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Synthesis, Antimicrobial and Antioxidant activity of Carboxamides derived from Naphtha [2, 1-b] furan

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ABSTRACT

The starting materials 2-substituted-4H-naphtho[2,1-b]furan-m-oxazin-4-ones **1a-d** were synthesized by well established method in our laboratory, by cyclodehydration of corresponding 3-acylaminonaphtho[2,1-b]furan-2-carboxylic acids. Reaction of oxazinones **1a-d** with aliphatic amines and aromatic amines resulted in ring opening and yielded respective 3-acylaminonaphtho [2, 1-b] furan-2-N-alkyl/aryl-2-carboxamides **2a-d, 3a-d**, **4a-d**, and **5a-d**. Similar reaction of **1a-d** with piperidine and morpholine produced 3-acylaminonaphtho[2,1-b]furan-2-N-piperidino/morpholino-2-carboxamides **6a-d** and **7a-d**. The newly synthesized compounds are characterized by spectral studies and elemental analysis. The compounds were screened for antimicrobial activity by agar well diffusion method and antioxidant activity by DPPH scavenging method. Some of the compounds exhibited promising activity against bacteria *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and also against fungi *Aspergillus niger* and *Aspergellius flavus*. Evaluation of the compounds for antioxidant activity revealed that the compounds **5b, 6b** and **6c** have considerable EC_{50} value of 154.17 µg/ml 123.38 µg/ml and 154.42 µg/ml respectively.

Key words: Naphthofuran, carboxamides, oxazinones, antimicrobial activity, antioxidant activity.

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INTRODUCTION

Carboxamides have received considerable attention owing to their wide range of biological and pharmacological activities [1]. Moclobemide is one of the carboxamides which finds application in decreasing depression and structure activity relationship studies revealed the importance of carboxamides group as pharmacophore [2-3]. Some of the acridine derivatives having carboxamide functionality have been reported to possess wide range of biological activities [4,5]. Antioxidant studies on 3-oxindoline-5-carboxamides indicated the relevance of carboxamide group in displaying such activity [6]. Such compounds are also known to act as polymerase (PARP) inhibitors [7]. Many of the derivatives of naphtho[2, 1-b]furan synthesized in our laboratory have been shown to possess biological and pharmacological activities such as antimicrobial, anti inflammatory, analgesic, antipyretic activities [8-14]. The presence of morpholine moiety has been shown to enhance biological profile of the compounds many folds [15]. Similarly, carboxamides of piperidine have been reported to exhibit antihypertensive and spasmolyic activities [16-17]. Hence, it was contemplated to synthesize carboxamide derivatives enclosing biologically active naphthofuran, piperidine and morpholine nuclei and evaluate the compounds for antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Melting points were determined in open capillary tube and are uncorrected. The IR spectra were recorded in KBr on SHIMADZU FT-IR - 8400 Spectrophotometer. The ^{1}H NMR were recorded in $Cdcl_3$ on Bruker Supercon FT NMR instrument using trimethylsilane (TMS) as an internal standard and DMSO, $CDCl_3$ as solvent. The mass spectra were recorded on SHIMADZU – LCMS 2010 Spectrometer. Compounds were analysed for elemental analysis and all compounds showed satisfactory elemental analysis. The chemicals and solvents used were of laboratory grade. The purity of the compounds was checked by TLC using Silica gel-G. Column chromatography was performed on Silica gel- G (60-120 mesh).

Synthesis of 3-acylaminonaphtho[2,1-b]furan-2-N-alkyl-2- carboxamides (2a-d, 3a-d)

A mixture of oxazinone **1a** (0.251g, 0.001 mol) and ethyl amine (0.005 mol) was warmed on a water bath for 1hour. The contents were cooled, excess of amine was removed by treating with dilute hydrochloric acid. The product **2a** that separated as a solid was collected and recrystallised from ethanol. Similarly the compounds **2b-d** were synthesized by using **1b-d.**

The compounds **3a-d** were synthesized from **1a-d** similarly by using n-propyl amine in place of ethyl amine.

Synthesis of 3-acylaminonaphtho[2,1-b]furan-2-N-aryl -2-carboxamides (4a-d, 5a-d)

A solution of oxazinone **1a** (0.251g, 0.001 mol) and aniline (0.005 mol) in glacial acetic acid (5 ml) was heated under reflux for 3 hours. The reaction mixture was cooled and left for

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some time when a fine crystalline compound **4a** separated out as solid. It was collected and recrystallised from ethanol. Similarly the compounds **4b-d** were synthesized by using **1b-d.**

The compounds **5a-d** were synthesized from **1a-d** similarly by using 4-methoxy aniline in place aniline.

Synthesis of 3-acylaminonaphtho[2,1-b]furan-2-N-piperidino-2-carboxamide(6a-d)

To a solution of oxazinone **1a** (0.251g, 0.001mol) in 1,4-dioxane (5 ml), piperidine (0.005 mol) was added and heated under reflux for 5 hours. The contents were allowed to cool and then poured in to crushed ice. The compound **6a** that separated as colourless solid was collected and recrystallised from ethanol **6a**.

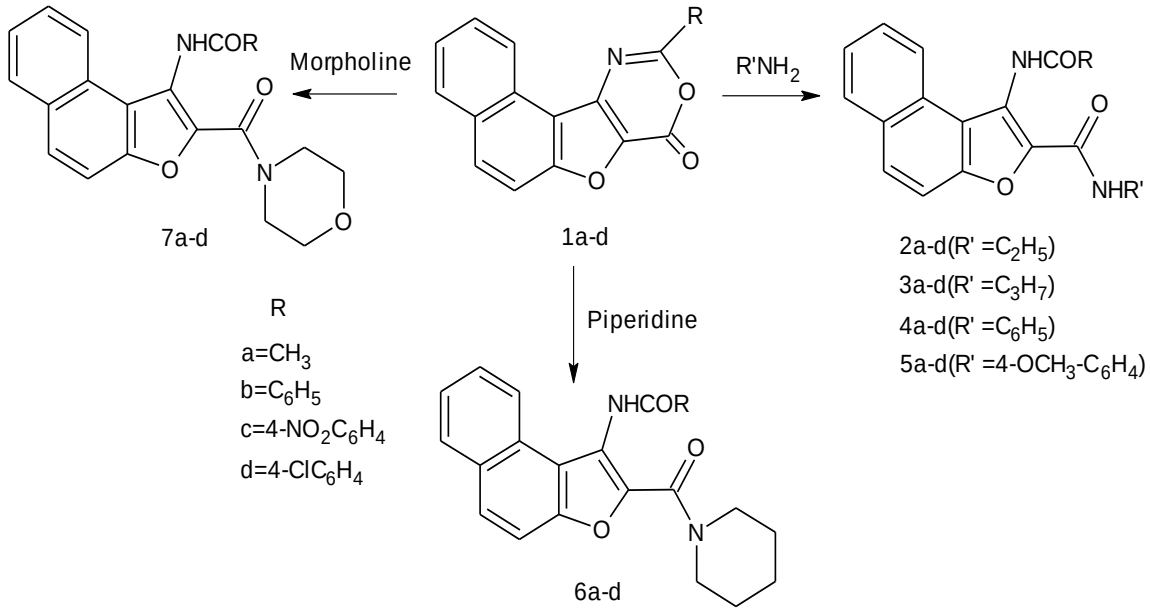
Similarly the carboxamides **(6b-d)** were synthesized from **(1b-d)**.

Synthesis of 3 -acylaminonaphtho[2,1-b]furan-2-N-morpholino-2-carboxamides (7a-d)

The oxazinone **1a** (0.251g, 0.001 mol) was dissolved in 1,4-dioxane (5 ml), morpholine (0.005 mol) was added and the reaction mixture was heated under reflux for 5 hours. The contents were allowed to cool and poured in to crushed ice. The colourless solid of **7a** that separated was collected and recrystallised from ethanol.
Similarly the carboxamides **(7b-d)** were synthesized from **(1b-d)**.

The sequence of the reactions is depicted in the scheme.

SCHEME



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Physical and analytical data of all the synthesized compounds are summarized in Table1

Table 1 – Physical and analytical data of synthesized compounds

Comp.	R	Molecular Formula	Melting point	Yield%	Found%(Calculated)		
					C	H	N
2a	CH_3	$C_{17}H_{16}N_2O_3$	145	59	68.88 (68.91)	5.37 (5.40)	9.40 (9.45)
2b	C_6H_5	$C_{22}H_{18}N_2O_3$	170	64	73.75 (73.74)	5.00 (5.02)	7.78 (7.82)
2c	4-$NO_2C_6H_4$	$C_{22}H_{17}N_3O_5$	229	67	65.46 (65.50)	4.17 (4.21)	10.44 (10.42)
2d	4-ClC_6H_4	$C_{22}H_{17}N_2O_3Cl$	195	63	67.30 (67.34)	4.25 (4.33)	7.10 (7.14)
3a	CH_3	$C_{18}H_{18}N_2O_3$	148	52	69.62 (69.67)	5.75 (5.80)	9.02 (9.03)
3b	C_6H_5	$C_{23}H_{20}N_2O_3$	178	59	74.24 (74.19)	5.35 (5.37)	7.49 (7.52)
3c	4-$NO_2C_6H_4$	$C_{23}H_{19}N_3O_5$	236	65	66.13 (66.18)	4.47 (4.55)	10.04 (10.07)
3d	4-ClC_6H_4	$C_{23}H_{19}N_2O_3Cl$	207	60	67.90 (67.98)	4.68 (4.67)	6.85 (6.89)
4a	CH_3	$C_{21}H_{16}N_2O_3$	172	58	73.23 (73.25)	4.61 (4.65)	8.19 (8.13)
4b	C_6H_5	$C_{26}H_{18}N_2O_3$	192	62	76.84 (76.84)	4.40 (4.43)	6.80 (6.89)
4c	4-$NO_2C_6H_4$	$C_{26}H_{17}N_3O_5$	243	67	69.10 (69.17)	3.75 (3.76)	9.22 (9.31)
4d	4-ClC_6H_4	$C_{26}H_{17}N_2O_3Cl$	216	64	70.75 (70.90)	3.81 (3.86)	6.35 (6.36)
5a	CH_3	$C_{22}H_{18}N_2O_4$	195	61	70.55 (70.58)	4.73 (4.81)	7.40 (7.48)
5b	C_6H_5	$C_{27}H_{20}N_2O_4$	215	65	74.20 (74.31)	4.56 (4.58)	6.36 (6.42)
5c	4-$NO_2C_6H_4$	$C_{27}H_{19}N_3O_6$	259	68	67.29 (67.35)	3.86 (3.95)	8.71 (8.73)
5d	4-ClC_6H_4	$C_{27}H_{19}N_2O_4Cl$	227	62	68.85 (68.93)	4.00 (4.04)	5.92 (5.95)
6a	CH_3	$C_{20}H_{20}N_2O_3$	166	55	71.35 (71.42)	5.90 (5.95)	8.29 (8.33)
6b	C_6H_5	$C_{25}H_{22}N_2O_3$	184	51	75.33 (75.37)	5.48 (5.52)	6.99 (7.03)
6c	4-$NO_2C_6H_4$	$C_{25}H_{21}N_3O_5$	230	59	67.66 (67.72)	4.73 (4.74)	9.42 (9.48)
6d	4-ClC_6H_4	$C_{25}H_{21}N_2O_3Cl$	202	57	69.40 (69.44)	4.81 (4.86)	6.39 (6.48)
7a	CH_3	$C_{19}H_{18}N_2O_4$	171	50	67.37 (67.45)	5.29 (5.32)	8.23 (8.28)
7b	C_6H_5	$C_{24}H_{20}N_2O_4$	189	52	69.97 (72.00)	4.92 (5.00)	6.97 (7.00)
7c	4-$NO_2C_6H_4$	$C_{24}H_{19}N_3O_6$	235	58	64.66	4.22	9.38

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					(64.71)	(4.26)	(9.43)
7d	4-ClC_6H_4	$C_{24}H_{19}N_2O_4Cl$	194	56	66.34 (66.35)	4.32 (4.37)	6.38 (6.45)

Antimicrobial activity

In vitro antimicrobial activity was determined by agar well diffusion method. For antibacterial activity 24 hours old cultures of Gram positive bacterium *Staphylococcus aureus* (ATCC 11632) and Gram negative bacteria *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 10145) were used. The compounds were tested at concentration of 0.01 g/ml and 0.005 g/ml in dimethyl sulphoxide. The zone of inhibition was measured after 24 hours incubation at 37^0 C and compared with the standard drug Streptomycin at same concentration. The results of antibacterial activity are presented in Table 2.

Similarly antifungal activity was carried out against 24 hr old cultures of *Aspergillus niger* and *Aspergellius flavus* at concentration of 0.01 g/ml and 0.005 g/ml in dimethyl sulphoxide. The zone of inhibition was measured after 72 hr incubation at 28^0 C and compared with the standard drug Fluconazole at same concentration. The results of antifungal activity are presented in Table 3.

Table 2 – Antibacterial activity of synthesized compounds

	Zone of inhibition in mm					
Compounds	**E.c**		**P.a**		**S.a**	
	0.01 g/ml	0.005 g/ml	0.01 g/ml	0.005 g/ml	0.01 g/ml	0.005 g/ml
Standard	21	16	20	15	23	18
Distilled water	Nil	Nil	Nil	Nil	Nil	Nil
DMSO	Nil	Nil	Nil	Nil	Nil	Nil
2a	15	10	14	11	14	10
2b	11	06	12	08	18	12
2c	18	15	16	13	09	07
2d	10	08	09	07	11	05
3a	15	12	10	07	14	10
3b	18	14	13	11	15	12
3c	14	10	12	09	13	08
3d	09	10	14	10	10	13
4a	15	08	15	09	14	12
4b	18	13	08	05	18	16
4c	12	06	12	07	16	09
4d	09	05	09	06	09	11
5a	14	10	10	08	15	10
5b	18	12	15	12	13	06
5c	11	08	10	05	08	06
5d	12	06	14	12	10	11
6a	19	14	17	13	18	13
6b	12	10	06	09	12	10

ISSN: 0975-8585

6c	10	07	08	06	10	05
6d	08	12	09	07	14	13
7a	15	13	16	14	09	13
7b	18	09	12	08	12	09
7c	09	07	10	06	16	13
7d	13	11	13	11	10	07

Standard : Streptomycin, E.c : *Escherichia coli,* P.a : *Pseudomonas aerugenosa,* S.a : *Staphylococcus aureus*

Table 3 – Antifungal activity of synthesized compounds

	Zone of inhibition in mm			
Compounds	**A. n.**		**A. f.**	
	0.01 g/ml	0.005 g/ml	0.01 g/ml	0.005 g/ml
Standard	18	13	20	16
Distilled water	Nil	Nil	Nil	Nil
DMSO	Nil	Nil	Nil	Nil
2a	16	11	18	12
2b	13	06	13	07
2c	09	07	16	10
2d	12	09	17	15
3a	15	12	13	10
3b	16	10	16	09
3c	09	05	16	11
3d	12	08	09	06
4a	15	11	17	13
4b	14	10	18	15
4c	15	09	14	11
4d	12	10	09	05
5a	09	06	10	08
5b	13	07	09	06
5c	16	12	16	11
5d	09	07	12	09
6a	16	09	17	11
6b	14	11	13	09
6c	15	10	08	05
6d	10	08	14	11
7a	16	12	09	07
7b	13	09	12	09
7c	08	06	15	11
7d	10	07	09	05

Standard : Fluconazole, A.n : *Aspergillus niger,* A.f : *Aspergellius flavus*

Antioxidant activity

ISSN: 0975-8585

Antioxidant activity of the synthesized compound was determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay method. The solution of DPPH was prepared by dissolving DPPH (2 mg) in methanol (50 ml). Ascorbic acid (4 mg) was dissolved in distilled water (10 ml) and the solution obtained was used as standard. The stock solution of test compounds was prepared by dissolving the test compounds (4 mg) in methanol (10 ml) and diluted by serial dilution method to obtain the solutions of different concentrations. The test solution (1 ml) and the solution of DPPH (2 ml) were taken is a test tube and the absorbance was recorded at 517 nm using spectrophotometer (SP-2012 UVPC SPECTRUM UV-Visible Spectrophotometer) and readings obtained are tabulated in Table 4.

Percentage scavenging DPPH free radical was calculated by using the following equation

$$\text{Scavenging Activity} = \frac{\%\ \text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

The graphs (Fig 1 to 4) were obtained by plotting % inhibition against concentration. The activity has been expressed in EC_{50} which was calculated from the graphs.

Table 4 – Antioxidant activity of synthesized compounds

Compounds	Percentage of scavenging activity				EC_{50} µg/ml (from graph)
	50µg/ml	100µg/ml	200µg/ml	400µg/ml	
Standard	48.37	60.21	76.28	97.74	58.88
2a	34.04	40.29	48.49	64.66	214.28
2b	22.20	28.57	39.64	52.58	357.95
2c	22.01	34.13	40.17	57.83	306.64
2d	25.46	39.00	46.79	61.67	237.73
3a	29.15	40.22	49.94	54.90	198.15
3b	32.23	38.90	50.32	59.57	195.22
3c	34.04	42.29	52.49	64.26	174.70
3d	19.70	27.16	40.67	50.53	385.80
4a	20.61	29.35	38.89	52.02	368.21
4b	28.01	37.18	50.82	63.06	193.75
4c	22.83	30.47	44.90	59.85	269.99
4d	34.21	45.18	52.82	61.06	164.43
5a	31.62	40.35	53.24	68.94	171.76
5b	33.07	42.04	56.12	69.00	154.17
5c	26.19	31.32	47.40	54.07	272.92
5d	30.95	42.29	54.05	62.30	162.97
6a	22.64	35.90	48.39	60.06	223.07
6b	32.04	46.17	60.43	69.28	123.38
6c	35.38	41.53	56.80	64.09	154.42
6d	30.22	43.09	52.11	70.74	174.94
7a	26.61	31.35	40.89	55.02	324.47

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7b	31.58	38.32	46.11	58.78	258.50
7c	32.83	40.47	48.00	63.85	220.39
7d	20.00	28.18	36.82	50.98	381.65

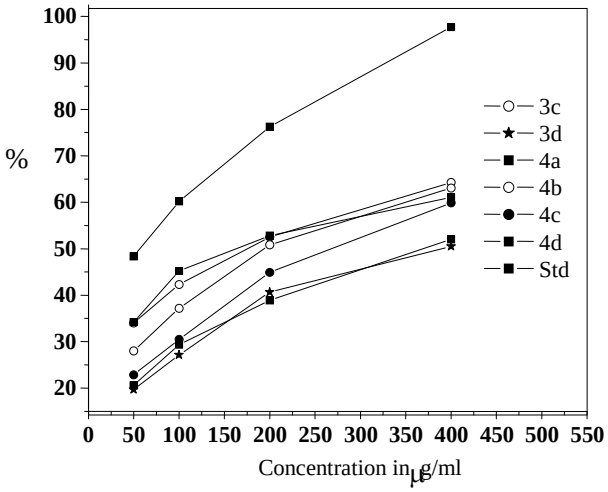

Figure 1

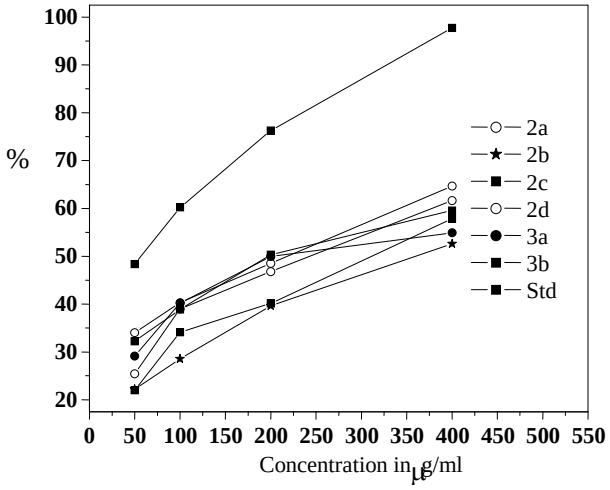

Figure 2

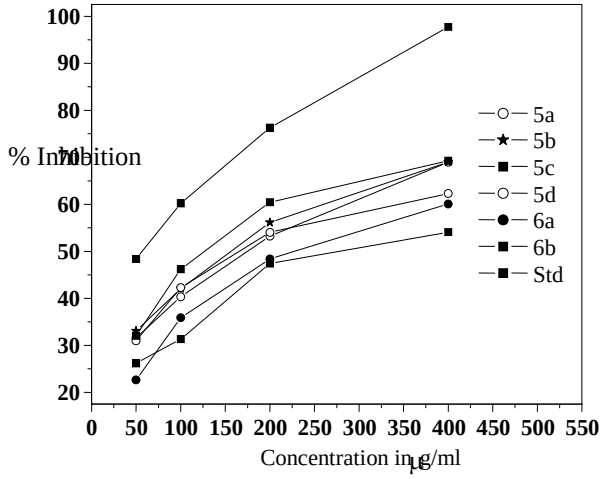

Figure 3

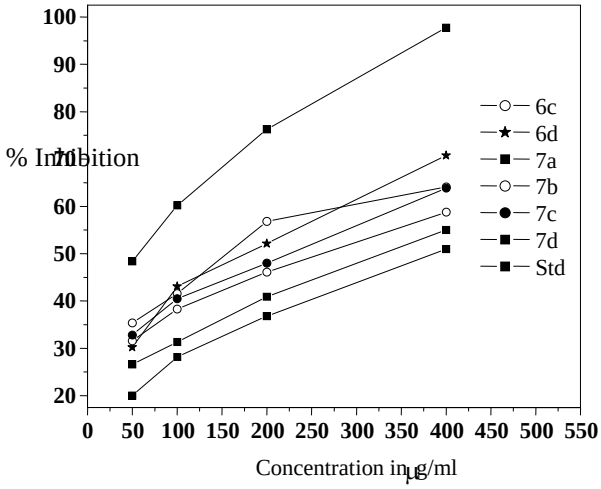

Figure 4

RESULTS AND DISCUSSION

For the synthesis of desired carboxamide, 2-substituted-4H-naphtho[2,1-b]furan-m-oxazin-4-ones **1a-d** served as excellent starting materials, which was prepared by well established procedure in our laboratory[17]. It involved cyclodehydration of 2-acylaminonaphtho[2,1-b]furan-2-carboxylic acids using acetic anhydride. The oxazinones **1a-d** on reaction with aliphatic amines viz., ethyl amine and n-propyl amine resulted in ring opening and produced 3-acylaminonaphtho[2,1-b]furan-2-N-alkylcarboxamide **2a-d** and **3a-d** respectively. The structure of compound **2a** was confirmed by spectral data. Its IR spectrum

exhibited absorption band at 1662 and 1621cm^{-1} due to two carbonyl groups. It's ^{1}H NMR spectrum showed a triplet, and a quartet at δ 1.2 and δ 4.0 due to –CH_3 and –CH_2 protons respectively. It also exhibited multiplet at δ 7.2-8.1 due to aromatic protons. Two singlets (D_2O exchangeable) due to two –NH protons were observed at δ 11.5 and δ 6.8.

Similar reaction of oxazinones when carried out with aromatic amines viz., aniline and 4-methoxyaniline resulted in the formation of 3-acylaminonaphtho[2,1-b]furan-2-N-arylcarboxamides **4a-d** and **5a-d**. Our interest was to introduce morpholine and piperidine ring system in naphthofuran nucleus to study the effect of these nucleus in biological and pharmacological activities. Hence it was thought to make use of oxazinones **1a-d** for this purpose. Thus oxazinones **1a-d** on treatment with piperidine yielded 2-ayclaminonaphtho[2,1-b]furan-2-N-piperidinocarboxamides **6a-d**. Similar reaction with morpholine produced 2-acylaminonaphtho[2,1-b]furan-2-N-morpholinocarboxamides **7a-d** in good yield. The structure assigned to compound **7a** was well supported by IR and ^{1}H NMR spectra. The IR spectrum exhibited two absorption bands at 1625 and 1647cm^{-1} due to two carbonyl groups and broad band at 3411cm^{-1} due to stretching frequency of -NH groups. In ^{1}H NMR spectrum of **7a** a singlet at δ 1.9 due to three protons of –CH_3 group and multiplet at δ 7.3-9.6 due to aromatic protons was observed. In addition to it showed D_2O exchangeable singlet at δ 11.5 due to NH proton. The spectral data of other compounds was in agreement with the assigned structure.

Investigation of antimicrobial activity revealed that the compounds **2c, 3b, 4b, 5b, 7b** exhibited promising activity against *Escherichia coli*. Whereas compounds **2c, 6a, 7a** were active against *Pseudomonas aeruginosa* and **2b, 4b, 6a** were moderately active against *Staphylococcus aureus*. Compounds **2a, 3b, 5c, 7a** were active against Aspergillus niger and **2a, 4b** were active against *Aspergellius flavus*.

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of synthetic compounds. In the present study, the compounds were investigated in comparison with the known antioxidant ascorbic acid. The EC_{50} values for DPPH radical with compounds **5b**, **6b** and **6c** were found to be 154.17 µg/ml, 123.38 µg/ml and 154.42 µg/ml.

CONCLUSION

The oxazinone ring system could be used for the synthesis of various substituted caroxamides involving naphtho[2,1-b]furan nucleus. Some of the synthesized compounds exhibited promising activity against both Gram-positive and Gram-negative bacteria as well as fungi. Few of the synthesized compounds were found to exhibit considerable antioxidant activity. Hence, there is plenty of scope for systematic study to evaluate the compounds for

other pharmacological activities such as antiviral, anti inflammatory, analgesic activities. The substitutions could be varied to obtain more potent compounds.

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REFERENCES

[1] Konopa J. Pure Appl Chem 2001; 73(9): 1421.
[2] Hadizadeh F, Hosseinzadeh H, Shariaty VSM, Seidi M, Kazemi S. Iranian J Pharma Res 2008; 7(1): 29.
[3] Pessoa-Mahana H, Gajardo GR, Araya-Maturoma R, Carcamo JK, Pessoa-Mahana CD. Syn Commun 2004; 34: 2513.
[4] Skladanowski A, Kanopa J. Br J Cancer 2000; 82: 1300.
[5] Atwell GJ, Cain BF, Baguley BC, Finlay GJ, Denny WA. J Med Chem 1984; 27: 1481.
[6] Kishor kumar C, Vijay Kumar H, Vijay Kumar MR, Naik N. J Pharmacuetical Sci Tech 2010; 2(12): 380.
[7] Gandhi YB, Luo Y, Liu X, Shi Y, Klighoter V, Johnson EF, Park C, Giranda VL, Penning TD, Zhu GD. Bioorg Med Chem Lett 2010; 20: 1023.
[8] Kumarswamy MN, Vaidya VP. Indian J Heterocyclic Chem 2001; 14: 193.
[9] Mahadevan KM, Padmashali B, Vaidya VP. Indian J Heterocyclic Chem 2001; 11: 15.
[10] Latha KP, Vaidya VP, Keshavavya J, Vijaykumar ML, Shreedhara CS. Nat Aca Sci Letters 2002; 25: 153.
[11] Shashikaladevi K, Ramaiah M, Roopa DL, Vaidya VP. E-Journal Chem 2011; 7(S1): 358.
[12] Mahadevan KM, Vaidya VP, Vagdevi HM. Indian J Chem 2003; 42B: 1931.
[13] Vaidya VP, Shreedhara CS, Gopal M, Shahabuddin MS, Shenoy PS. Indian J Pharm Sci 2003; 65(6): 880.
[14] Nagaraj GK, Kumarswamy MN, Vaidya VP, Mahadevan KM. ARKIVOC 2006; X: 211.
[15] Ghanbarpour A, Hadizadeh F, Piri F, Rashidi-Ranjbar P. Pharm Acta Helv 1987; 72: 119.
[16] Akhtar S, Saify ZS, Arif M, Mushtaq N, Dar A, Ahmed A. Pak J Physiol 2006; 2(1): 42.
[17] Nagashree AS, Lohith Kumar PJ, Kusuma K, Vaidya VP. Res J Pharm Bio Chem Sci 2011; 2(2): 855.