistry, NMKRV College for Women, Bangalore, Karnataka, India

^{§*}Department of Chemistry, Kuvempu University, Jnana Sahyadri
Shankaraghatta-577451, Dist: Shimoga, Karnataka, India
vaidyavijaya@hotmail.com

Received 23 February 2010; Accepted 1 May 2010

Abstract: Ethyl-3-nitronaphtho[2,1-*b*]furan-2-carboxylate (**2**) has been synthesized from ethyl naphtho [2,1-*b*]furan-2-carboxylate (**1**). This nitro product on reaction with hydrazine hydrate formed 3-nitronaphtho [2,1-*b*] furan-2-carbohydrazide (**3**). Various Schiff bases 3-nitro-*N*¹(aryl-methylene)-substituted-naphtho [2,1-*b*]furan-2-carbohydrazides **4(a-g)** were obtained by treating hydrazide (**3**) with different aldehydes. These Schiff bases on reaction with chloro acetyl chloride in presence of triethylamine in dioxane yielded 3-nitro-*N*-(3-chloro-2-oxo-substituted-phenyl-azetidine-1-yl) naphtho [2,1-*b*]furan-2-carboxamides **5(a-g)**. All the newly synthesized compounds have been characterized by spectral and analytical studies. The Schiff bases, **4a-g** and azetidine derivatives, **5a-g** have been studied for antioxidant and antimicrobial activities.

Keywords: Azetidinone, Naphthofuran, Antioxidant activity, Antimicrobial activity.

Introduction

The 2-azetidinone (β -lactam) ring is the essential feature of a large number of biologically active compounds namely penicillins, cephalosporins, carumonam, aztreonam, thienamycine and the nocardicins¹. Recently, besides the antibacterial activity, antifungal, antitubercular, antitumor, cholesterol absorption inhibition and enzyme inhibition activity have been reported in compounds containing 2-azetidinone ring²⁻⁴. Due to their relevance in both clinic and economic fields, variously substituted 2-azetidinones represent a very attractive target for contemporary organic synthesis⁵⁻⁸.

The various naphtho[2,1-*b*]furan derivatives, synthesized in our laboratory, have been shown to possess wide spectrum of biological and pharmacological activities⁹⁻¹². There are several evidences that the presence of nitro group enhance the biological activities of the compounds many folds. Thus 2-nitrobenzofuran, 2-nitro-3-methoxynaphtho[2,1-*b*]furan and 2-nitro-7-methoxynaphtho[2,1-*b*]furan have been synthesized and evaluated for possible antimalarial and mutagenic activities and they also find application in veterinary medicine¹³⁻¹⁵.

Hence, with a view to further assess the pharmacological profile of this class of compounds, it was thought worthwhile to synthesize some new congeners of β -lactam heterocycles by incorporating the naphtho[2,1-*b*]furan nucleus bearing nitro group and azetidinone moieties in a single molecular framework. The present work deals with the synthesis of the title compounds starting from ethyl naphtho[2,1-*b*]furan-2-carboxylate, followed by their antioxidant and antimicrobial screening.

Experimental

Melting points were recorded in open capillary tubes and are uncorrected. IR (cm^{-1}) were recorded in KBr on FT-IR (Research spectrometer) Shimadzu 8201 PC ($4000\text{--}400\text{cm}^{-1}$) and NMR on Bruker DRX-300 (300MHz-FT-NMR with low and high temperature facility -90° to $+80^\circ\text{C}$). Standard chemical shifts are given in δ ppm values. Compounds were checked for their purity by TLC on silica gel plates using chloroform and ethyl acetate of varying polarities and spots were located by iodine vapour.

*Synthesis of ethyl 3-nitronaphtho[2,1-*b*]furan-2-carboxylate (2)*

A cooled nitrating mixture of con HNO_3 and con H_2SO_4 (1:2, 15 mL) was added very slowly to a cooled solution of ester **1** (2.4 g, 0.01 mol) in glacial acetic acid (4 mL) and the mixture was stirred for about 30 minutes at 0 to 15°C . The stirring was continued for 2 h and the reaction mixture was poured on to crushed ice. The product, that separated as solid, was collected, dried and recrystallised from aqueous ethanol.

*Synthesis of 3-nitronaphtho[2,1-*b*]furan-2-carbohydrazide (3)*

A mixture of ethyl 3-nitronaphtho[2,1-*b*]furan-2-carboxylate (2.55 g, 0.01 mol) and hydrazine hydrate (2.5 mL, 99%) in ethanol (10 mL) was heated under reflux for 5 h, cooled to room temperature and the solid thus separated was filtered, washed with ethanol and recrystallised from aqueous DMF to obtain the product as solid.

*Synthesis of 3-nitro-*N*¹(aryl-methylene)-substituted-naphtho[2,1-*b*]furan-2-carbohydrazide 4(a-g)*

To a solution of 3-nitronaphtho[2,1-*b*]furan-2-carbohydrazide (0.42 g, 0.001 mol) in DMF (50 mL), aryl aldehydes (6 mL) were added, the mixture was refluxed on water bath for 5 h, and then poured into ice-cold water. The product that separated as solid was filtered, dried, and recrystallised from aqueous DMF.

*Synthesis of 3-nitro-*N*-(3-chloro-2-oxo-substituted-phenyl-azetidine-1-yl)naphtho[2,1-*b*]furan-2-carboxamide 5(a-g)*

A solution of chloro acetyl chloride (0.6 mL, 0.0055 mol) in dioxane (10 mL) was cooled to -10°C using ice-salt bath and kept for stirring. To this, triethyl amine (0.5 g, 0.005 mol) was added drop wise maintaining the temperature below 0°C , while white solid separated out. To this reaction mixture, solution of *N*-[(substituted)methylene]naphtho[2,1-*b*]furan-2-carbohydrazides (0.0025 mol) in dioxane (10 mL) was added drop wise regulating the

temperature less than 0 °C with stirring. After the addition was over the reaction mixture was refluxed for 16 h and was poured into ice cold water. The solid that separated out was filtered dried and recrystallised from dioxane.

Results and Discussion

The required starting materials, to accomplish the synthesis of title compounds, ethyl naphtho[2,1-*b*]furan-2-carboxylate (**1**) and ethyl 3-nitronaphtho[2,1-*b*]furan-2-carboxylate (**2**) were synthesised according to the procedure reported in the literature¹⁶. The reaction of ethyl 3-nitronaphtho[2,1-*b*]furan-2-carboxylate (**2**) with hydrazine hydrate in presence of acid catalyst in ethanol produced 3-nitronaphtho[2,1-*b*]furan-2-carbohydrazide (**3**). The structure of the carbohydrazide (**3**) was established by comparing its m.p. and R_f value with an authentic sample¹⁶.

The carbohydrazide (**3**) was treated with various substituted aromatic aldehydes in refluxing ethanol to obtain corresponding Schiff bases, 3-nitro-*N*¹(aryl-methylene)-substituted-naphtho[2,1-*b*]furan-2-carbohydrazides (**4a-g**) in good yield. The structures of the Schiff bases (**4a-g**) were established by IR spectral data, showing carbonyl absorption band in between 1680-1695 cm⁻¹ and C=N stretching frequency at 1620-1621 cm⁻¹. The structure assigned, was supported by ¹H NMR spectra in which a multiplet at δ 8.3 to 8.5 due to aromatic protons was observed.

Synthesis of 3-nitro-*N*-(3-chloro-2-oxo-substituted-phenyl-azetidine-1-yl)naphtho[2,1-*b*]furan-2-carboxamide **5(a-g)** was accomplished by the reaction between 3-nitro-*N*¹(aryl-methylene)-substituted-naphtho[2,1-*b*]furan-2-carbohydrazides **4a-g** with chloro acetyl chloride in presence of triethyl amine in dioxane. The structure of **5a** was confirmed by elemental analysis and spectral studies. The IR spectrum of **5a** exhibited the sharp adsorption band at 1709 cm⁻¹ due to amide carbonyl group and at 1657 cm⁻¹ due to carbonyl group of β-lactam ring. The ¹H NMR spectrum showed a singlet integrating for three protons at δ 3.8 due to OCH₃ protons. A multiplet at δ 7.0-8.5, two doublets one at δ 7.4 and another at δ 7.6 were attributed to ten aromatic protons, one CHPh and one CHCl protons respectively. The CONH proton appeared as a singlet downfield at δ12.1. The physical and analytical data of the newly synthesised compounds is presented in Table 1.

Table 1. The physical and analytical data of the newly synthesised compounds

Compd	R	Yield, %	M.P, °C	Found (calculated) %		
				C	H	N
4a	-C ₆ H ₅	50.00	209	66.82,(66.85)	3.60,(3.62)	11.65, (11.69)
4b	4-OCH ₃ -C ₆ H ₄	85.00	180	64.77,(64.78)	3.55,(3.59)	11.62, (11.69)
4c	-C ₄ H ₃ O	63.49	180	61.68,(61.71)	3.40,(3.42)	11.99, (12.00)
4d	3-NO ₂ -C ₆ H ₄	79.15	170	59.37,(59.40)	2.94,(2.97)	13.82, (13.86)
4e	2-Cl-C ₆ H ₄	64.73	>250	60.88, (60.99)	3.01,(3.04)	10.66, (10.67)
4f	-CH=CH- C ₆ H ₅	98.54	230	68.55,(68.57)	3.86,(3.89)	10.87, (10.90)
4g	4-Cl-C ₆ H ₄	55.09	229	60.87,(60.99)	3.00,(3.04)	10.63, (10.67)
5a	-C ₆ H ₅	24.24	>250	58.20,(58.21)	3.51,(3.52)	9.23, (9.26)
5b	4-OCH ₃ -C ₆ H ₄	29.41	>250	59.28,(59.29)	3.41,(3.43)	8.97, (9.02)
5c	-C ₄ H ₃ O	70.96	>250	56.38,(56.40)	2.81,(2.82)	9.85, (9.87)
5d	3-NO ₂ -C ₆ H ₄	46.71	180	54.93,(54.96)	2.67,(2.70)	11.64, (11.65)
5e	2-Cl-C ₆ H ₄	52.94	101	56.14,(56.17)	2.75,(2.76)	3.73, (3.75)
5f	-CH=CH-C ₆ H ₅	26.47	104	62.38,(62.40)	3.42,(3.46)	9.07, (9.10)
5g	4-Cl-C ₆ H ₄	26.01	110	56.13,(56.17)	2.74,(2.76)	3.72, (3.75)

Scheme 1

Antioxidant activity

The antioxidant activity of azetidinones and Schiff bases was determined by DPPH radical method. The compounds were taken at 100 µg concentration by their dry weight and mixed with 5 mL of 0.1 mM solution of DPPH radical in methanol and incubated at 20 °C for 20 min in darkness. The control was prepared as above without the compound and methanol was used for the baseline correction. The absorption of each solution was measured at 517 nm using “Shimadzu 175” spectrophotometer. The % of radical scavenging activity was expressed as percentage activity using the following formula.

$$\% \text{ of Radical scavenging activity} = \frac{(\text{Control absorbance} - \text{sample absorbance})}{\text{Control absorbance}} \times 100$$

Where control absorbance is the measurement of DPPH radical solution without compound and sample absorbance is the measurement of DPPH radical solution with the compound. Ascorbic acid (5 mg/mL) was used as standard for the comparison of antioxidant activity. The results revealed that, the compounds **4g** and **5c** exhibit moderate activity when compared to the standard ascorbic acid whose concentration was 5 mg/mL.

Antimicrobial activity

The antimicrobial activity of newly synthesized compounds was determined by cup-plate method. The *in vitro* antimicrobial activity was carried out against 24 h old culture of four bacteria and three fungi. The bacteria used were *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus* and *Staphylococcus aureus* and fungi used were *Aspergillus niger*, *Trichoderma spp* and *Penicillium spp*. The compounds were tested at a concentration of 500 µg/mL in DMF against both the organisms. The chloroamphenicol and fluconazole were used as standard for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with the standard drug after 24 h and incubation at 37 °C for antibacterial activity and 72 h at 25 °C for antifungal activity.

The compound **5f** showed promising activity against *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*. The compounds **5d** and **5e** exhibited moderate activity against *E. coli*. For antifungal activity, there was no zone of inhibition. The compounds did not exhibit activity against *Aspergillus niger*, *Trichoderma spp* and *Penicillium spp* organisms.

Acknowledgment

We thank the Principal, NMKRV College for Women, Bangalore, for providing laboratory facilities. The authors are also thankful to the Head, Department of Microbiology, Maharani's Science College, Bangalore, Head, Department of Biochemistry for helping in carrying out biological activities and I.I.Sc. Bangalore for spectral analysis.

References

1. Mata E G, Fraga M A and Delpiccolo C M L, *J Comb Chem.*, 2003, **5**, 208-210.
2. Singh G S, *Mini-Rev Med Chem.*, 2004, **4**, 69-92.
3. Singh G S, *Mini-Rev Med Chem.*, 2004, **4**, 93-109.
4. (a) Deshmukh A R, Bhawal B M, Krishnaswami D, Govande V V, Shinkre B A and Jayanthi A, *Curr Med Chem.*, 2004, **11**, 1889-1920; (b) Alcaide B and Almendros P, *Curr Med Chem.*, 2004, **11**, 1921-1949.
5. Georg G I, Ed., *The Organic Chemistry of β -Lactam*, VCH: New York, 1993.
6. Sammes P G, *Chem Rev.*, 1976, **76**, 113-155.
7. Southgate R *Contemp Org Synth.*, 1994, **1**, 417.
8. Mahadevan K M, Basavaraja K M, Prathima Mathias D A and Vaidya V P, *Indian J Chem.*, 2005, **44B**, 789-793.
9. Ravindra K C, Vagdevi H M and Vaidya V P, *ARKIVOC*, 2008, **XI**, 1.
10. Vaidya V P, Chandrashekhara C, Kumaraswamy M N and Basavaraja K M, *Indian J Heterocyclic Chem.*, 2007, **16**, 341.
11. Kumaraswamy M M, Prathima Mathias D A, Chandrashekhara C and Vaidya V P, *Indian J Pharma Chem.*, 2007, **68(6)**, 731-736.
12. Padmashali B, Mahadevan K M, Vaidya V P and Latha K P, *Indian J Chem.*, 2005, **44B**, 1446-1451.
13. Chiarini A, Ferranti A, Giovanninetti G and Metteuzzi D, *Farmaco II*, 1980, **35**, 413-417.
14. Tozkoparan B, Gokhan N, Aktay G, Yesilada E and Ertana M, *Eur J Med Chem.*, 2000, **35**, 743-750.
15. Mistry K and Desai K R, *Indian J Chem.*, 2005, **44B**, 1452.
16. Rajashekhara H, Ramesh D, Chandrashekhara C, Mahadevan K M and Vaidya V P, *Indian J Heterocyclic Chem.*, 2007, **16**, 353.