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Synthesis of Esters and Carbamothioates Contaminating Tramadol Moiety and Their HPLC Applications

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ABSTRACT

In the present study, a variety of novel tramadol esters (**2-6**) were synthesized *via* the reaction of (±)-*cis*-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl)cyclohexanol (tramadol) (**1**) with acid chlorides and triethylamine. Treatment of compound **1** with epichlorohydrin afforded corresponding ether derivative **7**. The reaction of compound **1** with isothiocyanates gave the novel carbamothioate derivatives **8-12**. Tramadol derivatives were found more sensitive for detection with (HPLC-DAD) than tramadol itself.

KEYWORDS

Tramadol, Esters, Carbamothioates, Isothiocyanate, HPLC.

INTRODUCTION

Tramadol hydrochloride (T), (±)-*cis*-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)cyclohexanol (**1**) [1-7] is a centrally acting analgesic agent used in the treatment of moderate to severe pain and as an alternative to opiates [8]. The sensitive analysis for drugs of abuse in seized materials or biological fluids has become important for forensic and toxicological laboratories. High performance liquid chromatography (HPLC) with ultraviolet (UV) [9-13] is one of the methods of choice for the analysis of drugs in biological samples. Tramadol contains a weakly absorping chromophore in its molecule [14]. Subsequently, UV detection is not suitable for the determination of low tramadol concentrations. Therefore, the development of a derivatization procedure that improves both chromatographic detection (sensitivity) and separation is an area of major interest in the analysis of tramadol. Usually, highly conjugated aromatic compounds such as acid chlorides are used in UV-visible derivatization [15-18]. In view of the previous applications and in continuation of our interest in synthesis of some fused and spiro-heterocycles [19-26], the aim of this work was to synthesize new tramadol derivatives to improve their sensitivity for detection with HPLC.

EXPERIMENTAL

All melting points were determined on a Koffler melting points apparatus and are uncorrected. Infrared spectra were

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obtained on a Nicolet 710 FT-IR spectrometer. ^{1}H NMR spectra are measured at 400 MHz and ^{13}C NMR spectra, at 100 MHz were recorded on a Bruker Avance III-400 MHz instruments, using $CDCl_3$ as solvent. The mass spectra were scanned on a Varian Mat CH-7 instruments at 70 eV. Elemental microanalysis was performed on a Perkin-Elmer CHN- 2400 elemental analyzer. All compounds were checked for their purity on TLC plates. The HPLC instrument (Agilent, USA) consisted of an Agilent technologies 1200 Series quaternary pump combined with an Agilent 1200 series photodiode array detector (USA), an Agilent 1200 series vacuum degasser (USA) and an Agilent auto sampler injector. Chromatographic separation was performed on a Zorbax SB-C8 (250 × 4.6 mm, 5 µm) column (USA) maintained at 25 °C. The mobile phase consisted of acetonitrile: buffer (0.01 M) potassium dihydrogen phosphate (50:50, v/v) with the addition of 0.1 % triethylamine adjusted to pH 5.5 with 0.1 M sodium hydroxide) at a flow rate of 1.00 mL/min. The detector was set to scan from 200 to 800 nm and had a discrete channel set at 218 nm [27].

Standards and reagents

• Tramadol (1.0 mg/mL) stock solutions were prepared by dissolving 10 mg of tramadol free base (11.38 mg tramadol HCl) in 10 mL methanol.

• Working solution of tramadol at concentration (10 µg/mL) was prepared by diluting 100 µL of 1 mg/mL of tramadol with distilled water in 10 mL voltametric flask.

• Phosphate buffer (0.01 M) was prepared fresh by dissolving 1.36 g (0.01 mol) of potassium dihydrogen phosphate in 1000 mL deionized water.

• Mobile phase was a mixture of 60 % phosphate buffer (0.01 M), 40 % acetonitrile with the addition of 0.1 % triethylamine (v/v). The pH of the final mixture was adjusted to pH 5 with 0.1 M phosphoric acid.

All stock solutions were stored at -20 °C and no change in the stability of the stock solution over one month was observed.

Extraction of tramadol from water: To 5 mL glass tubes added 1 mL of stock solution of tramadol in water (10 µg/mL), 100 µL of conc. ammonia (33 %) and 3 mL of MTBE. The tubes were vortexed for 0.5 h and centrifuged at 320 rpm for 3 min. The organic layer was evaporated to dryness. The dried extract was reconstituted in 200 µL acetonitrile, vortex mixed for 30-s and 50 µL was injected into HPLC.

Synthesis of esters compounds (2-6): Tramadol **1** (0.50 g, 0.1 mol) and 0.12 mol of the appropriate acid chlorides namely benzoyl chloride (0.28 mL), *p*-nitro benzoyl chloride (0.30 g), 3,5-dinitrobenzoylchloride (0.38 g), 2,4-dichlorobenzoyl chloride (0.52 mL) or *N*-phthalylglycylechloride (0.37 g) were dissolved in chloroform (20 mL), drops of triethylamine were added. The reaction mixture was stirred for 1-3 h over different periods of time (monitoring the reaction by TLC). Shaked the mixture with sodium carbonate solution (1 M) in separating funnel, washed the organic layer with water, separated it and dried over anhydrous sodium sulphate. Evaporated the layer, collected the precipitate, washed with diethyl ether and crystallized from ethanol.

1-(3-Methoxyphenyl)-2-[(dimethylamino)methyl]cyclohexylbenzoate (2): Yield 90 %, m.p.: 87 °C, IR (ν_{max}, cm^{-1}): 1100 (C-O-C), 1716 $(C=O)_{ester}$. ^{1}H NMR ppm: 1.42-1.76 (m, 9H cyclohexyl), 2.16 [s, 6H, N $(CH_3)_2$], 2.27 (s, 2H, NCH_2), 3.68 (s, 3H, OCH_3), 6.53-6.90 (m, 4H aromatic), 7.49-8.2 (m, 5H aromatic). Anal. calcd. for $C_{23}H_{29}NO_3$ (367.48): C, 75.17; H, 7.95; N, 13.06. Found: C, 75.34; H, 7.73; N, 13.30 %.

1-(3-Methoxyphenyl)-2-[(dimethylamino)methyl]cyclohexyl-4-nitrobenzoate (3): Yield 80 %, m.p.: 168-170 °C, IR (ν_{max}, cm^{-1}): 1100 (C-O-C), 1745 $(C=O)_{ester}$, 1460.3-1546 (NO_2). ^{1}H NMR ppm: 1.18-1.78 (m, 9H, cyclohexyl), 2.44 [s, 6H, N $(CH_3)_2$], 2.93 (s, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 6.71-6.20 (m, 4H, aromatic), 8.07-8.13 (m, 4H, aromatic). Anal. calcd. for $C_{23}H_{28}N_2O_5$ (412.48): C, 66.97; H, 6.64; N, 6.79. Found: C, 66.69; H, 6.83; N, 6.92 %.

1-(3-Methoxyphenyl)-2-[(dimethylamino)methyl]cyclohexyl-3,5-dinitrobenzoate (4): Yield 65 %, m.p.: 96 °C, IR (ν_{max}, cm^{-1}): 1100 (C-O-C), 1460, 1545 (NO_2), 1738 $(C=O)_{ester}$. ^{1}H NMR ppm: 1.18-1.77 (m, 9H, cyclohexyl), 2.03 [s, 6H, N $(CH_3)_2$], 2.34 (s, 2H, NCH_2), 3.75 (s, 3H, OCH_3), 6.67-7.19 (m, 4H, aromatic), 9.1-9.15 (m, 3H, aromatic). Anal. calcd. for $C_{23}H_{27}N_3O_7$ (457.47): C, 60.39; H, 5.95; N, 9.19. Found: C, 60.66; H, 5.70; N, 9.3.

1-(3-Methoxyphenyl)-2-[(dimethylamino)methyl]cyclohexyl-2,4-dichloro benzoate (5): Yield 95 %, m.p.: 56-60 °C, IR (ν_{max}, cm^{-1}): 800 (C-Cl), 1100 (-O-), 1742 (C=O). ^{1}H NMR ppm: 1.53-1.81 (m, 9H, cyclohexyl), 2.02 [s, 6H, N $(CH_3)_2$], 2.34 (s, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 6.18-6.99 (m, 4H, aromatic), 7.10-7.17 (m, 3H, aromatic). Anal. calcd. for $C_{23}H_{27}Cl_2NO_3$ (436.37): C, 63.30; H, 6.24; N, 3.21. Found: 63.64; H, 6.46; N, 3.37 %.

1-(3-Methoxyphenyl)-2-[(dimethylamino)methyl]-cyclohexyl[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acetate (6): Yield 40 %, m.p.: 160 °C, IR (ν_{max}, cm^{-1}): 1100 (-O-), 1734 (C=O). ^{1}H NMR ppm: 0.76-1.95 (m, 9H, cyclohexyl), 2.02 [s, 6H, N $(CH_3)_2$], 2.34 (s, 2H, NCH_2), 3.76 (s, 5H, OCH_3 + NCH_2), 6.71-7.32 (m, 4H, aromatic), 7.70-7.80 (m, 4H, aromatic). Anal. calcd. for $C_{23}H_{27}N_3O_7$ (450.53): C, 69.32; H, 6.71; N, 6.22. Found C, 69.65; H, 6.93; N, 6.46 %.

1-Chloro-3-({1-(3-methoxyphenyl)-2-[(dimethylamino)methyl]-cyclohexyl}oxy)propan-2-ol (7): A mixture of compound **1** (0.1 mol, 0.26 g) and epichlorohydrin (0.12 mol, 0.13 mL) in dioxane (20 mL), drops of triethylamine were added. The reaction mixture was stirred for 3 h at room temperature, evaporated the solvent, collected the precipitate, washed with diethyl ether and crystallized from ethanol. IR (ν_{max}, cm^{-1}): 750 (C-Cl), 3480 (OH). ^{1}H NMR ppm: 1.32-1.99 (m, 9H, cyclohexyl), 2.02 [s, 6H, N $(CH_3)_2$], 2.34 (s, 2H, NCH_2), 3.56 (d, 2H, OCH_2), 3.74 (s, 3H, OCH_3), 3.96 (d, 2H, CH_2-Cl), 4.61 (m, 1H, CH), 6.67-7.19 (m, 4H, aromatic). ^{13}C NMR: 22.29, 26.88, 27.94, 40.27, 41.32, 44.79, 47.80, 55.18, 61.61, 76.72, 77.04, 77.36, 110.99, 111.24, 117.39, 128.94, 152.03, 159.52. GC-MS, *m/z* (Ir %): 355.1 (49.8) (M^+), 281 (15.4), 147.1 (18.5), 58.1 (100). Anal. calcd. for $C_{19}H_{30}NO_3Cl$ (355.1): C, 64.12; H, 8.50; N, 3.94. Found: C, 64.39; H, 8.31; N, 3.71 %.

Carbamothioate derivatives (8-12): Ammonium thiocyanate (0.08 g, 0.001 mol) and the appropriate acid chlorides (0.001 mol) namely benzoyl chloride (0.56 mL), 4-nitro benzoyl chloride (0.35 mL), 3,5-dinitro benzoyl chloride (0.56 g), 2,4-dichloro benzoyl chloride (0.22 mL) and *N*-

phthalylglycyl chloride (0.22 g) in acetone (30 mL) was stirred for 1.5 h. Then compound **1** (0.01 mol, 0.5 g) and few drops of pyridine were added. The reaction mixture was refluxed for 7 h, left to cool and poured into crushed ice containing HCl solution (100 mL, 3 %). The precipitated solid was filtered off, washed with water, dried and crystallized from hexane.

3-(Methoxyphenyl)[(dimethylamino)methyl]cyclohexyl-(phenylcarbonyl)carbamothioate (8): Yield (90 %), m.p.: 116 °C, IR (ν, cm^{-1}): 1174 (C=S), 1698 (C=O), 3238 (NH). ^{1}H NMR ppm: 0.85-1.95 (m, 9H, cyclohexyl), 2.40 [s, 6H, N $(CH_3)_2$], 2.50 (s, 2H, NCH_2), 3.79 (s, 3H, OCH_3), 7.28-7.55 (m, 4H, aromatic), 7.58-7.91 (m, 5H, aromatic), 9.05 (s, 1H, NH). Anal. calcd. for $C_{24}H_{30}N_2O_3S$ (426.57): C, 67.58; H, 7.09; N, 6.57; S, 7.81. Found: C, 67.79; H, 7.19; N, 6.69; S, 7.74 %.

3-(Methoxyphenyl)[(dimethylamino)methyl]cyclohexyl-(4-nitro-phenylcarbonyl)carbamothioate (9): Yield (90 %), m.p.: 110 °C, IR (ν_{max}, cm^{-1}): 1170.9 (C=S), 1480, 1525 (NO_2), 1690 (C=O), 3302 (NH). ^{1}H NMR ppm: 1.51-1.85 (m, 9H, cyclohexyl), 2.49 [s, 6H, N $(CH_3)_2$], 2.65 (s, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 6.75-7.20 (m, 4H, aromatic), 8.03-8.24 (m, 4H, aromatic), 8.95 (s, 1H, NH). Anal. calcd. for $C_{24}H_{29}N_2O_5S$ (471. 56): C, 61.13; H, 6.20; N, 8.91; S, 7.04. Found C, 61.40; H, 6.40; N, 8.78; S, 7.24 %.

3-(Methoxyphenyl)[(dimethylamino)methyl]cyclohexyl(3,5-dinitro-phenylcarbonyl)carbamothioate (10): Yield (87 %), m.p.: 128 °C, IR (ν_{max}, cm^{-1}): 1165 (C=S), 1461-1544 (NO_2), 1629 (C=O), 3165 (NH). ^{1}H NMR ppm: 0.86-1.81 (m, 9H, cyclohexyl), 2.51 [s, 6H, N $(CH_3)_2$], 3.34 (s, 2H, NCH_2), 3.76 (s, 3H, OCH_3), 6.28-7.27 (m, 4H, aromatic), 8.18-8.92 (m, 3H, aromatic), 9.05 (s, 1H, NH). MS, *m/z* (*I*r %) : 516 (4.8) (M^+), 514 (5.4) (M-2), 498 (3.9), 471 (6.7), 458 (4.5), 415 (9.38), 400 (33.23), 354 (4.83), 338 (10.99), 292 (2.62), 251 (6.25), 195 (6.67), 135 (23.48), 118 (17.37), 103 (21.06), 91 (10.59), 80100), 58 (97.07). Anal. calcd. for $C_{24}H_{28}N_4O_7S$ (516.56): C, 55.80; H, 5.46; N, 10.85; S, 6.41. Found: C, 55.63; H, 5.68; N, 10.60; S, 6.70 %.

3-(Methoxyphenyl)[(dimethylamino)methyl]cyclohexyl(2,4-dichloro-phenylcarbonyl)carbamothioate (11): Yield (90 %), m.p.: 142 °C, IR (ν_{max}, cm^{-1}): 1178 (C=S), 1683 (C=O), 3264 (NH).^{1}H NMR ppm: 1.35-1.53 (m, 9H, cyclohexyl), 2.16[s, 6H, N $(CH_3)_2$], 2.19 (s, 2H, NCH_2), 3.74 (s, 3H, OCH_3), 6.50-6.91 (m, 4H, aromatic), 7.44-8.00 (m, 3H, aromatic), 10.25 (s, 1H, NH). Anal. calcd. for $C_{24}H_{28}N_2O_3SCl_2$ (495.46): C, 58.18; H, 5.70; N, 5.65; S, 6.69. Found: C, 58.46; H, 5.69; N, 5.58; S, 6.85 %.

3-(Methoxyphenyl)[(dimethylamino)methyl]cyclohexyl[(1,3-dioxo-1,3-di-hydro-2*H*-isoindol-2-yl)acetyl]-carbamothioate (12): Yield (90 %), m.p.: 124 °C, IR (ν_{max}, cm^{-1}): 1175 (C=S), 1677 (C=O), 1773 (C=O) anhyd., 3210 (NH). ^{1}H NMR ppm: 1.54-1.76 (m, 9H, cyclohexyl), 2.51 [s, 6H, N $(CH_3)_2$], 3.40 (s, 2H, NCH_2), 3.76 (s, 3H, OCH_3), 7.10-7.38 (m, 4H, aromatic), 7.99-8.19 (m, 4H, aromatic), 9.12 (s, 1H, NH). Anal. calcd. for $C_{27}H_{31}N_3O_5S$ (509.617): C, 63.64; H, 6.13; N, 8.25; S, 5.94. Found: C, 63.81; H, 6.31; N, 8.48; S, 5.71 %.

Extraction of derivatized tramadol from water: To 5 mL glass tubes added 1 mL of stock solution of tramadol in water (10 µg/mL), 100 µL of conc. ammonia (33 %) and 3 mL of MTBE. The tubes were then vortexed for 0.5 h and centrifuged at 320 rpm for 3 min. To the organic layer, 100 µL of appropriate derivatizing agents and 10 µL triethylamine were added which followed by vortex and mix for 15 min. 1 mL sodium carbonate (1 M) was added to the mixture, vortexed, mixed and centrifuged. The organic layer was transferred to glass tube and evaporated to dryness. The dried extract was reconstituted in 200 µL acetonitrile, vortex mixed for 30 s and 50 µL was injected into HPLC.

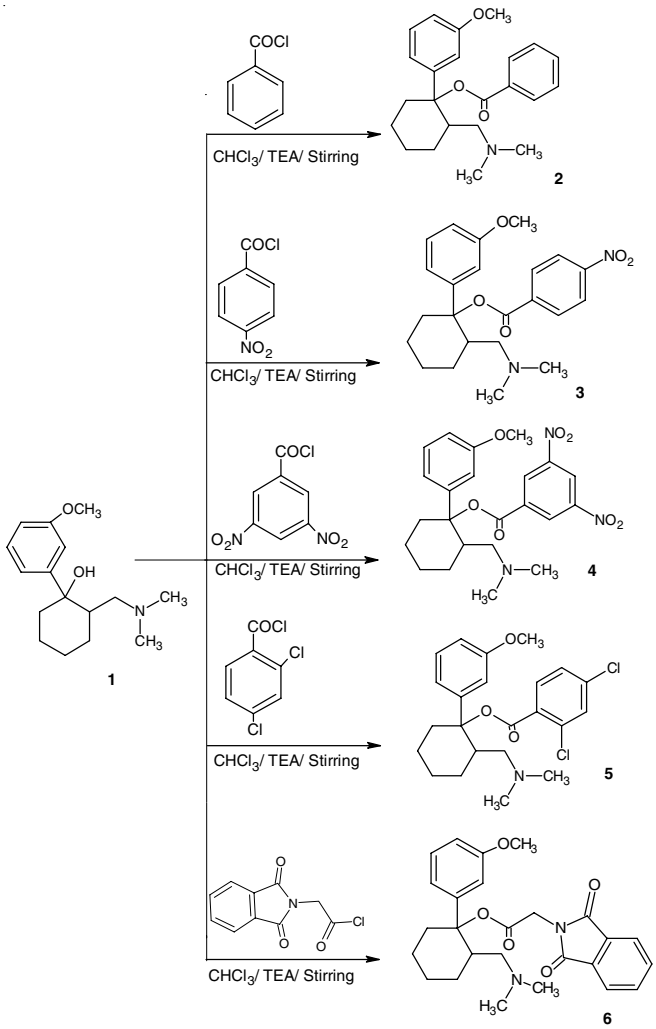
Analysis on HPLC: All stocks solution of tramadol and its derivatives were analyzed with HPLC-DAD for determination of peak height counts and UV spectra. The peak heights of tramadol without derivatization were compared with derivatized compounds.

RESULTS AND DISCUSSION

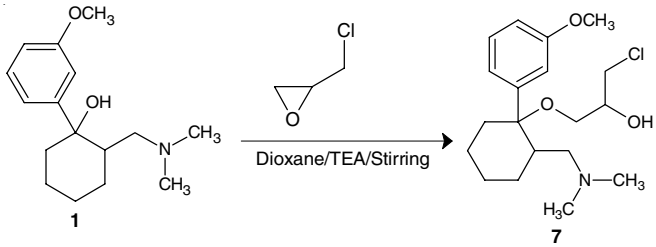
Reaction of (±)-*cis*-2-[(dimethylamino)methyl]-1-(3-methoxy-phenyl)cyclohexanol (tramadol) (**1**) with benzoyl chloride, *p*-nitro benzoyl chloride, 2,4-dichloro benzoyl chloride or *N*-phthalylglycyl chloride in presence of triethylamine in chloroform afforded ester derivatives **2-6** (**Scheme-I**). IR spectra (ν, cm^{-1}) for these compounds showed disappearance of OH group absorption band at 3310 cm^{-1} and appearance of new bands corresponding to nitro groups at 1545, 1350, carbonyl groups 1745 ester and 1690 amidic, C-O-C group at 1100 and C-Cl group at 800 cm^{-1}, respectively. ^{1}H NMR spectra showed disappearance of signal corresponding to OH group at δ 5.99 ppm and appearance of new singlet signal corresponding to -CH_2 group at δ 3.76 ppm in **6** and beside increasing in aromatic signals (c.f. experimental).

The reaction of tramadol **1** with epichlorohydrin and triethylamine in dioxane afforded 1-chloro-3-({1-(3-methoxy-phenyl)-2-[(dimethyl-amino)methyl]cyclohexyl}oxy)propan-2-ol (**7**) (**Scheme-II**). Its IR spectrum (ν_{max}, cm^{-1}) showed new absorption bands corresponding to OH group at 3480 and C-Cl group at 750 cm^{-1}, respectively. Its ^{1}H NMR ppm: showed the following signals at δ 3.56, 4.61 and 3.96 corresponding to OCH_2-, CH-OH and CH_2-Cl, respectively. ^{13}C NMR showed the following signals at 22.29, 26.88, 27.94, 41.32, 40.27, 44.79, 47.80, 55.18, 61.61, 76.72, 77.04, 77.36, 110.99, 111.24, 117.39, 128.94, 152.03, 159.52. Its mass spectrum recorded the molecular ion peak M^+ at *m/z* 355.1 which agreed well with the proposed molecular formula $C_{19}H_{30}NO_3Cl$ (Fig. 1).

Compound **1** was subjected to react with benzoylisothiocyante, *p*-nitrobenzoylisothiocyante, 3,5-dinitrobenzoylisothiocyante, 2,4-dichloro benzoylisothiocyante or *N*-phthalylglycylisothiocyante in pyridine/acetone mixture to yield carbamothioate derivatives **8-12** (**Scheme-III**). Their IR spectra (ν_{max}, cm^{-1}) showed new absorption bands corresponding to -NH groups at 3302-3134, -C=O- groups at 1698-1683 and C=S groups at 1175-1104 cm^{-1}, respectively. Their ^{1}H NMR spectra (δ, $CDCl_3$) showed disappearance of OH singlet signal and appeared new NH group signals at δ 8.50-10.25 ppm, beside increasing in aromatic signals. The mass spectrum of compound **10** recorded the molecular ion peak M^+ at *m/z* 516.56 which agree well with the proposed molecular formula $C_{24}H_{28}N_4O_7S$ (*c.f.* experimental).



Scheme-I: Reaction of tramadol with acid chlorides (**2-6**)



Scheme-II: Reaction of tramadol and epichlorohydrin

Application on HPLC: Screening and quantitative analysis of drugs of abuse and prescription drugs such as tramadol and pseudoephedrine is part of any systematic toxicological analysis. One of the major techniques used at present for the analysis of tramadol and pseudoephedrine is HPLC-DAD or HPLC-UV [28].

As reported, tramadol contains a weakly absorbing chromophore in their molecule. Subsequently, UV detection is not suitable for the determination of their low concentrations [27,29].

In this study, a pre-column derivatization with chromophoric reagents such as: benzoyl chloride, *p*-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, 2,4-dichlorobenzoyl chloride and *N*-phthalylglycyl chloride were tested to improve the sensitivity of detection.

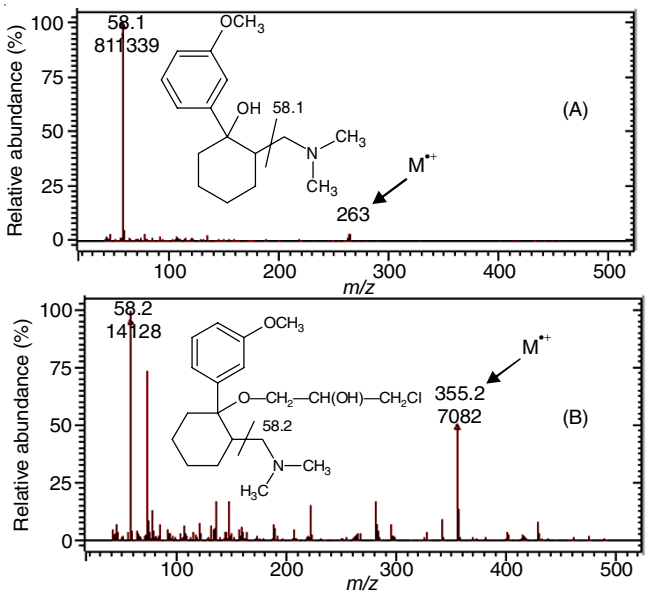
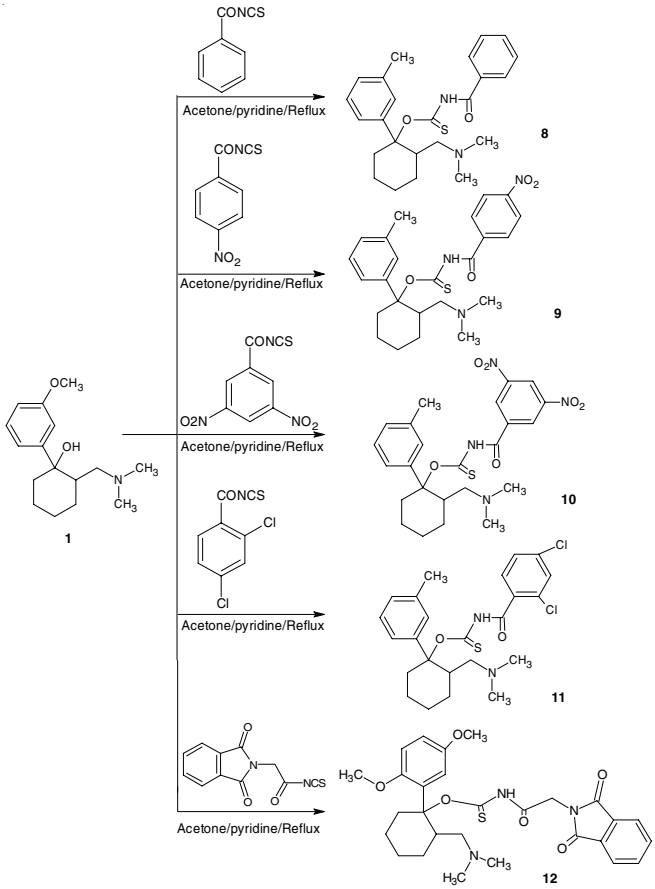


Fig. 1. Full scan MS-spectra of (A) Tramadol **1** and (B) its derivative **7**



Scheme-III: Reaction of tramadol and isothiocyanate

The derivatized products of tramadol such as, 1-(3-methoxyphenyl)-2-[(dimethylamino)methyl]cyclohexyl-2,4-dichlorobenzoate (**2**), 1-(3-methoxyphenyl)-2-[(dimethyl-amino)methyl]cyclohexylbenzoate (**3**), 1-[3-methoxyphenyl)-2-[(di-methylamino)methyl]cyclohexyl-4-nitro-benzoate (**4**) and 1-[3-methoxyphenyl)-2-[(dimethyl-amino)methyl]-cyclohexyl-3,5-dinitrobenzoate (**5**) at concentration of 10 µg/mL were prepared and analyzed with HPLC-DAD. Fig. 2

illustrates the chromatograms for tramadol (**1**) and its derivatives **2**-**5** at concentrations (10 µg/mL). As shown in the figure, the peak height response of tramadol derivatives giving more counts than tramadol itself. These results indicate that the detection of tramadol after pre-column derivatization were more sensitive than tramadol itself. Fig. 3 showed that UV spectra of tramadol derivatives were shifted to longer wavelengths (Bathochromic shift - a red shift), as a result of different chemical structures of tramadol derivatives.

Conclusion

The method for derivatization of tramadol with 2,4-dichlorobenzoyl chloride, benzoyl chloride, *p*-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride are simply prepared with highly peak height response than tramadol itself. Therefore, these derivatives could be used for the analysis of tramadol by HPLC to improve the sensitively of detection.

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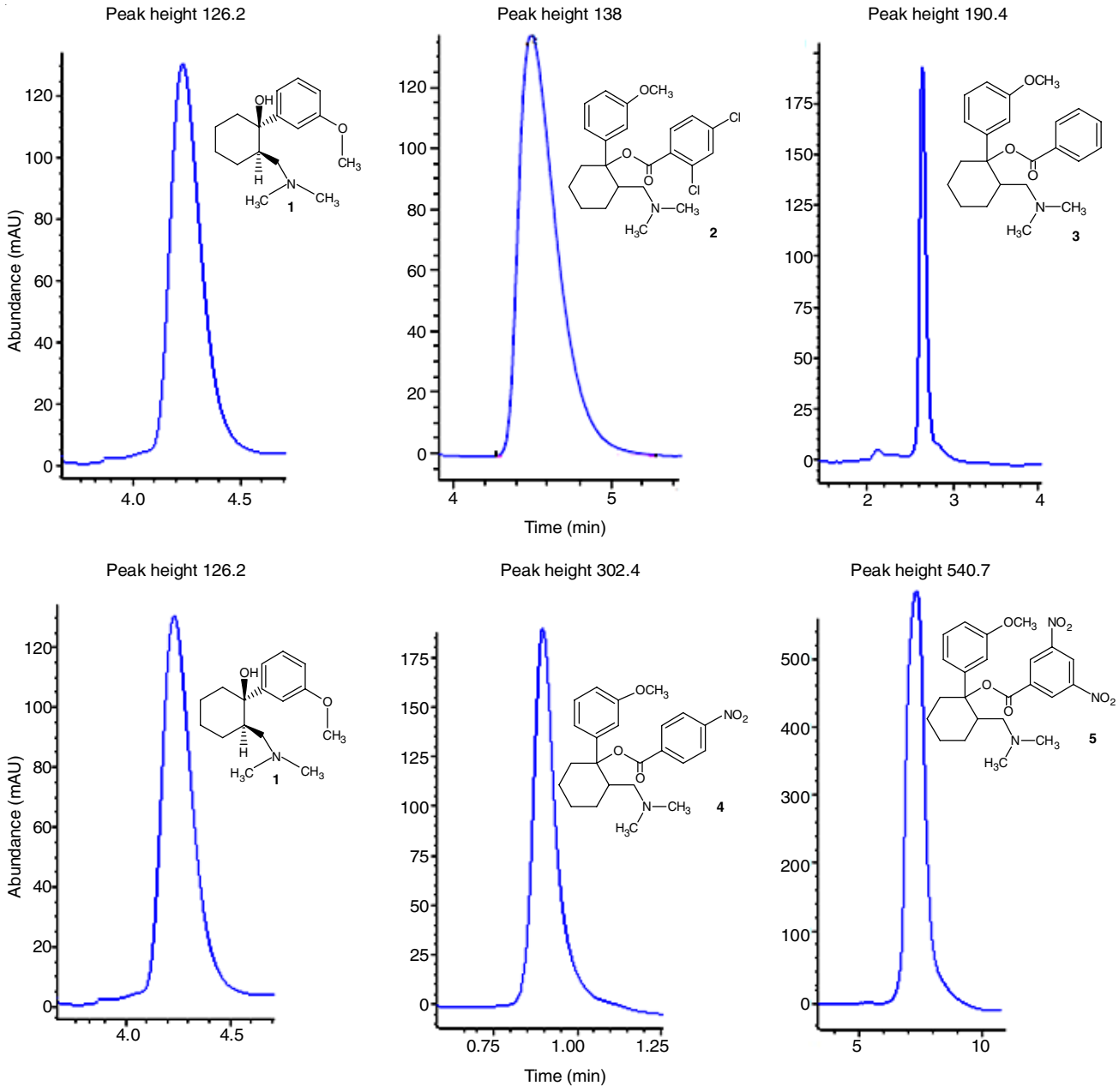

Fig. 2. HPLC chromatogram of tramadol (**1**) and its derivatives (**2**, **3**, **4** and **5**)

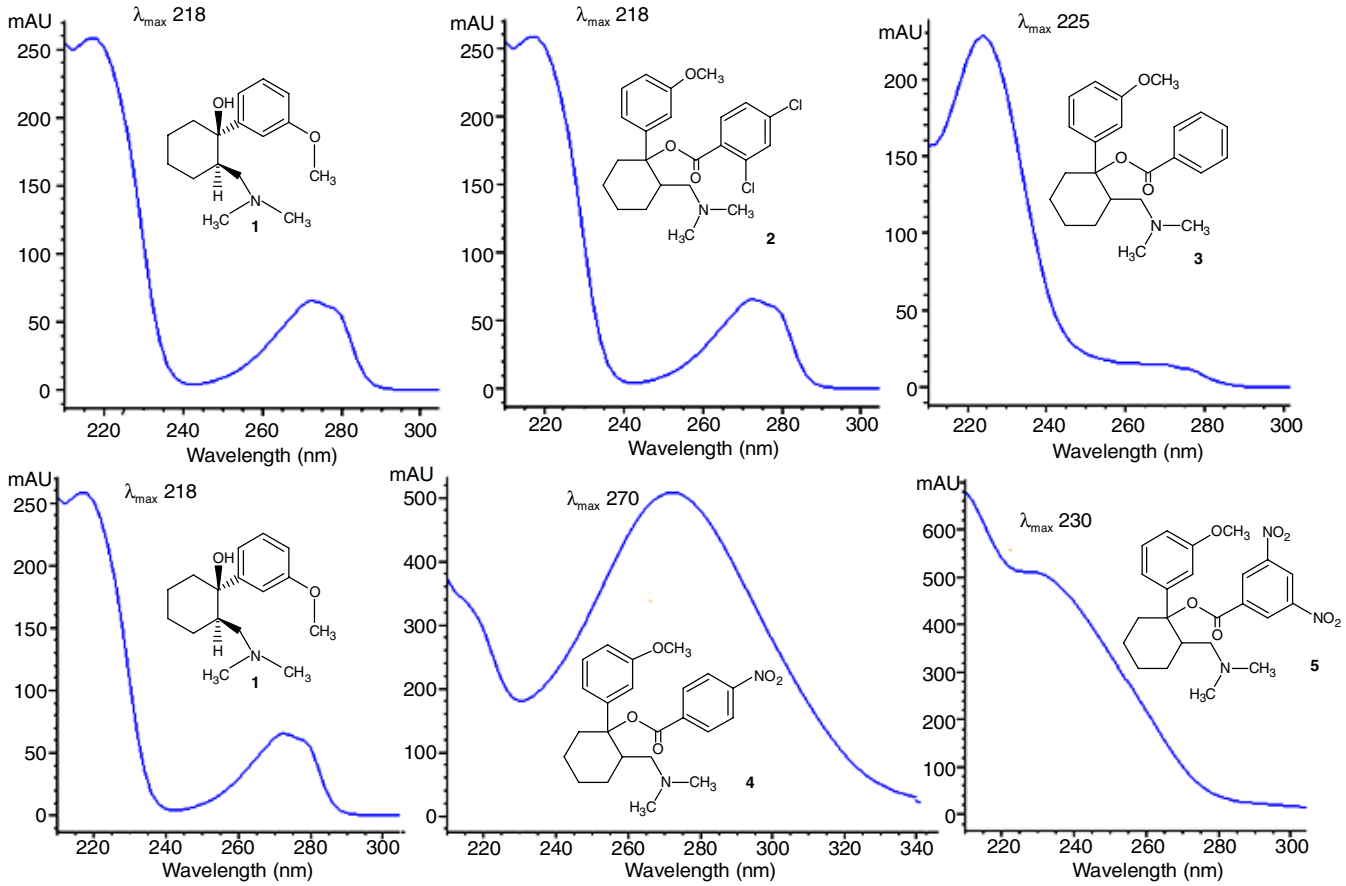

Fig. 3. UV spectra of tramadol (**1**) and its derivatives (**2**, **3**, **4** and **5**)

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