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Synthesis, spectral characterization and chymotrypsin activity of various *O*-phenyl-*N*-aryl carbamates

Aziz-ur-Rehman[1]*, Shaista Khaliq[1], Muhammad Athar Abbasi[1], Tehseen Ilyas[1], Faiz-ul-Hassan Nasim[2], Noreen Khalid[3], Rafia Manazer[3]

1 Department of Chemistry, Government College University, Lahore-54000, Pakistan.
2 Department of Biochemistry and Biotechnology;
3 Department of Pharmacy; The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.

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***Corresponding author:**

Email : azizryk@yahoo.com
Tel : (+92)-42-111000010 Ext.449

ABSTRACT

The sole purpose of this research work was to synthesize different *O*-phenyl-*N*-aryl carbamates and to evaluate their chymotrypsin activity. A series of *O*-phenyl-*N*-aryl carbamates has been synthesized by reacting different disubstituted aromatic amines (2a-e) with phenylchloroformate in basic aqueous media. Further brominated and nitrated carbamates (4a-d, 5d) were synthesized by bromination and nitration of *O*-phenyl-*N*-aryl carbamates. Purity of all the compounds was checked by TLC. All these synthesized compounds were characterized by IR, EI-MS, and ^{1}H-NMR and then screened against chymotrypsin enzyme. The screening of the synthesized derivatives revealed their effectiveness against chymotrypsin enzyme. Among these compounds, *O*-Phenyl-*N*-(3,5-dimethylphenyl)carbamate (3a) was found to be the most potent inhibitor for chymotrypsin while compound (3d) was found to be the least inhibitor.

INTRODUCTION

Carbamates is a class of organic compounds of developing interest because of their vast applications as protective groups for alcohols, thiols and amines and they have been found in various agrochemicals (fungicides, herbicides and pesticides) and Pharmaceuticals [1]. Carbamates can be used as drug intermediates, in polyurethane and in peptide synthesis. Among the several amine protecting groups, carbamates are preferred because of their resistive nature and stability in acidic and basic media [2]. Carbamates are mainly used to protect crops, livestock, communities and homes by working against insects. But they are also responsible for severe and life-threatening problems. Carbamate poisoning is believed to be caused by inhibition of acetyl cholinesterase. They are absorbed by ingestion, inhalation and may be by skin penetration, hence direct contact with them should be avoided [3,4]. Literature reported that carbamates have been synthesized as anticonvulsant against epilepsy which is neurological disorder. Treatment of disease is effected by resistance caused by antiepileptic drugs (AEDs) and adverse effects associated with it. Hence, for the development of safer and more effective AEDs, there is need to design safer anticonvulsants [5]. Among the enzymes involved in extracellular matrix degradation, certain serine proteases (Elastase, Collagenase, Cathepsin G, and Chymotrypsin) are able to solubilize fibrous proteins such as elastin and collagen [6]. Potent inhibitors have the potential to be developed as new therapeutic agents. In vertebrates, serine protease inhibitors have been studied for many years and they are known to be involved in phagocytosis, coagulation, complement activation, fibrinolysis and blood pressure regulation. In the last decade, it became obvious that in invertebrates, serine proteases and their inhibitors are also involved in parallel physiological processes, for example the blood clotting cascade in Limulus [7] and the innate immune response [8]. Our current investigations on the *α*-chymotrypsin inhibition, we describe here the in vitro α-chymotrypsin inhibitory activity of the synthesized various substituted *O*-phenyl-*N*-aryl carbamates.

MATERIALS AND METHOD

Silica gel coated plates were used for thin layer chromatography to confirm the purity of synthesized derivatives by using different ratios of ethyl acetate and *n*-hexane as solvent system. Detection was made at wavelength 254 nm in UV lamp. IR spectra were taken by the help of Jasco-320-A spectrophotometer, in KBr by applying pallet method. Melting point of compounds was recorded by Griffin & George apparatus by using open capillary tube method. ^{1}H-NMR spectra of all synthesized compounds was recorded in $CDCl_3$ as solvent at 400 MHz frequency using Bruker spectrometers. Further EIMS were taken by using a JMS-HX-110 spectrometer, with data system.

General Procedure for the synthesis of carbamates (3a-e):

Disubstituted aromatic amines (4.9 mmol; 2a-e) were taken in

round bottom flask and aqueous solution of sodium bicarbonate was added to maintain pH 9.0 to 10. Further phenylchloroformate (4.9 mmol; **1**) was added and stirred the contents vigorously at room temperature. Reaction completion was confirmed by thin layer chromatography (TLC). Finally precipitates were filtered, washed with distilled water and dried to get the corresponding substituted *O*-phenyl-*N*-aryl-carbamates (3a-e). Precipitates obtained were recrystallized by using methanol to obtain pure products.

Procedure for the synthesis of brominated carbamates:

In round bottom flask, 0.4 mmol of different synthesized carbamates (3a-3d) were taken and tried to dissolve in 5ml of glacial acetic acid. Reaction flask was set to stirring and on complete dissolution equimolar bromine water was introduced and continued stirring until reaction completion. Reaction coordinates were monitored by TLC. After reaction completion, reaction mixture was poured in excess of ice cold water, precipitates formed were filtered, washed with distilled water, dried and recrystallized by methanol to get pure products.

Procedure for the synthesis of nitrated carbamates

0.4 mmol of synthesized carbamates (3a-3e) was taken in round bottom flask; 3-5 ml of concentrated sulfuric acid was then introduced to dissolve them. Reaction flask was then kept on ice bath to cool the contents to 0-5 °C then 0.2 ml of concentrated nitric acid was added to the reaction mixture. Reaction flask was then set to stir for one hour. On reaction completion, reaction contents were poured in a beaker containing ice cold water. Only nitration of *O*-Phenyl-*N*-(2,5-dimethylphenyl)carbamate (3d) was successful which resulted into *O*-Phenyl-*N*-(4-nitro-2,5-dimethylphenyl) carbamates. Precipitates formed were filtered out, washed with excess distilled water and dried to get product.

In vitro α-chymotrypsin assay

The chymotrypsin inhibitory activity of the compounds was performed by the method [9] of Cannell, Kellam, Owsianka, and Walker (1988). Chymotrypsin (9units/ml of 50mMTrisHCl buffer pH 7.6; Sigma Chemical Co. USA) was pre-incubated with the compounds for 20 min at 258°C. 100mL of substrate solution (1mg of *N*-succinyl-phenylalanine-*p*-nitroanilide/ml of 50 mM TrisHCl buffer pH 7.6) was added to start the enzyme reaction. The absorbance of released *p*-nitroaniline was continuously monitored at 410nm until a significant color change was achieved. The final DMSO concentration in the reaction mixture was 7%. The percentage (%) inhibition was calculated as follows (ES)/E x 100, where E is the activity of the enzyme without test compound and S is the activity of enzyme in the presence of the test compound. The concentrations of test compounds that inhibited the hydrolysis of substrate upto 50% (IC_{50}) were determined by monitoring the effect of various concentrations of these compounds in the assays on the inhibition values. The IC_{50} value was then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Characterization of the synthesized compounds

O-Phenyl-*N*-(3,5-dimethylphenyl)carbamate(3a):

White granules; Yield: 91%; Melting Point: 72-74°C; Molecular formula: $C_{15}H_{15}NO_2$; Mol. Wt. 241; IR (KBr, ν_{max} cm^{-1}): 3445 (N-H stretching), 3022 (C-H aromatic stretching), 1719 (C=O stretching), 1449 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.37 (br.t, J = 8.0 Hz, 2H, H-3' & H-5'), 7.23-7.21 (m, 1H, H-4'), 7.17 (dd, J = 8.4, 1.2 Hz, 2H, H-2' & H-6'), 7.06 (s, 2H, H-2 & H-6), 6.79 (br s, 1H, -NH), 6.73 (s, 1H, H-4), 2.28 (s, 6H, Me-3 & Me-5); EIMS: *m/z* 241 $[M]^+$, 147 $[C_8H_9NCO]^+$, 119 $[C_8H_9N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-Phenyl-*N*-(2,3-dimethylphenyl)carbamate(3b)

Off-white granules; Yield: 80%; Melting Point: 86-88°C; Molecular formula: $C_{15}H_{15}NO_2$; Mol. Wt. 241; IR (KBr,ν_{max} cm^{-1}): 3440 (N-H stretching), 3024 (C-H aromatic stretching), 1710 (C=O stretching), 1445 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.57 (br s, 1H, H-6), 7.37 (br.t, J=8.0 Hz, 2H, H-3' & H-5'), 7.24-7.17(m, 3H, H-2', H-4' & H-6'), 7.10 (t, J = 8.0 Hz, 1H, H-5), 6.98 (br.d, J = 7.2 Hz, 1H, H-4), 6.68 (br s, 1H, -NH), 2.22 (s, 3H, Me-3), 2.30 (s, 3H, Me-2); EIMS: *m/z* 241 $[M]^+$, 147 $[C_8H_9NCO]^+$, 119 $[C_8H_9N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

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

White granules; Yield: 76%; Melting Point: 84-86°C; Molecular formula: $C_{15}H_{15}NO_2$; Mol. Wt. 241; IR (KBr, ν_{max} cm^{-1}): 3442 (N-H stretching), 3024 (C-H aromatic stretching), 1715 (C=O stretching), 1449 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.66 (br.s, 1H, H-6), 7.37 (br.t, J = 8.0 Hz, 2H, H-3' & H-5'), 7.24-7.17 (m, 3H, H-2', H-4' & H-6'), 7.02 (br.d, J = 8.4 Hz, 1H, H-5), 7.00 (s, 1H, H-3), 6.62 (br s, 1H, -NH), 2.28 (s, 6H, Me-2 & Me-4); EIMS: *m/z* 241 $[M]^+$, 147 $[C_8H_9NCO]^+$, 119 $[C_8H_9N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-Phenyl-*N*-(2,5-dimethylphenyl)carbamate(3d)

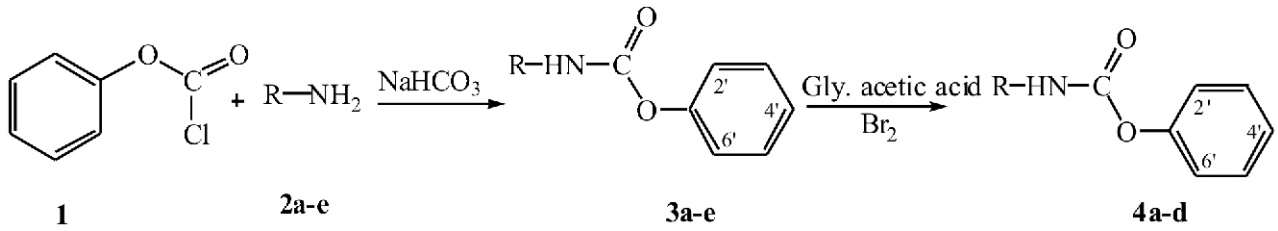
Silver grey granules; Yield: 90%; Melting Point: 112-114°C; Molecular formula $C_{15}H_{15}NO_2$; Mol. Wt. 241; IR (KBr, ν_{max} cm^{-1}): 3445 (N-H stretching), 3020 (C-H aromatic stretching), 1720 (C=O stretching), 1447 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.70 (br s, 1H, H-6), 7.37 (br.t, J = 8.0 Hz, 2H, H-3' & H-5'), 7.23-7.17 (m, 3H, H-2', H-4' & H-6'), 7.06 (d, J = 7.6 Hz, 1H, H-4), 6.87 (br.d, J = 7.6 Hz, 1H, H-3), 2.30 (s, 3H, Me-2), 2.27 (s, 3H, Me-5); EIMS: *m/z* 241 $[M]^+$, 147 $[C_8H_9NCO]^+$, 119 $[C_8H_9N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

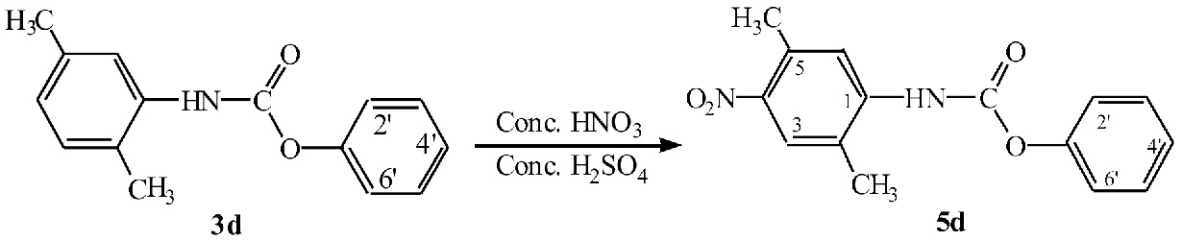
O-Phenyl-*N*-(2,6-dimethylphenyl)carbamate(3e)

White granules; Yield: 78%; Melting Point: 90-92°C; Molecular formula: $C_{15}H_{15}NO_2$; Mol. Wt. 241; IR (KBr, ν_{max} cm^{-1}): 3440 (N-H stretching), 3022 (C-H aromatic stretching), 1715 (C=O stretching), 1445 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.36-7.19 (m, 5H, H-2' to H-6'), 7.09 (br.s, 3H, H-3, H-4 & H-5), 6.33 (br s, 1H, -NH), 2.33 (s, 6H, Me-2, Me-6); EIMS: *m/z* 241 $[M]^+$, 147 $[C_8H_9NCO]^+$, 119 $[C_8H_9N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-Phenyl-*N*-(2,4-dibromo-3,5-dimethylphenyl) carbamate(4a)

White granules; Yield: 88%; Melting Point: 116-118°C; Molecular formula: $C_{15}H_{13}NO_2Br_2$; Mol. Wt. 399; IR (KBr, ν_{max} cm^{-1}): 3447 (N-H stretching), 3026 (C-H aromatic stretching), 1720 (C=O stretching), 1449 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.99 (s, 1H, H-6), 7.52 (br s, 1H, -NH), 7.39 (br.t, J =8.0 Hz, 2H, H-3' & H-5'),7.24-7.17 (m, 3H, H-2', H-4' & H-6'), 2.64 (s, 3H, Me-3), 2.37 (s, 3H, Me-5); EIMS: *m/z* 399 $[M]^+$, 227 $[C_8H_9BrNCO]^+$, 199 $[C_8H_9BrN]^+$, 174 $[C_6H_5BrOH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.





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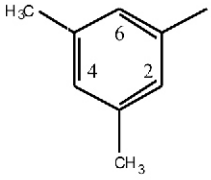
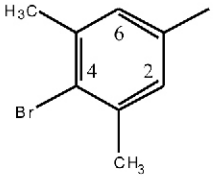
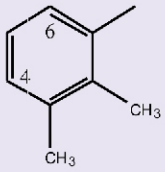
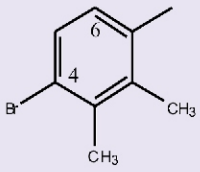
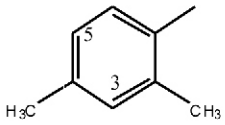
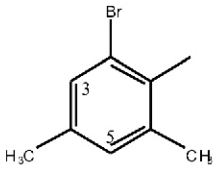
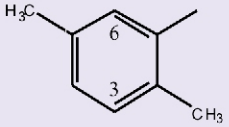
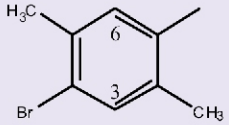
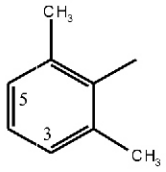
3a		4a	
3b		4b	
3c		4c	
3d		4d	
3e			

Scheme-1: Outline for the synthesis of various substituted O-phenyl-N-aryl carbamates

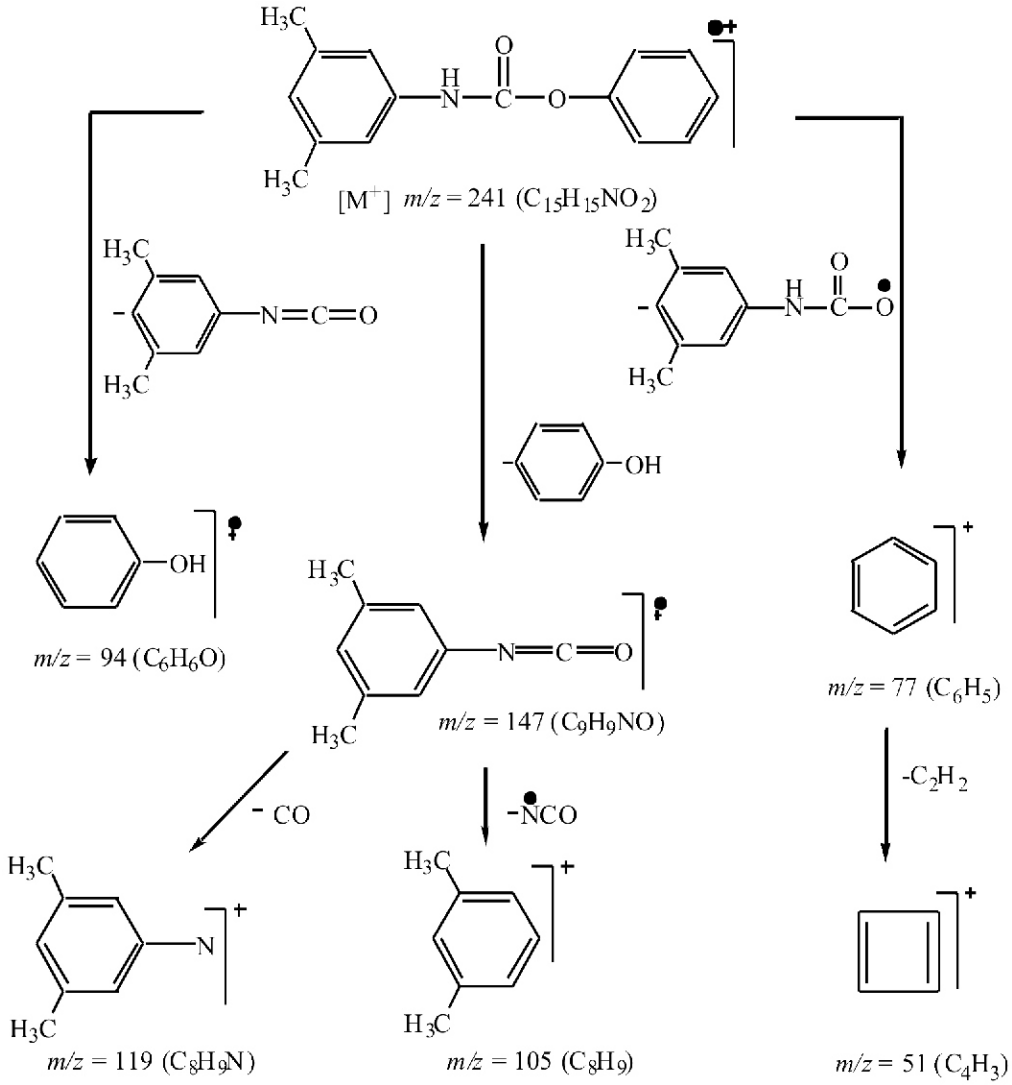


Figure-1: Mass fragmentation pattern of O-Phenyl-N-(3,5-dimethylphenyl)carbamate

O-Phenyl-*N*-(4-bromo-2,3-dimethylphenyl)carbamate (4b)

Light pink granules; Yield: 83%; Melting Point: 140-142°C; Molecular formula: $C_{15}H_{14}NO_2Br$; Mol. Wt. 320; IR (KBr, ν_{max} cm^{-1}): 3445 (N-H stretching), 3022 (C-H aromatic stretching), 1719 (C=O stretching), 1449 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$,400 MHz): δ (ppm) 7.49 (d, J = 8.8 Hz, 1H, H-5), 7.42-7.16 (m, 5H, H-2' to H-6'), 7.06 (d, J = 8.0 Hz, 1H, H-6), 2.41 (s, 3H, Me-3), 2.28 (s, 3H, Me-2); EIMS: *m/z* 320 $[M]^+$, 227 $[C_8H_9BrNCO]^+$, 199 $[C_8H_9BrN]^+$, 174 $[C_6H_5BrOH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-(4-bromophenyl)-*N*-(2,3-dibromo-4,6-dimethylphenyl) carbamate (4c)

Off-white granules; Yield: 78%; Melting Point: 122-124°C; Molecular formula: $C_{15}H_{12}NO_2Br_3$; Mol. Wt. 478; IR (KBr, ν_{max} cm^{-1}): 3440 (N-H stretching), 3021 (C-H aromatic stretching), 1717 (C=O stretching), 1446 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.49 (d, J = 8.8 Hz, 2H, H-3' & H-5'), 7.08 (d, J = 8.4 Hz, 2H, H-2' to H-6'), 7.04 (s, 1H, H-3), 2.31 (s, 3H, Me-4), 2.22 (s, 3H, Me-2); EIMS: *m/z* 478 $[M]^+$, 227 $[C_8H_9BrNCO]^+$, 199 $[C_8H_9BrN]^+$, 174 $[C_6H_5BrOH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-(4-bromophenyl)-*N*-(2-bromo-3,6-dimethylphenyl) carbamate (4d)

Light pink granules; Yield: 87%; Melting Point: 130-132°C; Molecular formula: $C_{15}H_{13}NO_2Br_2$; Mol. Wt. 399; IR (KBr, ν_{max} cm^{-1}): 3442 (N-H stretching), 3023 (C-H aromatic stretching), 1714 (C=O stretching), 1444 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.50 (d, J = 8.8 Hz, 2H, H-3' & H-5'), 7.38 (d, J = 8.0 Hz, 1H, H-5), 7.34 (br s, 1H, -NH), 7.18 (d, J = 7.6 Hz, 1H, H-4), 7.08 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 2.33 (s, 1H, Me-3), 2.25 (s, 1H, Me-6); EIMS: *m/z* 399 $[M]^+$, 227 $[C_8H_9BrNCO]^+$, 199 $[C_8H_9BrN]^+$, 174 $[C_6H_5BrOH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

O-(4-nitrophenyl)-*N*-(4-nitro-2,5-dimethylphenyl) carbamate (5d)

Yellow granules; Yield: 77%; Melting Point: 100-102°C; Molecular formula: $C_{15}H_{13}N_3O_6$; Mol. Wt. 331; IR (KBr, ν_{max} cm^{-1}):

Table 1: Biological studies of various substituted O-phenyl-N-aryl carbamates

Sample code	Chymotrypsin		
	Conc. (mM)	Inhibition (%)	IC_{50} (µmol.)
3a	0.5	87.85±0.11	44.11±0.12
3b	0.5	62.36 ±0.17	291.51±0.04
3c	0.5	58.29±0.14	<400
3d	0.5	51.22±0.15	212.45±0.06
3e	0.5	73.91±0.16	132.41±0.21
4a	0.5	74.46± 0.11	205.41±0.06
4b	0.5	54.08±0.19	<400
4c	0.5	60.33±0.13	133.31±0.55
4d	0.5	74.86±0.15	210.45±0.07
5d	0.5	56.93±0.17	<400
Control	Chymostatin	93.50±0.91	8.24±0.11

Note: IC50 values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZFit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

3440 (N-H stretching), 3022 (C-H aromatic stretching), 1719 (C=O stretching), 1449 (C=C stretching of aromatic ring); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.97 (s, 1H, H-3), 7.89 (s, 1H, H-6), 7.61 (d, J = 8.8 Hz, 2H, H-3' & H-5'), 7.59 (d, J = 8.8 Hz, 2H, H-2' & H-6'), 2.49 (s, 1H, Me-3), 2.28 (s, 1H, Me-5); EIMS: *m/z* 331 $[M]^+$, 192 $[C_8H_8N_2O_2CO]^+$, 164 $[C_8H_8NO_2N]^+$, 94 $[C_6H_5OH]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

RESULTS AND DISCUSSION

A series of substituted aromatic carbamates was synthesized and screened all the derivatives against chymotrypsin activity. The structures of all these synthesized substituted aromatic carbamates were confirmed by IR and mass spectral data and ^{1}H-NMR. Mass spectral data confirmed molecular formula of compound 3a to be $C_{15}H_{15}NO_2$ by giving *m/z 241 as molecular ion peak. IR spectrum bands appeared at* 3445 for N-H stretching, 3022 for C-H aromatic stretching, 1719 for C=O stretching and 1449 for C=C str. of aromatic ring. *EIMS showed characteristic peaks like base peak at* 147 $[C_8H_9NCO]^+$ *due to loss of phenol moiety and fragment peaks at* 119 $[C_8H_9N]^+$ due to loss of phenyl formate, at 94 $[C_6H_5OH]^+$ due to loss of 1-isocyanato-3,5-dimethylbenzene moiety as shown in figure-1. In ^{1}H-NMR spectrum of compound, two singlet peaks appeared at δ 7.06 (s, 2H, H-2 & H-6) and at δ 6.73 (s, 1H, H-4). These protons were appeared to be protons of tri-substituted benzene ring of amine as two protons had signal at one position due to symmetry of structure and the third (H-4) proton had signal slightly up-field than other two because both H-2 and H-6 protons are close to electron withdrawing amino group as compare to third proton hence their shift value is downfield. A signal appeared at δ 7.17 as doublet of doublet having both ortho & meta coupling values of 8.4 and 1.2 Hz integrated for two protons. Signal appeared at δ 7.37 as br.t with J value of 8.0 Hz, showing ortho coupling, integrated for two protons while signals at δ 7.23-7.21 appeared as multiplet for one proton. Signals appeared at δ 2.28 as singlet integration of six proton confirmed the presence of 2 methyl groups. On basis of above collective evidences, compound 3a came out to be *O*-Phenyl-*N*-(3,5-dimethylphenyl) carbamate.

The screening of the synthesized derivatives showed that these were active against chymotrypsin enzyme. Among these compounds, *O*-Phenyl-*N*-(3,5-dimethylphenyl)carbamate (3a) was found to be the most potent inhibitor for chymotrypsin having IC_{50} value 44.11±0.12 µmoles/L relative to Chymostatin reference standard with IC_{50} value of 8.24±0.11 µmoles/L respectively. This indicates that position of the substituent has great effect on inhibitory activity of these derivatives. Compound (3d) was found to be the least inhibitor having IC_{50} value <400. Similarly 4b and 5a were also found to have least inhibitory potential.

CONCLUSION

The projected structures of the synthesized compounds are well supported by spectroscopic data. The newly synthesized compounds showed varying degree of Chymostatin activity.

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