Research Article

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Synthesis and structural characterization of some lipoxygenase-inhibiting *O*-aryl-*O*-phenyl carbonates.

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A practically simple method for the production of various *O*-aryl-*O*-phenyl carbonates was executed by treating different phenols (2a-g) with phenyl chloroformate (1) in basic solution with the resultant production of title carbonates (3a-g). The structures of these carbonates were confirmed through their ^{1}H-NMR spectra. These were screened against acetylcholinesterase, butyrylcholinesterase and lipoxygenase enzymes and all were found to be good inhibitors of lipoxygenase except 3e. However, 3c and 3f were also active against acetylcholinesterase enzyme.

Key words: *O*-aryl-*O*-phenyl carbonates, phenyl chloroformate, phenols, acetylcholinesterase, lipoxygenase.

Aryl phenyl carbonate (diaryl carbonate) is the product of esterification of hemicarbonic acid with phenolic compounds (Shaikh & Sivaram 1996). They are unstable with respect to hydrolysis and decompose to corresponding phenol and carbon dioxide when they are exposed to optimum atmospheric conditions (Shaikh & Sivaram 1992; Shaikh & Sivaram 1996). Diaryl carbonates ware previously prepared by the use of highly toxic chemical, phosgene (Choppin & Rogers 1948). To avoid the use of phosgene a new method was proposed in 1975 wherein chloroacetic acid esters were reacted with phenolic compounds to produce aryl phenyl carbonate (Fountain & Pierschbacher 1975) Direct oxidative coupling of phenols with carbon dioxide (Kondo *et. al.* 1971; Murayama *et. al.* 2011), and the reaction between oxirane and carbon dioxide were some other methods for the synthesis of aryl phenyl carbonates with both the methods involving the use of different catalysts (Peppel 1958). The best and industrially most favorable method for the production of aryl phenyl carbonate is the carbon-interchange reaction wherein one organic carbonate is converted to aryl phenyl carbonate by displacing one or both the oxygens of acyl group by an appropriate aryl oxide i.e. the conversion of dialkyl carbonate to aryl phenyl carbonate or diaryl carbonate (Shaikh & Sivaram 1992; Shaikh & Sivaram 1996; Haubrock *et. al.* 2008) and the conversion of alkyl aryl carbonate to aryl phenyl carbonate or diaryl carbonate . The diphenyl carbonate is used as a monomer for the production of polycarbonate, an excellent thermoplastic that can be employed as an alternative for glass and metals because of its strength and transparency (Shaikh & Sivaram 1992; Shaikh & Sivaram 1996). It is also used in the production of methyl isocyanate which holds great agricultural significance for the

production of crop protecting agents. Diphenyl esters, the starting material for the production of high molecular weight polyesters, are formed when dicarboxylic acids react with diphenyl carbonate with the elimination of carbon dioxide (Shaikh & Sivaram 1996). In the present study a very facile method was employed for production of various *O*-aryl-*O*-phenyl carbonates (3a-g) by treating different phenols (2a-g) with phenyl chloroformate (1) in basic solution with very good yield. The synthesized carbonates were then screened against acetylcholinesterase, butyryl-cholinesterase and lipoxygenase to ascertain their inhibitory potential against these enzymes.

MATERIALS AND METHODS

General:

TLC was performed on pre-coated silica gel G-25-UV_{254} plates. Detection was carried out at 254 nm, and by ceric sulphate reagent. Purity was checked on TLC with different solvent systems using ethyl acetate and *n*-hexane giving single spot. The ^1H NMR spectra were recorded in CD_3OD on a Bruker spectrometers operating at 500 MHz. Chemical shifts are measured in ppm. The melting points were recorded on a Griffin & George melting point apparatus by open capillary tube and were uncorrected.

General Procedure

Calculated amount (10.0 mmol; 1.27 mL) of the given phenolic compound (**2a-g**) was dissolved in as minimum as possible amount of 10% NaOH solution (in distilled water) in a stoppered conical flask (Iodine flask). For the thorough dissolution of the phenolic compound, the reaction mixture was vigorously shaken for at least five minutes. To this solution, 0.75mL (0.02mmol) of phenyl chloroformate (**1**) was then added with extra care by the use of micro pipette. The flask was stoppered immediately so as to avoid the outflow of reacting agent, which has the strong tendency to react with moisture in air. The reaction mixture was then shaken excessively for at least 15 minutes with constant degassing at definite intervals till it was ensured that reaction has gone to completion. The precipitates of *O*-Aryl-*O*-phenyl carbonates were filtered. Recrystallization of these precipitates was carried out with methanol to remove any impurity. The TLC test of the resulting precipitates, using *n*-hexane and ethyl acetate in 3:1 ratio, gave single spot and hence confirmed its purity.

Acetylcholinesterase Assay

The AChE inhibition activity was performed according to the method of Ellman *et. al*. (1961) with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2H PO_4 buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM $well^{-1}$) was added, followed by the addition of 10 µL (0.005 unit $well^{-1}$) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM $well^{-1}$ substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM $well^{-1}$). After 15 min of incubation at 37ºC, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as a positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition}\ (\%) = \frac{Control - Test}{Control} \times 100$$

Butyrylcholinesterase Assay

The BChE inhibition activity was performed according to the method of Ellman *et. al*. (1961) with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, Na_2H PO_4 buffer, 50 mM and pH 7.7. Ten µL test compound 0.5 mM $well^{-1}$, followed by the addition of 10 µL (0.5 unit $well^{-1}$) BChE. The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 mins at 37ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM $well^{-1}$ substrate (butyrylthiocholine bromide) followed by the addition of 10 µL DTNB, 0.5 mM $well^{-1}$. After 15 min of incubation at 37ºC, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{Control - Test}{Control} \times 100$$

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Lipoxygenase Assay

Lipoxygenase activity was assayed according to the reported method (Baylac & Racine 2003) but with slight modifications. A total volume of 200 µL assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15µL purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and preincubated for 10 minutes at 25°C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Quercetin (0.5 mM $well^{-1}$) was used as a positive control. The percentage inhibition was calculated by formula given below.

$$\text{Inhibition (\%)} = \frac{Control - Test}{Control} \times 100$$

***O*-Phenyl-*O*-(4-methylphenyl) carbonate (3a)**

Yield 83% , m.p. 103 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.42 (br t, *J* = 8.0 Hz, 2H, H-3 & H-5), 7.27 (m, 1H, overlapped, H-4), 7.25 (m, 2H, H-2 & H-6), 7.21 (d, *J* =8.0 Hz, 2H, H-3′ & H-5′), 7.13 (d, *J* =8.5 Hz, 2H, H-2′& H-6′), 2.34 (s, 3H, H-7′).

Diphenyl carbonate (3b)

Yield 86%. m.p. 74 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.43 (br t, *J* = 8.5 Hz, 4H, H-3, H-5, H-3′ & H-5′), 7.28 (br t, *J* = 8.5 Hz, 6H, H-2, H-6, H-2′, H-6′, H-4 & H-4′).

***O*-Phenyl-*O*-(2,4-dimethylphenyl) carbonate (3c)**

Yield 89%. m.p. 40 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.43 (br t, *J* = 8.0 Hz, 2H, H-3 & H-5), 7.28 (br t, *J* = 8.0 Hz, 3H, H-2, H-6 & H-4, merged), 7.25 (br d, *J* = 8.5 Hz, 1H, H-6′), 7.09 (br s, 1H, H-3′), 7.04 (dd, *J* = 3.0, 8.5, 1H, H-5′), 2.30 (s, 3H, H_3-7′), 2.23 (s, 3H, H_3-8′).

***O*-Phenyl-*O*-(4-tert-butylphenyl) carbonate (3d)**

Yield 81%. m.p. 44 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.46 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 7.43 (br t, *J* = 7.5 Hz, 2H, H-3 & H-5), 7.28 (br t, *J* = 7.5 Hz, 2H, H-2 & H-6), 7.27 (br t, *J* = 7.5 Hz, 1H, H-4), 7.18 (d, *J* =8.5 Hz, 2H, H-2′ & H-6′), 1.32 (s, 9H, H_3-8′, H_3-9′ & H_3-10′).

***O*-Phenyl-*O*-(1-naphthyl) carbonate (3e)**

Yield 88%. m.p. 71 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 8.03 (br d, *J* = 8.0 Hz, 1H, H-8′), 7.95 (br d, *J* = 8.0 Hz, 1H, H-5′), 7.84 (br d, *J* = 8.5 Hz, 1H, H-2′), 7.61 (br t, *J* = 7.0 Hz, 1H, H-6′), 7.57 (br t, *J* = 7.0 Hz, 1H, H-7′), 7.54 (br d, *J* = 8.5 Hz, 1H, H-4′), 7.52 (br t, *J* = 8.0 Hz, 1H, H-3′), 7.43 (br t, *J* = 8.0 Hz, 2H, H-3, H-5), 7.28 (br t, *J* = 8.0 Hz, 3H, H-2, H-6 & H-4).

***O*-Phenyl-*O*-(2-naphthyl) carbonate (3f)**

Yield 87%. m.p. 86 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.95 (br d, *J* = 9.0 Hz, 1H, H-4′), 7.88 (br t, *J* = 9.0 Hz, 1H, H-5′), 7.85 (m, 1H, H-3′), 7.77 (d, *J* = 2.0 Hz, 1H, H-1′), 7.69 (br t, *J* = 8.5 Hz,1H, H-7′), 7.60 (d, *J* = 8.5 Hz, 1H, H-8′), 7.53 (br t, *J* = 8.5 Hz, 1H, H-6′), 7.43 (br t, *J* = 8.0 Hz, 2H, H-3 & H-5), 7.31 (br t, *J* = 8.0 Hz; overlapped, 3H, H-2, H-6 & H-4).

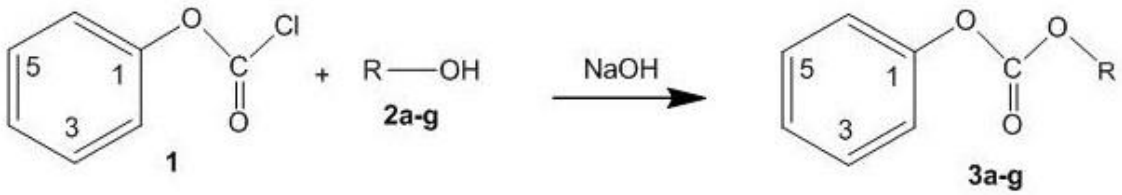
***O*-Phenyl-*O*-(4-bromophenyl) carbonate (3g)**

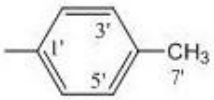
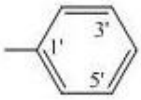
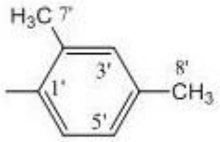
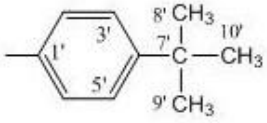
Yield 83%. m.p. 83 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 7.56 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), 7.43 (br t, *J* = 8.0 Hz, 2H, H-3 & H-5), 7.28 (br t, *J* = 8.0 Hz, overlapped, 3H, H-2, H-6 & H-4), 7.24 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′).

RESULTS AND DISCUSSION

Compound 3a was synthesized as brown powder. The molecular formula $C_{14}H_{12}O_3$ was established with the help of ^{1}H-NMR spectrum by counting the number of protons. In its ^{1}H-NMR spectrum, two *ortho* coupled doublets in aromatic region appearing at δ 7.21 (d, *J* = 8.5 Hz, 2H, H-3′ & H-5′), and 7.13 (d, *J* = 8.5 Hz, 2H, H-2′ & H-6′) were assigned to the protons of the di-substituted ring derived from *p*-cresol, whereas the signals appearing at δ 7.42 (br t, *J* = 8.0 Hz, 2H, H-3 & H-5), 7.27 (m, 1H, H-4) and 7.25 (m, 2H, H-2 & H-6)

Scheme-1: Synthesis of *O*-phenyl-*O*-aryl carbonates (3a-g).



Compound	R
3a	
3b	
3c	
3d	

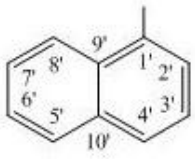
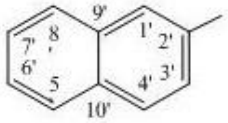
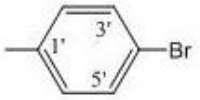
Compound	R
3e	
3f	
3g	

Table 1: Enzyme inhibition studies on *O*-phenyl-*O*-aryl carbonates (3a-g). (n=3, mean ± SE).

Compounds No.	DPPH		AChE		BChE		LOX	
	(%) at 0.5 mM	(IC_{50}) µmoles/L	(%)at 0.5mM	(IC_{50}) µmoles/L	(%) at 0.5mM	(IC_{50}) µmoles/L	(%) at 0.5mM	(IC_{50}) µmoles/L
	54.15±0.59	<400	22.87±0.47	Nil	38.71±0.98	Nil	78.91±0.66	102±0.91
3b	9.74±0.78	Nil	28.24±0.95	Nil	32.87±0.87	Nil	73.41±0.45	119±0.47
3c	26.68±0.75	Nil	66.11±0.83	232±0.93	50.90±0.45	<500	87.13±1.06	87±0.83
3d	14.90±0.91	Nil	55.93±1.01	<400	34.21±1.02	Nil	97.54±0.92	59±0.71
3e	4.98±0.84	Nil	22.96±0.93	Nil	50.81±1.11	<500	42.61±0.72	Nil
3f	50.32±0.73	<500	67.31±0.89	198±0.71	51.76±0.98	<500	95.76±0.76	65±0.67
3g	3.78±1.02	Nil	25.74±0.77	Nil	16.07±0.65	Nil	50.74±0.84	<500
Control			Eserine	0.04±0.001	Eserine	0.85±0.001	Quercetin	37.12±0.07

Note: DPPH = 1, 1-diphenyl-2-picrylhydrazyl radical

were typical for the protons of the mono-substituted ring derived from phenylchloroformate (**1**). Moreover, a characteristics singlet at δ 2.34 (s, 3H, H-7′) also revealed the presence of a methyl group in the structure of the molecule. On the basis of above cumulative evidences, the structure of **3a** was assigned as *O*-phenyl *O*-(4-methylphenyl) carbonate. Similarly, on the basis of such spectral evidences, the structures of other carbonates were also deduced as shown in scheme-1. These synthesized *O*-aryl-*O*-phenyl carbonates (**3a-g**) were screened for 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity as well as their inhibitory potential was also studied against three enzymes namely acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX). From the results (Table-1), it was inferred that these were good inhibitors of lipoxygenase except **3e** which was inactive against this enzyme. However, **3c** and **3f** were also active against acetylcholinesterase enzyme having IC_{50} values of 66.11 ± 0.83 and 67.31±0.89 µmoles/L, respectively, relative to eserine, a reference standard having IC_{50} value of 0.04 ± 0.001 µmoles/L (Table-1).

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