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Synthesis, characterization and antimicrobial studies of novel N-substituted napthofuran carboxamides

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Abstract

Prompted by the varied biological activities of carboxamides and napthofurans, a series of N-substituted napthofuran carboxamides **(4a-f)** derived from napthofurans were prepared by treating aromatic primary amines with naphofuroic acids **(3a-c)** employing $POCl_3$. The structures of these novel compounds were confirmed by elemental analysis and spectral data. All title compounds were screened for their antimicrobial activities. The screening data indicated that tested compounds were found to be less active than the standard drugs. A comparative study of antimicrobial activities of synthesized carboxamides is done.

Key words: Naphtho[2,1] furan, naphthoroic acids, carboxamides, antifungal and antibacterial activities.

Introduction

Naphthofurans possess a broad range of biological activities and are constituents of many important natural products [1-7]. Naphthofuran derivatives isolated from various natural sources like Fusarium oxysporum[8], Gossypium barbadense[9] are well known for various biological activities like anticancer[10], antifertility[11], mutagenic[12], growth inhibitory[13] and oestrogenic[14]. These derivatives have also been shown to exhibit cytotoxic activity[15]. Various derivatives of naphtho [2,1- *b*]furan fused with pyrimidine ring were synthesized and evaluated for antibacterial, antifungal, diuretic, and anthelmintic activities[16-18]. Amides and their heterocyclic derivatives can be used for the, prevention and treatment of tissue damage, involvement in inflammatory sites, the treatment of psoriasis and ulcerative colitis[19], etc.

In view of the various biological activities of napthofurans and substituted amides, the attempt has been made to synthesize various novel N-substituted napthofuran carboxamides carrying naphthofuryl ring and to study their antimicrobial activities. The present work describes the synthesis, characterization and antimicrobial studies of novel N-substituted napthofuran carboxamides.

Materials and Methods

Melting points were determined in open glass capillaries and were found uncorrected. The purity of the compounds was checked by TLC. IR spectra were recorded in KBr on a Perkin Elmer Spectrometer. ^1H NMR spectra were recorded on Brucker 400 MHz instrument in DMSO-d_6 as solvent and TMS as an internal standard.

Ethyl naphtho-[2,1-b]furan-2-carboxylate 2a

To a solution of 2-hydroxy-1-naphthaldehyde **1** (5.16 g, 0.03 mol) in dry N,N-dimethylformamide (25 ml), ethylchloroacetate (3.66 g, 0.03 mol) and anhydrous potassium carbonate (12.4 g, 0.9 mol) were added and the reaction mixture was refluxed on water bath for 24 h. The reaction mixture was then poured into ice cold water, to obtain the product ethyl naphtho-[2,1-*b*]furan-2-carboxylate **2a** as solid, which was collected by filtration, dried and recrystallised from ethanol.

5-Bromo-2-ethylnaphtho-[2,1-b]furan-2-carboxylate 2b

To a solution of 2-ethyl naphthofuran-2-carboxylate **2a** (0.1mol) in glacial acetic acid was added a solution of bromine (0.1mol) in acetic acid (20 ml) with stirring during 1h at 10-20^0 C and the stirring was continued for 3h. The reaction mixture was poured into ice-cold water and the solid obtained was filtered out. It was washed with water, dried and the product was recrystallised from ethanol.

5-Nitro -2-ethyl naphtho- [2,1-b] furan 2-carboxylate 2c

To a solution of 2-ethyl naphthofuran 2-carboxylate **2a** (0.1mol) in glacial acetic acid, nitrating mixture was added with stirring during 1h at 10-20 ^{o}C and the stirring was continued for 3h. The reaction mixture was poured into ice-cold water and the solid obtained was filtered out. It was washed with water, dried and the product was recrystallised from ethanol.

5-Substituted-naphtho[2,1- *b*]furan-2-carboxylic acids (3a-c)

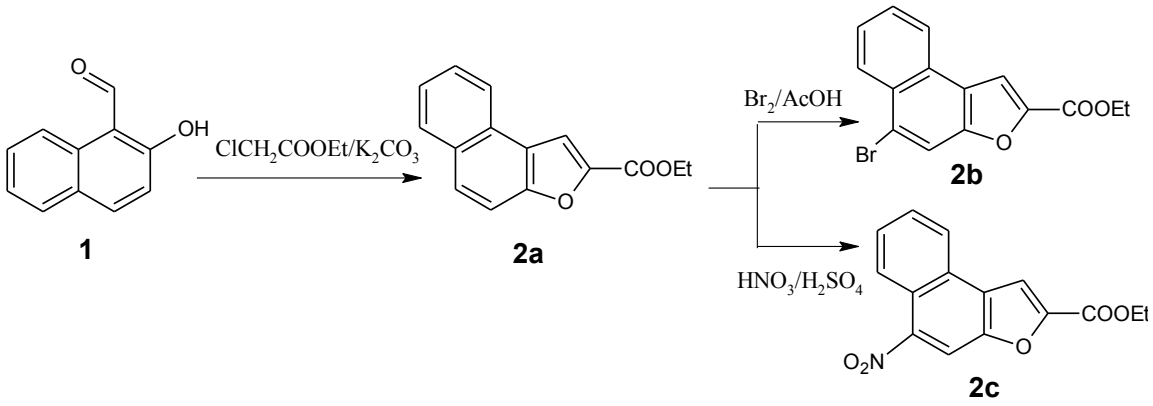
Ethyl naphtho[2,1-b]furan 2-carboxylate was dissolved in methanol and mixed with 10% NaOH solution. The mixture was refluxed for 2h. After the completion of the hydrolysis, the reaction mixture was poured into ice cold water and acidified with hydrochloric acid. Solid separated is filtered and recystallised from ethanol. The 5-substituted naphthofuran-2-carboxylic acids **(3a-c)** were prepared and melting points were recorded (Table 1).

Table 1: Physical characterization data of compounds **(3a-c)**

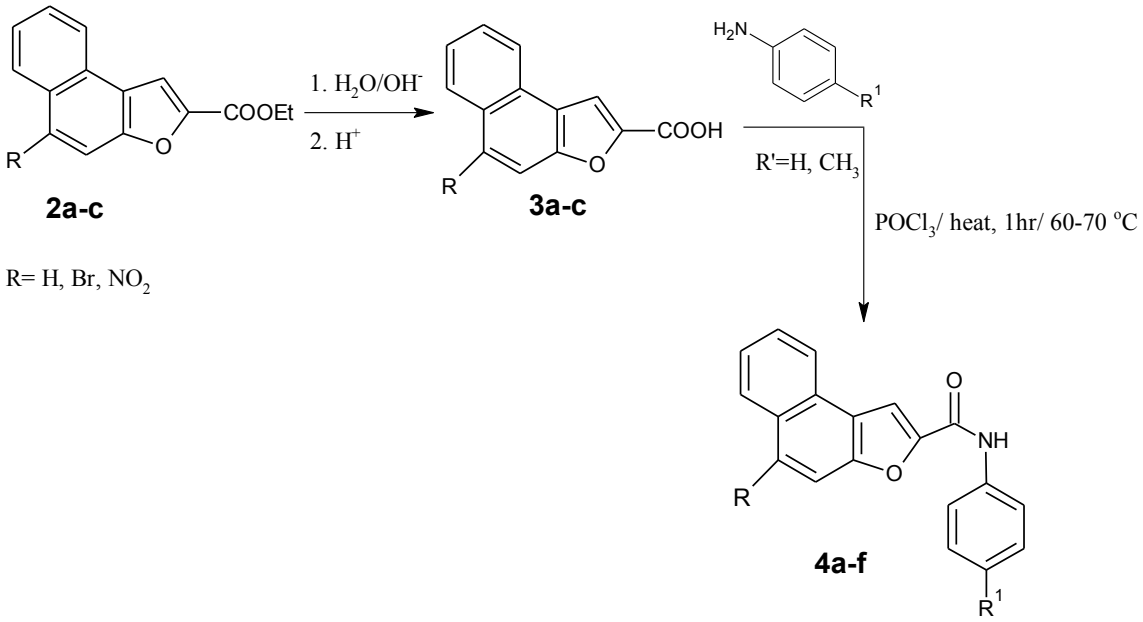
Compd.	R	Molecular formula	m.p. ^{o}C	Yield (%)	Elemental Analysis(%) Calculated (Found)		
					C	H	N
3a	H	$C_{13}H_8O_3$	174	85	73.5(73.2)	3.0(2.98)	-
3b	Br	$C_{13}H_7BrO_3$	228	88	53.6(53.22)	2.4(2.33)	-
3c	NO_2	$C_{13}H_7NO_5$	>280	75	60.7(60.31)	2.7(2.53)	5.44(5.32)

5-substituted-naphtho[2,1-b]furoyl-4-substituted benzene carboxamide (4a-f)

To an equimolecular mixture of suitable substituted aromatic amine (10 mmol) and naphthofuroic acid **(3a-c)** (10 mmol), phosphorus oxychloride (2ml, 20 mmol) was added. The resulting mixture was refluxed for 1h on water bath. The reaction mixture was poured into crushed ice with stirring. The resultant solid was collected, washed with water and filtered off to obtain the solid product (**4a-f)**. These compounds were purified by recrystallisation from ethanol. The characterization data of compounds **4a-f** are recorded in Table 2. The sequence of the reaction is depicted in the **Scheme 1** and **Scheme 2.**



Scheme 1: Synthesis of 2-ethyl naphthofuran-2-carboxylate derivatives



Scheme 2: Reaction pathway for the synthesis of N-substituted napthofuran carboxamides

Table 2: Physical characterization data of compounds **(4a-f)**

Compd.	R	R^1	Molecular formula	m.p. °C	Yield (%)	Elemental Analysis (%) Calculated (Found)		
						C	H	N
4a	H	H	$C_{19}H_{13}NO_2$	103	85	79.44(79.38)	4.52(4.43)	4.87(4.86)
4b	H	CH_3	$C_{20}H_{15}NO_2$	85	84	79.73(79.65)	4.98(4.88)	4.65(4.62)
4c	Br	H	$C_{19}H_{12}BrNO_2$	260	86	77.50(77.33)	3.27(3.26)	3.82(3.79)
4d	Br	CH_3	$C_{20}H_{14}BrNO_2$	180	85	63.15(63.04)	3.68(3.66)	3.68(3.60)
4e	NO_2	H	$C_{19}H_{12}N_2O_4$	240	86	68.67(68.56)	3.61(3.59)	8.43(8.41)
4f	NO_2	CH_3	$C_{20}H_{14}N_2O_4$	>32	84	69.36(69.23)	4.04(4.05)	8.09(8.02)

Results and Discussion

Ethyl naphtho-[2,1-b]furan-2-carboxylate **(2a)** was prepared by treating 2-hydroxy-1-naphthaldehyde (1) with ethylchloroacetate in presence of potassium carbonate in dimethylformamide. This compound **(2a)** was brominated to get compound **(2b)** and nitrated to get compound **(2c)**. These esters were hydrolyzed in alkaline medium to obtain their respective carboxylic acids **(3a, 3b, 3c)**. The resulting carboxylic acids were then warmed with 4-substituted aromatic amines, employing phosphorus oxychloide on a water bath maintained at 40-45 ^{o}C to yield 5-substituted-nahtho[2,1] furanoy1-4-subituted aromatic amines **(4a-f)**. Title compounds were confirmed on the basis of elemental analyais and spectral data.

In the IR spectrum of compound **4d**, C=O stretching frequency appeared at 1720 cm-^{1} and NH stretching frequency observed at 3300 cm-^{1}. The 400 MHz ^{1}H NMR spectrum of compound **(4d)** showed a singlet corresponding to methyl group at δ, 1.9. The NH proton of the amide group appeared as a singlet at δ, 3.54 integrating for one proton. The aromatic protons of the p-tolyl group appeared in the range of δ, 7.80-8.04 integrating for four protons. The four aromatic protons of naphthalene ring appeared as a complex multiplet in the range of δ, 7.56-7.72 and the remaining two protons of naphthalene ring appeared as two doublets at δ, 8.31 and 8.51 respectively.

The mass spectrum of compound **(4d)** showed a molecular ion peak at m/z 380 consistent with its molecular formula $C_{20}H_{14}BrNO_2$ thus confirming the formation of N-(3-bromo)naphthofuroyl-p-toluidine.

All the title compounds **(4a-f)** were screened for their antimicrobial activities according to tube dilution method [20, 21].

a) Antibacterial activity: All the newly synthesized compounds were screened for their *in vitro* antibacterial activities against Gram positive bacteria viz., *Staphyllococcus aureus* and Gram negative bacteria viz., *E.coli* at concentrations of 0.0625 mg, 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg. The minimum inhibitory concentrations (MIC values) were determined. Gentamycin was used as the standard drug at concentrations of 25 µg, 50 µg, 100 µg, 200 µg, 400 µg and 800 µg for comparison and the solvent control was kept. The screening data indicated that all the synthesized N-substituted napthofuran carboxamides showed appreciable antibacterial activity against *E.coli and S.aureus e*xcept compounds **4d** and **4e.** The results of antibacterial activity are shown in Table 3 and 4.

b) Antifungal activity: The newly synthesized compounds were also tested for their antifungal activities against *Aspergillus niger* and *Candida albicans* according to tube dilution method at concentrations of 0.0625 mg, 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg. Amphotericin was used as the standard drug at concentrations of 25 µg, 50 µg, 100 µg, 200 µg, 400 µg and 800 µg for comparison. The minimum inhibitory concentrations (MIC values) were determined. The screening data indicated that N-substituted napthofuran carboxamides did not show any appreciable antifungal activity except **4a, 4b, 4c** against *Candida albican,* but it was less than the standard drug. The results of antifungal activity are shown in Table 3 and 4.

Table-3: Antimicrobial activity screening data at 2 mg concentration of N-substituted napthofuran carboxamides

	Antibacterial activity		Antifungal activity	
Compound	*Staphyllococcus aureus*	*Escherichia coli*	*A.niger*	*C.albicans*
4a	1	0.3	0.5	1.5
4b	0.9	0.9	0	1.4
4c	0.3	0.5	0	1.2
4d	0	0	0	0
4e	0	0	0	0
4f	0.8	0.7	0	0.6

Table-4: Antibacterial and antifungal activities of the selected samples of N-substituted napthofuran carboxamides with zone of inhibition >0.5 cm at different concentrations.

E. coli

samples	**0.0625 mg**	**0.125 mg**	**0.25 mg**	**0.5 mg**	**1.0 mg**	**2.0mg**	**MIC mg**
4a	0	0	0	0	0.2	0.4	1
4b	0	0	0	0	0.1	0.3	1
4c	0	0	0	0	0	0.2	2
4f	0	0	0	0	0.3	0.8	1
	25 µg	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**MIC µg**
Gentamycin	1.8	2	2.3	2.6	2.8	3.1	25

S. aureus

samples	**0.0625 mg**	**0.125 mg**	**0.25 mg**	**0.5 mg**	**1.0 mg**	**2.0 mg**	**MIC mg**
4a	0	0	0	0.2	0.5	0.7	0.5
4b	0	0	0	0	0	0.3	2
4c	0	0	0	0	0	0.1	2
4f	0	0	0	0	0	0.2	2
	25 µg	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**MIC µg**
Gentamycin	1.3	1.8	2.1	2.5	2.7	3.4	<25

A. niger

samples	**0.0625 mg**	**0.125 mg**	**0.25 mg**	**0.5 mg**	**1.0 mg**	**2.0mg**	**MIC mg**
4a	0	0.9	1	1.5	1.6	2.3	0.125
4b	0	0	0	0	0	1.1	2
4c	0	0	0	0	0.6	0.8	1
4f	0	0.5	0.6	0.7	1.1	2.3	0.125
	25 µg	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**MIC µg**
Amphotericin	0	0	0.2	0.3	0.5	0.7	100

C.albicans

samples	0.0625 mg	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0mg	MIC mg
4a	0	0	0	0	1	1.2	1
	25 µg	**50 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**MIC µg**
Amphotericin	0	0.2	0.7	0.9	1.3	1.5	50

Conclusion

In the present work we synthesized N-substituted napthofuran carboxamides **(4a-f)** and evaluated their *in vitro* antibacterial activities against *Staphyllococcus aureus* and *Escherichia coli* and antifungal activities against *Aspergillus niger* and *Candida albicans*. Results indicated that compound **4a** and compound **4f** (except for *C. albicans*) show appreciable activity against these microorganisms in mg level.

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