British Journal of Pharmaceutical Research
8(1): 1-12, 2015, Article no.BJPR.19068
ISSN: 2231-2919

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Synthesis and Evaluation of Novel Mannich Bases of N-methyl-3-phenyl-3-[4-trifluoromethyl) phenoxy] propan-1-amine (Fluoxetine)

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Authors' contributions

This work presented is the outcome of joint efforts of all authors. The study was designed by author GAM as a part of the assignments and tasks to be accomplished by the students working for M. Phil. degree program in Pharmaceutical Chemistry. The details were shared and discussed with author AHS. The work was done by author NW under close supervision and mentorship. All the data on chromatography, spectroscopy and biological assays of the newly synthesized compounds were presented by author NW, which was evaluated and approved in joint sessions. The final manuscript was prepared by mutual understanding of all the authors.

Article Information

DOI: 10.9734/BJPR/2015/19068
Editor(s):
(1) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
Reviewers:
(1) Fatma Kandemirli, Biomediacal Engineer, Kastamonu University, Turkey.
(2) Anonymous, Medical University of Vienna, Austria.
(3) Anonymous, India.
(4) Spasojevic Natasa, University of Belgrade, Belgrade, Serbia.
Complete Peer review History: http://sciencedomain.org/review-history/10337

Original Research Article

Received 23rd May 2015
Accepted 4th July 2015
Published 26th July 2015

ABSTRACT

Three new Mannich bases of Fluoxetine (FA-1, FA-2, and FA-3) were synthesized and screened for their antidepressant activity through forced swim test. These new Mannich bases were synthesized in reflux condenser. Although none of the new compounds showed extra activity compare to its parent compound, nonetheless, they maintained antidepressant activity. The Mannich Bases were synthesized by attaching formaldehyde and different ketones to the secondary amine of fluoxetine at position-7. Their chemical structures have been confirmed by means of ^{1}H-NMR, ^{13}C-NMR and MS data.

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Keywords: Fluoxetine; mannich bases; antidepressant; carbonyl groups; forced swim test.

1. INTRODUCTION

Depression is a recurring, inveterate and possibly life-hazard illness victimizing about 20% of the world population. According to a World Health Organization (WHO) survey, it is one of the top ten causes of morbidity and mortality worldwide and 40–50% of the risks are genetic in nature. However, the particular genes that form the base for these risks are not yet known. Whist the remaining 50–60% non genetic risks are not well defined too, generic views are confined to identifying early childhood trauma, emotional stress, physical illness, and even viral infections as the possible causatives. Most of the experts agree on categorizing depression as a syndrome and not a disease [1-4]. Currently effective antidepressant drugs are available but their main problem is severe side effects and thus about 70 percent patients using antidepressants discontinue them pre-maturely resulting in severe withdrawal effects [5-7]. Fluoxetine ($C_{17}H_{18}F_3NO$) which is an early member of a newer class of antidepressant drugs commonly known as selective serotonin-reuptake inhibitors (SSRIs), got approval as a drug for the treatment of depression from FDA on 29 December 1987 [8]. It is in common use as a treatment for major depressive disorders (including pediatric depression), obsessive–compulsive disorder, bulimia nervosa and panic disorder but in the meantime it also possess severe side effects including sexual dysfunction, anorgasmia, delayed ejaculation, decreased libido, gastrointestinal disturbances, anxiety, sedation, nightmares and insomnia as well as suicidal attempts. These side effects causing the discontinuation of fluoxetine therapy. Due to these reasons, a greater need for faster acting, safer and more effective drugs for depression has always been felt [9-14].

Mannich reaction could be very helpful for synthesizing new effective drugs. The amino alkylation of CH-acidic compounds was explained by many chemists at the advent of 19th century; however, it was Professor Carl Mannich who for the first time recognized the significance of this reaction and deduced its mechanism resulting in this reaction being labeled after him. Mannich reaction is one of the most significant C-C bond forming reaction and has been used as a classical method for the preparation of Beta amino ketones and aldehydes which are called 'Mannich Bases'. Mannich bases are synthetic building blocks, which can easily be converted into a range of useful and valuable derivatives. In essence, it is a condensation reaction where amino alkylation of the acidic proton occurs just next to the carbonyl group with aldehydes (mostly formaldehyde) and amines either primary or secondary which result in β-amino-carbonyl compound (Mannich Base). Mannich reaction plays an important role in pharmaceutical chemistry and it is amongst the commonly used reaction in the preparation of several drug products, therefore we decided to use this reaction on fluoxetine [15-19].

2. EXPERIMENTAL

2.1 Materials and Methods

Fluoxetine was gifted by Wilson Pharmaceuticals Islamabad, Pakistan in its standard packing. All the chemicals and solvents used in this research were analytical grade, dried and purified before use. Gallen Kamp apparatus was used for recording the melting points through capillary method. The compounds were purified by recrystallization technique in suitable solvents. Reactions were performed in reflux condensers; the progresses of reactions were monitored through Thin Layer Chromatography (TLC) on silica Gel Plates GF-254 (Macherey-Nagel, Germany) and were visualized by using ultraviolet light at 254 nm and 366 nm on HP-UVIS Desaga (Heidelberg, Germany). Nuclear Magnetic Resonance spectra were recorded on AVANCE Spectrophotometer AV 300. Solvent used for NMR analysis was of spectroscopy grade Dimethyl Sulfoxide (DMSO). Chemical shifts are given in parts per million (ppm), and tetramethylsilane (TMS) was used as internal standard. Mass spectra were recorded by using JEOL JMS 600-H system high resolution Mass spectrometry.

2.2 General Procedure

Equimolar fluoxetine and paraformaldehyde were dissolved separately in 10 ml ethanol. Both solutions were mixed and kept on reflux condenser with gentle heating and stirring for 30 min. Ketone was added then to the reaction mixture and refluxed. Progress of reaction was monitored through TLC. After completion, the mixture was precipitated by cooling, the precipitate was filtered, washed and

recrystallized to obtain pure product. The structure of each compound is given in Fig. 1-3.

2.2.1 Synthesis of 4-(methyl(3-Phenyl-3-(4(trifluromethyl)phenoxy)propyl)amino) butane-2-one (FA-1)

Fluoxetine 2.78 g, 9 mmoles reacted with paraformaldehyde (9 mmoles) in ethanol absolute (10 ml) for 30 min, half pellet of NaOH was added to facilitate the solubility of paraformaldehyde and the mixture was placed on reflux condenser , then acetone (9 mmoles) was added to the reaction mixture and refluxed at 75Cº for 12 hrs. Progress and purity were checked through TLC (Methanol: Chloroform: 25%Ammonia=43:43:14). Finally the mixture was kept in refrigerator overnight (8 hrs) to get precipitate, the precipitate was then filtered through whatman filter paper, washed with ethanol and recrystallized in hot ethanol resulted in final crystals.

2.2.1.1 Product

Black sticky crystals; Melting Point=145Cº; Molecular Formula=$C_{21}H_{24}F_3NO_2$; Molecular Weight: 379.41 g/mole; %Yield=75%.

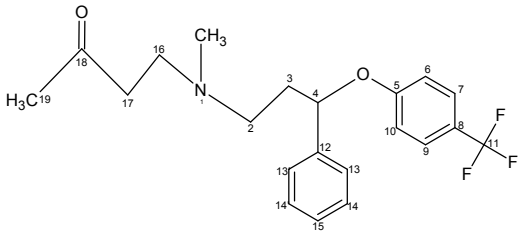


Fig. 1. Compound FA-1

The molecular ion peak of m/z 379.1 confirmed that the Mannich base has been formed. While the Proton NMR peak at C-16 was 3.35 ppm which gave strong evidence for Mannich Base as no other peak comes in this range and also the peak at C-17 was 2.08 ppm and C-19 at 2.07 ppm which further strengthened the evidence.

^{1}H NMR: (d6-DMSO, 300MHz) δ: 1.90 (s, 3H); 2.24 (d, 2H); 2.11 (t, 2H); 5.51 (d, 1H); 7.03 (s, 1H); 7.52 (s, 1H); 7.55 (s, 1H); 7.06 (s, 1H); 7.37 (s, 1H); 7.35 (d, 1H); 7.26 (d, 1H); 7.33 (d, 1H); 7.39 (s, 1H); 3.35 (d, 2H); 2.08 (d, 2H); 2.07 (s, 3H).

^{13}C NMR; DMSO (39.75); 30.12, 32.33, 43.17, 47.59, 53.14, 77.47, 115.19, 124.10, 128.26, 141.12, 160.63, 202.94 (see Table 1)

EIMS m/z (%): 379.41, 58.0, 72.0, 91.0, 104.0, 117.0, 135.0, 176.0, 218.0, 251.0, 309.0, 322.1, 337.0, 365.1, 379.1

Table 1. ^{1}H NMR and ^{13}CNMR data for compound FA-1

Carbon number	^{1}H NMR data	^{13}C NMR data
N-1	1.905	47.59
C-2	2.241	52.85
C-3	2.111	32.33
C-4	5.514	77.47
C-5	--------	160.63
C-6	7.039	114.90
C-7	7.525	126.77
C-8	--------	124.10
C-9	7.547	126.80
C-10	7.060	115.19
C-11	--------	125.99
C-12	--------	141.12
C-13	7.375	128.26
C-14	7.352	128.77
C-15	7.265	127.62
C-16	3.354	53.14
C-17	2.089	43.17
C-18	--------	202.94
C-19	2.074	30.12

4-(methyl(3-Phenyl-3-(4(trifluromethyl)phenoxy) propyl)amino)butane-2-one (FA-1)

2.2.2 Synthesis of 3-(Methyl (3-Phenyl-3(4-trifluromethyl)Phenoxy)Propylamino)1-Phenylpropan-1-one (FA-2)

Fluoxetine 2.78 g, 9 mmoles reacted with paraformaldehyde (9 mmoles) in ethanol absolute (10 ml) for 30 min, half pellet of NaOH was added to facilitate the solubility of paraformaldehyde and the mixture was placed on reflux condenser, then acetophenone(9 mmoles) was added to the reaction mixture and refluxed at 75C for 12 hrs. Progress and purity were checked through TLC (Methanol: Chloroform: 25%Ammonia=43:43:14). Finally the mixture was kept in refrigerator overnight (8 hrs) to get precipitate, the precipitate was then filtered through Whatman filter paper, washed with ethanol and recrystallized in hot ethanol resulted in final crystals.

The results of reaction are given in Scheme 1 ^{1}H NMR, C-13 NMR, and MS data for each compound is shown in Tables 1-4; & spectra for each compound are added for ready reference.

2.2.2.1 Product

Light yellowish crystals; Melting Point=260C°; Molecular Formula=$C_{26}H_{26}F_3NO_2$; Molecular Weight=441.48 g/mole; %Yield=77%.

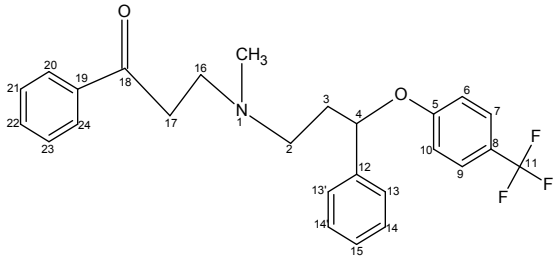


Fig. 2. Compound FA-2

The molecular ion peak of m/z 440.3 confirmed that Mannich base has been formed. Whilst the Proton NMR peak at C-16 was 3.38 ppm which gave strong evidence for Mannich Base as no other peak comes in this range and also the peak at C-17 was 3.62 ppm which further strengthened the evidence.

^{1}H NMR: (d_6-DMSO, 300MHz) δ: 2.02 (s, 3H); 2.22 (d, 2H); 2.06 (t, 2H); 4.03 (d, 1H); 7.46 (s, 1H); 7.54 (s, 1H); 7.57 (s, 1H); 7.48 (s, 1H); 7.38 (s, 1H); 7.32 (d, 1H); 7.30 (d, 1H); 7.34 (d, 1H); 7.40 (s, 1H); 3.38 (d, 2H); 3.62 (d,2H); 7.93 (s,1H); 7.69 (d,1H); 7.60 (d,1H); 7.63 (d,1H); 7.86 (s, 1H).

^{13}C NMR; DMSO (39.70); 30.27, 37.91, 40.89, 52.81, 54.63, 77.46, 126.0, 127.43, 128.38, 135.21, 161.25, 202.12 (see Table 2)

EIMS m/z (%): 441.48, 51.0, 77.0, 105.0, 133.0, 171.1, 199.1, 233.0, 273.0, 291.1, 303.0, 335.1, 378.0, 408.0, 423.1, 440.3

2.2.3 Synthesis of 2-((Methyl(3-Phenyl-3(4 trifluromethyl)Phenoxy)Propyl) amino) methyl) Cyclohexanone (FA-3)

Fluoxetine 2.78 g, 9 mmoles reacted with paraformaldehyde (9 mmoles) in ethanol absolute (10ml) for 30 min, half pellet of NaOH was added to facilitate the solubility of paraformaldehyde and the mixture was placed on reflux condenser , then cyclohexanone (9 mmoles) was added to the reaction mixture and refluxed at 75C for 12hrs. Progress and purity were checked through TLC (Methanol: Chloroform: 25% Ammonia=43:43:14). Finally the mixture was kept in refrigerator overnight (8hrs) to get precipitate, the precipitate was then filtered through Whatman filter paper, washed with ethanol and recrystallized in hot ethanol which resulted in final crystals.

Table 2. ^{1}H NMR and ^{13}CNMR data for compound FA-2

Carbon number	^{1}H NMR data	^{13}C NMR data
N-1	2.027	40.89
C-2	2.225	52.81
C-3	2.060	30.27
C-4	4.039	77.46
C-5	-------	161.25
C-6	7.465	126.00
C-7	7.546	127.99
C-8	-------	127.62
C-9	7.572	127.83
C-10	7.489	126.77
C-11	-------	127.43
C-12	-------	135.46
C-13	7.387	128.26
C-14	7.327	128.56
C-15	7.309	127.77
C-16	3.385	54.63
C-17	3.623	37.91
C-18	-------	202.12
C-19	-------	135.24
C-20	7.932	128.82
C-21	7.699	128.74
C-22	7.601	133.23
C-23	7.637	128.66
C-24	7.865	128.77

3-(Methyl(3-Phenyl-3(4-trifluromethyl)Phenoxy) Propylamino)1-Phenylpropan-1-one (FA-2)

2.2.3.1 Product

Off white crystals; Melting Point=130C°; Molecular Formula=$C_{24}H_{28}F_3NO_2$; Molecular Weight: 419.47 g/mole; %Yield=72%.

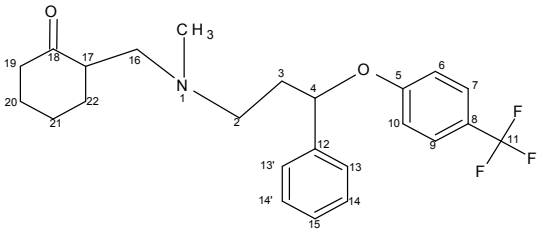


Fig. 3. Compound FA-3

The molecular ion peak of m/z 419.3 confirmed that Mannich base has been formed. While the Proton NMR peak at C-16 was 3.15 ppm which gave strong evidence for Mannich Base as no other peak comes in this range and also the peak

of C-17, C-19, C-20, C-21, C-22 were respectively at 2.19, 2.20, 2.08, 2.00, 2.06 ppm. The free proton 'NH' of fluoxetine is also a target.

Table 3. ^{1}H NMR and ^{13}C NMR data for FA-3

Carbon number	^{1}NMR data	^{13}C NMR data
N-1	2.107	42.21
C-2	2.243	53.89
C-3	2.226	31.50
C-4	4.791	77.42
C-5	------	160.80
C-6	7.542	115.98
C-7	7.615	125.21
C-8	------	120.89
C-9	7.608	125.96
C-10	7.528	116.10
C-11	------	123.71
C-12	------	141.13
C-13	7.410	127.16
C-14	7.348	128.57
C-15	7.332	126.76
C-16	3.157	58.48
C-17	2.195	48.57
C-18	------	210.46
C-19	2.204	42.15
C-20	2.082	28.61
C-21	2.009	24.96
C-22	2.068	31.66

2-((Methyl(3-Phenyl-3(4-trifluromethyl)Phenoxy) Propyl)amino)methyl)Cyclohexanone(FA-3)

^{1}H NMR: (d6-DMSO, 300MHz) δ: 2.10 (s, 3H); 2.24 (d, 2H); 2.2 (t, 2H); 4.79(d, 1H); 7.54 (s, 1H); 7.61 (s, 1H); 7.60 (s, 1H); 7.52 (s, 1H); 7.41 (s, 1H); 7.34 (d, 1H); 7.33 (d, 1H); 7.38 (d, 1H); 7.45 (s, 1H); 3.15 (d, 2H); 2.19 (m,2H); 2.20 (d,2H); 2.08 (m, 2H); 2.00 (m, 2H); 7.06 (m, 2H).

^{13}C NMR; DMSO (39.75) 24.96, 31.50, 42.21, 48.57, 53.89, 58.48, 77.42, 116.10, 123.71, 125.96, 128.97, 141.13, 160.80, 210.46 (see Table 3)

EIMS m/z (%): 419.47, 44.0, 58.0, 91.0, 110.0, 148.0, 174.9, 217.9, 232.0, 276.1, 309.0, 321.9, 338.1, 352.1, 367.1, 386.1, 419.3.

3. RESULTS AND DISCUSSION

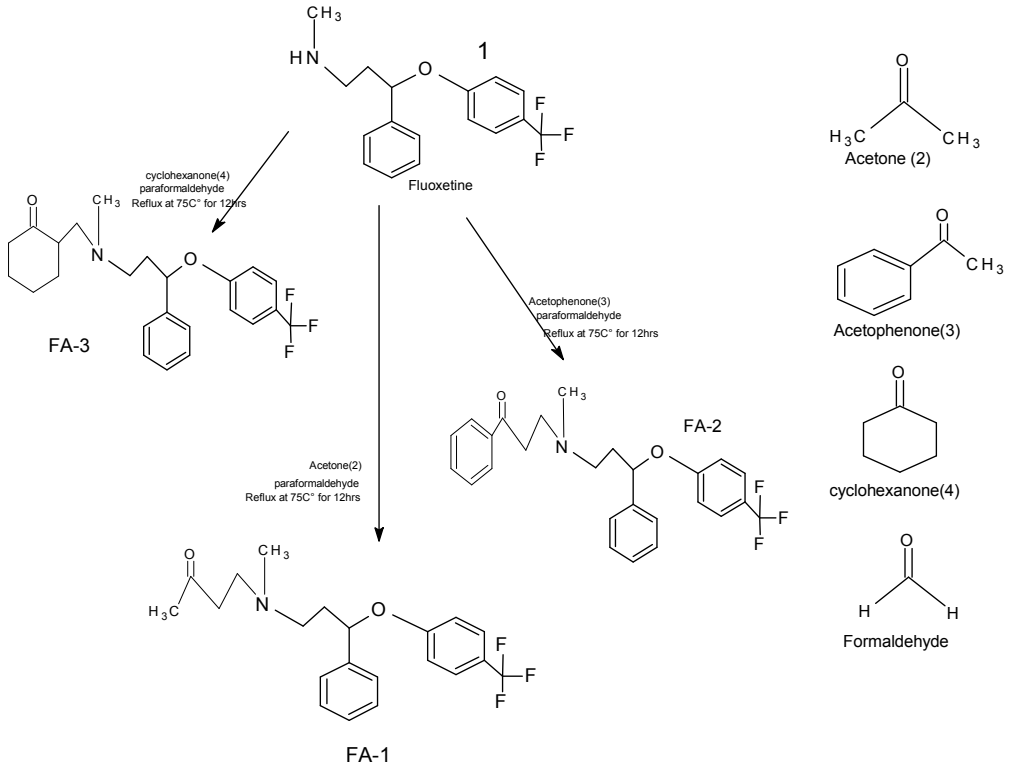
The free proton 'NH' of fluoxetine (Scheme: 1) is a potential point for synthesizing Mannich bases. In this present research work three novel Mannich bases (Fig. 1: FA-1, Fig. 2: FA-2, and Fig. 3: FA-3) were synthesized by reacting fluoxetine, paraformaldehyde with different ketones i.e. Acetone, Acetophenone and Cyclohexanone by using the standard procedure. The synthesized compound's purity was checked by TLC (Thin layer chromatography) on silica gel plates which gave a single spot, confirming purity of the product. The chemical structure of the compounds was determined by [1] H-NMR, [13] C-NMR, and Mass spectroscopy and finally antidepressant activity was performed on mice using Forced Swim Test (FST).

3.1 Antidepressant Activity of New Compounds in Mice

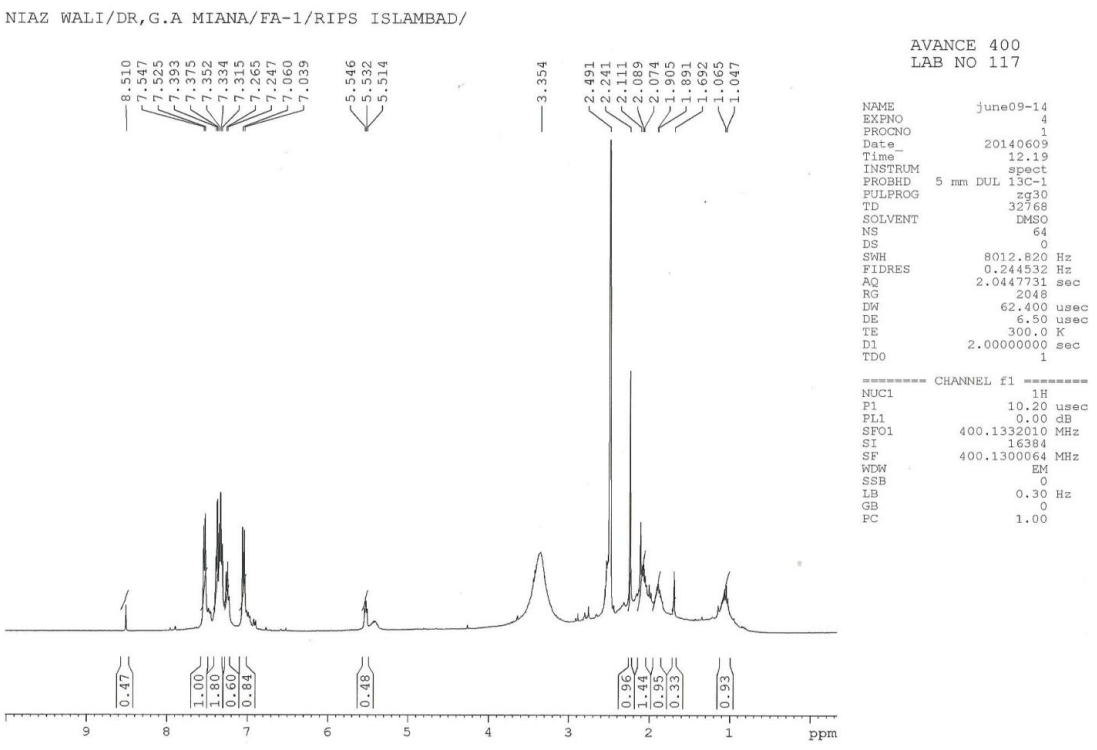
3.1.1 Procedure

The antidepressant activity of test samples was conducted using the forced swim test (FST). This test was based on stressful stimulus in which mice were put in water-jar about 25cm long and filled with water up to 10cm. The mice tried to escape by swimming and climbing from water jar but after a certain time it started floating on the surface of water without any further endeavor to escape, the situation being known as immobility. This condition could be described as "behavioral despair", where the animal loses hope to escape the stressful environment. So by administering antidepressant drugs, the immobility time will decrease. This immobility time is considered as an attribute of antidepressant effect. Total 48 albino mice with body weights between 20-25 g were divided into 08 groups each consisting of 6 mice. All these mice were subjected to daily treatment for a period of 14 days. On 14th day, instantly after intraperitoneal administration, each animal was separately allowed to swim freely in a transparent glass vessel (25 cm high, 10 cm diameter) filled with 10 cm of water at room temperature for a period of 05 minutes, as a pre test session, without recording any parameters. After 24 hours, forced swim test was performed in the same cylindrical vessel for 05 minutes [20]. The results of FST for Group 1-8 are presented in Table 4. The standard and treatment groups are explained as follows:

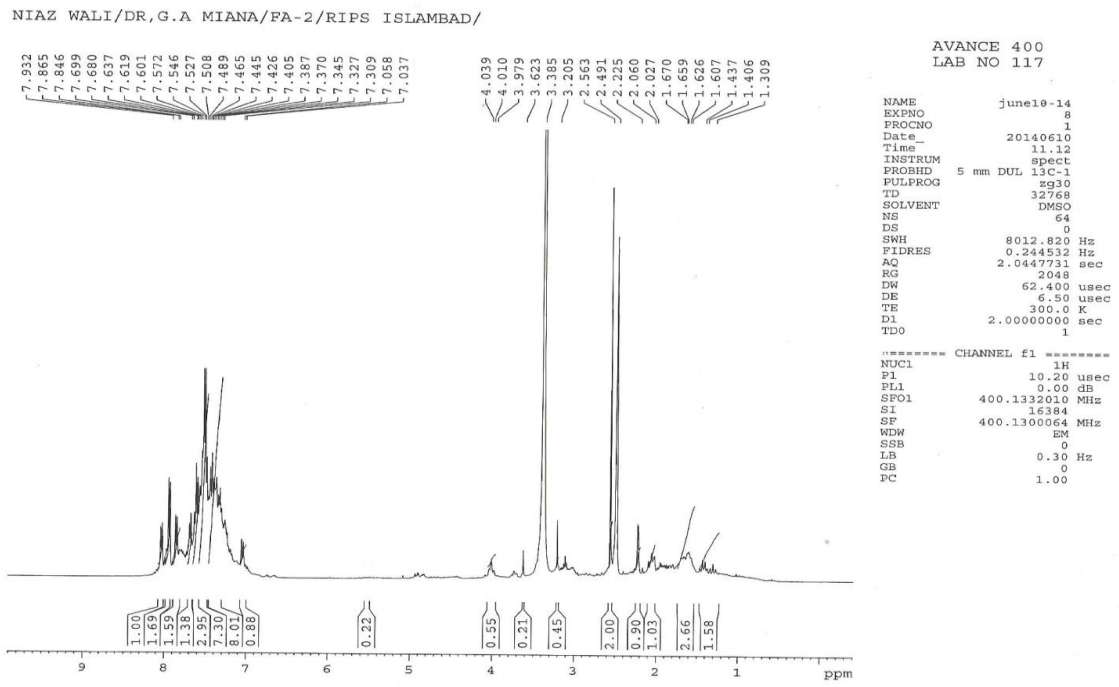
Group 1- Negative control (10 ml/kg, vehicle)
Group 2- FA-1- 10 mg/kg
Group 3- FA-1- 20 mg/kg
Group 4- FA-2- 10 mg/kg
Group 5- FA-2- 20 mg/kg
Group 6- FA-3- 10 mg/kg
Group 7- FA-3- 20 mg/kg
Group 8- *Fluoxetine* -10 mg/kg.



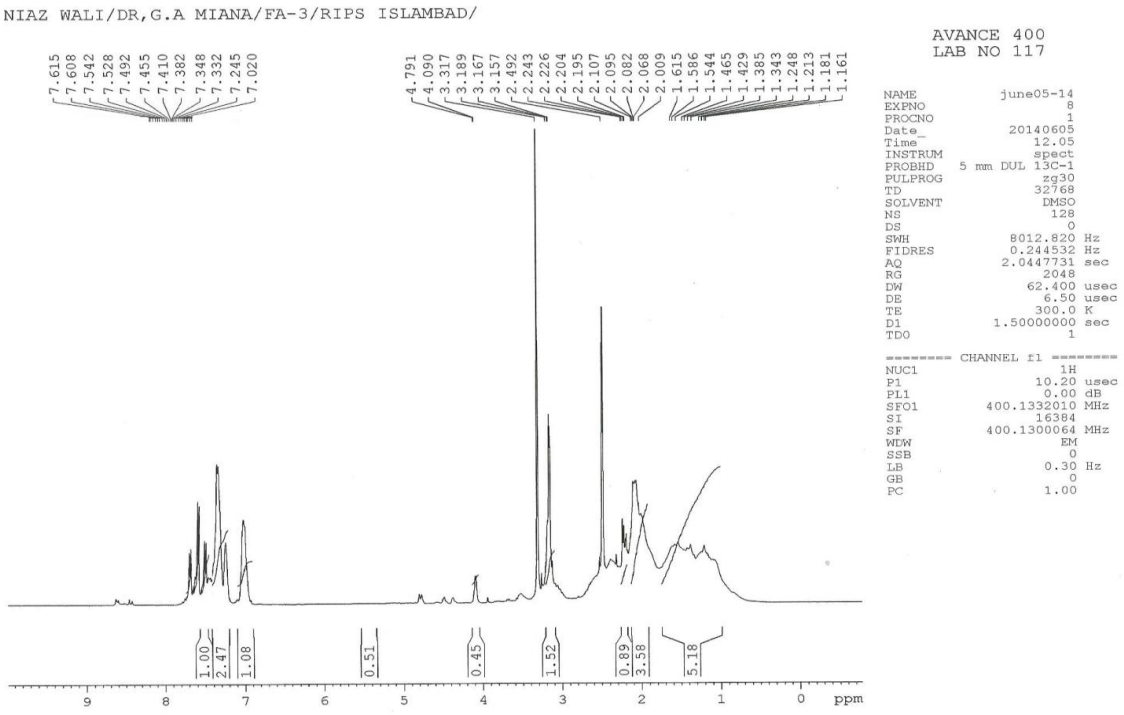
Scheme 1. Reaction of fluoxetine with different ketones resulting in Mannich bases.



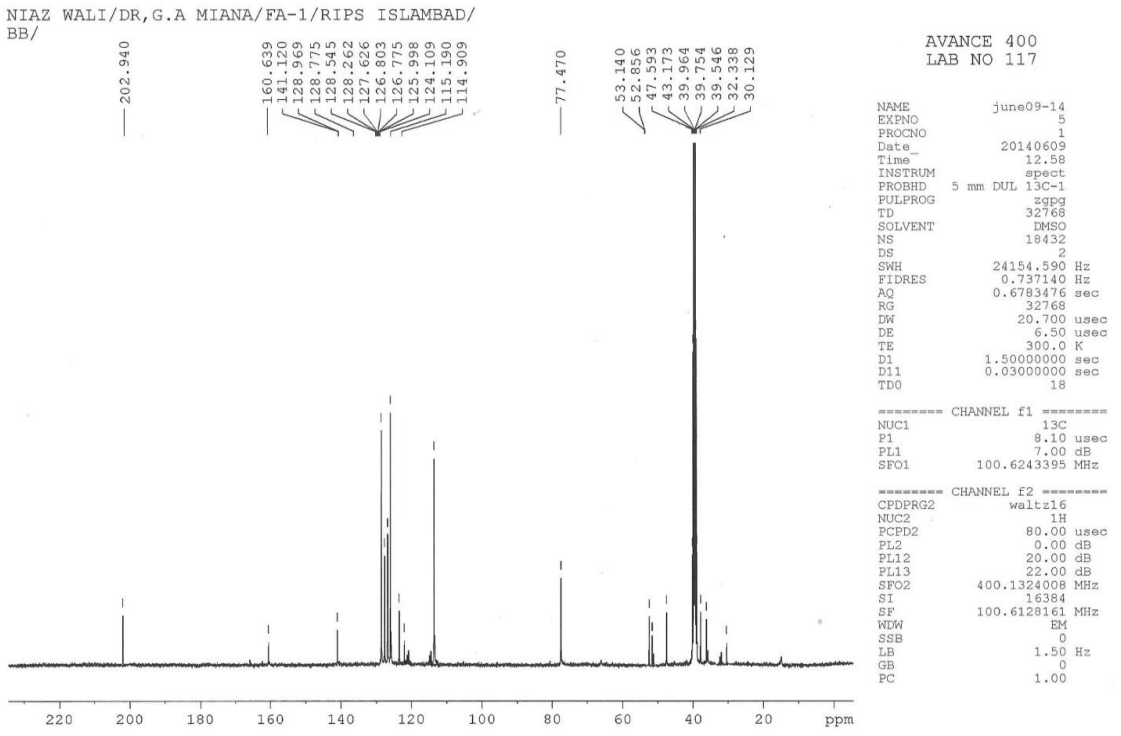
^{1}H NMR spectrum of compound FA-1



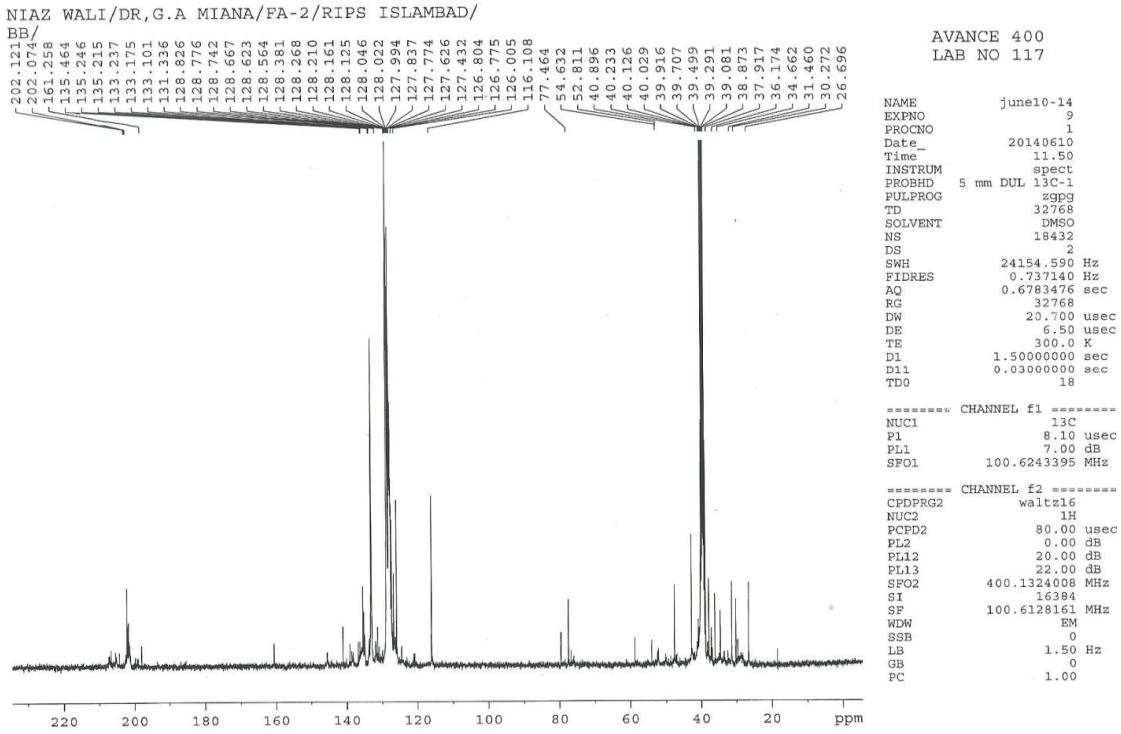
^{1}H NMR spectrum of compound FA-2



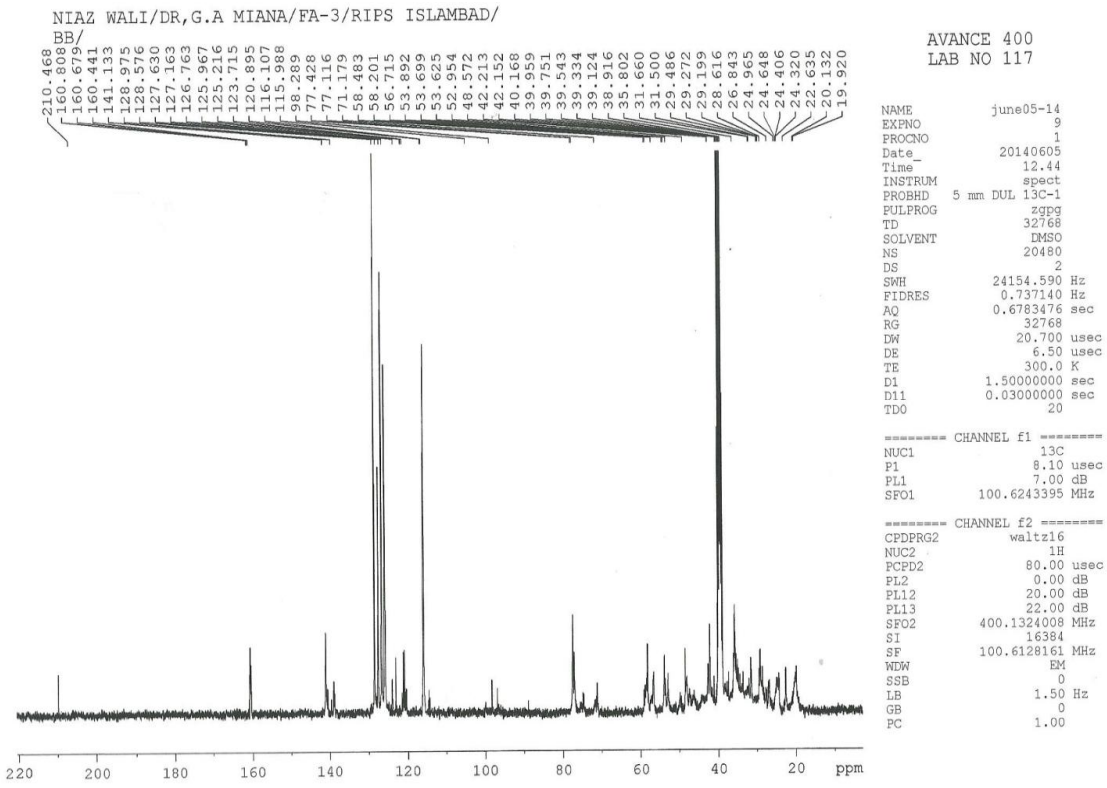
^{1}H NMR spectrum of compound FA-3



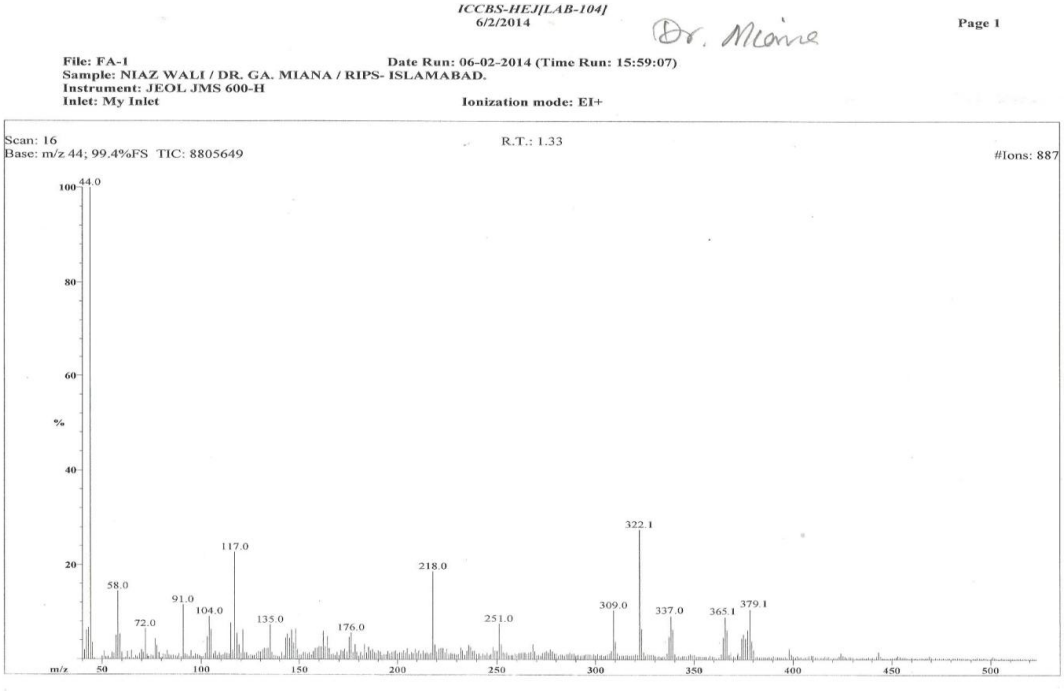
C-13 NMR spectrum of compound FA-1



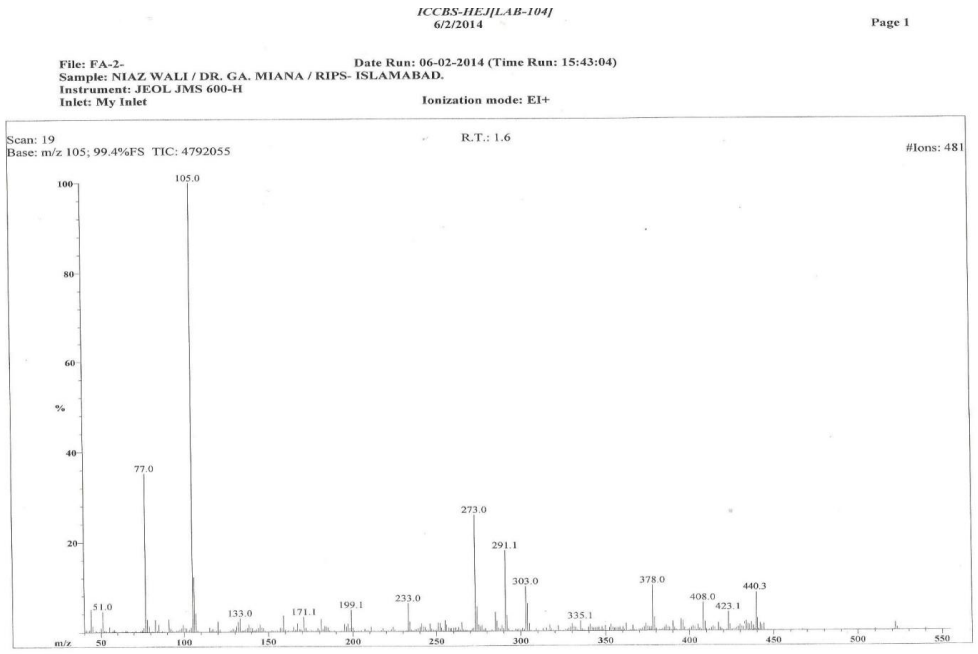
C-13 NMR spectrum of compound FA-2



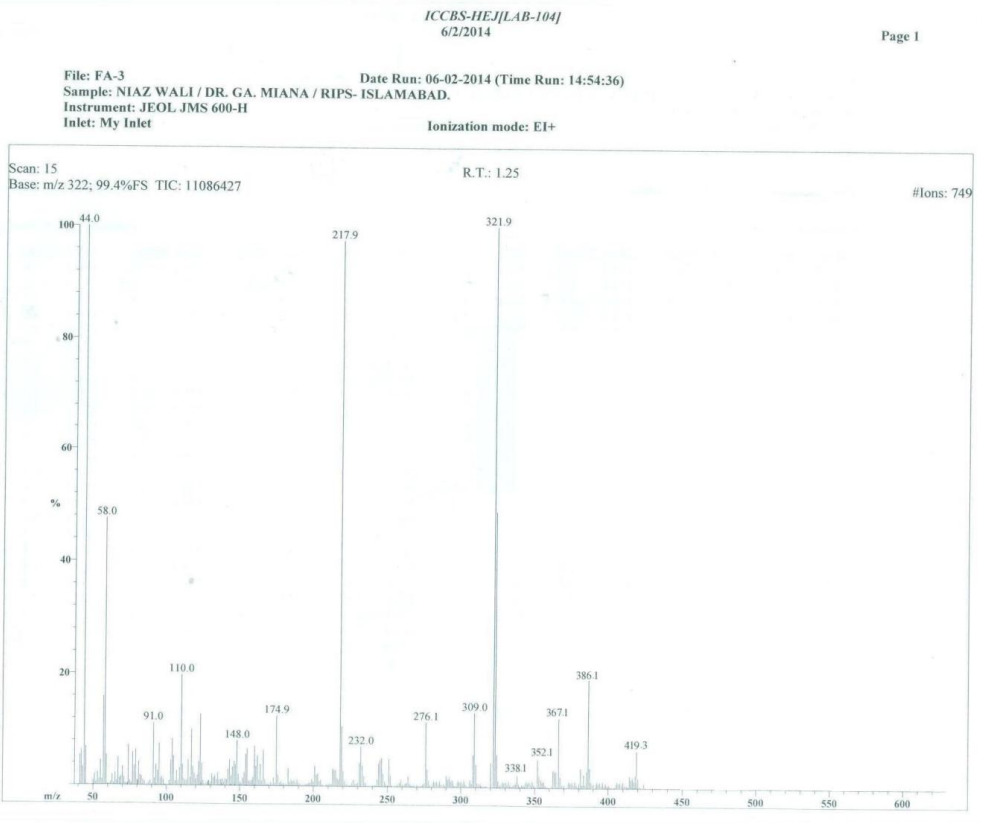
C-13 NMR spectrum of compound FA-3



Mass spectrum of compound FA-1



Mass spectrum of compound FA-2



Mass spectrum of compound FA-3

Table 4. Antidepressant effect of compounds on duration of immobility, climbing and swimming of mice in forced swimming test (FST)

Treatment (mg/kg)	Treatment (mg/kg)	Immobility (second)	Climbing (second)	Swimming (second)
Control	-	107.41±3.78	93.18± 4.92	98.61±3.27
FA-1	10	96.20±3.38	98.21±2.34	101.73±2.69
FA-1	20	92..47±2.09*	102.41±2.14*	107.52±3.08
FA-2	10	99.73±4.05	100..87±3.63	102.64±2.17
FA-2	20	93..97±3.65*	101.54±2.77*	105.97±2.56
FA-3	10	104.34±3.79	94..48±3.18	100.86±3.52
FA-3	20	100.22±2.61	99.04±2.56*	102.72±2.79
Fluoxetine	10	77.93±1.28**	106.71±2.14**	117.03±3.27

*Statistical significance *P<0.05 and **P<0.01*

4. DISCUSSION AND CONCLUSION

Mannich bases of antidepressant fluoxetine (Selective serotonin reuptake inhibitor) were synthesized in reflux condenser by using paraformaldehyde and different ketones like acetone, acetophenone, cyclohexanone. Percentage yield of all Mannich bases (FA-1, FA-2, and FA-3) was good i.e. 75%, 77% and 72% respectively. Antidepressant activity was performed by adopting the standard procedure of forced swim test and recorded the different measures of depression like Swimming (Active movements of extremities and circling in the cylinder), Climbing/Trashing (Active upward directed movements of forelimbs on the container wall) and Immobility (floating in water without swimming i.e. mice did not attempt to escape except the movement which was necessary for its head to keep it above the water) were recorded as a measure of depression. Although none of the new compound showed extra activity compared to its parent compound nonetheless, they maintained antidepressant activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the animal house ethics committee, Rifah University, Islamabad, Pakistan.

ACKNOWLEDGEMENT

The authors are thankful to Wilson Pharmaceuticals Islamabad, Pakistan for the supply of Fluoxetine in its standard packing which was used in the current experiment as a reference.

COMPETING INTERESTS

The work presented is for academic affairs. The authors have no conflict of interest or any competing interest with any institution or industry or organization. The authors have nothing to declare.

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