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Synthesis, characterization, antianxiety activity and 3D-QSAR study of some novel indole bearing azetidinone derivatives

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Abstract : The manuscript describes synthesis of some novel indole bearing azetidinone derivatives and their evaluation for antianxiety activity. The compounds were synthesized following three step reaction to yield twenty derivatives as 3{[3-chloro-2-substituted-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one **(23-42)**. All the final structures were assigned on the basis of IR, ^{1}H NMR, mass spectra and elemental analyses.The final derivatives 3{[3-Chloro-2-(4-hydroxy,3-methoxy phenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one **(28)** and 3{[3-Chloro-2-(4-(Dimethylamino)phenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one **(29)** were found to be promising molecule in the series. The dimethyl amino and hydroxy substitution on the para position of phenyl ring system provided with active compounds having percentage preferences of open arm with 69.44and 83.33 respectively at 50 mg/kg dose level when compared to the standard drug. 3D-QSAR results revealed that addition of electropositive and bulky groups at the phenyl ring will contribute towards increase in the antianxiety activity of the molecules while electronegative groups decreases the activity.
Keywords : Indole, Azetidinone, Antianxiety activity, 3D-QSAR.

Introduction

Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behaviour, such as pacing back and forth, somatic complaints, and rumination[1]. It is also a generalized mood or condition that occurs without an identifiable triggering stimulus. As such, it is distinguished from fear, which occurs in the presence of an observed threat. Additionally, fear is related to the specific behaviours of escape and avoidance, whereas, anxiety is the result of threats that are perceived to be uncontrollable or unavoidable[2]. Anxiety can be appropriate, but when experienced regularly the individual may suffer from an anxiety disorder. People facing anxiety may withdraw from situations which have provoked anxiety in the past [3-5]. The physiological symptoms of anxiety may include, headache, vertigo, nausea, palpitations, itchy skin and frequent urination[6,7]. The physiological reason for anxiety have not been completely established, but some facts suggested that there is decrease in the level of GABA or relative deficiency in the GABA neurotransmission, which can be augmented by agents acting on different components of the GABA system[8].

In an attempt to synthesize and evaluate new compounds as antianxiety agents, we report herein, synthesis, biologicalevaluation and 3D-QSAR studies of a number of indole bearing azetidinone derivatives. Indoles are considered as the most promising bicyclic heteroaromatic nucleus in the field of medicinal

chemistry. The Indole framework is a medicinally relevant scaffoldand has become widely identified as a privileged structure orpharmacophore due to its different pharmacological actions[9-13]. Literature survey also suggests the potential of azetidinone derivatives as antianxiety agents[14]. Compounds containing the azetidinonenucleus have been reported to possess diverse pharmacologicalactivities[15-18]. The indole moiety is present in approvedas well as experimental drugs and the azetidinone moiety in experimental drugs[19-21]. Therefore, in the present investigation, it was envisagedthat if these two pharmacophores are linked together thiswould generate new molecular templates as shown in **Figure1**, whichare likely to exhibit antianxiety-like action in animalmodels.The synthetic strategy for the final derivatives involved in the reaction between schiff bases with chloroacetyl chloride in the presence of a catalytic amount of triethylamine. The scheme of the present study is outlined in**Figure 2**along with the substitutions shown in **Table 1.**

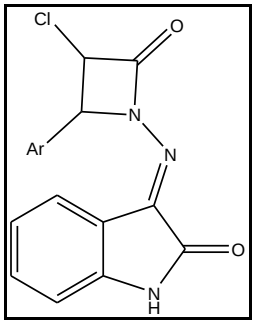

Figure 1: General structure of proposed scaffold as antianxiety agents

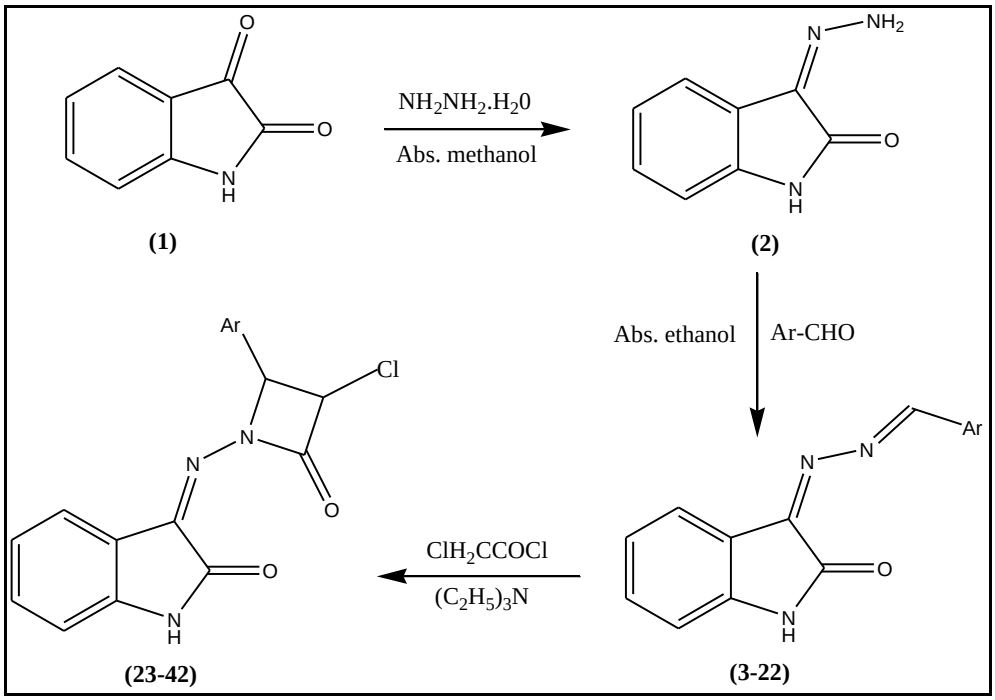

Figure 2: Scheme synthetic route of the titled compounds

Table 1: List of substitutions

Compound No.		**-Ar**	**Compound No.**		**-Ar**
3	**23**	C_6H_5-	**13**	**33**	4-CH_3-C_6H_5-
4	**24**	4-Cl-C_6H_5-	**14**	**34**	2-OCH_3-C_6H_5-
5	**25**	4-C_6H_5-CH_2O-C_6H_5-	**15**	**35**	4-OCH_3-C_6H_5-
6	**26**	2-NO_2-C_6H_5-	**16**	**36**	2-Cl-C_6H_5-
7	**27**	4-OH-C_6H_5-	**17**	**37**	3-Br-C_6H_5-

8	**28**	4-OH,3-OCH_3-C_6H_5-	**18**	**38**	2,4-$(OCH_3)_2$-C_6H_5-
9	**29**	4-$(CH_3)_2$-N-C_6H_5-	**19**	**39**	4-NO_2-C_6H_5-
10	**30**	3-NO_2-C_6H_5-	**20**	**40**	2,3-$(OCH_3)_2$-C_6H_5-
11	**31**	3,4,5-$(OCH_3)_3$-C_6H_5-	**21**	**41**	3-Cl-C_6H_5-
12	**32**	2,4-$(Cl)_2$-C_6H_5-	**22**	**42**	C_4H_3O- (2-furyl)

Experimental work

Chemistry

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA), S D Fine-Chem (Mumbai, MH, India) and Merck (Darmstadt, Germany), unless specified. Melting points (m.p.) were detected with open capillaries using ThermoNik precision melting point cum boiling point apparatus (model C-PMB-2, Mumbai, MH, India) and are uncorrected. Infrared (IR) spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Tokyo, Japan) at the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharai (RTM) Nagpur University. Proton (1H) and carbon 13 (13C) nuclear magnetic resonance (NMR) were obtained using a BrukerAvance II 400 MHz spectrometer (Billerica, MA, USA), using tetramethylsilane (TMS) as internal standard. All chemical shift values were recorded as d (ppm), coupling constant value *J* is measured in hertz, the peaks are presented as s (singlet), d (doublet), t (triplet), brs (broad singlet), dd (double doublet), m (multiplet). The purity of compounds was controlled by thin layer chromatography (silica gel HF254e361, type 60, 0.25 mm; Merck, Darmstadt, Germany). Electrospray ionization mass spectrometry (ESI-MS) was recorded at Waters Q-TOF spectrometer (Waters, Milford, MA, USA) at the Sophisticated Analytical Instrumentation Facility (SAIF), Punjab University (Chandigarh, PB, India).

Synthesis of 3-hydrazinylidene-1,3-dihydro-2H-indol-2-one (2)

A mixture of isatin (**1)** (1mmol) and hydrazine hydrate (99%, 0.055 g, 1.1 mmol) in absolute methanol (25 ml) was refluxed for 1 h and then cooled to room temperature. The precipitate of hydrazones was filtered and dried. The crude product was recrystallized from ethanol to give hydrazones (**2)**.

Yield: 70 %; mp 248-250 ºC; R_f0.39 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3411-3319, (NH, NH_2), 1687 (C=O), 1609 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.87-7.37 (m, 4H, Ar-H), 10.57 (s, 2H, NH_2), 9.39 (s, 1H, NH); EI-MS: m/z $[M+H]^+$ 162.24

General synthesis of 3-[(substituted benzylidene)hydrazinylidene]1,3-dihydro-2H-indol-2-one (3-22)

To a solution of compound **2** (0.01 mol) in ethanol (60 mL), substituted aromatic aldehyde (0.01 mol) along with few drops of glacial acetic acid were added. Then resulting mixture was 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 was recrystallized from 70% ethanol.

General synthesis of 3{[3-chloro-2-substituted-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (23-42)

A mixture of Schiff base **(3-22)**(0.01 mol) and triethyl amine (0.02 mol) was dissolved in 1, 4-Dioxane (15 m1). To this, a solution of chloroacetyl chloride (0.02 mol) was added in portion wise 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.

3{[3-Chloro-4-oxo-2-phenylazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (23)

Yield: 63%; mp 172-174 ºC; R_f0.53 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3367 (NH), 1704 (C=O), 1542 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.03-8.37 (m, 9H, Ar-H), 8.66 (s, 1H, NH), 6.88 (d, 1H, CH-Ar); 5.48 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 326.74. Anal. Calcd for $C_{17}H_{12}ClN_3O_2$: C, 62.76; H, 3.69; N, 12.92. Found: C, 61.45; H, 2.79; N, 11.77.

3{[3-Chloro-2-(4-chlorophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (24)

Yield: 71%; mp 191-193ºC; R_f0.61 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3369 (NH), 1699 (C=O), 1521 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.10-7.95 (m, 8H, Ar-H), 8.65 (s, 1H, NH), 5.88 (d, 1H, CH-Ar); 5.04 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 361.94. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_2$: C, 56.66; H, 3.05; N, 11.66. Found: C, 55.21; H, 2.79; N, 10.75.

3{[3-Chloro-2-(4-benzoyloxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (25)

Yield: 61%; mp 135-137 ºC; R_f0.55 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3267 (NH), 1698 (C=O), 1545 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.05-8.89 (m, 13H, Ar-H), 9.56 (s, 1H, NH), 4.78 (d, 1H, CH-Ar); 6.12 (d, 1H, CH-Cl); 5.20 (d, 2H, CH_2); EI-MS: m/z $[M+H]^+$ 432.87. Anal. Calcd for $C_{24}H_{18}ClN_3O_3$: C, 66.70; H, 4.16; N, 9.72. Found: C, 65.61; H, 3.89; N, 9.15.

3{[3-Chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (26)

Yield: 63%; mp 168-170ºC; R_f0.42 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3395 (NH), 1675 (C=O), 1586 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.84-8.26 (m, 8H, Ar-H), 8.97 (s, 1H, NH), 5.07 (d, 1H, CH-Ar); 5.49 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 371.74. Anal. Calcd for $C_{17}H_{11}ClN_4O_4$: C, 55.13; H, 2.97; N, 15.13. Found: C, 54.33; H, 1.97; N, 14.94.

3{[3-Chloro-2-(4-hydroxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (27)

Yield: 72%; mp 211-213ºC; R_f0.58 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3324 (NH), 1723 (C=O), 1576 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.66-7.92 (m, 8H, Ar-H), 8.45 (s, 1H, NH), 5.66 (d, 1H, CH-Ar); 6.71 (d, 1H, CH-Cl); 9.56 (s, 1H, OH); EI-MS: m/z $[M+H]^+$ 342.74. Anal. Calcd for $C_{17}H_{12}ClN_3O_3$: C, 59.82; H, 3.51; N, 12.31. Found: C, 58.51; H, 2.98; N, 11.87.

3{[3-Chloro-2-(4-hydroxy,3-methoxy phenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (28)

Yield: 67%; mp 178-180ºC; R_f0.44 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3289 (NH), 1689 (C=O), 1557 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.72-8.02 (m, 7H, Ar-H), 8.75 (s, 1H, NH), 5.21 (d, 1H, CH-Ar); 6.55 (d, 1H, CH-Cl); 2.76 (s, 3H, OCH_3); 9.87 (s, 1H, OH); EI-MS: m/z $[M+H]^+$ 372.77. Anal. Calcd for $C_{18}H_{14}ClN_3O_4$: C, 58.22; H, 3.77; N, 11.32. Found: C, 57.45; H, 2.94; N, 10.92.

3{[3-Chloro-2-(4-(Dimethylamino)phenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (29)

Yield: 76%; mp 182-184 ºC; R_f0.61 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3295 (NH), 1745 (C=O), 1577 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.76-8.70 (m, 8H, Ar-H), 10.72 (s, 1H, NH), 5.05 (d, 1H, CH-Ar); 5.45 (d, 1H, CH-Cl); 3.57 (s, 6H, CH_3); EI-MS: m/z $[M+H]^+$ 369.81. Anal. Calcd for $C_{19}H_{17}ClN_4O_2$: C, 61.95; H, 4.61; N, 15.21. Found: C, 60.12; H, 4.12; N, 14.63.

3{[3-Chloro-2-(3-nitrophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (30)

Yield: 58%; mp 204-206 ºC; R_f0.43 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3292 (NH), 1672 (C=O), 1543 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.78-8.09 (m, 8H, Ar-H), 9.22 (s, 1H, NH), 5.23 (d, 1H, CH-Ar); 5.98 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 371.74. Anal. Calcd for $C_{17}H_{11}ClN_4O_4$: C, 55.13; H, 2.97; N, 15.13. Found: C, 54.37; H, 2.56; N, 14.61.

3{[3-Chloro-2-(3,4,5-trimethoxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (31)

Yield: 61%; mp 221-223 ºC; R_f0.52 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3342 (NH), 1626 (C=O), 1537 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.66-8.16 (m, 6H, Ar-H), 8.78 (s, 1H, NH), 5.43 (d, 1H, CH-Ar); 5.51 (d, 1H, CH-Cl); 3.93 (s, 9H, CH_3); EI-MS: m/z $[M+H]^+$ 416.82. Anal. Calcd for $C_{20}H_{18}ClN_3O_5$: C, 57.83; H, 4.33; N, 10.12. Found: C, 56.37; H, 4.12; N, 9.82.

3{[3-Chloro-2-(2,4-dichlorophenyl)-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (32)

Yield: 64%; mp 224-226 ºC; R_f0.62 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3286 (NH), 1723 (C=O), 1529 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.23-8.15 (m, 7H, Ar-H), 9.45 (s, 1H, NH), 5.23 (d, 1H, CH-Ar); 5.03 (d,

1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 395.63. Anal. Calcd for $C_{17}H_{10}Cl_3N_3O_2$: C, 51.77; H, 2.53; N, 10.65. Found: C, 50.45; H, 1.98; N, 9.72.

3{[3-Chloro-2-(4-Methylphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (33)

Yield: 61%; mp 173-175 ºC; R_f0.57 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3294 (NH), 1741 (C=O), 1521 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.34-8.51 (m, 8H, Ar-H), 9.23 (s, 1H, NH), 5.13 (d, 1H, CH-Ar); 5.43 (d, 1H, CH-Cl); 2.42 (s, 3H, CH_3); EI-MS: m/z $[M+H]^+$ 340.77. Anal. Calcd for $C_{18}H_{14}ClN_3O_2$: C, 63.71; H, 4.12; N, 12.38. Found: C, 63.53; H, 3.23; N, 11.43.

3{[3-Chloro-2-(2-Methoxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (34)

Yield: 69%; mp 166-168 ºC; R_f0.41 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3334 (NH), 1698 (C=O), 1543 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.89-7.81 (m, 8H, Ar-H), 8.99 (s, 1H, NH), 5.07 (d, 1H, CH-Ar); 5.65 (d, 1H, CH-Cl); 2.31 (s, 3H, OCH_3); EI-MS: m/z $[M+H]^+$ 356.77. Anal. Calcd for $C_{18}H_{14}ClN_3O_3$: C, 60.84; H, 3.94; N, 11.83. Found: C, 59.57; H, 2.53; N, 10.43.

3{[3-Chloro-2-(4-Methoxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (35)

Yield: 61%; mp 172-174 ºC; R_f0.54 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3296 (NH), 1679 (C=O), 1521 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.89-8.61 (m, 8H, Ar-H), 10.88 (s, 1H, NH), 5.03 (d, 1H, CH-Ar); 5.34 (d, 1H, CH-Cl); 3.61 (s, 3H, OCH_3); EI-MS: m/z $[M+H]^+$ 356.77. Anal. Calcd for $C_{18}H_{14}ClN_3O_3$: C, 60.84; H, 3.94; N, 11.83. Found: C, 59.77; H, 2.61; N, 10.52.

3{[3-Chloro-2-(2-chlorophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (36)

Yield: 81%; mp 158-160 ºC; R_f0.61 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3345 (NH), 1709 (C=O), 1582 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.90-8.98 (m, 8H, Ar-H), 10.97 (s, 1H, NH), 5.48 (d, 1H, CH-Ar); 6.91 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 361.19. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_2$: C, 56.66; H, 3.05; N, 11.66. Found: C, 55.51; H, 2.61; N, 10.72.

3{[3-Chloro-2-(3-bromophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (37)

Yield: 67%; mp 182-184 ºC; R_f0.63 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3297 (NH), 1739 (C=O), 1567 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.15-8.65 (m, 8H, Ar-H), 9.77 (s, 1H, NH), 5.16 (d, 1H, CH-Ar); 6.32 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 405.64. Anal. Calcd for $C_{17}H_{11}BrClN_3O_2$: C, 50.49; H, 2.72; N, 10.39. Found: C, 49.32; H, 1.75; N, 9.84.

3{[3-Chloro-2-(2.4-dimethoxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (38)

Yield: 73%; mp 156-158 ºC; R_f0.43 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3345 (NH), 1716 (C=O), 1513 (C=N); ^{1}H NMR (DMSO, ppm): δ 6.48-8.92 (m, 7H, Ar-H), 10.42 (s, 1H, NH), 6.13 (d, 1H, CH-Ar); 5.89 (d, 1H, CH-Cl); 3.87 (s, 6H, OCH_3); EI-MS: m/z $[M+H]^+$386.80. Anal. Calcd for $C_{19}H_{16}ClN_3O_4$: C, 59.22; H, 4.15; N, 10.90. Found: C, 58.59; H, 3.98; N, 9.55.

3{[3-Chloro-2-(4-nitrophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (39)

Yield: 59%; mp 165-167 ºC; R_f0.65 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3298 (NH), 1675 (C=O), 1572 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.07-8.87 (m, 8H, Ar-H), 9.33 (s, 1H, NH), 5.31 (d, 1H, CH-Ar); 6.04 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 371.74. Anal. Calcd for $C_{17}H_{11}ClN_4O_4$: C, 55.13; H, 2.97; N, 15.13. Found: C, 54.65; H, 1.87; N, 14.75.

3{[3-Chloro-2-(2,3-dimethoxyphenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (40)

Yield: 59%; mp 165-167 ºC; R_f0.65 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3298 (NH), 1675 (C=O), 1572 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.12-8.91 (m, 7H, Ar-H), 9.51 (s, 1H, NH), 4.98 (d, 1H, CH-Ar); 6.13 (d, 1H, CH-Cl); 2.41 (s, 6H, OCH_3); EI-MS: m/z $[M+H]^+$ 386.80. Anal. Calcd for $C_{19}H_{16}ClN_3O_4$: C, 59.09; H, 4.14; N, 10.88. Found: C, 58.68; H, 3.87; N, 10.11.

3{[3-Chloro-2-(3-chlorophenyl) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (41)

Yield: 71%; mp 172-174 ºC; R_f0.43 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3223 (NH), 1702 (C=O), 1573 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.09-8.78 (m, 8H, Ar-H), 9.23 (s, 1H, NH), 5.34 (d, 1H, CH-Ar); 6.13 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 361.19. Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_2$: C, 59.09; H, 3.09; N, 11.66. Found: C, 58.65; H, 2.87; N, 11.02.

3{[3-Chloro-2-(furfuran) -4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one (42)

Yield: 59%; mp 165-167 ºC; R_f0.65 (Methanol: Toulene 1:4); IR (KBr, cm^{-1}): 3298 (NH), 1675 (C=O), 1572 (C=N); ^{1}H NMR (DMSO, ppm): δ 7.12-8.82 (m, 7H, Ar-H), 9.13 (s, 1H, NH), 5.21 (d, 1H, CH-Ar); 5.88 (d, 1H, CH-Cl); EI-MS: m/z $[M+H]^+$ 316.71. Anal. Calcd for $C_{15}H_{10}ClN_3O_3$: C, 57.09; H, 3.16; N, 13.30. Found: C, 56.87; H, 2.87; N, 12.85.

Pharmacology

The experimental protocols for the pharmacological screening on mice were done with Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj (RTM) Nagpur University, Nagpur, India (Reg. no.: IAEC/UDPS/2016/21).

Antianxiety activity

Animals

Female 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 micehad been acclimatized to the above environment for at least 7 days. Each mouse received a single i.p. 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[22-23]. 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(50 mg/kg) and diazepam (2 mg/kg) 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the centre 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. All the results of the antianxiety activity are given in **Table 2**.

Table 2: Effect of *3{[2-chloro-3-substituted-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one*series of compounds in EPM

Sr. No.	Compound	% preference of open arm	No. of entries in open arm	Average time spent in open arm (s)
1.	23	58.33	5.25 ± 0.73	27.00± 1.14
2.	24	29.54	3.25 ± 0.64	28.25± 0.65
3.	25	62.50	6.25 ±0.54	33.50± 0.12
4.	26	41.66	5.00 ±0.35	35.50± 1.11
5.	27	55.00	5.50±0.54	38.50± 0.45
6.	28	69.44	6.25 ±0.73	34.00± 0.67
7.	29	83.33	7.50±0.53	42.25± 0.12
8.	30	37.50	4.50±0.55	34.25± 0.85
9.	31	67.50	6.75 ±0.41	39.25± 0.75
10.	32	43.75	5.25 ±0.21	27.75± 0.45

11.	33	52.50	5.65±0.41	28.50± 0.65
12.	34	67.50	6.75 ±0.41	32.50 ± 1.23
13.	35	11.36	1.25 ±0.21	41.50 ± 0.22
14.	36	25.00	3.00 ±0.35	35.75 ± 0.76
15.	37	22.91	2.75 ±0.73	29.25 ± 1.54
16.	38	47.50	4.75 ±0.41	36.00 ± 0.94
17.	39	13.63	1.50±0.25	32.50 ± 0.65
18.	40	38.46	5.00±0.50	26.25 ± 0.87
19.	41	50.00	4.50±1.03	15.25 ± 0.65
20.	42	12.50	1.25 ±0.21	22.00 ± 0.22
21.	Vehicle/control	0.0	1.75 ±0.41	09.75 ± 0.45
22.	Diazepam (2mg/kg)	85.00	8.50±0.55	42.50 ± 0.54

Data analyzed by using one-way analysis of variance (ANOVA) with post hoc Tukey test. n = 6; dose = 50 mg/kg. Values are represented as mean ± S.E.M. Values are significant at $***P< 0.05$, compared with control group.

3D-QSAR Study

The 3D-QSAR was performed using the molecular modeling software package VLife Molecular Design Suite (VLifeMDS) version 4.3.1 on HP-PC (HPLV1911) with a Pentium IVprocessor and Windows 7 operating system.The results obtained from antianxiety activity were employed for carrying out the 3D QSAR studies, the percentage preference to open armvalues of twenty compounds**Table 2** employed to obtain the statistical analysis on basis of various descriptors such as steric and electrostatic which were calculated and used as independent variables.The dataset of twenty molecules in were divided into training and test set by random selection method for MLR, PCR, PLSR and kNN-MFA model. The seventy eight percentages of total data selected as training set (15 molecules) and remaining as test set (5 molecules).The Modules >>QSARPlus>> 3D-QSAR from the main menu of MDS was selected to launch the worksheet. By default all the molecules in a directory were considered for QSAR. QSAR tool was chosen from which molecules were opened, the subfolder containing set of molecules was selected. Activity data which was stored as 'activity.txt' was inserted by selecting File >> Insert Data. The field parameters electrostatic, hydrophobic and steric were computed by selecting QSAR Tools >> Compute Field window. The Gasteiger-Marsili charge was selected for the computation and invariable columns were removed. The data selection was done by choosing QSAR Tools >> Data Selection. Training data set selection method was applied to create training and test set for this random selection was done. The data was selected in the range of 65% to 85%. Finally, Variable Selection and Model Building Wizard tool was selected for the application of statistical methods like kNN, PLSR, MLR and PCR from Advanced Methods >> Method. The statistical data was generated which results in coefficient of determination (r^2), cross validated coefficient of determination (q^2), r^2 for external test set (pred_r^2) fitness plot and points of distribution. The best model was selected on basis of co-relation coefficient (r^2) and various statistical parameters such as standard error of co-relation coefficient (r^2se), Sequential Fischer test (F), predicted co-relation coefficient for external test set (pred_r^2), and standard error of predicted external co-relation coefficient (pred_r^2se). The models were cross-validated by 'leave one out' scheme and cross-validation co-relation coefficient q^2 was calculated. The model with high q^2 value is said to have high predictability.

Results and discussion

Chemistry

The reported investigation deals with synthesis and characterization of several indole bearing azetidinone nucleuses to form final twenty derivatives. To achieve these, three different steps were carried out. In the first step, isatin (1*H*-indole-2,3-dione) **1**was reacted with hydrazine hydrate in presence of methanol under conditions of reflux to yield the 3-hydrazinylidene- 1,3-dihydro-2*H*-indol-2-one **2.** NMR spectra of this compound exhibited prominent signals at δ 9.39 ppm and 10.57 ppm corresponding to the secondary amide proton and primary amine protons respectively. The aromatic protons belonging to fused benzene ring was

exhibited around d 6.87 to 7.37 ppm presenting four protons. The major spectral change was observed in the IR spectrum which provides with an appearance of primary amine functional group at 3411. cm−1. In the next step, Schiff bases **(3-22)** were formed by refluxing **2** with various substituted aromatic aldehydes in the presence of few drops of glacial acetic acid. These compound were confirmed on the basis of spectral studies;^{1}HNMR spectra showed, in each case, the signals as multiplet at 6.11-8.55 ppm attributed to Ar-H in addition to the singlet at the N-H group in the region 8.77-10.89 ppm. The singlet also appeared at d 8.93–10.86 ppm attributed toone proton of N=CH. Thus, it confirmed the formation ofSchiff bases. The final derivatives of this series*3{[2-chloro-3-substituted-4-oxoazetidin-1-yl]imino}-1,3-dihydro-2H-indol-2-one***(23-42)**were synthesized by carrying out cyclization of compounds (**3-22)**with chloroacetyl chloridein presence of triethylamine.These products were obtained in satisfactory yield and purity as studied on the thin layer chromatography and melting point studies. The structural confirmation was carried out on the basis of spectral studies,the IR spectra of these compounds exhibited absorbance for important functional groups, such as secondary amide at 3267-3395 cm^{-1}; the carbonyl group is indicated at 1699-1745 cm^{-1}and the C=N bond is reflected around 1521-1586 cm^{-1}.These groups are common to all the molecules from final derivatives.The ^{1}H NMR spectra of these compounds exhibited several characteristic NMR shifts.The ^{1}H NMR spectra showed, in each case the signals as multiplet at δ 7.07-8.87 ppm attributed to Ar-H in addition to the singlet of the N-H group in the region δ 8.45-9.45 ppm. The singlet appeared for C-2 of the azetidinonering in the regions δ 5.07-6.88 ppm integrating for one proton. The singlet also appeared at δ 5.04-6.91 ppm attributed to one proton of CH-Cl. Thus, it confirmed the formation of indole ring containing azetidinonederivatives.EI-MS of all compounds displayed the $[M + H]^{+}$ confirming their molecular weight. The elemental (CHN) analyses were found within the limit of theoretical values. (cm^{-1})

Pharmacological screening

The compounds (**23-42)**were evaluated for antianxiety activity by elevated plus maze test (EPM) in mice at dose of (50 mg/kg) and compared with the standard drugdiazepam(2 mg/kg). There were no mortality and noticeablebehavioural changes in acute oral toxicity for all the groups tested. The synthesized compounds were found to be safeup to 2000 mg/kg body weight. Initially, dose-dependentstudy of compound **23**at different doses (25, 50, 100, and200 mg/kg, i.p.) were performed to ensure the maximumeffective dose for new synthesized compounds as antianxiety in EPM. From this study, we found that 50 mg/kg is themaximum effective dose and therefore it was selected for further pilot study of antianxiety-like effects of compounds (**23-42)**in EPM. Antianxiety activity was assessed as number of entries in open arm, and data has been presented as**Table 2**.

The standard diazepam had percentage preference to open arm 85.00% at a dose level of2mg/kg. In our research all the synthesized derivatives canproduce significant percentage preference to open arm when comparedto the standard drug.Compounds **28**and **29**were found to be the most potent derivatives fromthe series, showing preference to open arm 69.44 and 83.33 respectively. At the same time, compounds **23, 25, 27, 31**and**34** showed moderate activity while compounds **24, 35**and **42**showed poor activity.The preliminary SAR of indole bearing azetidinone ring suggested that substitution of the phenyl ring by electron releasing groups on ortho and para position lead to an increase of antianxiety activity **(28, 29)**.Whereas compounds containing electron withdrawing groups at ortho and para position of the aromatic core showed good antianxiety activity (**23, 25, 27, 31 and 34).**Moreover,compounds having electron withdrawing group specially chloro at ortho and para position of the aromatic nucleus caused decrease in antianxiety activity **(24, 42)**. Therefore,such compounds would represent a useful matrix for thedevelopment of a new class of clinically useful antidepressantagents and deserve further investigation and derivatization.

3D-QSAR Study

In the present study, Partial least square regressionmodel (PLSR-SW) is developed coupled withstepwise variable selection method to develop 3D-QSAR models ofindole bearing azetidinone derivatives as antianxiety agents based on steric and electrostatic fields. The total dataset was divided into training and test sets using the sphere exclusionalgorithm for diversity of the sampling procedure. This approachresulted in selection of compounds **23, 26, 27, 30** and **31**as thetest set and the remaining 15 compounds as the training set **Table 3**.Selection of molecules in the training set and test is a key andimportant feature of any QSAR model. Therefore the care was takenin such a way that biological activities of all compounds in test liewithin the maximum and minimum value range of biologicalactivities of trainingset of compounds. The UniColumn

statistics fortraining set and test set were generated to check correctness ofselection criteria for trainings and test set molecules and resultreflected the correct selection of test and training sets **Table 4**.Several statistically significant 3D-QSAR models using stepwisevariable selection method were generated, of which thecorresponding best model is reported herein. The best 3D-QSAR PLSR-SWmodel selected based on the value of statisticalparameters has a q2 = 0.8442 and pred_r2 = 0.0712 **Table 5**.

From **Table 3**, it is evident that predicted activities of all thecompounds are in good agreement with their correspondingexperimental activities. The plots of observed versus predictedactivity of both training & test sets molecules helped in crossvalidationof **PLSR-SW** QSAR model and are depicted in **Figure 3**.The contribution plot and the 3D-QSAR graphical interface provides with thepoints, points generated in the model are E_698, E_671 and S_394 accountingfor positive electrostatic, negative electrostatic, positivesteric fieldsrespectively at the lattice points on the grid as shown in the **Figure 3,4,5**,these points suggestthe significance and requirements or removal of the various substitutions on the structure tomaximise the antianxiety activity.It shows that the removal of E_671 at thephenyl ring i.e., the electropositive group(electrostatic descriptor) which is in the negative range and thus suggests that removalof these would help in increase in the activity.In a similar fashion the positive contributorswould be the electrostatic and steric descriptors E_698 and S_394respectively. Addition of electropositive and bulky group at thephenyl ring will contributetowards enhancing the antianxiety activity of the molecules.Theseresults are in close agreement with the experimental observationsthat compounds **28** and **29** with electropositive groups showed greater antianxiety activity while compounds **24, 35**and **42** with electronegative groups showed poor activity.

Table 3: Observed and predicted activity by QSAR equation along with residuals

Compounds	Sets	Activity		Residual
		Experimental	Predicted	
23	Test	58.33	41.53	16.80
24	Training	29.54	41.01	-11.47
25	Training	62.50	64.26	-1.76
26	Test	41.66	33.68	7.98
27	Test	55.00	42.75	12.25
28	Training	69.44	70.77	-1.33
29	Training	83.33	82.21	1.12
30	Test	37.50	10.90	26.6
31	Test	67.50	49.44	18.06
32	Training	43.75	36.21	7.54
33	Training	52.50	70.26	-17.76
34	Training	67.50	61.25	6.25
35	Training	11.36	10.71	0.65
36	Training	25.00	27.50	-2.5
37	Training	22.91	32.58	-9.67
38	Training	47.50	52.08	-4.58
39	Training	13.63	20.12	-6.49
40	Training	38.46	42.06	-3.6
41	Training	50.00	31.84	18.16
42	Training	12.50	29.68	-17.18

Table 4: Uni Column statistics of the training and test sets for QSAR model

Data Set	Average	Maximum	Minimum	Standard Deviation	Sum
Training set	45.1076	83.3330	11.3630	22.0918	676.6140
Test set	46.1666	67.5000	12.5000	21.7354	230.8330

Table 5: Statistical results of 3D-QSAR PLSR-SW model generated by stepwise variable selection method

Sr. No.	Parameters	Results
1.	n	15
2.	Degree_of_freedom	12
3.	r2	0.8825
4.	q2	0.8442
5.	F_test	45.0539
6.	r2_se	8.1802
7.	q2_se	9.4201
8.	pred_r2	0.0712
9.	pred_r2se	20.9780

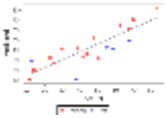

Figure 3:Comparison of observed activity versus predictedactivity for training set & test set compounds according to 3D-QSARmodel by PLSR-SW method

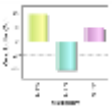

Figure 4: Contribution Plot for Steric and Electrostatic Descriptors Selected in 3D-QSARmodel by PLSR-SW method

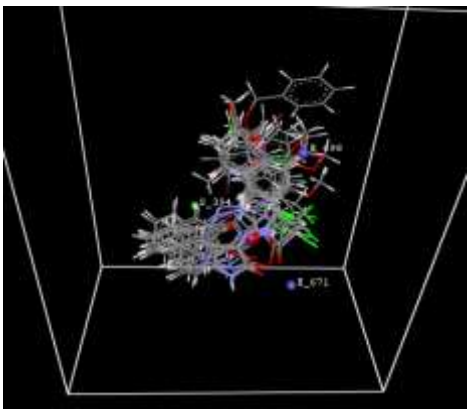

Figure 5: Stereo view of molecular rectangular field gridaround the superposed molecular units of indole bearing azetidinone derivatives using PLSR-SW method

Conclusion

In summary, we have described the synthesis of novel indole bearing azetidinone derivatives by conventional method with high purity and better yields of product. All the spectral studies were in good agreement with the final structures of the titled derivatives. All synthesized compounds were evaluated for antianxiety activity in EPM. Among all derivatives tested in the present study, compounds 28 and 29 exhibiting promising antianxiety activity comparable to that of the diazepam. The substitution of electron releasing groups on the para position of phenyl ring system provided with active compounds having percentage preference to open arm of 69.44 and 83.33 respectively. The 3D-QSAR study results revealed that addition of electropositive and bulky group at thephenyl ring will contributetowards enhancing the antianxiety activity of the molecules while electronegative groups showed poor activity and theseresults are in close agreement with the experimental observations. Hence, these studies are useful inunderstanding the structural requirements for design of novel and potent antianxiety agents.

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