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Curtius Rearrangement Reactions using 7-Methoxy benzofuran-2- Carbonylazide

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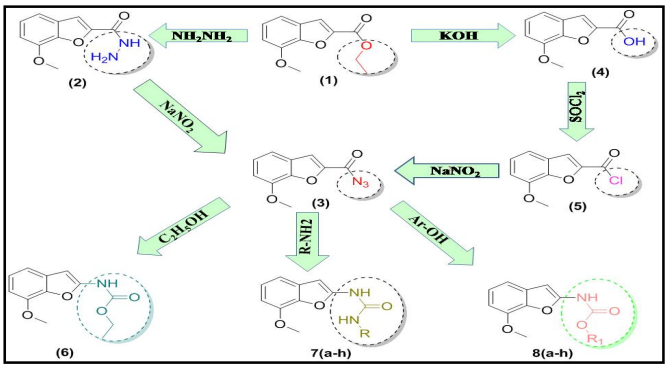
ABSTRACT

Our continued search for biologically active benzofuran derivatives involving carbamates and carbamides, we now report the synthetic investigation of 7-methoxybenzofuranyl-carbamates ***(6*** *and* ***8a-h)*** *and carbamides* ***(7a-h)*** *via the Curtius rearrangement of 7-methoxybenzofuran-2-carbonylazide* ***(3)****. The required intermediate carbonyl azide was synthesised from ethyl-7-methoxybenzofuran-2-carboxylate* ***(1)*** *by two established synthetic routes. One through the carboxylic acid* ***(4)*** *and acid chloride* ***(5)*** *and the other through carbonyl hydrazide* ***(2)****. The carbonyl azide was subjected to Curtius rearrangement in anhydrous medium with ethanol and various aromatic phenols to obtain carbamates* ***(6*** *and* ***8a-h)*** *while with primary amines and cyclohexylamine to obtain carbamides* ***(7a-h)****. The structures of all the synthesized compounds were confirmed by their IR,* ^{1}*HNMR and Mass spectral data. All the newly synthesized compounds were screened for anti bacterial activity and antifungal activity. Few selected compounds were screened for their anti oxidant properties and DNA cleavage studies. Few compounds exhibited appreciable activity.*

Graphical Abstract

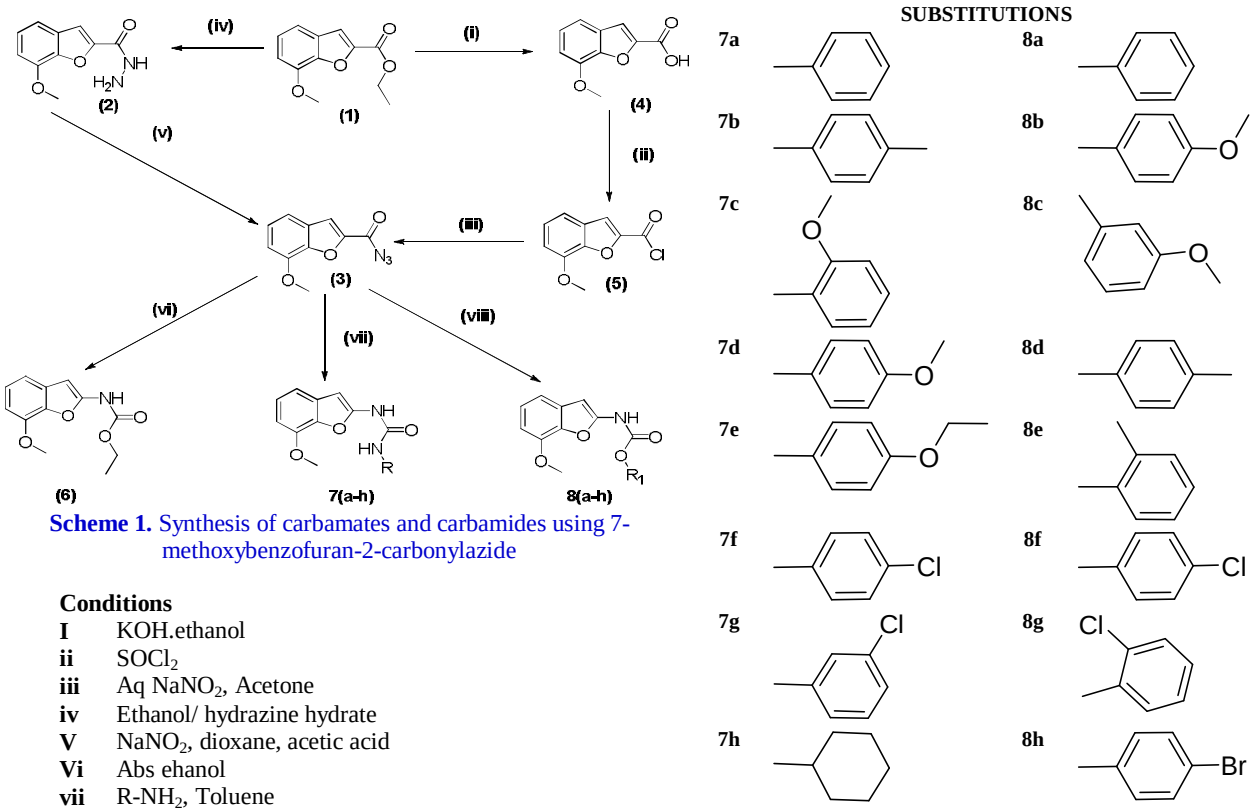


Synthesis of carbamates and carbamides using 7- methoxybenzofuran-2-carbonylazide

Keywords: Carbamates, Carbamides, Benzofuran, Curtius rearrangement, Antibacterial, Anti-oxidant properties, DNA cleavage

INTRODUCTION

Organic carbamates are a stable class of compounds derived from the unstable carbamic acid (-HN-COOH) by substitution of the amino and carboxyl moieties with various kinds of structurally diverse alkyl/aryl, or substituted alkyl/aryl and are identified by the presence of the carbamates and carbamides linkage respectively (R-NH-CO-O-R' and R-NH-CO-NH-R'). Organic carbamates represent an important class of compounds showing various interesting properties. They find wide utility in areas, such as pharmaceuticals [1], agrochemicals such as pesticides, herbicides, insecticides, fungicides [2-4], as intermediates in organic synthesis [5-8], for the protection of amino groups in peptide chemistry [9, 10]. These carbamates have been extensively used as intermediate for the synthesis of structurally diverse synthetic intermediates/molecules of biological significance [11-15]. Therefore, considerable interest has been generated in the recent past in the development of efficient and safe methodologies for carbamate ester synthesis. These have frequently been employed as



Scheme 1. Synthesis of carbamates and carbamides using 7-methoxybenzofuran-2-carbonylazide

Conditions

I	KOH.ethanol
ii	$SOCl_2$
iii	Aq $NaNO_2$, Acetone
iv	Ethanol/ hydrazine hydrate
V	$NaNO_2$, dioxane, acetic acid
Vi	Abs ehanol
vii	R-NH_2, Toluene
viii	Phenol, Toluene

pharmaceuticals in the forms of drugs and prodrugs [16]. In recent years, several reports have indicated that the carbamate linkage present in the active pharamacophores of various structurally diverse molecules increases the biological activities of semi synthetic/synthetic/natural molecules [17-19]. Basavaraja K. M. *et.al* reported the synthesis and antimicrobial activity of 3-methoxybenzo furanyl-carbamates and carbamides [20]

Chemistry: Thus, keeping the above facts and views under considerations and in continuation of our search for benzofuranyl carbamates and carbamides [21-24], we now report the synthesis of various carbamates and carbamides bearing benzofuran by using 7-methoxybenzofuran-2-carbonylazide(3). The synthesis was achieved by Curtius rearrangement reaction of 7-methoxy benzofuran-2-carbonylazide (3). Few synthesized compounds are screened for their antibacterial and anti fungal activity. Some selected compounds of present investigation were screened for anti-oxidant properties and DNA cleavage.

MATERIALS AND METHODS

All chemicals were from Sigma-Aldrich, Molchem Chemical Company, India and solvents were used without further purification. Melting points were determined in open capillary tubes and are uncorrected. The purity of all the synthesized compounds was checked by TLC. IR spectra were recorded on Perkin Elmer-237 spectrophotometer by KBr disc method. The ^{1}H NMR spectra were recorded on a Bruker Avance Spectrometer (400MHz) using TMS as an internal standard. $CDCl_3$ and DMSIO-d_6 as solvent, chemical shifts (delta) are given in ppm. The Mass spectra (MS) were recorded on a Jeol GC mate GC-MS. Elemental analysis (C, H, N) was performed on Perkin Elmer 240 analyser. The purity of the compounds were checked on a Silica gel G coated on Aluminum plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed in UV light.

(a) Preparations

7-Methoxy-benzofuran-2-carbonyl azide (3), Method-A: The 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (10 g, 0.048 mol) was suspended in a mixture of dioxane (60 mL) and acetic acid (60 mL) cooled to 0^{o}C in a freezing mixture. An ice cold solution of sodium nitrite (5.2 g in 20 mL water) was introduced in small portion with vigorous stirring. The temperature of the reaction mixture was maintained below 2°C. After the complete addition, the reaction was allowed to stand at room temperature for 30 min and the pale yellow solid thus separated was collected, washed with cold water. The product was dried over phosphorus pentoxide in vacuum (9 g, 85%).

7-Methoxy-benzofuran-2-carboxylic acid (4) Method –B: To a solution of compound **1** (0.02 mol) in absolute ethanol (30 mL), ethanolic potassium hydroxide (2 g in 20 mL absolute ethanol) was added and the reaction mixture was heated under reflux for 2 h on a water bath. The excess of ethanol was distilled off under reduced pressure and the residual solution was diluted with cold water. The clear solution thus obtained was cooled and acidified with dilute hydrochloric acid carefully to precipitate the carboxylic acid. It was collected, washed with water and crystallized from a mixture of benzene and petroleum ether as colorless needles. Yield 88%, melting point 205^{o}C. Calculated: C (62.50), H (4.20), Found: C (62.65), H (3.00), N(4.21).

7-Methoxy-benzofuran-2-carbonyl chloride (5): A mixture of **4** (5 g) and thionyl chloride (10 mL) was refluxed on a water bath for 2 h. The excess of thionyl chloride was removed under reduced pressure. The residual solid was washed with petroleum ether. The crude acid chloride **5** thus obtained was used in the next step without further purification.

7-Methoxy-benzofuran-2-carbonyl azide (3): To a stirred solution of the acid chloride **5** (2 g) in acetone (50 mL), a solution of sodium azide (0.6 g in 2 mL water) was added drop wise at 0°C. After the complete addition of sodium azide solution, the temperature of the reaction mixture was raised to 25 ^{o}C and this temperature was maintained for 30 min to ensure the completeness of the reaction. The reaction mixture was diluted with cold water (100 mL) and the pale yellow azide which separated was collected after washing with cold water. It was dried over phosphorus pentoxide in vacuum (1.6 g, 77 %). The azide obtained was used for further step without further purification melting point = 113^{o}C (d). Mixed melting point of the compound with the sample obtained by method-A was not depressed.

(7-Methoxy-benzofuran-2-yl)-carbamic acid ethyl ester (6): A suspension of azide **3** (0.01 mol) in absolute ethanol (10 mL) was refluxed on steam bath for 3 h. The reaction mixture was concentrated under reduced pressure and then diluted with water. The product that separated was collected and crystallized from mixture of benzene and petroleum ether as colorless needles. Yield 78%, melting point 211°C. Calculated: C (61.27), H (5.57), N (5.95) Found: C (61.20), H (5.65), N(5.90).

1-(7-Methoxy-benzofuran-2-yl)-3-aryl-ureas (7a-g) and 1-Cyclohexyl-3-(7-methoxy-benzofuran-2-yl)-urea(7h): A mixture of azide **3** (0.001 mol) and appropriate amine (0.001 mol) in anhydrous toluene (15 mL) was heated under reflux (120°C) in an oil bath for 5 h. The crystalline products **7**

separated out from the reaction mixture was collected, washed with toluene and petroleum ether. The analytical sample was obtained by crystallisation from suitable solvent. The physical constant, percentage yield, solvent for crystallisation and analytical data of the products **7a-h** are given in the table 1.

Table 1. Analytical data of compounds 7a-h

Comp.	Substituent 'R'	M.P. (°C)	Yield (%)	Solvent	Mol. formula	Found (calculated) %		
						C	H	N
7a	C_6H_5	200	80	Aq. ethanol	$C_{16}H_{14}N_2O_3$	68.00 (68.07)	5.08 (5.00)	9.99 (9.92)
7b	$C_6H_4CH_3$ (p)	215	79	Ethanol	$C_{17}H_{16}N_2O_3$	68.99 (68.91)	5.50 (5.44)	9.39 (9.45)
7c	$C_6H_4OCH_3$ (o)	186	81	Methanol	$C_{17}H_{16}N_2O_4$	65.45 (65.38)	5.16 (5.16)	8.90 (8.97)
7d	$C_6H_4OCH_3$ (p)	192	78	Ethanol	$C_{17}H_{16}N_2O_4$	65.45 (65.38)	5.22 (5.16)	8.92 (8.97)
7e	$C_6H_4OC_2H_5$ (p)	180	80	Ethanol	$C_{18}H_{18}N_2O_4$	66.20 (66.25)	5.50 (5.56)	8.64 (8.58)
7f	C_6H_4Cl (p)	197	71	Aq. ethanol	$C_{16}H_{13}ClN_2O_3$	60.62 (60.67)	4.09 (4.14)	8.90 (8.84)
7g	C_6H_4Cl (m)	210	75	Ethanol	$C_{16}H_{13}ClN_2O_3$	60.76 (60.67)	4.14 (4.14)	8.90 (8.84)
7h	C_6H_{11} (Cyclohexyl)	219	77	Ethanol	C16H20N2O3	66.70 (66.65)	6.90 (6.99)	9.79 (9.72)

(7-Methoxy-benzofuran-2-yl)-carbamic acid aryl ester (8a-h): A mixture of azide **3** (0.001 mol) was suspended in anhydrous toluene (30 mL) and heated in an oil bath at 70- 80°C till the evolution of nitrogen gas stopped (nearly 1h). Then the appropriate phenol (0.001 mol) in toluene (10 mL) was added and the reaction mixture was heated at 110-120°C for 3 h. After the removal of toluene under reduced pressure, the residue was dissolved in ether, the ethereal solution was washed with 10% aqueous solution of sodium hydroxide to remove any unreacted phenol and finally with water. The organic layer was dried over anhydrous calcium chloride. The removal of solvent furnished resinous mass which solidified on cooling. Further purification was achieved by crystallisation from suitable solvent.

The physical constant, percentage yield, solvent for crystallisation and analytical data of the products **8(a-h)** are given in the table 2.

Table 2. Analytical data of compounds (8a-h)

Comp.	Substituent 'R'	M.P. (°C)	Yield (%)	Solvent	Mol. formula	Found (calculated) %		
						C	H	N
52a	C_6H_5	222	79	Ethanol	$C_{16}H_{13}NO_4$	67.90 (67.84)	4.64 (4.63)	4.91 (4.94)
52b	C_6H_4-OCH_3 (p)	216	70	Ethanol	$C_{17}H_{15}NO_5$	65.22 (65.17)	4.90 (4.83)	4.52 (4.47)
52c	C_6H_4-OCH_3(m)	200	69	Methanol	$C_{16}H_{12}ClNO_4$	60.40 (60.48)	3.87 (3.81)	4.45 (4.41)
52d	$C_6H_4CH_3$ (p)	210	71	Ethanol	$C_{16}H_{12}ClNO_4$	60.40 (60.48)	3.90 (3.81)	4.48 (4.41)
52e	$C_6H_4CH_3$ (o)	197	70	Aq. ethanol	$C_{16}H_{12}BrNO_4$	53.03 (53.06)	3.34 (3.34)	3.94 (3.87)
52f	C_6H_4Cl (p)	205	78	Aq. ethanol	$C_{16}H_{12}ClNO_4$	60.50 (60.48)	3.80 (3.81)	4.48 (4.41)
52g	C_6H_4Cl (o)	189	80	Ethanol	$C_{16}H_{12}ClNO_4$	60.50 (60.48)	3.86 (3.81)	4.39 (4.41)
52h	C_6H_4Br (p)	213	75	Ethanol	$C_{16}H_{12}BrNO_4$	53.10 (53.06)	3.30 (3.34)	3.87 (3.87)

RESULTS AND DISCUSSION

7-Methoxy-benzofuran-2-carbonyl azide (3): The desired carbonyl azide **3** was prepared from ethyl-7-methoxybenzofuran-2-carboxylate **(1)** by two different methods.

One of the method (A) involved the nitrosation of the carbohydrazide 2 was obtained from **1** with sodium nitrite in dioxane and acetic acid at 0-5^{o}C. The carbonyl azide **3** was thus produced in 85% yield and was sufficiently pure for further Curtius rearrangement. The diagnostic azide peak was observed at 2153 cm^{-1} indicated the formation carbonyl azide 3 (Figure 1).

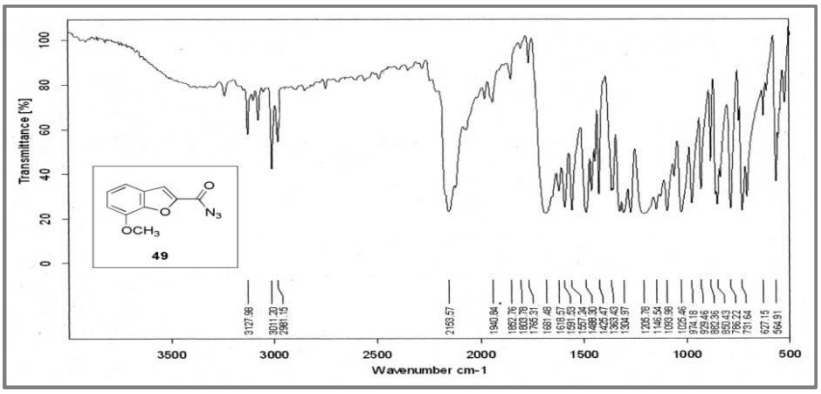


Figure 1. IR spectrum of compound 3.

The same compound **3** was also prepared from **1** following an alternative procedure method B. Thus the careful hydrolysis of the ester **1** in ethanolic potassium hydroxide solution gave carboxylic acid **4,** which was then treated with thionyl chloride, to get acid chloride **5**, which was then treated with an aqueous solution of sodium azide at 0^{o}C provided the carbonyl azide **3**. The identity of the product **3** from both the methods was established by super imposable IR spectra and mixed melting points. The IR spectrum of **4** contained a broad band in the region of 3400-2525 cm^{-1} and sharp band at 1690 cm^{-1} due to carboxylic acid -OH and >C=O group frequencies respectively (Figure 2).

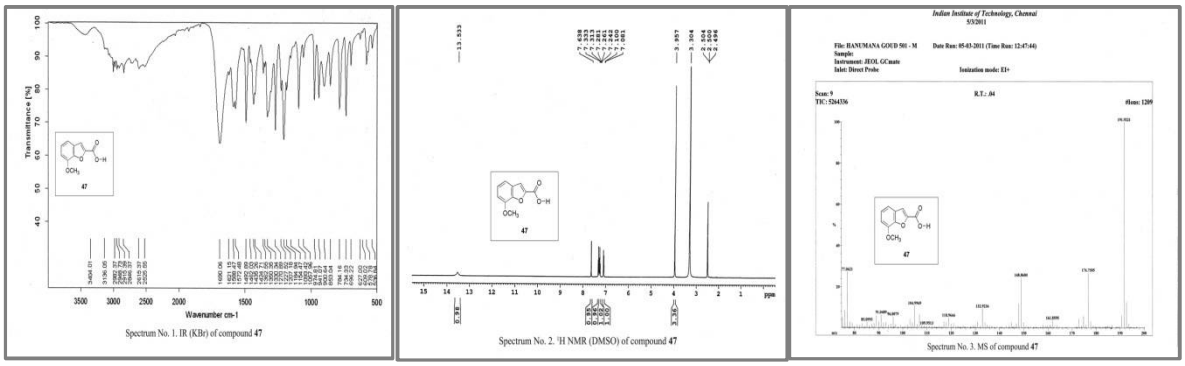


Figure 2. IR, NMR and Mass spectrum of compound 4.

To provide an additional evidence for the proposed structure, the ^{1}H NMR and mass spectrum of **4** were recorded. The ^{1}H NMR spectrum in DMSO-d_6 was exhibited a singlet at δ3.95 ppm due to -OCH_3 protons, a multiplet in the range of δ7.08-7.33 ppm were due to the C4-C6 aromatic protons and a single was observed at δ7.63 ppm due to -CH_3. The carboxylic acid proton was resonated as a singlet at δ13.53 ppm. The molecular ion peak was observed at *m/z* 192 confirmed the formation of **4.**

(7-Methoxy-benzofuran-2-yl)-carbamic acid ethyl ester (6): When carbonyl azide **3** was subjected to thermal Curtius rearrangement in an ethanolic solution afforded the carbamate **6**. The

disappearance of azide band (C≡N) at 2153 cm^{-1} and appearance of new sharp band at 3267 cm^{-1} due to -NH indicated the formation of carbamate **6** in its IR spectrum (Figure 3).

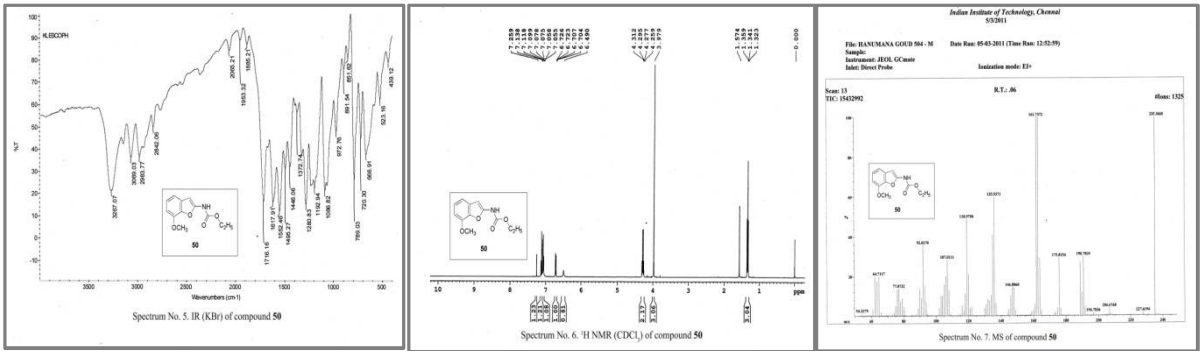


Figure 3. IR, NMR and Mass spectrum of compound 06.

To provide an additional evidence for the proposed structure, the ^{1}H NMR and mass spectrum of 6were recorded. The ^{1}H NMR spectrum in $CDCl_3$ was exhibited a triplet atδ 1.34 ppm due to -CH_3 protons of ethyl group, a singlet at 3.97 due to- OCH_3 protons and a quartet at δ 4.27 ppm due to -CH_2 protons of ethyl group. The NH proton was resonated as a broad singlet at δ 6.49 ppm. The aromatic protons were resonated in the range of δ 6.70-7.25 ppm. The molecular ion peak was observed at *m/z* 235 confirmed the formation of **6.**

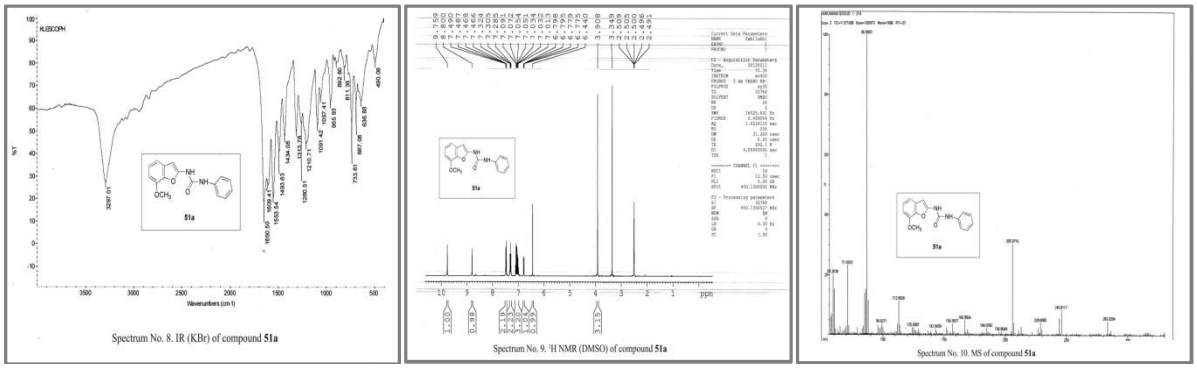


Figure 4. IR, NMR and Mass spectrum of compound 7a.

1-(7-Methoxy-benzofuran-2-yl)-3-aryl-ureas(7a-g)/1-Cyclohexyl-3-(7-methoxy-benzofuran-2-yl)-urea (7h): The Curtius rearrangement of **3**, in presence of various amines such as aniline, *p*-toluidine, *o*-anisidine, *p*-anisidine, *p*-phenetidine, *p*-chloro aniline, *m*-chloro aniline and cyclohexylamine in dry toluene afforded the respective carbamides **7a-h** in excellent yields. The disappearance of azide band at 2153 cm^{-1} and appearance of new sharp band at 3290-3450 cm^{-1} due to -NH indicated the formation of carbamides **7a-h** in their IR spectrum (Table 3).

To provide the additional evidences for the proposed structures, the ^{1}H NMR and mass spectrum of **7a** were recorded. The ^{1}H NMR spectrum in DMSO-d_6 was exhibited a singlet at δ 3.90 ppm due to -OCH_3 protons, a multiplet in the range of δ 6.44-7.49 ppm were due to the aromatic protons and two singlets were observed at δ 8.80 and 9.75 ppm due to two NH protons. The molecular ion peak was observed at *m/z* 282 confirmed the formation of **7a** (Figure 4).

(7-Methoxy-benzofuran-2-yl)-carbamic acid aryl ester 8a-h; A series of aryl carbamates **8a-h** were prepared by carrying out the Curtius rearrangement of **3** in presence of various phenols like phenol, *p*-methoxy, *m*-methoxy, *p*-methyl, *o*-methyl, *p*-chloro, *o*- chloro and *p*-bromo phenols in dry toluene.

The disappearance of azide band at 2153 cm^{-1} and appearance of new sharp band at 3259-3350 cm^{-1} due to -NH indicated the formation of carbamides **8a-h** in their IR spectrum (Table 4).

Table 3. IR data of compounds 7a-h

Compound	Substituent 'R'	IR data (cm^{-1})	
		NH	C=O
7a	C_6H_5	3297	1650
7b	$C_6H_4CH_3$ (p)	3356	1646
7c	$C_6H_4OCH_3$(o)	3341	1658
7d	$C_6H_4OCH_3$ (p)	3384	1642
7e	$C_6H_4OC_2H_5$ (p)	3391	1661
7f	C_6H_4Cl (p)	3364	1667
7g	C_6H_4Cl (m)	3410	1650
7h	C_6H_{11} (cyclohexyl)	3352	1644

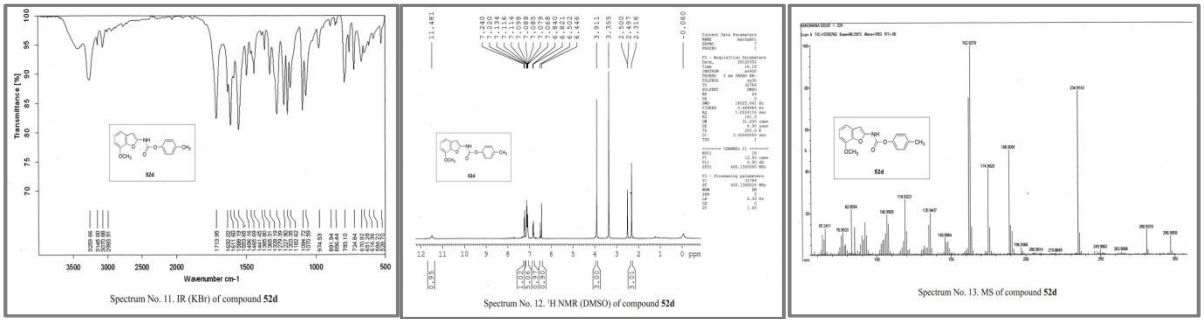


Figure 5. IR, NMR and Mass spectrum of compound 8h.

To provide the additional evidences for the proposed structures, the ^1H NMR and mass spectrum of **8d** were recorded. The ^1H NMR spectrum in DMSO-d_6 was exhibited two singlets at δ 2.31 and δ 3.91 ppm due to -CH_3 and -OCH_3 protons respectively. The aromatic protons were resonated as a multiplet in the range of δ 6.44-7.24 ppm and a singlet was observed at δ 11.48 ppm due to -NH proton. The molecular ion peak was observed at *m/z* 297 confirmed the formation of **8d** (Figure 5).

Table 4. IR data of compounds 8a-h

Compound	Substituent 'R'	IR data (cm^{-1})	
		NH	C=O
8a	C_6H_5	3275	1716
8b	C_6H_4-OCH_3 (p)	3264	1721
8c	C_6H_4-OCH_3(m)	3314	1708
8d	$C_6H_4CH_3$ (p)	3259	1713
8e	$C_6H_4CH_3$ (o)	3310	1718
8f	C_6H_4Cl (p)	3284	1711
8g	C_6H_4Cl (o)	3268	1707
8h	C_6H_4Br (p)	3350	1704

Biological Activities

Antimicrobial studies: The compounds **6, 7(d** and **f)** and **8(f** and **h)** were screened for their antibacterial and antifungal activity at 50 µg $disc^{-1}$ by the disc diffusion method Further MIC of these compounds was determined by micro broth dilution method.

The antibacterial and antifungal data (Table 5 to 8) revealed that the compounds exhibited moderate to good activity. The compounds bearing chlorine substituent on **7 f** and **8h** showed potent activity.

Table 5. Results of antibacterial activity of the compounds 6, 7(d and f) and 8(f and h) at 50 µg mL^{-1}

Comp.	Diameter of the zone of inhibition in mm (Relative inhibition %)				
	Gram negative			Gram positive	
	E. Coli	*P. Aeruginosa*	*K. Pneumoniae*	*S. aureus*	*I. S. II. FAECALIS*
6	13 (72.2)	21 (84)	16 (80)	12 (63.1)	14 (70)
7d	12 (66.6)	20 (80)	14 (70)	11 (57.8)	15 (75)
7f	**15 (83.3)**	**23 (92)**	**19 (85)**	**17 (89.4)**	**19 (95)**
8f	14 (77.7)	19 (75)	15 (75)	13 (68.4)	16 (80)
8h	**17 (94.4)**	**24 (96)**	**18 (90)**	**16 (84.2)**	**18 (90)**
Ciprofloxacin	18 (100)	25 (100)	20 (100)	19 (100)	20 (100)

Table 6. Results of antifungal activity of the compounds 6, 7(d and f) and 8(f and h) at 50 µg mL^{-1}

Comp.	Diameter of the zone of inhibition in mm (Relative inhibition %)				
	A. niger	*A. fumigates*	*C. albicans*	*P. notatum*	*III. RHIZOPUS*
6	24 (80)	19 (79.1)	17 (70.8)	19 (73.0)	16 (61.5)
7d	21 (70)	18 (75)	16 (66.6)	18 (69.2)	14 (53.8)
7f	**26 (86.6)**	**20 (83.3)**	**21 (87.5)**	**21 (80.7)**	**20 (76.9)**
8f	20 (66.6)	17 (70.8)	20 (83.3)	20 (76.9)	18 (69.2)
8h	**28 (93.3)**	**21 (87.5)**	**22 (91.6)**	**23 (88.4)**	**22 (84.6)**
Fluconazole	30 (100)	24 (100)	24 (100)	26 (100)	26 (100)

Table 7. Results of antibacterial activities of compounds 6, 7(d and f) and 8(f and h) MICs (µg mL^{-1})

Comp.	Gram negative			Gram positive	
	E. Coli	*P. Aeruginosa*	*K. pneumoniae*	*S. aureus*	*S. Faecalis*
6	62.5	31.25	16	62.5	125
7d	125	62.5	125	125	62.5
7f	**2**	**8**	**4**	**2**	**2**
8f	8	125	62.5	16	31.25
8h	**1**	**2**	**2**	**8**	**16**
Ciprofloxacin	1	1	1	1	1

Table 8. Results of antifungal activities of compounds 6, 7(d and f) and 8(f and h) MICs (µg mL^{-1})

Comp.	*A. Niger*	*A. fumigates*	*C. albicans*	*P. notatum*	*IV. RHIZOPUS*
6	31.25	16	62.5	125	62.5
7d	62.5	31.25	125	62.5	125
7f	**16**	**16**	**8**	**8**	**16**
8f	125	125	31.25	31.25	31.25
8h	**8**	**8**	**16**	**16**	**8**
Fluconazole	8	8	8	8	8

Antioxidant studies: *In vitro* antioxidant activity of the synthesised compounds performed by ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method. ABTS solution **I** (2 mM of ABTS solution) and solution **II** (17 mm of potassium per sulfate) were prepared using distilled water. Solution II (0.3 mL) was added to 50 ml of solution **I** and the reaction mixture was left to stand at room temperature overnight in dark before use. Test solutions were prepared by dissolving drug samples and the standard (ascorbic acid) was accurately weighed (10 mg) separately and dissolved in 1 mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions. Distilled DMSO (1 mL) was added to 0.2 mL of various concentrations of the drug

samples or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. After 20 min, the absorbance was measured spectrophotometrically at 734 nm using ELISA reader. Blank was maintained without ABTS. IC_{50} value obtained was the concentration of the sample required to inhibit 50% ABTS radical mono cation. The statistical analysis was performed by One way ANOVA followed by Tukey'spost-hoc test was employed to analyze the results (Graph Pad Prism Software). The difference below the probability level of 0.05 was considered as statistically significant.

Table 9. Results of antioxidant activity of compounds 6, 7(d and f) and 8(f and h) by ABTS method

Comp.	IC_{50} Value* Micromolar
6	81.21±1.56
7d	86.14±1.71
7f	**59.63±1.81**
8f	76.42±1.54
8h	**52.54±1.62**
Standard (Ascorbic acid)	12.10±0.51

*The results are presented as Mean±SEM, n=5; IC_{50} values of all the synthesized compounds are significantly different ($p<0.05$) from that of the standard (ascorbic acid).

The results (Table 9) indicated that, the synthesized compounds exhibited moderate to good antioxidant activity with ABTS method. Among the series, the compound **7f** and **8h** showed potent activity. Ascorbic acid showed potent ability to inhibit free radicals with IC_{50} values of 12.10±0.51 micro molar concentration.

DNA cleavage studies: The similar procedure is followed as mentioned in the literature [**25, 26**].

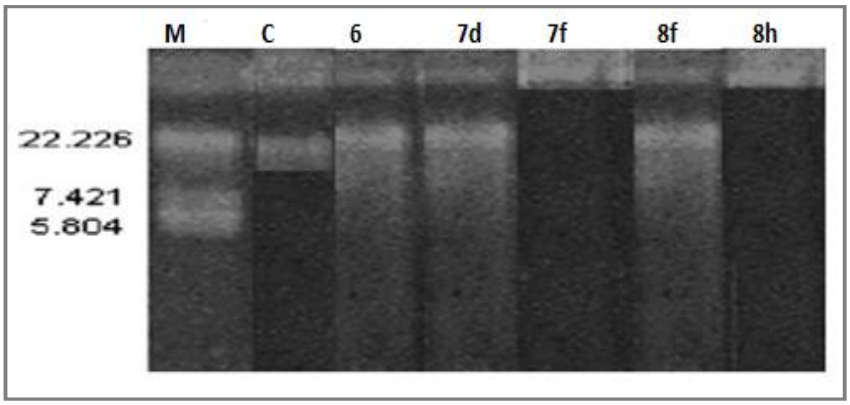


Figure 6. Gel electrophoresis of compounds**6, 7d, 7f, 8f** and**8h** on DNA of *E.coli* at 25µg Lane M: DNA marker; Lane C: Untreated DNA.

The compounds **7f** and **8h** act as potent nuclease agents. As the compounds were observed to cleave the DNA, it can be concluded that, the compound inhibits the growth of the pathogenic organism by cleaving the genome. The gel containing *E. coli* DNA treated with compounds shows that after treatment, the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage was observed with **7f** and **8h** (Figure 6).

DNA Protection studies: The similar procedure is followed as mentioned in the literature [**26**]. The compounds **7f**, and **8h** showed better activity than trolox regarding protection against 2, 2'-azobis (2-amidinopropane hydrochloride) (AAPH) induced DNA strand (Figure 7).

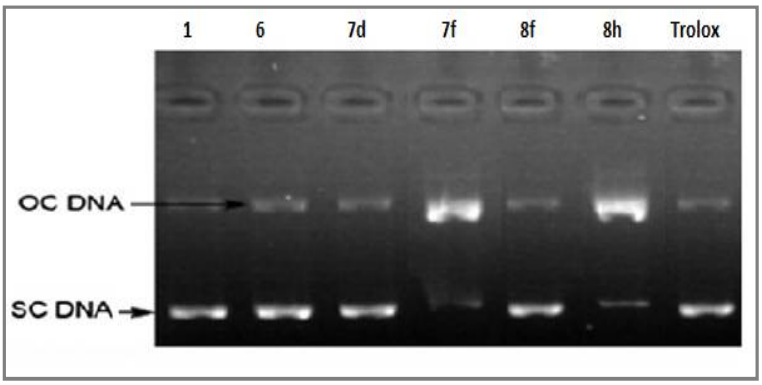


Figure 7. Protection against AAPH-induced pBR322 DNA strand breakage by compounds.
Lane 1: blank, native DNA, Lanes **6, 7(d,f), 8(f,h)** test compounds and trolox.

APPLICATION

The synthetic work carbamate and carbamides as side chain at position 2 of benzofuran ring will be encouraging due to their biological activity. Particularly the substitutions **d, f and h (scheme)** on aryl ring of carbamates and carbamides had exhibited enhanced activity. This will help in designing the drugs of suitable activity described under biological activity.

CONCLUSION

The structures of all the new compounds synthesized in the present investigation were in consistent with the structures assigned and were supported by their spectral data.

The compounds **6, 7(d** and **f)** and **8(f** and **h)** were screened for their antibacterial and antifungal activity at 50 µg/disc by the disc diffusion method. Among the compounds screened for antibacterial activity, compounds **7f** and **8h** have shown appreciable activity against standard drug ciprofloxacin and others shown moderate activity (Table 5 and 7).

Compounds **7f** and **8h** have exhibited appreciable antifungal activity against standard drug fluconazole and the remaining have appears to be having moderate activity (Table 6 and 8).

The antibacterial and antifungal data (Table 5 to 8) revealed that the compounds exhibited moderate to good activity. The compounds bearing chlorine substituent on **7 f** and **8h** showed potent activity.

The results shown in table No..9 indicated that, the synthesized compounds exhibited moderate to good antioxidant activity with ABTS method. Among the series, the compound **7f** and **8h** showed potent activity. Ascorbic acid showed potent ability to inhibit free radicals with IC_{50} values of 12.10±0.51 micro molar concentration.

The compounds **7f** and **8h** act as potent nuclease agents. As the compounds were observed to cleave the DNA, it can be concluded that, the compound inhibits the growth of the pathogenic organism by cleaving the genome. The gel containing *E. coli* DNA treated with compounds shows that after treatment, the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The complete cleavage was observed with **7f** and **8h**.

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REFERENCES

[1]. S. J. Shaw, Structure activity relationship of discodermolide analogues, *Mini-Rev. Med. Chem.* **2008**, 8, 276.
[2]. The Pesticidal Manual, 10th ed.; Thomlin. C. D. S. Ed.*; Crop. Protection Publication*; Farnham, UK, **1994**.
[3]. T. Goto, Y. Ito, S. Yamada, H. Matsumoto, H. Ok, H. Nagase, The high throughput analysis of N-methyl carbamate pesticides in fruits and vegetables by liquid chromatography electro spray ionization tandem mass spectroscopy, *Anal. Chim. Acta.*, **2006**, 555, 225.
[4]. J. Ma, N. Lu, W. Qin, R. Xu, Y. Wang, X. Chen, Differential responses of eight cyanobacterial and green algai species, to carbamate insecticides, *Ecotoxicol. Environ. Saf.* **2006**, 63, 268.
[5]. A. J. Wills, Y. K. Ghosh, S. Balasubramanian, Synthesis of a polymer supported oxazolidine aldehyde for asymmetric chemistry, *J. Org. Chem.* **2002**, 67, 6646.
[6]. C. Han, R. Shen, S. Su, J. A.Porco, Copper mediated synthesis of N-acyl vinylogous carbamic acids and derivatives; synthesis of antibiotic CJ-15,801, *Org. Lett.*, **2004**, 6, 27.
[7]. A. B. Smith, B. S. Freez, M. J. LaMarche, T. Hirose, I. Brouard, R. V. Rucker, M. Xian, K. F. Sundermann, S. J. Shaw, M. A. Burlingame, S. B. Horwitz, D. C. Myles, Design, synthesis and evaluation of carbamate-substituted analogues of (+)-discodermolide, *Org. Lett.* **2005**, 7, 311.
[8]. E. M. Dangerfield, M. S. Timmer, B. L. Stocker, Total synthesis without protecting groups: pyrrolidines and cyclic carbamates, *Org. Lett.***2009**, 11, 535.
[9]. T. W. Greene, P. G. Wuts, M. Protective Group in Organic Synthesis, 4th ed.; John: New York, NY, **2007**.
[10]. P. J. Kociensiki, Protective Groups, 3rd ed.; *Thieme: Stuttgart*, **2003**.
[11]. R. B. Chedid, M. Brummer, B. Wibbeling, R. Fohlich, D. Hoppe, Stereo-and Regiochemical divergence in the substitution of a Lithiated Alk-1-en-3-yn-yl carbamate: synthesis of highly enantioenriched vinylallenes or Alk-3, *Angew. Chem., Int. Ed.*, **2007**, 46, 3131.
[12]. J. Becker, S. Grimme, R. Frohlich, D. Hoppe, Estimation of the kinetic acidity from substrate conformation stereochemical course of the deprotanation of cyclohexenyl carbamates, *Angew. Chem., Int. Ed.*, **200**7, 46, 1645.
[13]. H. Qin, N. Yamagiva, S. Matsunaga, M. Shibasaki, Bismuth-catalysed direct substitution of the hydroxy group in alcohols with sulfonamides, carbamates and carboxamides, *Angew. Chem., Int. Ed.***200**7, 46, 409.
[14]. X. Han, R. A. Widenhoefer, Gold (I)-catalysed intramolecular hydroamination of alkenyl carbamates, *.Angew. Chem., Int. Ed.*, **2006**, 45, 1747.
[15]. K. C. Nicolaou, J. N. Mathison, Synthesis of imides, N-acyl vinylodous carbamates and ureas and nitriles by oxidation of amides and amines with Dess-Martin periodinane, *Angew. Chem., Int. Ed.*, **2005**, 44, 5992.
[16]. S. M. Rahmanthullan, R. R. Tidwell, S. K. Jones, J. E. Hall, D. W. Boykin, Carbamate prodrugs of N-alkylfuramidines, *Eur. J. Med. Chem.***2008**, 43, 174.
[17]. K. Takaoka, Y. Tatsu, N. Yumoto, T. Nakajima, K. Shimamoto, Synthesis of carbamate-type caged derivatives of a novel glutamate transporter blocker, *Bioorg. Med. Chem.*, **2004**, 12, 3687.

[18]. C. Borrel, S.Thoret, X. Cachet, D. Guenard, F. Tillequin, M. Koch, S. Michel, Newantitubilin-derivatives in the combretastatin A4 series: synthesis and biological evaluation, *Bioorg. Med. Chem.,* **2005**, 13, 3853.
[19]. K. M. Basavaraja, Y. S. Agasimundin, Synthesis, Characterization and Biological Screening of 5-Bromo-Benzofuranyl Aryl Ureas and Carbamates, *Indian J. Heterocyclic Chem.,* **2002**, 12, 1.
[20]. H. M. Naveenakumari, Manjunatha Harihara Mathada, Mahesh Kumar and K. M. Basavaraja**,** Synthesis, characterisation, biological screening of 5-bromo-benzofuranyl arylureas and carbamates, *J. Applicable Chem.,* **2019**, 8(5), 2067-2073.
[21]. H. M. Naveena Kumari, Manjunatha Harihara Mathada, Mahesh Kumar, K. T Sudha. and K. M. Basavaraja**,** Synthesis, Characterization, Antimicrobial screening of 5-Bromobenzofuranyl-Aryl ureas and carbamates, *Asian Journal of Organic and Medicinal Chemistry,***2019**, 4(4), 232-23.
[22]. H. M. Naveena Kumari, Manjunatha Harihara Mathada, Mahesh Kumar, K. T. Sudha and K. M. Basavaraja**,** Synthesis, characterization, biological activity of 5-bromobenzofuran-2-carboxylic acid(substituted-benzylidene)hydrazides and their derivatives, *J. Applicable Chem.*, 2020, 9(2), 223-228.
[23]. Manjunatha Harihara Mathada, Mahesh Kumar, H. M. Naveena Kumari, S. V. Bharathi, L. N. Netravati and K. M. Basavaraja, Synthesis and antimicrobial activity of 5-bromo-7-methoxybenzofuranyl-2-carbonylazide derivatives, *J Applicable Chem.*, **2021**, 10(04), 445-454.
[24]. T. A. Brown, Essential Molecular Biology: A Practical Approach, Vol. I; Oxford University Press: NewYork, **1990**, 51–52.
[25]. J. Yang, G. Y. Liu, F. Dai, X. Y. Cao, Y. F. Kang, L. M. Hu, J. J. Tang, X. Z. Li, Y. Li, X. L. Jin, B. Zhou, Synthesis and biological evaluation of hydroxylated 3-phenylcoumarins as antioxidants and antiprolifirative agents, *Bioorg. Med. Chem. Lett.*, **2011**, 21, 6420.