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Synthesis, characterization and pharmacological screening of various impurities present in Opipramol, Pargeverine and Propiverine bulk drugs

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Abstract

*Impurities identified by R.L. Fine Chemical, Bangalore, during the manufacturing of bulk drugs like opipramol, pargeverine and propiverine were synthesized and characterized by various analytical techniques such as TLC, FTIR, [1]H-MNR, FABMS and Elemental analysis. Impurities **(1a-e)** were synthesized as per **scheme 1** which were identified during manufacturing of opipramol bulk drug and screened for their anti-anxiety activity by elevated plus-maze method. The pharmacological screening showed that impurities **(1a-e)** have good anti-anxiety activity comparable to diazepam and opipramol. The other impurities **(1f-i),** structurally similar to pargeverine and propiverine were synthesized by **scheme 2** and evaluated for their antispasmodic activity using pargeverine and propiverine as standard drug. Pharmacological methods involve the in vitro antispasmodic activity studies on a freshly removed guinea pig ileum using a force displacement transducer amplifier connected to a physiograph. Among the synthesized impurities **(1f-i)** in the present study, a promising compound **1g**, a potent muscle relaxant as compared to standard drugs was obtained.*

Keywords: Opipramol, pargeveine, propiverine, anti-anxiety and antispasmodic activity

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INTRODUCTION

Proper characterization of manufactured pharmaceuticals requires that every detectable impurity in the finished product be positively identified and it's toxicity determined. A description of the identified and unidentified impurities present in new drug substance is known as impurity profile [1, 2]. Identification of impurities below apparent levels of 0.1% is generally not considered necessary. However, identification should be attempted for those potential impurities that are expected to be unusually potent, producing toxic or pharmacologic effects at levels lower than

0.1%. The laboratory studies conducted to detect impurities in the drug substance include test result of materials manufactured during the development process of batches from the proposed commercial process, as well as results of intentional degradation studies used to identify potential impurities that arise during storage [3,4]. Iminostilbene is reacted with 1-bromo-3-chloropropane in the presence of a weak base selected from a hydrogen phosphate salt and an acetate salt in the presence of a phase transfer agent to produce N-(3-halopropyl)iminostilbene, which is mixture of N-(3-chloropropyl)iminostilbene and N-(3-bromoropropyl)iminostilbene, and then N-(3-halopropyl)iminostilbene was reacted with N-(2-hydroxyethyl)piperazine to get opipramol [5-7]. Benzilic acid salts of α,α-diphenyl-α-hydroxyaceticacid β-dialkylaminoethyl esters were found to be useful in useful in the treatment of rhinitis [8, 9].

It has been suggested that an agent showing inhibitory action on the release of acetylcholine from the vagus nerve has antispasmodic potency comparable to antimuscarinic agents on the gastrointestinal system in experimental animals, and such an agent may be attractive for clinical use as a spasmolytic [10-13]. We have tried to find a novel type of spasmolytic agent having potent antispasmodic activity without an antimuscarinic effect. During the course of such a search, 1-methylpiperidin-4-ylhydroxy(diphenyl)acetate **(lg)** showed a marked suppressive activity on the response of isolated ileum from guinea pig to transmural electrical stimulation and a little inhibitory effect on the response to acetylcholine [14, 15].

RESULTS AND DISCUSSION

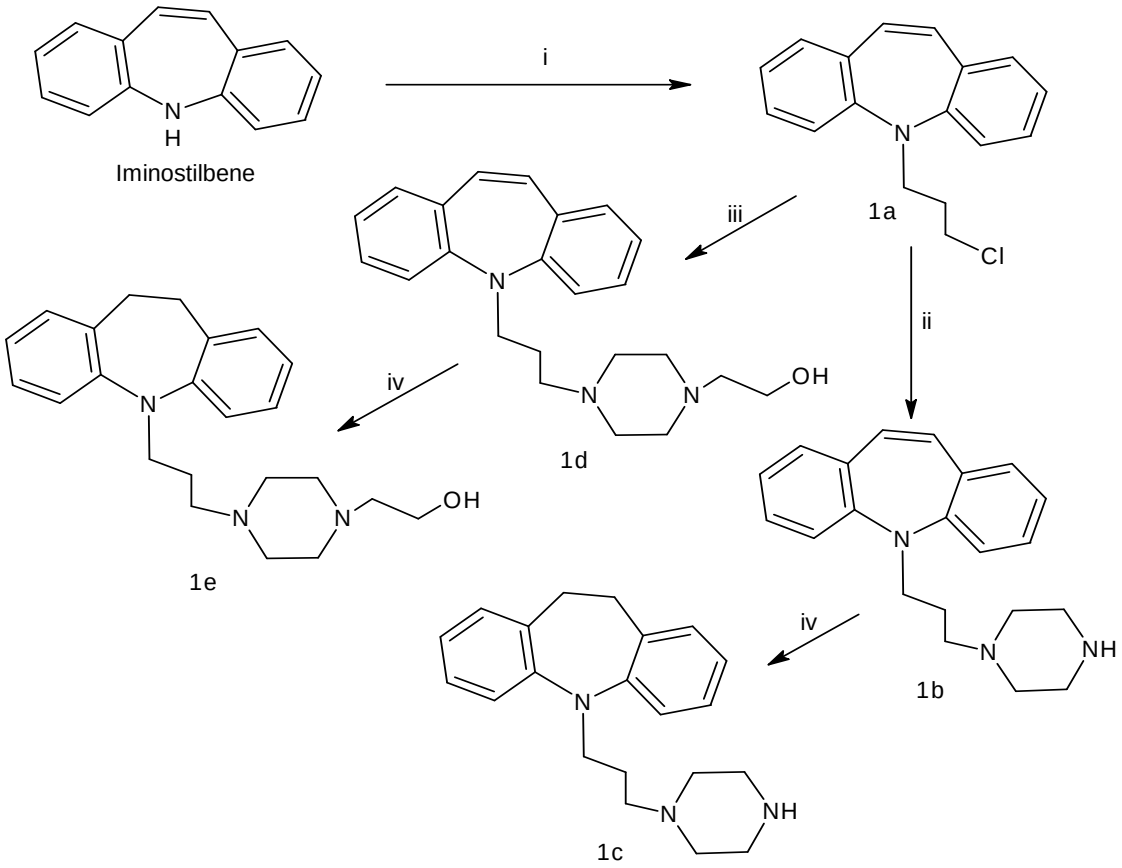
Chemistry

Various impurities **(1a-e)** identified during the manufacturing of Opipramol were synthesized by scheme 1 and scheme 2 represents the synthesis protocols of impurities **(1f-i)** identified during the bulk production of pargeverine and propiverine.

Pharmacological evaluation

Synthesized impurities (**1a-e)** were screened for anti-anxiety activity by elevated plus-maze apparatus using diazepam and opipramol as a standard drug. The parameters used to assess anti-anxiety activity were given first preference of mouse to open or enclosed arm, number of entries in open and enclosed arms and average time spent by animals in each arm (Average time=total duration in the arm/number of entries) was calculated by elevated plus-maze apparatus in albino mice. All the synthesized compounds and standard drug were given at the dose of 25mg/kg intraperitoneally. The all compounds showed the good % preference to open arms as compared to standard drug (diazepam and opipramol) and also were found to have less% preference to open arms as compared to standard drugs.

We have tried to find a novel type of spasmolytic agent having potent antispasmodic activity without an antimuscarinic effect in evaluation of synthesized impurities **(1f-i)** as per scheme 2. During the course of such a search, 1-methylpiperidin-4-yl-hydroxy(diphenyl)acetate **(lg)** showed a marked suppressive activity on the response of isolated ileum from guinea pig to transmural electrical stimulation and a little inhibitory effect on the response to acetylcholine.



Scheme 1. Reagents and conditions: (i) K_2CO_3, chlorobromopropane, trioctylmethyl ammonium bromide, 95 ºC, reflux, 12 h; (ii) Piperazine, toluene, 105-108 ºC, reflux, 8h; (iii) Piperazine ethanol, toluene, 110 ºC, reflux, 10h; (iv) HCOOH, Pd/C, ammonium formate, under N2 condition, stirring, 8 h.

MATERIALS AND METHODS

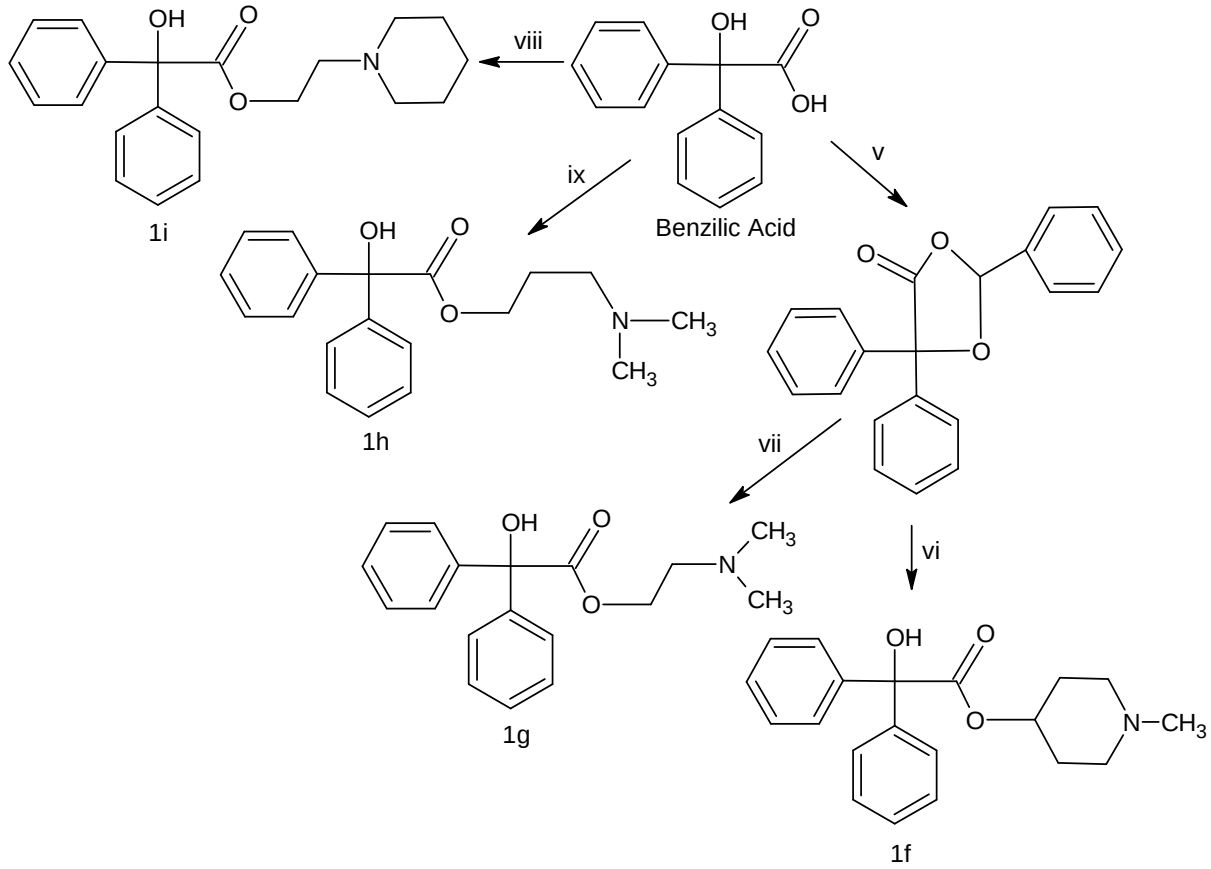
Chemistry

Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) plates (silica gel G) which were visualized by exposing to iodine vapors and UV light. The FT-IR spectra were recorded on Bio-rad FTS-135 spectrophotometer using KBr pellets; υ_{max} values are given in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale and coupling constants (J values) are expressed in Hz. The FAB Mass spectra were obtained on JEOL-JMS-DX 303 system, equipped with direct inlet probe system. Elemental analysis was carried out on CHNS Elementar (Vario EL III) using sulphanilic acid as a standard and tugsten (VI) oxide as a combusting agent and analyses for C, H, N were within ±0.4% of the theoretical values.

5-(3-chloropropyl)-5H-dibenzo[b,f]azepine (1a)

Iminostilbene (0.12 mol), chlorobromopropane (0.51 mol) and potassium carbonate (0.19 mol) transferred into 500 ml three necked round bottom flask. Then 2g of trioctylmethylammonium bromide (phase transfer catalyst) and 125 ml of toluene were added to the flask and the contents

were refluxed for for 12 hrs at 95 ºC. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1a**) was collected.



Scheme 2. Reagents and conditions: (v) Benzaldehyde, paratoluenesulfonic acid, toluene, 115 ºC, reflux, 8 h; (vi) 1-methylpiperidin-4-ol, sodium tertiary butoxide, under N_2 condition, 50 ºC, 48 h; (vii) Dimethylaminoethanol, sodium tertiary butoxide, under N_2 condition, RT stirring, 48 h; (viii) 1-(2-chloroethyl) piperidine, KOH, toluene, Trioctylmethylammonium bromide, 85 ºC, reflux, 18 h; (ix) Trioctylmethylammonium bromide, KOH, 26% 3-chloro-N,N-dimethylpropan-1-amine, 85 ºC, reflux, 10 h.

Cream solid: Yield was found to be 83.30%, mp 138-140 °C, R_f value 0.66. FTIR (KBr, ν_{max}) cm^{-1}: 2820-2760 (CH_2-N_{str}), 1600 (Ar C=C_{str} Bz), 1179 (C-N_{str}), 780(ArC-Cl $_{str}$), 705 (Ar C-H_{def} Bz), 667 (BzC-Br_{str}). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.80 (m, 2H, CH_2-alpha), 3.06 (t, 2H, CH_2-beta), 3.38(brt, 2H, CH_2), 6.99 (brs, 2H, -CH=CH-cycloheptane), 6.43-7.20 (4brm, 8H, Ar-H).). MS (*m/z)*: 269/270 (M^+/ M^++1). *Anal*. Calcd. for $C_{17}H_{16}NCl$: C, 75.69; H, 5.98; N, 5.19. Found: C, 75.67; H, 5.95; N, 5.17.

5-(3-piperazine-1-ylpropyl)-5H-dibenzo[b,f]azepine (1b)

In a 500 ml four necked round bottom150 ml of toluene was placed, to this 0.03 mol of **1a** and piperazine (0.01) were added and the reaction mixture was refluxed for 8 hours at 105-108 ºC. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1b**) was collected.

White solid, Yield was found to be 72%, mp 130-145 °C, R_f value, 0.70. FTIR (KBr, ν_{max},) cm^{-1}: 3220-3070 (N-H_{str}), 2820-2760 (CH_2-N_{str}), 1650 (C=N_{str}), 1590 (Ar C=C_{str} Bz), 1179 (C-N_{str}), 1050 (ArC-F_{str}), 780 (ArC-Cl $_{str}$), 705 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS,): δ 1.62 (m, 2H, CH_2-alpha), 2.36 (t, 2H, CH_2-Beta), 2.65(4brm, 8H, CH_2-pyrazine), 6.99 (brs, 2H, -CH=CH-cycloheptane), 6.43-7.20 (4brm, 8H, Ar-H). MS (*m/z*): 319/320 (M^+/ M^++1). *Anal.* Calcd. for $C_{21}H_{25}N_3$: C, 78.96; H, 7.89; N, 13.15. Found C, 78.95; H, 7.87; N, 13.13.

5-(3-piperazine-1-ylpropyl)-5H-dihydro-dibenzo[b,f]azepine (1c)

In a 250 ml of three necked round bottom flask 100 ml of methanol was placed, to this 3g of above product **(1b)**, 5g of ammonium formate, 5ml formic acid and 1g of palladium catalyst were added under nitrogen and the whole contents were stirred for 8 hours at room temperature under nitrogenous condition. After the completion of reaction the content was filtered off to remove the catalyst. Methanol was distilled off under vacuum at 65 ºC. To the residue 50 ml of water and 100 ml dichloromethane were added and extracted. Aqueous layer was discarded and dichloromethane layer was distilled off to get the final product (**1c**).

White solid, Yield was found to be 50%, mp 143-144 °C, R_f value, 0.72. FTIR (KBr, ν_{max}) cm^{-1}: 3220-3070 (N-H_{str}), 2820-2760 (CH_2-N_{str}), 1650 (C=N_{str}), 1590 (Ar C=C_{str} Bz), 1179 (C-N_{str}), 1050 (ArC-F_{str}), 780 (ArC-Cl $_{str}$), 705 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.40 (m, 2H, CH_2-alpha), 2.25 (t, 2H, CH_2-Beta), 2.65 (4brm, 8H, CH_2-pyrazine), 2.80 (m, 4H, CH=CH-cycloheptane), 6.38-6.90 (4brm, 8H, Ar-H). MS (*m/z*): 321/322 (M^+/ M^++1). *Anal.* Calcd. for $C_{21}H_{27}N_3$: C, 78.46; H, 8.47; N, 13.05. Found C, 78.45; H, 8.44; N, 13.02.

2-{4-[3-(10,11-dibenzo[b,f]azepine-5-yl)-propyl]-piperazin-1-yl}ethanol (1d)

In 250 ml four necked round bottom 40 ml of toluene was placed, to this 0.04 mol of **1c** and 0.02 mol of piperazine ethanol were added. The reaction mixture was refluxed for 10 hours at 110 °C. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1d**) was collected.

White solid, Yield was found to be 74%, mp 64-67 °C, R_f value, 0.52, FTIR (KBr, ν_{max}) cm^{-1}: 3560-3200 (ArO-H_{str}), 3220-3070 (N-H_{str}), 3010-3100 (=C-H_{str}), 1601 (C=N_{str}), 1514 (Ar C=C_{str} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.62 (m, 2H, CH_2-Alpha), 2.55 (t, 2H, CH_2-Beta), 2.46(4brm, 8H, CH_2-pyrazine), 3.06 (t, 2H, CH_2), 6.90 (brs, 2H, -CH=CH-cycloheptane), 6.50-7.10 (4brm, 8H, Ar-CH), 10.02(s, 1H, Ar-OH). MS (*m/z*): 363/364 (M^+/ M^++1). *Anal.* Calcd. for $C_{23}H_{29}N_3O$: C, 76.03; H 8.04; N, 11.56. Found C, 76.01; H, 8.02; N, 11.53.

2-{4-[3-(10,11-dihydro-dibenzo[b,f]azepine-5-yl)-propyl]-piperazin-1-yl}ethanol (1e)

In a 250 ml of three necked round bottom flask 100 ml of methanol was placed, to this 3g of above product **(1d)**, 5g of ammonium formate, 5ml formic acid and 1g of palladium catalyst were added under nitrogen and the whole contents were stirred for 8 hours at room temperature under nitrogenous condition. After the completion of reaction the content was filtered off to remove the catalyst. Methanol was distilled off under vacuum at 65 ºC. To the residue 50 ml of water and 100 ml dichloromethane were added and extracted. Aqueous layer was discarded and dichloromethane layer was distilled off to get the final product (**1e**).

White solid, Yield was found to be 65%, mp 74-76 °C, R_f value, 0.64. FTIR (KBr, ν_{max}) cm^{-1}: 3560-3200 (ArO-H_{str}), 3220-3070 (N-H_{str}), 3010-3100 (=C-H_{str}), 1601 (C=N_{str}), 1514 (Ar C=C_{str} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.60 (m, 2H, CH_2-Alpha), 2.36 (t, 2H, CH_2-Beta), 2.46(4brm, 8H, CH_2-pyrazine), 3.63 (t, 2H, CH_2), 2.88 (brs, 2H, -CH=CH-cycloheptane), 6.43-7.20 (4brm, 8H, Ar-H), 11.02(s,1H, Ar-OH). MS (*m/z*): 365/366 (M^+/ M^++1). *Anal.* Calcd. for $C_{23}H_{31}N_3O$: C, 75.58; H, 8.55; N, 11.50. Found C, 75.54; H, 8.54; N, 11.48.

Synthesis of 2-(dimethylamino)ethylhydroxy(diphenyl)acetate (1f)

It consist of two steps

(i) ***2,5,5-triphenyl-1,3-dioxolan-4-one (Intermediate B)***

In a 1000 ml four-necked round bottom flask (azeotropic setup) 200 ml of toluene was placed, to this 50 g of benzilic acid, 26g of crude benzaldehyde and 5g of para toluene sulphonic acid (PTSA) were added. This reaction mixture was refluxed for 8 hours at 110 ºC (completion of reaction was indicated by collection of 4 moles of water) and this reaction was carried out under nitrogenous condition. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**Intermediate B**) was collected.

(ii) ***2-(dimethylamino)ethylhydroxy(diphenyl)acetate (1f)***

In a 250 ml three-necked round bottom flask, 40 ml of dimethylaminoethanol was placed, to this 6g of 2,5,5-triphenyl-1,3-dioxolan-4-one and 1.5g of sodium tertiary butoxide were added. This reaction mixture was stirred for 48 hours at room temperature under nitrogenous condition. Completion of reaction was checked by TLC using acetone:chloroform (1:1) solvent system. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1f**) was collected.

Cream solid, Yield was found to be 64%, mp 86-88°C, R_f value, 0.73, FTIR (KBr, ν_{max}, cm^{-1}): 3580-3300(ArO-H_{str}), 3220-3070 (N-H_{str}), 3010-3050 (=C-H_{str}), 1662 (C=N_{str}),1650 (C=O_{str}), 1600 (Ar C=C_{str} Bz), 1231(C-N_{str}) 720 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.67 (t, 2H, CH_2-Alpha), 2.20 (s, 6H, N-2XCH_3), 4.08 (t, 2H, CH_2-Beta), 6.70-7.59 (4brm, 8H, Ar-H), 10.50(s, 1H, Ar-OH). MS (*m/z*): 299/300 (M^+/ M^++1). *Anal.* Calcd. for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found C, 72.20; H, 7.04; N, 4.67.

1-methylpiperidin-4-ylhydroxy(diphenyl)acetate (1g)

In a 250 ml four-necked round bottom flask, 80 ml of 1-methylpiperidin-4-ol was placed, to this 6g of 2,5,5-triphenyl-1,3-dioxolan-4-one and 2g of sodium tertiary butoxide were added. This reaction mixture was stirred for 48 hours at 50 ºC on water bath under nitrogenous condition. Completion of reaction was checked by TLC using acetone:chloroform (1:1) solvent system. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1g**) was collected.

Cream solid, Yield was found to be 54%, mp 216-218°C, R_f value, 0.45, FTIR (KBr, ν_{max}) cm^{-1}: 3580-3300 (ArO-H_{str}), 3220-3070 (N-H_{str}), 3010-3050 (=C-H_{str}), 1662 (C=N_{str}), 1650 (C=O_{str}), 1600 (Ar C=C_{str} Bz), 1231(C-N_{str}) 720 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.25 (s, 3H, methyl), 1.50 (brm, 2H, CH_2-piperidine), 2.24 (t, 2H, CH_2piperidine), 2.64 (t, 2H, CH_2-Alpha), 6.41-7.59 (4brm, 8H, Ar-H), 10.45(s, 1H, Ar-OH). MS (*m/z)*: 325/326 (M^+/ M^++1). *Anal.* Calcd. for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.32. Found C, 73.83; H, 7.10; N, 4.30.

3-(dimethylamino)propylhydroxy(diphenyl)acetate (1h)

In a 250 ml four-necked round bottom flask 100 ml of 26% 3-chloro-N,N-dimethylpropan-1-amine, to this 10g of benzilic acid, 2g of potassium hydroxide, 1g of trioctylmethylammonium bromide (phase transfer catalyst) and 20 ml of water were added. The reaction mixture was brought to pH 9 using 10% potassium hydroxide solution. This reaction mixture was refluxed for 18 hours at 85 ºC under nitrogenous conditon. Completion of reaction was checked by TLC using acetone:chloroform (1:1) solvent system. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1h**) was collected.

Cream solid, Yield was found to be 54%, mp 76-78°C, R_f value, 0.50. FTIR (KBr, ν_{max}) cm^{-1}: 3580-3300(ArO-H_{str}), 3220-3070 (N-H_{str}), 2950 (N-CH_{3str}), 2820 (CH_2-N_{str}), 1690 (C=O_{str}), 1601 (C=N_{str}), 1560 (Ar C=C_{str} Bz), 1250 (C-N_{str}), 712 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.80 (t, 2H, CH_2-Alpha), 2.35 (s, 6H, N-2XCH_3), 4.08 (t, 2H, CH_2-Beta), 6.63-7.50 (4brm, 8H, Ar-H), 10.01(s, 1H, Ar-OH). MS (*m/z)*: 313/314 (M^+/ M^++1). *Anal.* Calcd. for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found C, 72.80; H, 7.39; N, 4.45.

2-piperidin-1-ylethylhydroxy(diphenyl)acetate (1i)

In a 250 ml four-necked round bottom flask 50 ml of 1-(2-chloroethyl)-piperidine, to this 6g of benzilic acid, 2g of trioctylmethylammonium bromide (phase transfer catalyst) and 8 ml of water were added. The reaction mixture was brought to pH 9 using 10% potassium hydroxide solution. This reaction mixture was refluxed for 18 hours at 85 ºC under nitrogenous condition. Completion of reaction was checked by TLC using acetone:chloroform (1:1) solvent system. After the completion of reaction 100 ml of water was added to the reaction mixture and toluene layer was separated out. To the toluene layer, 50 ml of water was added and acidified to pH 2 with HCl. The aqueous layer was separated out and to this 50 ml of toluene was added. The mixture was adjusted to pH 10 using 10% sodium carbonate solution and was extracted. The

basic toluene layer was separated out and was washed with water (3X100 ml). Toluene layer was distilled off under vacuum; the final product (**1i**) was collected.

Cream solid, Yield was found to be 55%, mp 168-169 °C, R_f value, 0.54. FTIR (KBr, ν_{max}) cm^{-1}: 3580-3300 (ArO-H_{str}), 3220-3070 (N-H_{str}), 2820 (CH_2-N_{str}), 1654 (C=O_{str}), 1540 (C=N_{str}), 1452(CH_2 of C_6H_5), 1509 (Ar C=C_{str} Bz), 1290 (C-N_{str}), 720 (Ar C-H_{def} Bz). ^{1}H-NMR ($CDCl_3$-d_6, TMS): δ 1.05(t, 2H, CH_2-Alpha), 1.43-2.70 (3brm, 6H, CH_2-piperidine), 7.59-7.01 (4brm, 8H, Ar-H), 10.25(s, 1H, Ar-OH). MS (*m/z)*: 339/340 (M^+/ M^++1). *Anal*. Calcd. for $C_{21}H_{25}NO_3$: C, 74.31; H, 7.12; N, 4.13. Found C, 74.30; H, 7.10; N, 4.11

Anti-anxiety activity

Animals

Male mice weighing 25-30 g were housed in a cage with controlled room temperature at 22-25ºC. Food and water were available ad libitum. Tests were performed only after the mice had been acclimatized to the above environment for at least 7 days. Each mouse received a single intraperitoneal injection of drug or vehicle and was tested once in the elevated plus-maze (EPM).

Elevated plus-maze apparatus

The apparatus comprised of two open arms (35 × 5 cm) and two closed arms (30× 5× 15cm) that extended from a common central platform (5 × 5 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 50 cm above floor level as validated and described by Lister [16, 17]. Testing was conducted in a quiet room that was illuminated only by a dim light. Mice were given a single i.p. dose of various test compounds or diazepam 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5 min test period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels [18, 19]. The data were shown in Table I.

Table I. Anti-anxiety activity of synthesized compounds (1a-e)

Impurities	Dose (mg/kg;ip)	% preference of open arm	No. of entries in open arm	Average time spent in open arm
[y]Control	0.1 ml/10g	11	2 ± 0.31	7.5 ± 1.14
1a	25	50.33	5 ± 0.30	10 ± 0.23
1b	25	38.00	4 ± 0.45	8.2 ± 0.12
1c	25	14.66	3 ± 0.19	6.1 ± 1.11
1d	25	60.00	6 ±0.19	12 ± 0.65
1e	25	33.33	2 ± 0.30	7.7 ± 1.23
Diazepam	25	66.66	8 ± 0.75	13.6± 0.54
Opipramol	25	68.42	9±0.68	12.96±0.62

[y] *1% w/v gum acacia solution was used as vehicle; Six animals were used in each group; Standard drug: Diazepam and Opipramol*

Antispasmodic activity

The work includes muscle relaxation studies on isolated guinea pig ileum, contracted with acetylcholine14-15. Guinea pigs (n = 6) of both sexes (300 – 500 g) were used for this study. The animals were killed by a blow to the head, the ileum was removed immediately and placed in

aerated Krebs saline at 37 ^{o}C. This saline contained (in mM): NaCl, 120.7; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 15.5; and glucose, 11.5 at pH 7.3. For tension recording 2 cm ileal strips were mounted in a 10 mL organ bath and were connected to physiograph (Polyrite, Recorders and Medicare systems) through force tension transducer [20]. In the concentration range 10µM - 150µM papaverine and all its derivatives caused relaxation of spontaneous rhythmic contractions of both guinea pig ileum accompanied by a fall in resting tension [21-23].

The inhibition contraction was measured simply as percentage reduction in the height of spontaneous contractions. The percentage relaxation of all derivatives is compared in Table II.

Table II. Antispasmodic activity of compounds (1f-i)

Compound	% Relaxation at various concentration(µM, 500µL) 150	100	50	10
Pargeverine	68.46±3.49	43.90±2. 72	26.70±0.32	7.80±0.27
Propiverine	68.46±3.49	44.80±3.67	30.62±0.56	6.92±0.24
1f	55.50±2.12*	38.10±0.54*	20.80±1.17*	15.00±1.16*
1g	85.46±3.25*	65.29±2.15*	44.86±3.90*	19.89±1.13*
1h	79.48±1.59*	60.21±3.20*	40.42±2.27*	15.15±1.01*
1i	27.50±1.41*	14.50±0.50*	4.50±0.50*	1.20±0.20*

Values are mean ±S.E.M.
** P < 0.01, compared to pargeverine and propiverine*

The results are expressed as mean ± S.E. The statistical significance was treated with the paired student's t-test. P value < 0.01 was considered to be significant. Increase or decrease in tension was expressed as percent of maximal response to standard drugs.

Statistics
Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Tukey test. P<0.05 was considered significant. All data are expressed as mean ± standard error of mean (S.E.M.).

CONCLUSION

Impurities **(1a-e)** were exhibited significant anti-anxiety activity as compared to standard drugs (diazepam and opipramol). Anti-anxiety data of synthesized compounds revealed that compound possessing electron withdrawing group were found to good activity. Anti-anxiety data were shown in Table I. Among the synthesized impurities **(1f-i)** as reported scheme 2 in the present study, a promising compound **1g**, a potent muscle relaxant as compared to standard drugs was obtained. The data were shown in Table II.

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