Pak. J. Chem. 3(3): 129-133, 2013 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2013.v03.i03.p06

Nitrated and Brominated Narcotine and its Cleaved Adduct as Butyrylcholinesterase Inhibitors

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ABSTRACT
Narcotine is a very antitussive agent and its modification may lead to some more biological activities. In this presented paper, narcotine (**1**) was first subjected to nitration and bromination to yield nitrated narcotine (**2**) and brominated narcotine (**3**). It was further made to react with phenylchloroformate **(6)** to give a cleaved addition product **4**. This adduct **4** was further nitrated and brominated to yield substituted derivatives **5** and **6**, respectively. The structure elucidation of the synthesized compounds was processed *via* IR, EI-MS and ^{1}H-NMR spectra. These were also screened against butyrylcholinesterase enzyme and were found to the moderate inhibitors of butyrylcholinesterase except nitrated product, **2**, of narcotine **(1)**.

Keywords: Bromination, Butyrylcholinesterase, Narcotine, Nitration, Cleaved adducts.

1. INTRODUCTION
Among the variety of cancer ceasing agents, noscapinoids are getting appreciable attention[1]. Narcotine or noscapine is an antitussive agent and has potential anticancer effects[2]. First time it was reported in 1930 as antitussive agent and later on in 1954[3]. Up to the end of 1960, the further corroboration was furnished by the researchers, especially the bronchial asthma relief[4-7] and also the replacement of codeine by noscapine[8]. Because of less side effects and amended compliancy in patients in comparison of taxanes, noscapinoids are familiar for oral use[9-12].

Butyrylcholinesterase enzyme (BChE, EC 3.1.1.8) belongs to serine hydrolases enzymes. The interaction of this enzyme with the substrates and inhibitors relies on the differences in amino acid (AA) residing at the active sites. Acetylcholine is terminated by it at the cholinergic synapses so the main part of neuromuscular junctions and cholinergic brain synapses. The termination of the nerve impulse in cholinergic synapses and the hydrolysis of the neurotransmitter acetylcholine are catalyzed by it[13-14].

Literature survey of related structures exposed that the structural modifications of organic synthesized compounds result in varying qualitative and quantitative activity. Therefore in the undertaken investigation, the bromination[15] and nitration of narcotine **(1)** followed by the bromination and nitration of its addition product **4** were performed and their therapeutic potential was checked as butyrylcholinesterase enzyme inhibitors.

2. RESULTS AND DISCUSSION
2.1 *Chemistry*
The synthesis of some derivatives of narcotine after minor modification in its structure was carried out with an aim to elucidate their biological activity. The designed compounds derived from narcotine (**1**) were synthesized according to scheme 1. First the bromination in glacial acetic acid and nitration in a nitrating mixture of conc. HNO_3/H_2SO_4 was carried out. Then again both the reactions were processed for cleavage adduct **(4)** of narcotine **(1)**. The cleaved product was obtained by nucleophilic addition reaction of narcotine **(1)** with phenyl chloroformate in an organic media through stirring[16].

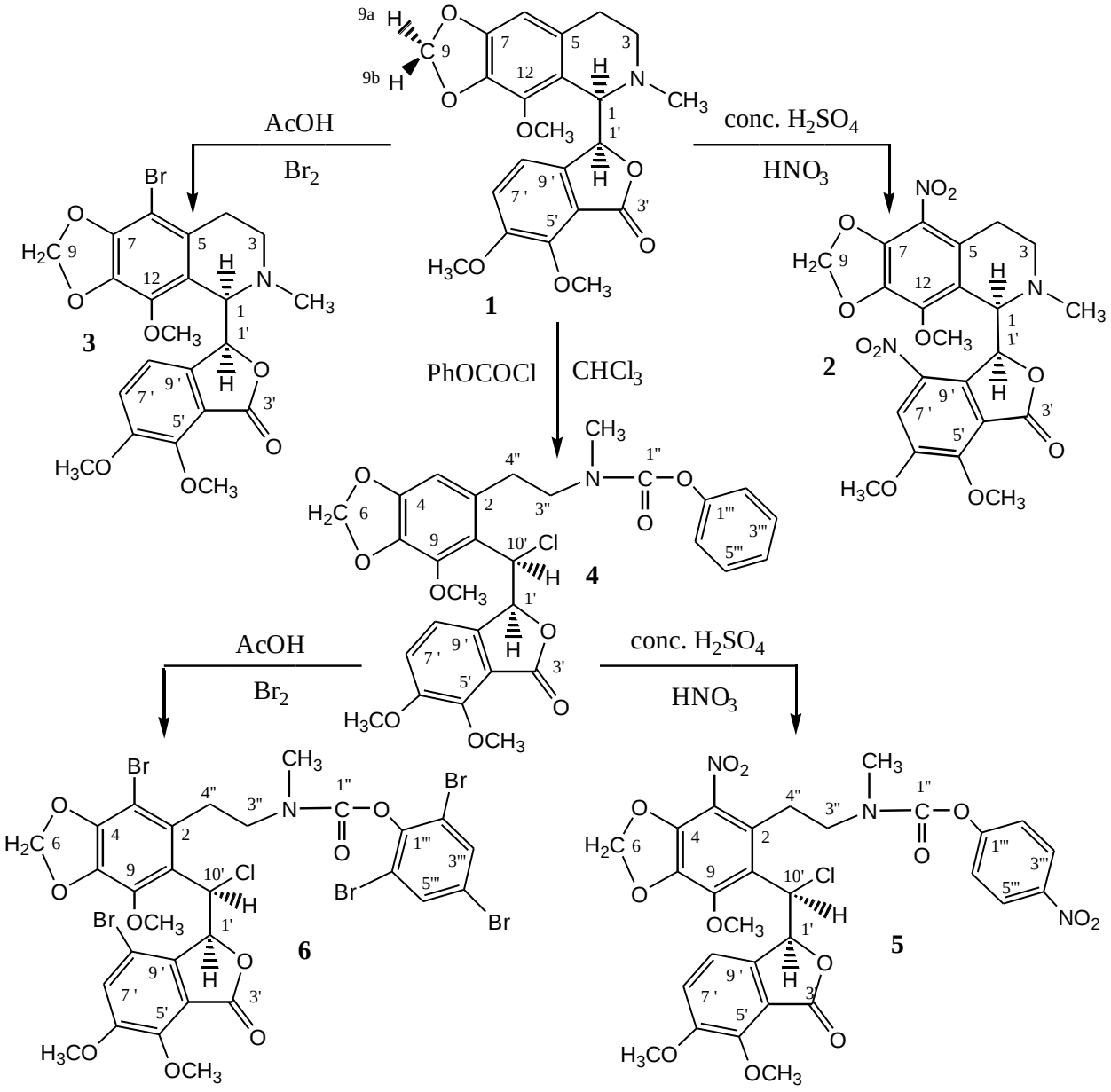
Table-1: Biological screening of different derivatives of narcotine against butyrylcholinesterase enzyme

Compound	BChE	
	%age at 0.5 mM	IC_{50} µmoles
1	20.58±0.25	-
2	36.38±0.38	-
3	97.11±0.18	38.91±0.77
4	73.78±0.22	115.31±0.45
5	80.61±0.89	68.11±0.21
6	73.23±0.67	112.31±0.21
Control	Eserine	0.85±0.0001

Narcotine (**1**) was a white crystalline solid and its structure was confirmed through extensive spectral studies. Its molecular formula**,** ($C_{22}H_{23}NO_7$) was supported by the different signals resonating along with integration value in ^{1}H-NMR spectrum and molecular ion peak in EI-MS at *m/z* 413. In the EI-MS spectrum, the cationic fragment observed at *m/z* 398 and 382 because of loss of two radicals i.e. CH_3 and OCH_3 respectively. Similarly a distinct peak at *m/z* 213

*Corresponding Author Received 14th May 2013, Accepted 12th September 2013

for a fragment ion after the removal of ($C_{10}H_9O_4$) group was also observed. In the aromatic region of its ^{1}H-NMR spectrum, two doublets at δ 7.16 (d, *J* = 8.4 Hz, 1H, H-7′), 6.17 (br d, *J* = 8.4 Hz, 1H, H-8′) and in the aliphatic region, one doublet at 5.63 (d, *J* = 3.6 Hz, 1H, H-1′) & two singlets at 3.98 (s, 3H, CH_3O-5′), 3.96 (s, 3H, CH_3O-6′) depicted the dimethoxy isobenzofuranone group in the molecule. The signals appearing at 6.34 (s, 1H, H-6), 5.92 (d, *J* = 0.9 Hz, 1H, H_b-9), 5.90 (d, *J* = 1.2 Hz, 1H, H_a-9), 3.85 (s, 3H, CH_3O-12), 2.58-2.65 (m, 2H, H-3), 2.52 (s, 3H, CH_3N-2) and 2.32-2.46 (m, 2H, H-4) were characteristics of other 4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline group. On the basis of these evidences, the structure of **1** was clearly assigned as 6,7-Dimethoxy-3-(4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one. The mass fragmentation pattern of compound **4** is drawn in figure-1. Likewise on the basis of structural manifests from EI-MS and ^{1}H-NMR, presented in experimental section, the structures of other derivatives (scheme-1) were elucidated.



Scheme-1: Nitration and bromination of narcotine and its cleaved adduct.

2.2 *Enzyme inhibition study in vitro*

The screening of the synthesized compounds against butyrylcholinesterase (BChE) enzyme revealed that out of five synthesized compounds only **2** was inactive and remaining all i.e. **3, 4, 5** and **6** showed moderate inhibitory potential against this enzyme (Table-1). However, among these the relatively enhanced activity of brominated product **3**, having IC_{50} value 38.91±0.77 μmoles/L, and nitrated product **5** with IC_{50} value 68.11±0.21 μmoles/L, relative to eserine, a reference standard having IC_{50} value of 0.85±0.0001 μmoles/L, could be attributed to bromination and nitration after nitrogen containing heterocyclic ring cleavage, respectively, in these molecules. Moreover, this study also urges the

researchers to investigate such cleaved adducts of other famous alkaloids against various enzymes to explore their therapeutic potentials for the ailment of diseases.

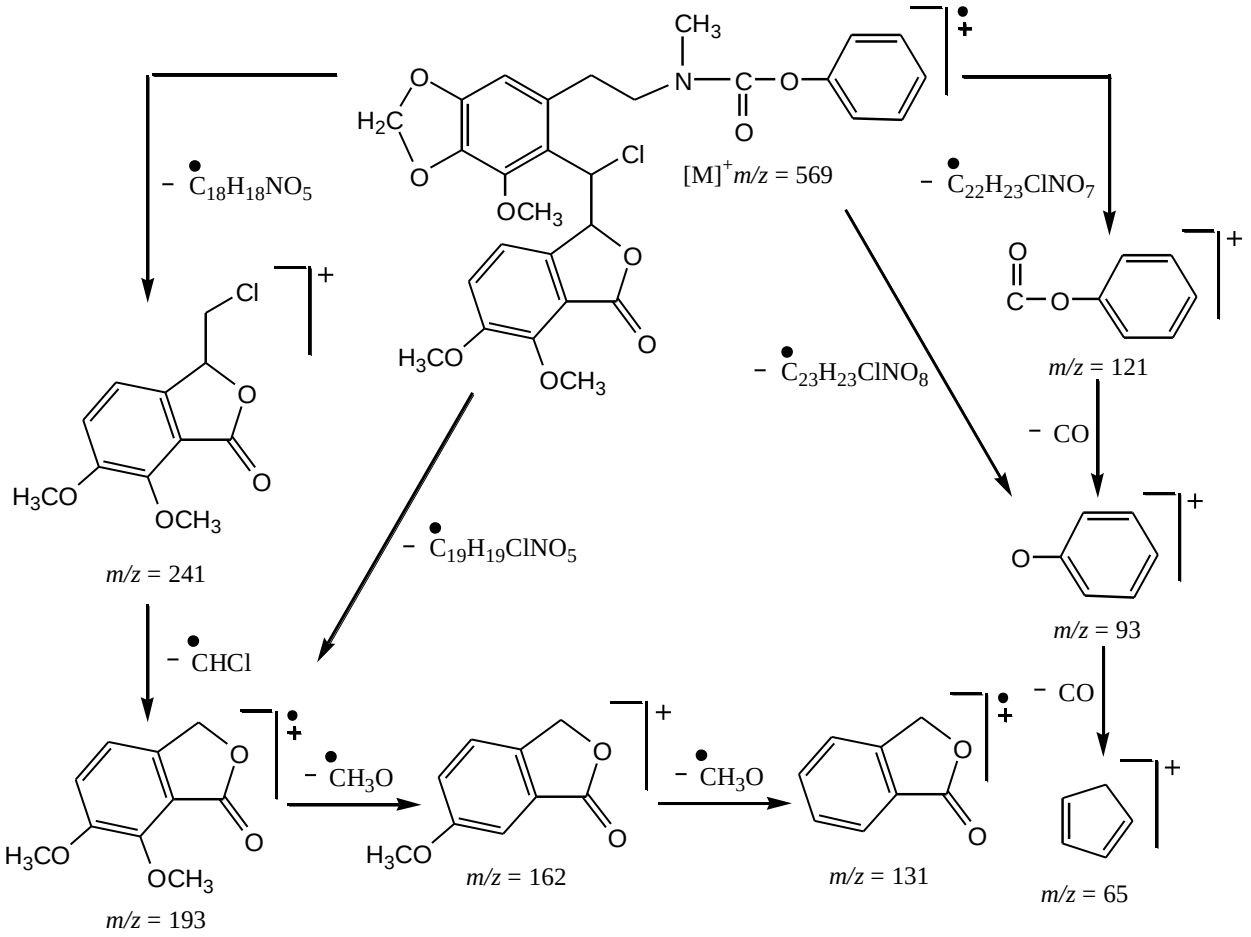


Fig-1: Mass fragmentation pattern of the synthesized compound **4**

3. CONCLUSION

Structure clarification of synthesized compounds was processed by spectroscopic analysis. The enzyme inhibition activity against butyrylcholinesterase enzyme rendered the compounds **3** and **5** as potent inhibitors, shown by their IC_{50} values as compared to eserine taken as reference standard. This evaluation may be helpful for medicinal chemistry and also for pharmaceutical industry as drug candidates in drug development programme.

4. EXPERIMENTAL

4.1 *General*

Narcotine was purchased from Sigma Aldrich in purified form for this study, yet it was further analyzed through spectral analysis. Purity of the synthesized compounds was checked on TLC (pre-coated silica gel G-25-UV_{254} plates, detected at 254 nm) with different solvent systems using ethyl acetate and *n*-hexane giving single spot. ^{1}H-NMR spectra (with chemical shift values in ppm and coupling constant values in hertz (Hz)) were commemorated in CD_3OD on a Burker Aspect AM-300 MHz spectrometer. The I.R. spectra (wave number in cm^{-1}) were commemorated through KBr salt pellet method on a Jasco-320-A spectrophotometer. Mass spectra (EI-MS) were sketched on Finnigan MAT-112 instrument.

4.2 *General procedure for nitration*

A sample of **1** (1.2 mmol; 0.5 g) or **4** (1.2 mmol, 0.7 g) was dissolved in conc. H_2SO_4 (2 mL) and HNO_3 (0.5 mL). The mixture was continuously stirred for 2-3 hours. The reaction progress was supervised *via* TLC. After confirming the single spot, the product was extracted using chloroform as solvent. The nitrated product was obtained after evaporation of solvent.

4.2.1 *6,7-Dimethoxy-3-(4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one (1)*

White crystalline solid; ^{1}H-NMR (300 MHz, CD_3OD): δ 7.16 (d, *J* = 8.4 Hz, 1H, H-7'), 6.34 (s, 1H, H-6), 6.17 (br d, *J* = 8.4 Hz, 1H, H-8'), 5.92 (d, *J* = 0.9 Hz, 1H, H_b-9), 5.90 (d, *J* = 1.2 Hz, 1H, H_a-9), 5.63 (d, *J* = 3.6 Hz, 1H, H-1'), 4.38 (d, *J* = 3.6 Hz, 1H, H-1), 3.98 (s, 3H, CH_3O-5'), 3.96 (s, 3H, CH_3O-6′), 3.85 (s, 3H, CH_3O-12), 2.58-2.65 (m, 2H, H-3), 2.52 (s, 3H, CH_3N-2), 2.32-2.46 (m, 2H, H-4); IR (KBr) ν_{max} (cm^{-1}): 3056 (Ar-H), 1740 (C=O), 1530 (Ar C=C),

1250 (Ar-O), 1175 (C-O), 1145 (C-N), 1050 (C-O); EIMS: *m/z* 413 $[M]^+$, 398 $[M-CH_3]^+$, 382 $[M-CH_3O]^+$, 367 $[M-CH_2O_2]^+$, 220 $[M-C_{10}H_9O_4]^+$, 193 $[M-C_{12}H_{14}NO_3]^+$.

4.2.2 *6,7-Dimethoxy-3-(4-methoxy-6-methyl-9-nitro-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-4-nitroisobenzofuran-1(3H)-one (2)*

Light brown crystalline solid; Yield: 90%; ^{1}H-NMR (400 MHz, CD_3OD): δ 6.95 (s, 1H, H-7'), 6.41 (s, 2H, H-9), 4.36 (d, *J* = 4.4 Hz, 1H, H-1'), 4.03 (d, *J* = 4.4 Hz, 1H, H-1), 3.68 (s, 3H, CH_3O-5'), 3.63 (s, 3H, CH_3O-6'), 2.72 (s, 3H, CH_3O-12), 2.69 (s, 3H, CH_3N-2), 2.26-2.32 (m, 2H, H-3), 1.58-1.63 (m, 2H, H-4); IR (KBr) ν_{max} (cm^{-1}): 3057 (Ar-H), 1742 (C=O), 1531 (Ar C=C), 1254 (Ar-O), 1180 (C-O), 1146 (C-N), 1051 (C-O), 1350 (Ar-NO_2); EIMS: *m/z* 503 $[M]^+$, 488 $[M-CH_3]^+$, 472 $[M-CH_3O]^+$, 457 $[M-CH_2O_2]^+$, 264 $[M-C_{10}H_9NO_6]^+$, 237 $[M-C_{12}H_{14}N_2O_5]^+$.

4.2.3 *4-Nitrophenyl-2-(6-(chloro-(4,5-dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)methyl) -7-methoxy-4-nitrobenzo[d][1,3]dioxolo-5-yl)ethyl(methyl)carbamate (5)*

Reddish brown crystalline solid; Yield: 84%; ^{1}H-NMR (400 MHz, CD_3OD): δ 8.10 (d, *J* = 8.8 Hz, 2H, H-3''' & H-5'''), 7.25 (d, *J* = 6.8 Hz, 1H, H-7'), 7.10 (d, *J* = 7.2 Hz, 1H, H-8'), 6.87 (d, *J* = 8.8 Hz, 2H, H-2''' & H-6'''), 6.20 (s, 2H, H-6), 3.68 (s, 3H, CH_3O-5'), 3.67 (d, *J* = 3.6 Hz, 1H, H-10'), 3.63 (d, *J* = 3.6 Hz, 1H, H-1'), 3.61 (s, 3H, CH_3O-6'), 3.55 (s, 3H, CH_3O-9), 3.45 (t, *J* = 6.4 Hz, 2H, H-3''), 2.69 (s, 3H, CH_3N-2'), 1.53 (t, *J* = 6.4 Hz, 2H, H-4''); IR (KBr) ν_{max} (cm^{-1}): 3058 (Ar-H), 1742 (C=O), 1532 (Ar C=C), 1352 (Ar-NO_2), 1252 (Ar-O), 1177 (C-O), 1147 (C-N), 1052 (C-O), 712 (C-Cl); EIMS: *m/z* 661 $[M+2]^+$, 659 $[M]^+$, 544 $[M-CH_3]^+$, 628 $[M-CH_3O]^+$, 613 $[M-CH_2O_2]^+$, 566 $[M-C_6H_5O]^+$, 538 $[M-C_7H_5O_2]^+$, 523 $[M-NO_2]^+$, 376 $[M-C_{10}H_9O_4]^+$.

4.3 *General procedure for bromination*

A sample of **1** (1.2 mmol; 0.5 g) or **4** (1.2 mmol, 0.7 g) was completely dissolved in glacial AcOH (2 mL) followed by the addition of bromine water (0.5 mL). The stirring was continued for 2-3 hours. The reaction completion was monitored with the help of TLC. After completion of reaction, the product was extracted with chloroform. On evaporation of this solvent, borminated product was obtained.

4.3.1 *6,7-Dimethoxy-3-(9-bromo-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)isobenzofuran-1(3H)-one (3)*

Light green crystalline solid; Yield: 91%; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.46 (d, *J* = 8.4 Hz, 1H, H-7'), 7.23 (d, *J* = 8.2 Hz, 1H, H-8'), 6.44 (s, 2H, H-9), 4.35 (d, *J* = 4.0 Hz, 1H, H-1'), 4.13 (d, *J* = 4.0 Hz, 1H, H-1), 3.96 (s, 3H, CH_3O-5'), 3.92 (s, 3H, CH_3O-6'), 3.84 (s, 3H, CH_3O-12), 2.97-3.07 (m, 2H, H-3), 2.87 (s, 3H, CH_3N-2), 2.64-2.85 (m, 2H, H-4); IR (KBr) ν_{max} (cm^{-1}): 3052 (Ar-H), 1737 (C=O), 1527 (Ar C=C), 1247 (Ar-O), 1173 (C-O), 1142 (C-N), 1048 (C-O), 549 (C-Br); EIMS: *m/z* 494 $[M+2]^+$, 492 $[M]^+$, 477 $[M-CH_3]^+$, 461 $[M-CH_3O]^+$, 446 $[M-CH_2O_2]^+$, 299 $[M-C_{10}H_9O_4]^+$, 193 $[M-C_{12}H_{13}NO_3Br]^+$.

4.3.2 *2,4,6-Tribromophenyl-2-(4-bromo-6-(chloro-(7-bromo-4,5-dimethoxy-3-oxo-1,3-dihydro isobenzofuran-1-yl)methyl)-7-methoxybenzo[d][1,3]dioxolo-5-yl)ethyl(methyl)carbamate (6)*

Dark brown crystalline solid; Yield: 87%; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.05 (br s, 2H, H-3''' & H-5'''), 6.40 (s, 1H, H-7'), 6.20 (s, 2H, H-6), 3.98 (s, 3H, CH_3O-5'), 3.91 (s, 3H, CH_3O-6'), 3.67 (d, *J* = 3.6 Hz, 1H, H-10'), 3.63 (d, *J* = 3.6 Hz, 1H, H-1'), 3.55 (s, 3H, CH_3O-9), 2.82 (t, *J* = 6.4 Hz, 2H, H-3''), 2.68 (s, 3H, CH_3N-2''), 1.27 (t, *J* = 6.4 Hz, 2H, H-4''); IR (KBr) ν_{max} (cm^{-1}): 3054 (Ar-H), 1739 (C=O), 1526 (Ar C=C), 1248 (Ar-O), 1176 (C-O), 1146 (C-N), 1051 (C-O), 716 (C-Cl), 550 (C-Br); EIMS: *m/z* 961 $[M+2]^+$, 959 $[M]^+$, 944 $[M-CH_3]^+$, 928 $[M-CH_3O]^+$, 913 $[M-CH_2O_2]^+$, 629 $[M-C_6H_2OBr_3]^+$, 601 $[M-C_7H_2O_2Br_3]^+$, 686 $[M-C_{10}H_9O_4Br]^+$.

4.4 *Procedure for the nucleophilic addition of phenylchloroformate to narcotine*

A sample of narcotine (**1**, 0.24 mmol; 0.1 g) was dissolved in chloroform and then phenylchloroformate (0.121 mL) was poured into it. The mixture was stirred for 5-6 hours and monitored by TLC after different intervals. At the single spot of reaction, the product was extracted with chloroform. On evaporation of this solvent, a ring cleaved adduct **4** was obtained[16].

4.4.1 *Phenyl-2-(6-(chloro-(4,5-dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)methyl)-7-methoxybenzo[d][1,3]dioxolo-5-yl)ethyl(methyl)carbamate (4)*

Light yellow crystalline solid; Yield: 87%; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.32 (br d, *J* = 6.8 Hz, 2H, H-2''' & H-6'''), 7.14 (br t, *J* = 7.6 Hz, 2H, H-3''' & H-5'''), 7.07 (br d, *J* = 7.8 Hz, 1H, H-4'''), 6.73 (d, *J* = 7.6 Hz, 1H, H-7'), 6.57 (s, 1H, H-3), 6.50 (d, *J* = 7.6 Hz, 1H, H-8'), 5.97 (s, 2H, H-6), 5.92 (br s, 1H, H-1'), 3.92 (br s, 1H, H-10'), 3.84 (s, 3H, CH_3O-5'), 3.80 (s, 3H, CH_3O-6'), 3.55 (s, 3H, CH_3O-9), 2.97-3.03 (m, 2H, H-3''), 2.94 (s, 3H, CH_3N-2''), 2.81-2.88 (m, 2H, H-4''); IR (KBr) ν_{max} (cm^{-1}): 3053 (Ar-H), 1743 (C=O), 1528 (Ar C=C), 1254 (Ar-O), 1177 (C-O), 1144 (C-N), 1049 (C-O), 710 (C-Cl); EIMS: *m/z* 571 $[M+2]^+$, 569 $[M]^+$, 554 $[M-CH_3]^+$, 538 $[M-CH_3O]^+$, 523 $[M-CH_2O_2]^+$, 476 $[M-C_6H_5O]^+$, 448 $[M-C_7H_5O_2]^+$, 376 $[M-C_{10}H_9O_4]^+$.

4.5 *Butyrylcholinesterase Assay*

The inhibition activity of this enzyme was executed as reported earlier[17] but with some variations. The reaction mixture was prepared by mixing Na_2HPO_4 buffer (0.060 mL, 50 mM, pH 7.7), test compound (0.010 mL, 0.5 mM well^{-1}) and BChE (0.010 mL (0.5 unit well^{-1}) followed by pre-reading at 405 nm and pre-incubation at 37 ºC for 0.16 hours. Butyrylthiocholine bromide (0.010 mL, 0.5 mM well^{-1}) was employed as substrate and reaction initiator. DTNB (0.010 mL, 0.5 mM well^{-1}) was also added. The measurement of absorbance at 405 nm after an incubation of 0.25 hours at 37 ºC was used as the key point in %age inhibition. Plate reader utilized was Synergy HT (BioTek, USA) 96-well. Triplicate of each experiment was performed. Eserine (0.5 mM well^{-1}) served as positive control. The %age inhibition was accounted as,

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

IC_{50} values (test compound conc. for 50% enzyme inhibition) of compounds were computed using EZ–Fitz Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

4.6 *Statistical analysis*

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± sem.

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