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Antidepressant molecules: Synthesis and evaluation of novel azetidinone compounds

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Abstract

In the effort to develop new potential molecule for the betterment of the health condition, a few of novel substituted azetidinones were synthesized and characterized for antidepressant action. All the synthesized compounds were characterized for their structure elucidation. Antidepressant studies of these compounds indicated that the compounds were having significant antidepressant activity. The synthesis of the compounds was performed in three steps that utilized SN_2 type substitution. The IR spectra of all the compounds exhibited the stretching vibration peaks due to C-N, C=O, C=N at 1400-1000 cm^{-1}, 1765-1645 cm^{-1} and 1690-1520 cm^{-1} (medium) respectively. The other vibrations that appeared in the spectra included those from aromatic C=C and C-H, C-H alkane, C-Cl **(4e),** C-O **(4d),** C-H (methoxy), C-C (cycobutyl) and C=O (cycobutyl). The ^{1}HNMR spectra obtained displayed the peaks of aliphatic CH and aromatic CH at 2-3.3, and 6.7-7.2 ppm respectively. The mass spectra displayed the molecular ion peak and the isotopic peaks as calculated. The reference drug fluoxetine and the test compounds **4b, 4d** and **4e** decreased the immobility of mice in FST whereas the swimming frequency was increased significantly. An activity profile similar to TST was seen and the compounds **4a, 4c** and **4f** did not give the expected results.

Keywords: Azetidinone, fluoxetine, CNS, depressant, FST, TST

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Introduction

Depression is one of the most common psychiatric disorders that has been characterized and classified in a several ways. The most commonly prescribed drug for depression such as imipramine, desipramine, fluoxetine etc. nowadays have been limited due to their potential side effects.

Azetidin-2-one**,** a four-membered cyclic lactam (ß-lactam) skeleton has been recognized as an attractive target of contemporary organic synthesis of a large number of organic molecules by exploiting the strain energy associated with it. It possesses good pharmacological and biological activities like antimicrobial (Pandya and Desai, 2013), antibacterial (Khan et al, 2018), antifungal (Patel and Mehta, 2006) anti- inflammatory (Rajasekaran et al, 2010), antitubercular (Dubey et al, 2013), anticancer and cytotoxic (Deep et al, 2016). Some attempts to design newer antidepressant molecules using azetidinone have also been reported (Kerzare et al, 2018; Thomas et al, 2016).

In view of the above limitations and increasing demand of antidepressant drugs it was hypothesized that utilizing a simple nitrogen bearing heterocycle like azetidinone as a part of larger molecule may be able to present antidepressant action and may also inculcate properties favourable for interaction with the enzymes involved in depression.

Material and Methods

The azetidinone derivatives were characterized for solubility, yield, physical appearance, melting point and spectral characteristics. The melting points were determined on melting point apparatus and are uncorrected. IR spectra are recorded on Bruker spectrophotometer, mass and proton NMR spectra were recorded on Jeol system. The solvent system used for TLC of the compound was Methanol : Ethyl Acetate in the ratio 4:6for the desired products and 8:2 / 7:3 for determining the purity (completion of reaction) ofthe intermediate products.

The scheme for synthesis of azetidinone was adapted from report by Kerzare et al (2018). The synthesis of compounds was accomplished according to the scheme depicted in Figure 1.

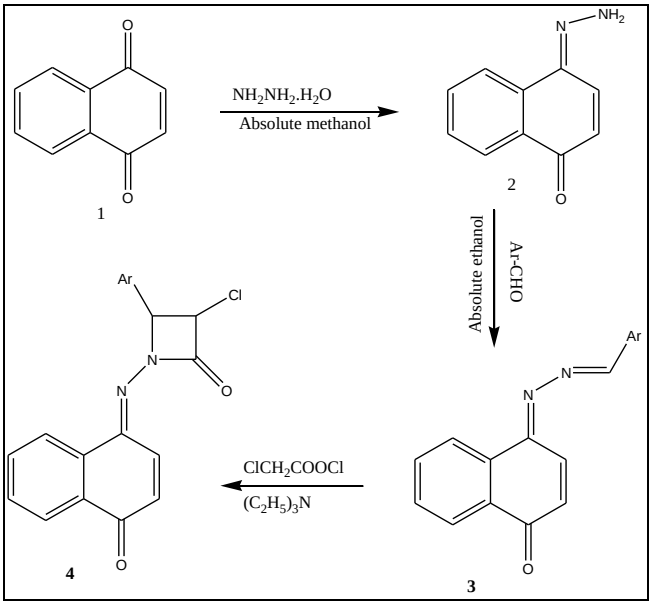

Figure 1 Scheme of the synthesis of Azetidinone Derivatives

Synthesis of (E)-4-hydrazononaphthalen-1(4H)-one (2)

A weighed quantity of napthoquinone (15.08 g, 0.1 mol) was added to hydrazine hydrate (99%, 5.05 g, 1.1 mol) in 100 ml of absolute methanol and the mixture was refluxed for 1 h followed by cooling to room temperature. Crystals of hydrazone that precipitated out was filtered and dried. The crude product was recrystallized using ethanol to obtain the pure hydrazone.

Synthesis of Substituted (E)-4-(2-methylenehydrazono)naphthalen-1(4H)-one (3a-f)

To a solution of compound **2** (0.01 mol) in ethanol (60 mL), substituted aromatic aldehyde (0.01 mol) and with a few drops of glacial acetic acid were added. The resulting mixture was then refluxed for 7-8 h. The excess of the ethanol was distilled off and the remaining mixture was cooled, poured onto crushed ice and filtered. The crude product obtained was recrystallized from 70% ethanol.

Synthesis of (E)-3-chloro-1-(4-oxonaphthalen-1(4H)-ylideneamino)azetidin-2-one (4a-f)

A mixture of Schiff base **(3a-f)** (0.01 mol) and triethylamine (0.02 mol) was dissolved in 1, 4-Dioxane (15 mL). To this, a solution of chloroacetyl chloride (0.02 mol) was added in portions with vigorous shaking at room temperature for 20 min. The reaction mixture was heated under reflux for 3 h and the content was kept at room temperature for 48 h and poured into ice-cold water. The resulting solid was filtered, washed several times with water and then recrystalised from 70% ethanol.

(E)-3-chloro-1-(4-oxonaphthalen-1(4H)-ylideneamino)-4-phenylazetidin-2-one1H, ***4a***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

(E)-3-chloro-4-(4-nitrophenyl)-1-(4-oxonaphthalen-1(4H)-ylideneamino)azetidin-2-one, ***4b***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

(E)-3-chloro-4-(2-nitrophenyl)-1-(4-oxonaphthalen-1(4H)-ylideneamino)azetidin-2-one, ***4c***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

(E)-3-chloro-4-(4-methoxyphenyl)-1-(4-oxonaphthalen-1(4H)-ylideneamino)azetidin-2-one, ***4d***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

(E)-3-chloro-4-(4-chlorophenyl)-1-(4-oxonaphthalen-1(4H)-ylideneamino)azetidin-2-one, ***4e***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

(E)-3-chloro-4-(4-(dimethylamino)phenyl)-1-(4-oxonaphthalen-1(4H)-ylideneamino) azetidin-2-one, ***4f***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3000-2900 cm^{-1} (C-C cyclic), 1600-1700 cm^{-1} (C-C Ar), 3100-3000cm^{-1} (CH Ar), 1500cm^{-1} (C=N), 1400-1000 cm^{-1} (C-N), 1100-1020 (C-Cl)

Evaluation of antidepressant action (Hsu et al, 2012)

The *in vivo* antidepressant action of the synthesized compounds was carried out in male albino mice weighing between 25–30 g by FST and TST method.

The animal were grouped and housed in poly acrylic cages (38x23x10 cm) in the animal house of the institute. Not more than four animals per cage were housed and maintained under standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week for acclimatization before and during the experiments.

Animal were divided into 7 groups of 6 animals each for conducting the study. Group I was administered with normal saline and served as control, group II, III, IV,V & VI were administered 40 mg/kg (i.p) of the test compounds, whereas group VII served as positive control and was administered with fluoxetine, 10 mg/kg (i.p).

Forced Swim Test

The synthesized compounds and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually placed in a glass cylinder (25 cm height, 10 cm diameter) filled with water (22-25°C) up to 10 cm height. Each mouse was allowed to swim for 6 minutes during the test, and the duration of immobility was observed and noted during the final 4 minutes of the test. The time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above water was regarded as the immobility period.

The animals were dried using tower and returned back to their housing conditions.

Tail Suspension Test

The synthesized compounds and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually suspended by tail using clamp (2 cm from the tip of the tail) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Minimal background noise was maintained and the testing was carried out in dark room. All animals were suspended for total 6 minutes, and the duration of immobility was observed and noted during the final 4 minutes of the test. Mice were considered immobile only when they hung passively and completely motionless.

The animals were used only once for this test.

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.05 ($P<0.05$) vs control.

Results and Discussion

The yield, melting point and molecular weight (calculated) of the synthesized compound were depicted in the table 1 while the solubility of the compounds is presented in table 2.

The IR spectra of all the compounds exhibited the stretching vibration peaks due to C-N, C=O, C=N at 1400-1000 cm^{-1}, 1765-1645 cm^{-1} and 1690-1520 cm^{-1} (medium) respectively. The other vibrations that appeared in the spectra included those from aromatic C=C and C-H, C-H alkane, C-Cl **(4e),** C-O **(4d),** C-H (methoxy), C-C (cycobutyl) and C=O (cycobutyl). The ^{1}HNMR spectra obtained displayed the peaks of aliphatic CH and aromatic CH at 2-3.3, and 6.7-7.2 ppm respectively. The mass spectra displayed the molecular ion peak and the isotopic peaks as calculated.

CNS depressant action

The antidepressant action of the synthesized compounds was testing using two animal models and the results obtained are depicted in Figure 5.1 and 5.2. The immobility time was recorded and statistically analyzed using one way ANOVA followed by Dunnett's multiple comparison test.

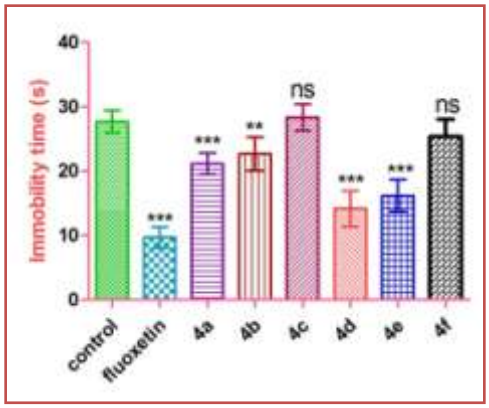

Figure 2 Effect of test compounds 4a-4f (40mg/kg) and fluoxetine (10mg/kg) on immobility time of mice in TST. *p<0.05, **p<0.01, ***p<0.001, Values are represented as mean ± SD, (n=6)

As it can be seen from Figure 2 that the immobility time for the compounds **4b, 4d**and **4e** was much lower than the control group and was comparable to that of fluoxetine at a dose on 10mg/kg. However the results obtained by compounds 4**a, 4c** and **4f** were not as effective as it was expected (p<0.05) signifying the presence of substitution on the piperazine nitrogen for antidepressant effect.

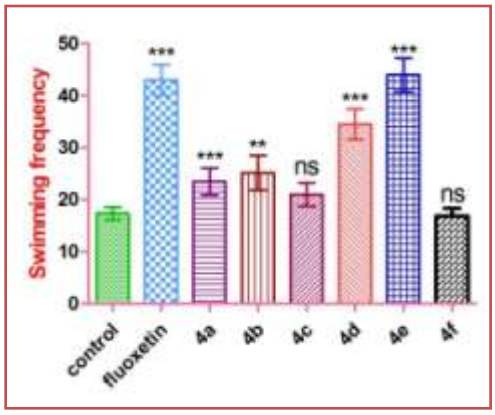

Figure 3 Effect of test compounds 4a-4f (40mg/kg) and fluoxetine (10mg/kg) on immobility time of mice in FST. **p<0.05, ***p<0.001, ns-not significant, Values are represented as mean ± SD, (n=6)

As it can be seen from Figure 2 and 3 that the reference drug fluoxetine and the test compounds **4b, 4d** and **4e** decreased the immobility of mice in FST whereas the swimming frequency was increased significantly. An activity profile similar to TST was seen and the compounds **4a, 4c** and **4f** did not give the expected results.

Conclusion

The present work focused on synthesizing new azetidinone derivatives possessing antidepressant potential. The synthesized compounds with diverse substitution pattern were able to exhibit antidepressant action. Further studies on new compounds of similar structure would be carried out in order to derive a relation between the structure and activity of the nucleus.

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Swimming Test and in the Tail Suspension Test in Mice. Evidence Based Complementary Alt Med Article ID 497302; https://doi.org/10.1155/2012/497302

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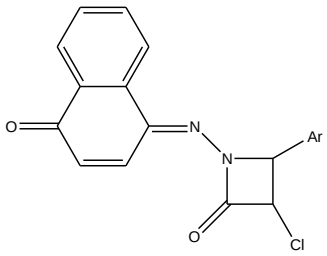
Table 1 Properties of the azetidinone compounds



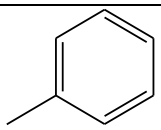
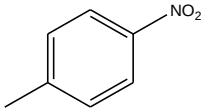
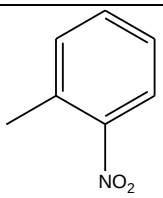
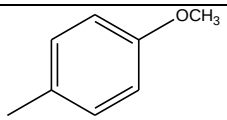
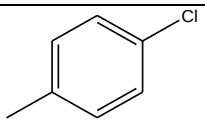
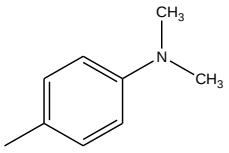
Code	----Ar	Color	M.P (˚C)	% Yield	Molecular weight (Calculated)
4a		Yellow	172-174	70	336
4b		Brown	191-193	68	381
4c		Brown	204-206	62	381
4d		Yellow	169-173	64	366
4e		Yellow	211-214	61	371
4f		Yellow	196-199	66	379

Table 2 Solubility profile of the synthesized azetidinones

Comp. Name	Solubility Profile			
	Water	**Methanol**	**$CHCl_3$**	**Acetone**
4a	Insoluble	Partially Soluble	Freely Soluble	Partially Soluble
4b	Insoluble	Partially Soluble	Freely Soluble	Partially Soluble
4c	Insoluble	Partially Soluble	Freely Soluble	Partially Soluble
4d	Insoluble	Partially Soluble	Partially Soluble	Freely Soluble
4e	Insoluble	Partially Soluble	Partially Soluble	Freely Soluble
4f	Insoluble	Partially Soluble	Freely Soluble	Partially Soluble