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SYNTHESIS AND ANTIDEPRESSANT ACTIVITY OF SOME NOVEL FLUOXETINE DERIVATIVES

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يتضمن هذا البحث تشييد مشتقات جديدة من الفلوكستين **(1a)** بهدف اختبارها كمضادات للاكتئاب وتقع هذه المركبات في ثلاث سلاسل رئيسية من مستبدلات الفلوكستين تتكون السلسلة الأولي من ١ ميثيل ١ [٣ فينيل ٣ (٤ ترای فلوروميثيل فينوكسى) بروبيل] ٣ مستبدل اليوريا والثيويوريا **(2a-c)(3a-m)**. وقد تم تحضيرها بتفاعل الفلوكستين **(1a)** مع الأيزوسيانات والأيزوثيوسيانات المختلفة علي التوالي. والسلسلة الثانية تتكون من ن اسيل/ارويل-ن-ميثيل ٣ فينيل ٣ [(٤ ترای فلورومثيل-فينوكسى)] بروبيل امين **(4a-d)** والتي تمت بتفاعل **(1a)** مع كلوريد الأسيل او الأرويل او ثلاثي فلورو أنهيدريد الخليك. أما السلسلة الثالثة وهي ن كلوروأسيل الفلوكستين **(5a-c)** فقد تم تحضيرها من خلال تفاعل **(1a)** مع كلوريد اسيل الكلورو. بالإضافة إلي تحضير مركب البروبيونيتريل **(8)** وذلك بتفاعل **(1a)** مع الأكريلونيتريل. هذا وقد تم اختبار فعالية جميع المركبات (٢٤ مركبا) في تثبيط ارتجاع السيروتونين وتم اختيار ثمانية منهم لاختبار فعاليتهم لتثبيط ارتجاع النورابينفرين وقد اثبت اربعة مركبات **(3e, 3h, 3i, 5b)** فاعلية مشابهة لفاعلية الفلوكستين.

*The present work involves the synthesis of three series of novel fluoxetine derivatives in order to evaluate their potential as antidepressants. The first series consists of 1-methyl-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]-3-substituted ureas **2a-c** and thioureas as their bioisosters **3a-m** which were prepared by reacting fluoxetine **1a** with different isocyanates and isothiocyanates respectively. The second series N-acyl/aroyl-N-methyl-3-phenyl-3-(4-trifluoromethylphenoxy)-propylamines **4a-d** were synthesized by refluxing **1a** with acyl/aroyl chloride and trifluoroacetic anhydride. The third one, N-chloroacyl-fluoxetine **5a-c** was obtained via the reaction of **1a** with chloroacyl chloride. In addition to a propionitrile derivative **8** which was achieved by*

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*refluxing **1a** with acrylonitrile. The twenty four final compounds were biologically screened throughout the work for their potential as serotonin reuptake inhibitors by measuring potentiation of 5-HTP induced neurotoxity and some as norepinephrine reuptake inhibitor by measuring yohimbine-induced mortality in mice to calculate5-HTP/NE ratio as a parameter for selectivity to inhibit serotonin reuptake. Four compounds **(3e, 3h, 3i, 5b)** were found to be as potent as fluoxetine.*

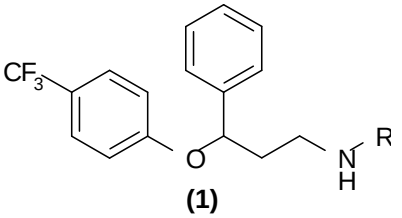
INTRODUCTION

Mood disorders are among the most common mental disorders encountered in clinical practice and are considered the diseases of the end of the twentieth century[1&2].

Depression is a complex of variable mental disorders that may be characterized by manic states as well as states of decreased motor activity. The biological etiology of depression is due to deficiency of biogenic neurotransmitters notably norepinephrine (NE) and serotonin (5-HT)[3&4].

Antidepressants include monoamine oxidase inhibitors (MAOIs)[5] tricyclic antidepressants (TCA)[3&6], serotonin and norepinephrine reuptake inhibitors[4&7], selective norepinephrine reuptake inhibitors (SNRIs)[8-10] and selective serotonin reuptake inhibitors (SSRIs)[4&5]. Fluoxetine **1a** (±) N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy] propan-

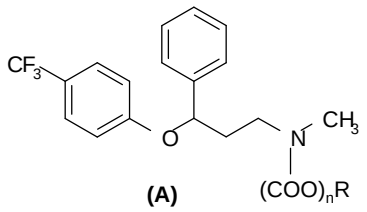
1-amine HCl is a selective and competitive inhibitor of serotonin-reuptake[11], its selectivity for the serotonin-uptake carrier versus other monoamine-uptake carriers appears to be greater than 50 fold[12]. It is still on the market since its approval[12]. The (S)-enantiomer and the major metabolite norfluoxetine **1b** are highly active against serotonin transport and also may have antimigraine effects not found in the (R)- enantiomer[4]. Moreover, fluoxetine**1a** has a delay in the onset of action for about 2-6 weeks, although it shows reduced side effects compared to other antidepressant drugs[11,13].



(1)

a) R = CH_3 , b) R= H

Literature survey declared the preparation procedure for some N-substituted derivatives of N-methyl-3-(4-trifluoromethylphenoxy)-3-phenyl-propylamine having the general formula **(A)** through the reaction with chloroformic acid esters, where n=1, R= alkyl, alkylaryl and aryl groups. Also, other N-substituted derivatives of **(A)** are included where n=0, R= alkyl and alkylaryl[14].



(A)

n = 0,1 R = alkyl, aryl, alkylaryl

As most (SSRIs) are aryl or aryloxyamines, the phenoxyphenyl-propanamine skeleton appears to be a suitable framework for preparation of a variety of substituted fluoxetine and screening their antidepressant activity[14]. Therefore, we are interested to substitute the (COO)n R side chain of **(A)** by different isosteric moieties including CONHR and CSNHR, where R= alkyl, aryl and alkylaryl to obtain novel N-substituted fluoxetine derivatives **2a-c** and **3a-m**. Further N-substitution is achieved using acid chloride, chloroacyl chloride or trifluoroacetic anhydride to give **4a-c**, **5a-c** or **4d** respectively. In addition, a cyanoethyl derivative **8** is performed in order to investigate the influence of the N-substitution of fluoxetine on its antidepressant activity.

EXPERIMENTAL

Chemistry

Melting points were uncorrected and were determined by open capillary tube method using Electrothermal 9100 digital melting point apparatus. Elemental microanalyses were carried out at the microanalytical centre, Faculty of Science, Cairo University. Infrared spectra were recorded on JASCO FT/IR-460Plus spectrophotometer and Bruker FT-IR spectrophotometer Vector 22 as potassium bromide discs or neat. ^{1}H NMR were recorded on Varian Gemini 200 spectrophotometer at 200 MHz, using TMS as internal standard and Varian Mercury spectrophotometer at 300 MHz. Chemical shift values (δ) are given in (ppm). Mass spectra were performed on Schimadzu GCMS-QP1000EX mass spectrophotometer, Hewlett Packard 5988A GC/MS mass spectrophotometer, and Fennigan MAT, SSQ 7000 GC/MS mass spectrophotometer at 70eV. Reaction time was determined by TLC using Macherey-Nagel Alugram Sil G/UV_{254} silica gel plates with fluorescent indicator UV_{254}, and carbon tetrachloride: methanol (9.5: 0.5) as the eluting system and the spots were visualized using Vilber Lourmet ultraviolet lamp at λ= 254nm.

1-Methyl-1-[3-phenyl-3-(4-trifluoromethylphenoxy)-propyl]-3-substituted ureas 2a-c

A mixture of fluoxetine base (0.45 g; 1.45 mmol), the appropriate isocyanate (1.59 mmol) and triethylamine (0.15 g, 1.45 mmol) in dry benzene (20 ml) was refluxed for 10-15 hours. The solvent was evaporated under reduced pressure and the residue was recrystallized from absolute ethanol. Table 1. **2a**: IR (KBr) 3327 (NH) and 1626 (CO). **2b**:

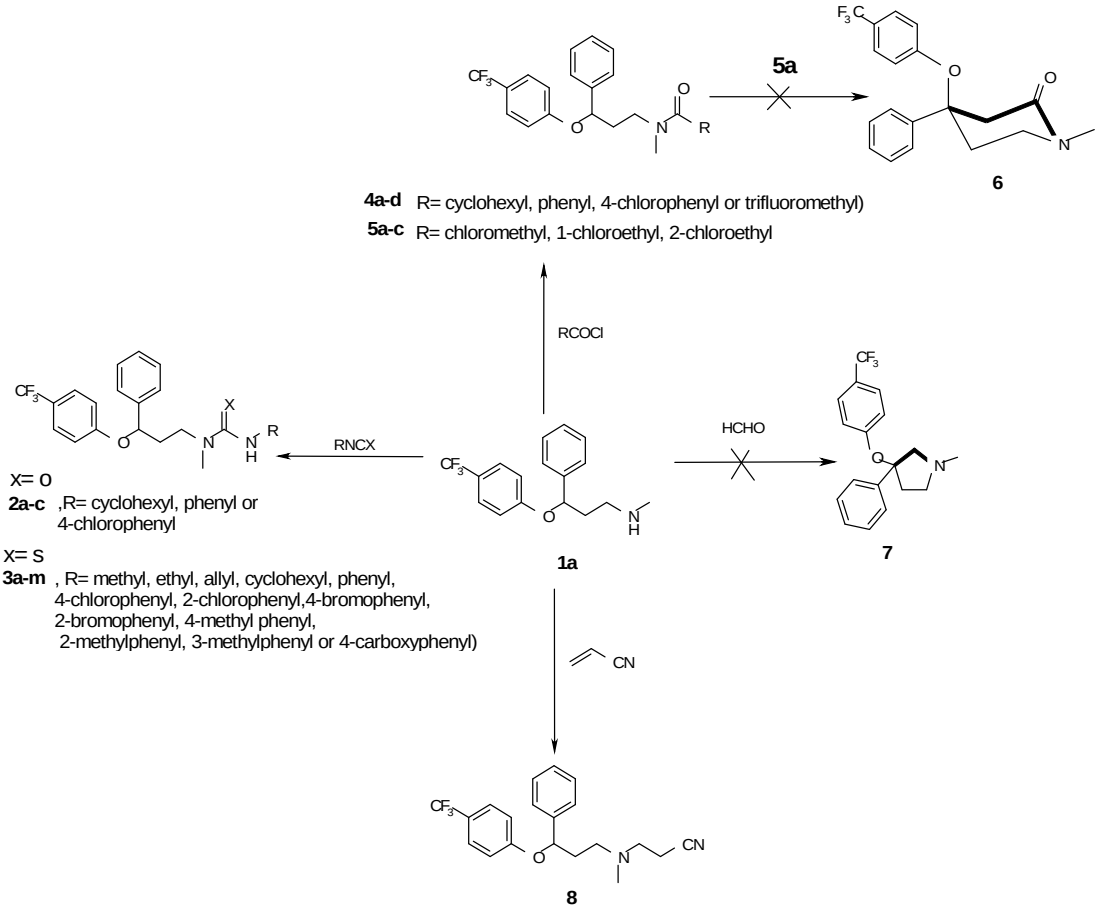
(KBr) 3346 (NH) and 1645 (CO). **2c**: (KBr) 3295 (NH) and 1632 (CO). **2b**: ^{1}H NMR ($CDCl_3$) δ = 2.18 (q, 2H at C_2 of the propanamine), 3.04 (s,3H, N$\underline{CH_3}$), 3.59 (m,2H at $C_1$of the propanamine), 5.27 (t,1H at C_3 of the propanamine), 7.25 (m,14H, aromatic protons), 8.92 (s,1H of $\underline{NH}COC_6H_5$). 2a: Ms: m/z (%)= M^+ 434 (13.30) and 273 (100). 2b: Ms: m/z (%)= M^+ 428 (11.68) and 267 (100). **2c**: Ms: m/z (%)= M^+ 462 (8.22) and 197 (100).

1-Methyl-1-[3-phenyl-3-(4-trifluoromethylphenoxy)-propyl]-3-substituted thioureas 3a-m

A mixture of fluoxetine base (0.45 g, 1.45 mmol), the appropriate isothiocyanate (1.59 mmol) and triethylamine (0.15 g, 1.45 mmol) in absolute ethanol (20 ml) was refluxed for 24 hours. The solvent was evaporated under reduced pressure and the residue was recrystallized from absolute ethanol or purified by flash chromatography using alumina gel as the stationary phase and chloroform: methanol (9.5: 0.5) as the mobile phase. Table1, **3a**: IR (KBr) 3362 (NH) and **3e**: IR(KBr) 3216 (NH). **3a**: ^{1}H NMR (DMSO) δ = 2.17 (m, 2H at C_2 propanamine), 2.89 (d, 3H, CH_3 CSNH$\underline{CH_3}$), 3.04 (s,3H, $\underline{NCH_3}$), 3.95 (m, 2H at C_1 propanamine), 5.49 (t,1H at C_3 propanamine), 7.39 (m, 10H, aromatic and NH protons). **3c**: ^{1}HNMR ($CDCl_3$) δ = 2.25 (q, 2H at C_2), 3.04 (s, 3H, N$\underline{CH_3}$), 3.94 (t, 2H at C_1), 4.20 (m, 2H at C_1 allyl), 5.05 (d, 2H C_3 allyl), 5.25 (t,1H at C_3), 5.75 (m,1H at C_2 allyl), 7.29 (m, 9H, aromatic protons) and 11.51 (s, 1H, $\underline{NH}$CSNallyl). **3f**: ^{1}H NMR ($CDCl_3$) δ= 2.34 (q, 2H at C_2), 3.28 (s, 3H, N$\underline{CH_3}$), 4.04 (m, 2H at C_1), 5.33(t, 1H at C_3), 7.25 (m, 14H, aromatic protons and NH proton). **3a**: Ms: m/z (%)= M^+ 382 (25.28) and 117 (100). **3c**: Ms: m/z (%)= M^+ 408 (26.78) and (100). **3e**: Ms: m/z (%)= M^+ 445 (7.14) and 91 (100) and **3f**: Ms: m/z (%)= M^+ 478 (21.6) and (100).

N-Methyl-N-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]-cyclohexanecarboxamide 4a, N-Methyl-N-[3-phenyl-3-(4-trifluoromethylphenoxy)-propyl]-4-chlorobenzamide 4c

A mixture of the corresponding carboxylic acid (1.45 mmol) and thionyl chloride (0.17 g, 1.45 mmol) in dry benzene (10 ml) was refluxed for 3 hours. Fluoxetine base (0.45 g, 1.45 mmol) and anhydrous potassium carbonate (0.20 g, 1.45 mmol) were added and reflux was continued for additional 6 hours. The mixture was filtered while hot, and the solvent was evaporated under reduced pressure. The residue was solidified by trituration with petroleum ether (40-60^{o}C) and recrystallized from ethanol. Table 2. **4a**: IR (KBr) 1627 (CO) and **4c**: IR (KBr) 1620 (CO). **4a**: ^{1}H NMR ($CDCl_3$) δ= 1.50 (m, 11H, cyclohexyl protons), 2.07 (q, 2H at C_2), 2.95 (d, 3H, N$\underline{CH_3}$), 3.48 (m, 2H at C_1), 5.17(t, 1H at C_3), 7.29 (m, 9H, aromatic protons).


5a
6
4a-d R= cyclohexyl, phenyl, 4-chlorophenyl or trifluoromethyl)
5a-c R= chloromethyl, 1-chloroethyl, 2-chloroethyl
RCOCl
RNCX
HCHO
7
1a
X= O
2a-c ,R= cyclohexyl, phenyl or 4-chlorophenyl
X= S
3a-m , R= methyl, ethyl, allyl, cyclohexyl, phenyl, 4-chlorophenyl, 2-chlorophenyl,4-bromophenyl, 2-bromophenyl, 4-methyl phenyl, 2-methylphenyl, 3-methylphenyl or 4-carboxyphenyl)
CN
8

Scheme

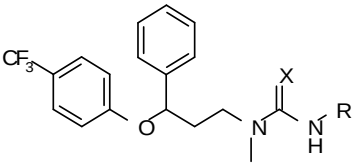


Table 1: Physical and analytical data of the prepared compounds **2a-c** and **3a-m**.

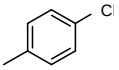
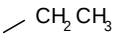
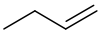
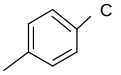
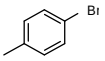
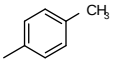
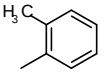
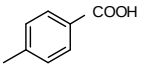
Comp. No.	R	X	Molecular formula (Mol. Wt)	Yield % M.P.°C	Microanalysis	
					Calculated %	Found %
2a		O	$C_{24}H_{29}F_3N_2O_2$ (434.50)	95 235-7	C 66.34 H 6.73 N 6.45	66.55 6.82 6.50
2b		O	$C_{24}H_{23}F_3N_2O_2$ (428.46)	94 135-8	C 67.28 H 5.41 N 6.54	66.90 5.50 6.80
2c		O	$C_{24}H_{22}ClF_3N_2O_2$ (462.90)	83 140-2	C 62.27 H 4.79 N 6.05	62.10 4.80 5.90
3a		S	$C_{19}H_{21}F_3N_2OS$ (382.45)	50 110	C 59.67 H 5.53 N 7.32 S 8.38	60.23 5.19 6.45 9.19
3b		S	$C_{20}H_{23}F_3N_2OS$ (396.48)	70 89	C 60.59 H 5.85 N 7.07	60.07 5.66 7.02
3c		S	$C_{21}H_{23}F_3N_2OS$ (408.49)	54.2 < 25	C 61.75 H 5.68 N 6.86	60.98 5.60 6.79
3d		S	$C_{24}H_{29}F_3N_2OS$ (450.57)	77 < 25	C 63.98 H 6.49 N 6.22	63.90 6.77 6.17
3e		S	$C_{24}H_{23}F_3N_2OS$ (444.52)	88.3 135	C 64.85 H 5.22 N 6.30 S 7.21	65.44 5.27 6.80 7.40
3f		S	$C_{24}H_{22}ClF_3N_2OS$ (478.97)	76 115-6	C 60.18 H 4.63 N 5.85	60.72 4.34 5.76
3g		S	$C_{24}H_{22}ClF_3N_2OS$ (478.97)	72.5 < 25	C 60.18 H 4.63 N 5.85	59.50 4.75 5.25

Table 1: Continue

Comp. No.	R	X	Molecular formula (Mol. Wt)	Yield % M.P.^{o}C	Microanalysis	
					Calculated %	Found %
3h		S	$C_{24}H_{22}BrF_3N_2OS$ (522.42)	86.7 125-6	C 55.17 H 4.21 N 5.35	55.67 4.54 5.20
3i		S	$C_{24}H_{22}BrF_3N_2OS$ (522.42)	38 85-90	C 55.07 H 4.24 N 5.35	55.41 4.57 5.24
3j		S	$C_{25}H_{25}F_3N_2OS$ (458.55)	54 105-7	C 65.48 H 5.50 N 6.11	66.18 5.52 6.12
3k		S	$C_{25}H_{25}F_3N_2OS$ (458.55)	36 128-32	C 65.48 H 5.50 N 6.11	64.87 6.00 6.02
3l		S	$C_{25}H_{25}F_3N_2OS$ (458.55)	99 55-60	C 65.48 H 5.50 N 6.11	66.14 4.75 5.95
3m		S	$C_{25}H_{23}F_3N_2O_3S$ (488.53)	57.5 150-3	C 61.47 H 4.75 N 5.73	61.08 4.72 5.63

N-Methyl-N-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]benzamide 4b

A mixture of sodium hydroxide (0.10 g, 2.60 mmol), fluoxetine base (0.62 g, 2.00 mmol) and water (10 ml) was treated with benzoyl chloride (0.28 g, 2.00 mmol) with continuous stirring during the course of one hour, keeping the temperature down by cooling with running water. The reaction mixture was extracted with benzene. The organic phase was dried over anhydrous magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using alumina gel as the stationary phase and carbon tetrachloride: methanol (9.8:0.2) as the mobile phase and separated as oily product. Table 2. IR (KBr) 1715.0 (CO). ^{1}H NMR ($CDCl_3$): δ= 2.24 (q, 2H at C_2), 3.08 (d, 3H, N$\underline{CH_3}$), 3.78 (m, 2H at C_1), 5.36(t, 1H, at C_3) and 7.05 (m, 14H, aromatic protons).

N-Methyl-N-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]–2,2,2-trifluoroacetamide 4d

A mixture of fluoxetine base (0.53 g, 1.70 mmol), trifluoroacetic anhydride (0.36 g, 1.70 mmol) and triethylamine (0.17 g, 1.70 mmol) in dry benzene (20 ml) was stirred at 0-5^{o}C for 2 hours, then at room temperature for 24 hours. The reaction mixture was poured onto ice; the oil separated was extracted with 2x10 ml chloroform. The organic

phase was washed with 3x10 ml brine solution, dried over anhydrous magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using alumina gel as the stationary phase and carbon tetrachloride: methanol (9.5:0.5) as the mobile phase and separated as oily product. Table 2. IR (KBr) 1695.8 (CO). ^{1}H NMR ($CDCl_3$): δ= 2.23 (m, 2H at C_2), 3.07 (d,3H, NCH_3), 3.64 (t, 2H at C_1), 5.320(t,1H, at C_3) and 7.15 (m,9H, aromatic protons). Ms: m/z (%)= M^+ 405 (0.10) and 140 (100).

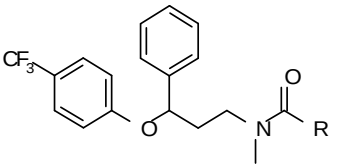
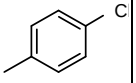
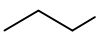


Table 2: Physical and analytical data of the prepared compounds **4a-5c**.

Comp. No.	R	Molecular formula (Mol. Wt)	Yield % M.P.°C	Microanalysis	
				Calculated %	Found %
4a		$C_{24}H_{28}F_3NO_2$ (419.49)	68.5 80-83	C 68.72 H 6.73 N 3.34	69.20 6.80 3.05
4b		$C_{24}H_{22}F_3NO_2$ (413.44)	62 Oil at RT	C 69.72 H 5.36 N 3.39	69.30 5.30 3.31
4c		$C_{24}H_{21}ClF_3NO_2$ (447.89)	60.5 80-82	C 64.36 H 4.73 N 3.13	64.54 4.92 3.07
4d		$C_{19}H_{17}F_6NO_2$ (405.34)	45 Oil at RT	C 56.30 H 4.23 N 3.46	56.87 5.21 3.35
5a		$C_{19}H_{19}ClF_3NO_2$ (385.81)	68 63-5	C 59.15 H 4.96 N 3.63 Cl 9.20	58.32 5.00 3.62 9.50
5b		$C_{20}H_{21}ClF_3NO_2$ (399.84)	71.5 60-1	C 60.08 H 5.29 N 3.50 Cl 8.87	60.20 6.00 3.77 8.40
5c		$C_{20}H_{21}ClF_3NO_2$ (399.84)	69 77-9	C 60.08 H 5.29 N 3.50 Cl 8.87	59.83 5.70 3.55 8.63

N-methyl-N-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]chloroacylamides 5a-c

A mixture of fluoxetine HCl (0.50 g, 1.45 mmol), the appropriate chloroacyl chloride (1.595 mmol) and anhydrous potassium carbonate (0.40 g, 2.90 mmol) in dry benzene, was refluxed for 6-24 hours. The hot solution was filtered, the solvent was evaporated under reduced pressure and the residue was recrystallized from absolute ethanol. Table 2. 5a: IR (KBr) 1657 (CO). **5b**: IR (KBr) 1648 (CO). **5c**: IR (KBr) 1643 (CO). **5a**: ^{1}H NMR ($CDCl_3$): δ= 2.20(m, 2H at C_2), 3.00(d, 3H, $N\underline{CH_3}$), 3.59 (t, 2H at C_1), 4.00(s, 2H, $CO\underline{CH_2}Cl$), 5.20(t,1H at C_3), 7.30(m,9H, aromatic protons). **5a**: Ms: m/z (%)= M^+ 386 (0.07) and 121(100). **5b**: Ms: m/z (%)= M^+ 399 (0.3) and 134 (100) and **5c**: Ms: m/z (%)= M^+ 399 (0.60) and 238 (100).

3-{Methyl-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]amino}propionitrile 8

A mixture of fluoxetine base (0.62 g, 2.00 mmol), acrylonitrile (0.14 ml, 2.00 mmol) in dry benzene (10 ml) was refluxed for 24 hours. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using alumina gel as the stationary phase and chloroform as the mobile phase to give compound 8. (Yield= 55%, m.p.= Oil at room temperature). Microanalysis for $C_{20}H_{21}F_3N_2O$ Calculated (%)C: 66.29; H: 5.80; N: 7.73. Found (%)C: 65.55; H: 5.80; N: 7.62. IR (KBr) 2247.9 (CN). ^{1}H NMR ($CDCl_3$): δ= 1.96 (m, 2H $CH_2\underline{CH_2}CN$), 2.15 (t, 2H, $\underline{CH_2}CH_2CN$), 2.25(s, 3H, $N\underline{CH_3}$), 2.38 (t,2H at C_1), 2.64 (m,2H at C_2), 5.41(t,1H at C_3), 7.15(m,9H, aromatic protons). Ms: m/z (%)= M^+ 362 (0.10) and 97 (100).

Antidepressant activity

All the twenty four newly synthesized compounds were studied for their serotonin-reuptake inhibition by measuring potentiation of 5-HTP (5-hydroxytryptophan) induced neurotoxicity in mice. Eight compounds **2a**, **2b**, **2c**, **3e**, **3h**, **3i**, **5a** and **5b** were tested for their norepinephrine-reuptake inhibition by measuring yohimbine-induced mortality to calculate 5-HT/NE ratio as a parameter for selectivity to inhibit serotonin reuptake. These compounds were chosen on the basis of the steep 5-HTP-induced neurotoxicity curve, except compounds **2a**, **2c** and **5a** which were included in this study although they showed less effect in the 5-HTP model in order to obtain a correlation about the SAR of these derivatives.

Potentiation of 5-HTP- induced neurotoxicity in mice in vivo[15]

Two control groups, six animals each, were used; one handeled alcohol-water as a solvent system, while the other used water-tween as a suspending agent. Three doses of each compound were administered to three groups (six animals each). Animals were injected i.p. with the compound or the vehicle. Thirty

minutes later, the mice received 75 mg/kg pargyline HCl s.c. in a loose fold of skin on the back of the neck. Ninety minutes after pargyline, the animals were injected with 5-HTP, (5 mg/kg, i.p.). The number of animals with head-shakes (neurotoxicity) is recorded for each group within 20 minutes. From the dose-response curve: The median toxic dose (TD_{50}) is calculated for each compound as well as its 95% confidence limit using the method of Litchfield and Wilcoxon[16].

Potentiation of yohimbine induced mortality[17]

A general control group was used for water-tween as a suspending agent, consisting of six animals. Three to five doses of each compound were administered to each animal of each group, each group comprises six animals. In case of fluoxetine, a dose-yohimbine mortality curve could not be established experimentally. Male mice (22-25 g) are randomly assigned to test groups. The compound or vehicle is given i.p. thirty minutes prior to s.c. injection of 25 mg/kg yohimbine (a sublethal dose). The groups are placed into cages with free access of food and water. The number of animals died within 24 hours is recorded for each group. From the dose-mortality curve, the median lethal dose (LD_{50}) is calculated for each compound as well as its 95% confidence limit using the method of Litchfield and Wilcoxon[16].

5-HT/NE selectivity ratio

The selectivity in inhibiting the reuptake of 5-HT and NE by using 5-HTP induced neurotoxicity and yohimbine induced mortality as a ratio of TD_{50} from 5-HTP model to the YLD_{50} of yohimbine model as mM is calculated and compared with that of fluoxetine and clomipramine.

RESULTS AND DISCUSSION

Chemistry

Fluoxetine **1a** was refluxed with isocyanates / isothiocyanates in presence of triethylamine to give the corresponding ureas **2a-c** and thioureas **3a-m** respectively. IR spectra of compound **2b** revealed the appearance of a band corresponding to (CO) at 1645 cm^{-1}, in addition to the (NH) band at 3346 cm^{-1}. ^1H NMR of **2b** showed an increase in the integration of aromatic protons (9 to 14 Hs) at 7.25 ppm and mass spectrum was in accordance with the structure.

Furthermore, refluxing **1a** with cyclohexanoyl or p-chlorobenzoyl chloride in dry benzene and in presence of anhydrous potassium carbonate afforded the amide derivatives **4a** and **4c**. The benzamide **4b** was prepared through reacting **1a** with benzoyl chloride on cold in aqueous sodium hydroxide. Preparation of the trifluoroacetamide **4d** was done by stirring **1a** with trifluoroacetic anhydride at 0-5^oC then at room temperature. IR of the amide derivatives **4a-d** revealed the lack of (NH) band and the appearance

of the (CO) band at ≈ 1620 cm-1. ^{1}H NMR of **4a** showed multiplet at 1.50 ppm assigned to (11Hs) of the cyclohexyl. ^{1}H NMR of 4b demonstrated an increase in the integration of aromatic protons at 7.05 ppm attributed to (m, 14 Hs).

Moreover, N-chloroacyl derivatives **5a-c** were achieved by refluxing **1a** with the appropriate choroacyl chloride in dry benzene and in presence of anhydrous potassium carbonate. IR spectrum of **5a** revealed the disappearance of (NH) band and the appearance of a new band at 1657 cm^{-1} corresponding to (CO). ^{1}H NMR spectrum of 5a showed a new singlet at 4.00 ppm (2Hs) assigned to the methylene protons of the chloroacetyl moiety. Mass spectra of **5a-c** were complying with their structures.

Many attempts were carried out to cyclize **5a** into a six membered ring (piperidin-2-one derivative) **6** using different bases. All trials failed to give the target compound **6** and ^{1}H NMR revealed the presence of the characteristic signal of the benzylic proton at 5.30 ppm. Another attempt was done to rigidify fluoxetine by locking free rotation of the propanamine chain in a five membered ring **7** (a pyrrolidine derivative). The trial was carried out by refluxing **1a** with 35% formaldehyde solution but unfortunately fluoxetine was recovered unchanged.

Further modification of fluoxetine nucleus was done by refluxing **1a** with acrylonitrile in dry benzene. IR of the obtained propionitrile derivative **8** showed a strong and sharp nitrile stretching band at 2247 cm^{-1} in addition to the disappearance of the (NH) band. ^{1}H NMR showed two new signals: a multiplet at 1.96 ppm and a triplet at 2.15 ppm corresponding to the two methylene groups of the propionitrile moiety. Mass spectrum was complying with the structure of the compound.

Pharmacology

The TD_{50} of fluoxetine (5.09 mM) and clomipramine (2.00 mM) that potentiated 5-HTP-induced neurotoxicity were not statistically significant (Table 3). Similarly 15 new compounds out of 24 showed TD_{50} not significantly different from that of fluoxetine. This might indicate that they have antidepressant potency in this model similar to fluoxetine. From Table 3, it can also be seen that the remaining 9 compounds were less potent than fluoxetine. They have TD_{50} significantly higher than that of fluoxetine.

Clomipramine was twice as potent as fluoxetine to potentiate yohimbine-induced mortality. The 8 new compounds chosen to be tested in this model showed potency lower than that of clomipramine but were not significantly different (Table 4).

Table 3: Effect of fluoxetine, clomipramine and newly synthesized compounds on 5-HTP-induced neurotoxicity in mice *in vivo*.

Compound	TD_{50} (mM)	95% C.L
Fluoxetine	5.09	2.72-9.51
Clomipramine	2.00	0.78-5.15
2a	39.95 [a]	10.89-146.48
2b	4.69	2.12-10.39
2c	49.36 [a]	14.83-164.29
3a	16.58 [a]	3.62-75.91
3b	2.56	0.33-19.85
3c	20.19 [a]	9.49-42.95
3d	4.04	1.56-10.49
3e	4.69	2.12-10.39
3f	8.15	2.89-22.95
3g	18.65 [a]	13.91-25.00
3h	4.04	1.56-10.49
3i	4.04	1.56-10.49
3j	16.58 [a]	3.62-75.91
3k	8.15	2.89-22.95
3l	5.43	2.83-10.41
3m	4.04	1.56-10.49
4a	15.26	7.95-29.29
4b	16.57 [a]	3.62-75.91
4c	11.76	5.15-26.84
4d	16.57 [a]	3.62-75.91
5a	20.19 [a]	9.49-42.95
5b	4.69	2.12-10.39
5c	5.43	2.83-10.41
8	5.43	2.83-10.41

$TD_{50:}$ 5-HTP Median neurotoxic dose (mM).
95% C.L.: 95% confidence limit.
[a] Significantly different from respective Fluoxetine treated group at $p < 0.05$.
Statistical comparisons between groups were carried out by using Litchfield and Wilcoxon method[16].

Table 4: Effect of fluoxetine, clomipramine and some newly synthesized compounds on yohimbine-induced mortality.

Compound	LD_{50} (mM)	95% C.L
Fluoxetine	>12.90*	-
Clomipramine	5.78	2.52-13.25
2a	32.47	4.24-248.88
2b	16.58	8.90-30.88
2c	22.71	19.44-26.54
3e	80.22	26.51-242.73
3h	29.59	16.99-51.54
3i	29.59	16.99-51.54
5a	6.45	2.30-18.07
5b	30.61	21.98-42.62

$YLD_{50:}$ Median yohimbine lethal dose (mM).
95% C.L.: 95% confidence limit.
*LD_{50} for fluoxetine was not exactly calculated because a dose yohimbibe mortality curve could not be established experimentally.
Statistical comparisons between effect on 5-HTP treatment and yohimbine treatment were carried out by using Litchfield and Wilcoxon method (except for Fluoxetine tested by Fisher's exact test).

The 5-HT/NE selectivity of fluoxetine, clomipramine and selected 8 new compounds is shown in Table 5 and presented in Figure (1). The ratio for fluoxetine was < 0.39 which was not calculated accurately in the present study because of the higher value of TD_{50} to potentiate yohimbine-induced mortality. It is expected, however, to be 0.1 from the literature[18]. Clomipramine showed a ratio of 0.35 which was not significantly different from one. Of the 8 new compounds tested, **2c** and **5a** showed ratios of 2.17 and 3.13, respectively ($p< 0.05$). Compounds **2a** and **2b** showed ratios of 1.23 and 0.28 which were not different than one. Compounds **2c** and **5a** might be considered as reuptake inhibitors of NE more than 5-HT.

The other 4 compounds, **3e**, **3h**, **3i** and **5b**, showed ratios significantly lower than one. These compounds, therefore, can be considered as reuptake inhibitors of 5-HT more than NE. Of particular interest is compound **3e** which showed the highest selectivity (ratio of 0.06).

Table 5: 5-HT/NE selectivity of fluoxetine, clomipramine and new compounds.

Compound	TD_{50} (mM) (5-HT) X	LD_{50} (mM) (NE) Y	5-HT/NE Selectivity X/Y
Fluoxetine	5.09	>12.90	<0.39
Clomipramine	2.00	5.78	0.35
2a	39.95	32.47	1.23
2b	4.69	16.58	0.28
2c	49.36	22.71	2.17[a]
3e	4.69	80.22	0.06[a]
3h	4.04	29.59	0.14[a]
3i	4.04	29.59	0.14[a]
5a	20.19	6.45	3.13[a]
5b	4.69	30.61	0.15[a]

[a] : Significantly different from that of fluoxetine at $p< 0.05$.
Statistical comparisons between effect on 5-HTP treatment and yohimbine treatment were carried out by using Litchfield and Wilcoxon[16] (except for fluoxetine tested by Fischer's exact test)

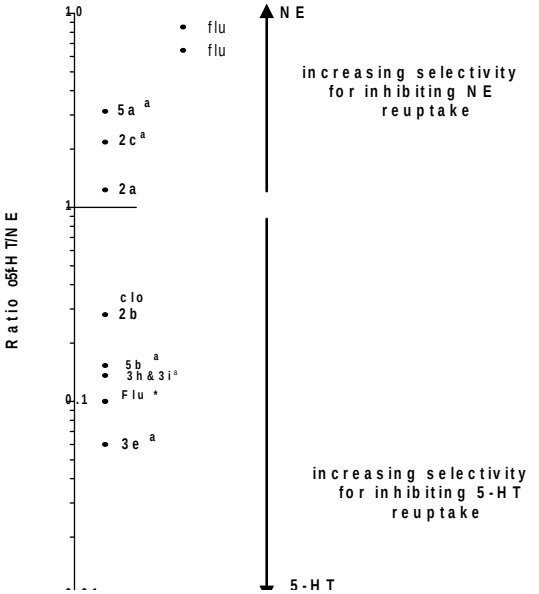


Fig. 1: Selectivity of Fluoxetine, Clomipramine (clo) and some of the newly synthesized compounds in inhibiting the teuptake of 5-HT and NE by using 5-HTP induced neurotoxicity and yohimbine induced mrtality as a ratio of TD_{50} in HTP model to that of yohimbine model.
a: significantly different from one at $p<0.05$
* Fluoxetine rati from the literature

Structure-Activity Relationship (SAR)

Antidepressant activity of the newly synthesized compounds showed that blockade of the secondary amino group of fluoxetine does not always decrease its activity or its selectivity. The urea derivative **2b** retained its activity as 5-HT-reuptake inhibitor while **2c** showed a significant selectivity towards NE reuptake inhibition. Thiourea **3a** decreases the activity of fluoxetine while increasing the length of the side chain or substitution with a cyclohexyl does not affect the activity of fluoxetine. Moreover, the majority of aromatic substituents of **3** did not change its activity. The phenyl derivative **3e** showed potency as a (SRI) comparable to fluoxetine and at the same time, it demonstrated a high 5-HT/NE selectivity ratio. The cholinergic, histaminergic and adrenergic blockade of **3e** were studied and found to display minimal effects as observed with fluoxetine[19]. Moreover **3e** showed a safety margin (LD_3/ED_{97}) equal to or even better than that of fluoxetine[19]. Acylation of fluoxetine **4a-d** and **5a** destroyed its activity as (SRI), while **5b** and **5c** retained (SRI) activity. **5a** showed a significant NE/5-HT selectivity. 2-Cyanoethyl derivative **8** showed a 5-HT reuptake inhibition comparable to **5c.** Calculated Log P values of the new compounds, however, showed that all derivatization made to fluoxetine did not alter its lipophilicity as was hoped for (unpublished observation) (Table 6). Further studies are needed to explain why certain derivatization of fluoxetine decreased its potency as SRI, particularly in relation to peripheral hydrolysis with consequent less CNS availability.

The minimum energy conformer for fluoxetine (Figure 2) and **3e** (Figure 3) was carried out. It was found that, as previously reported[10], the propanamine chain in fluoxetine folds towards the trifuoromethyl-phenoxy ring to obtain the proper spatial orientation between the phenoxy ring and the basic nitrogen. Also, the methyl group at the nitrogen atom is proximal to the trifluoro-methylphenoxy moiety. In compound **3e**, this orientation is retained, but the methyl group is lying remote (Figure 3). Therefore, it can be assumed that the minimum energy conformer is not necessarily, the most active conformer, at least with respect to the orientation of the methyl group in space.

Table 6: Calculated log P of the tested compounds.

Compound	C log P	Compound	C log P
Fluoxetine	4.27	**3j**	7.26
2a	5.37	**3k**	7.26
2b	5.49	**3l**	7.26
2c	6.05	**3m**	----------
3a	5.11	**4a**	5.89
3b	5.45	**4b**	5.82
3c	5.94	**4c**	6.37
3d	6.66	**4d**	5.06
3e	6.78	**5a**	4.44
3f	7.33	**5b**	4.94
3g	7.33	**5c**	4.74
3h	7.60	**8**	4.68
3i	7.60		

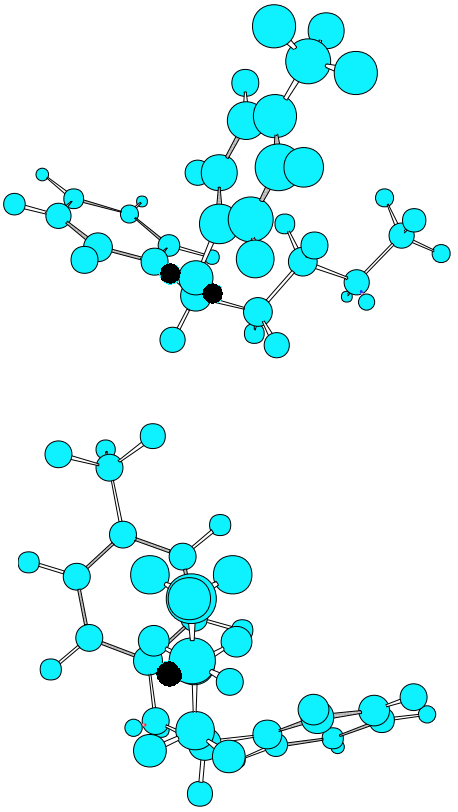


Fig. 2: 3D views of the minimum energy conformer of Fluxetine.

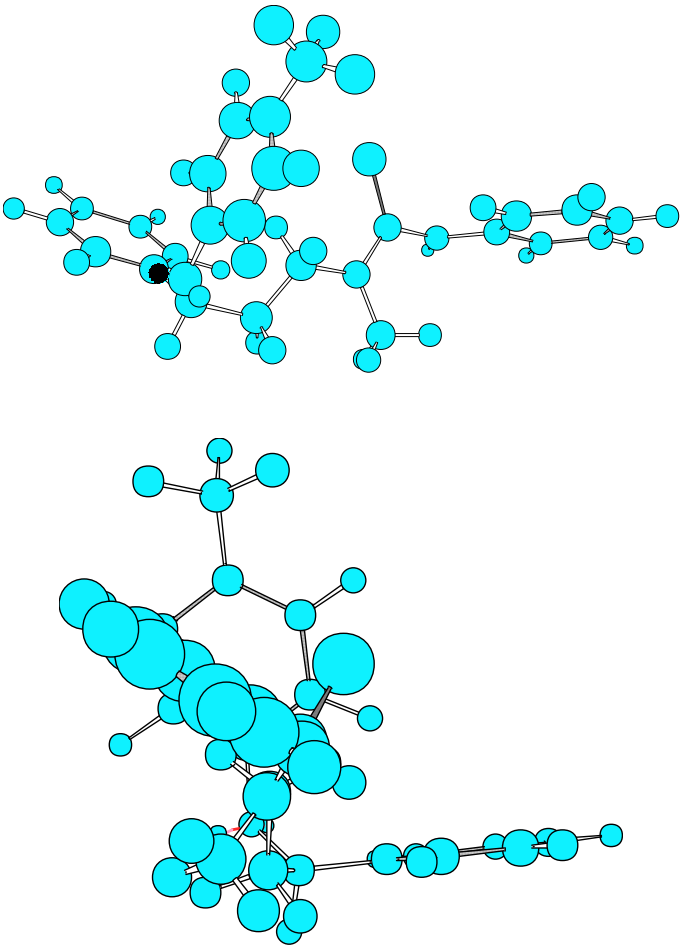


Fig. 3: 3D views of the minimum energy conformer of **3e**.

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