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Synthesis of Aryl anilinomaleimide based derivatives as glycogen synthase kinase-3β inhibitors with potential role as antidepressant agents

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Abstract

A series of aryl anilinomaleimide based derivatives has been synthesized and evaluated for *in vitro* glycogen synthase kinase-3β **(**GSK-3β) inhibitory activity. Large number of compounds from the series exhibited moderate to potent inhibitory activity against GSK-3β, with more than one-third of the compounds showing inhibition with IC_{50} values <1 μM. The molecular docking studies against GSK-3β (PDB: 1Q3D) revealed multiple H-bonding interactions by the synthesized molecules with important amino acid residues on the receptor site in addition to H-bond with water residue and π-cation interactions by some compounds. Given the potential role of GSK-3β inhibition in treatment of depression, compounds **8j**, **8b**, **8i**, **8l**, **8a** and **8n** exhibiting significant GSK-3β inhibition (IC_{50} values of **0.09**, **0.12**, **0.17**, **0.19**, **0.21** and **0.23 μM** respectively) were further investigated for antidepressant activity by the widely accepted forced swim test and tail suspension test (FST and TST) models. All the tested compounds displayed antidepressant-like effects, particularly compounds **8j** and **8b** which exhibited significant antidepressant activity, about 1.4-fold higher than fluoxetine, a standard antidepressant drug in both FST and TST. Preliminary structure-activity relationships have also been generated based on the experimental data obtained.

Introduction

Glycogen synthase kinase-3 (GSK-3), is a serine-threonine protein kinase, constitutively active and ubiquitously expressed in all mammalian tissues and subcellular organelles[1]. GSK-3 is involved in a multitude of key cellular and physiological events ranging from metabolism to immunity and behaviour by virtue of regulating more than 70 diverse substrates[1, 2]. Besides being involved in a number of human pathological conditions, including Alzheimer's disease, cancer and diabetes[3-5], GSK-3 dysfunction has been implicated in psychiatric disorders like bipolar disorder, mood disorders, depression and schizophrenia[1, 6, 7]. GSK-3 is present in two major isoforms, GSK-3α and GSK-3β. Among the two isoforms, GSK-3β is the most often investigated isoform and has received more interest from investigators studying mood disorders[8]. For example, lithium, a well-known GSK-3β inhibitor, used as a mood stabiliser in bipolar disorder as well as adjunct therapy for depression and schizophrenia for nearly 50 years has been proposed to exert its therapeutic action by inhibiting GSK-3β directly as well as indirectly through Akt/β-arrestin/protein phosphatase 2A complex[7, 9, 10].

The involvement of GSK-3β in depression has been linked to deficient serotonergic neurotransmission observed in depression, considering that serotonergic activity contributes to the inhibitory control of GSK-3β in mammalian brain *in vivo*[8]. The regulation of behaviour by various psychoactive medications including selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, tricyclic antidepressant and second-generation antipsychotics, involve a shared action of inhibition of GSK-3β in either the direct or downstream mechanism of action[11, 12]. GSK-3β is further validated as a viable target for antidepressant action by the reported anti-depressive effects of GSK-3β inhibitors in animal models. The substrate-competitive peptide inhibitor L803-mts displayed antidepressant effect in the mouse FST[13], AR-A014418 showed similar effects in rats[14] while as the thiadiazolidinone NP031115 also displayed antidepressant activity in the mouse FST after systemic administration[15]. Furthermore, pharmacological or genetic inhibition of GSK-3β was found to replicate behavioural effects of these drugs in rodents[16]. Given its potential role in psychiatric disorders and their treatment, pharmacological inhibition of GSK-3β represents a fascinating therapeutic strategy for treatment of human neurodegenerative and psychiatric conditions.

Among the GSK-3β inhibitors identified from remarkably different structural classes, maleimides like SB415286 (anilinomaleimide) and SB216763 (arylindolemaleimide) are well known representative compounds of potential importance[17-19]. Their applications in many pathologies including diabetes, Alzheimer's disease, cancer, inflammation, neuropathological and neuropsychiatric disorders have been explored[20]. LY2090314, a maleimide based GSK-3β

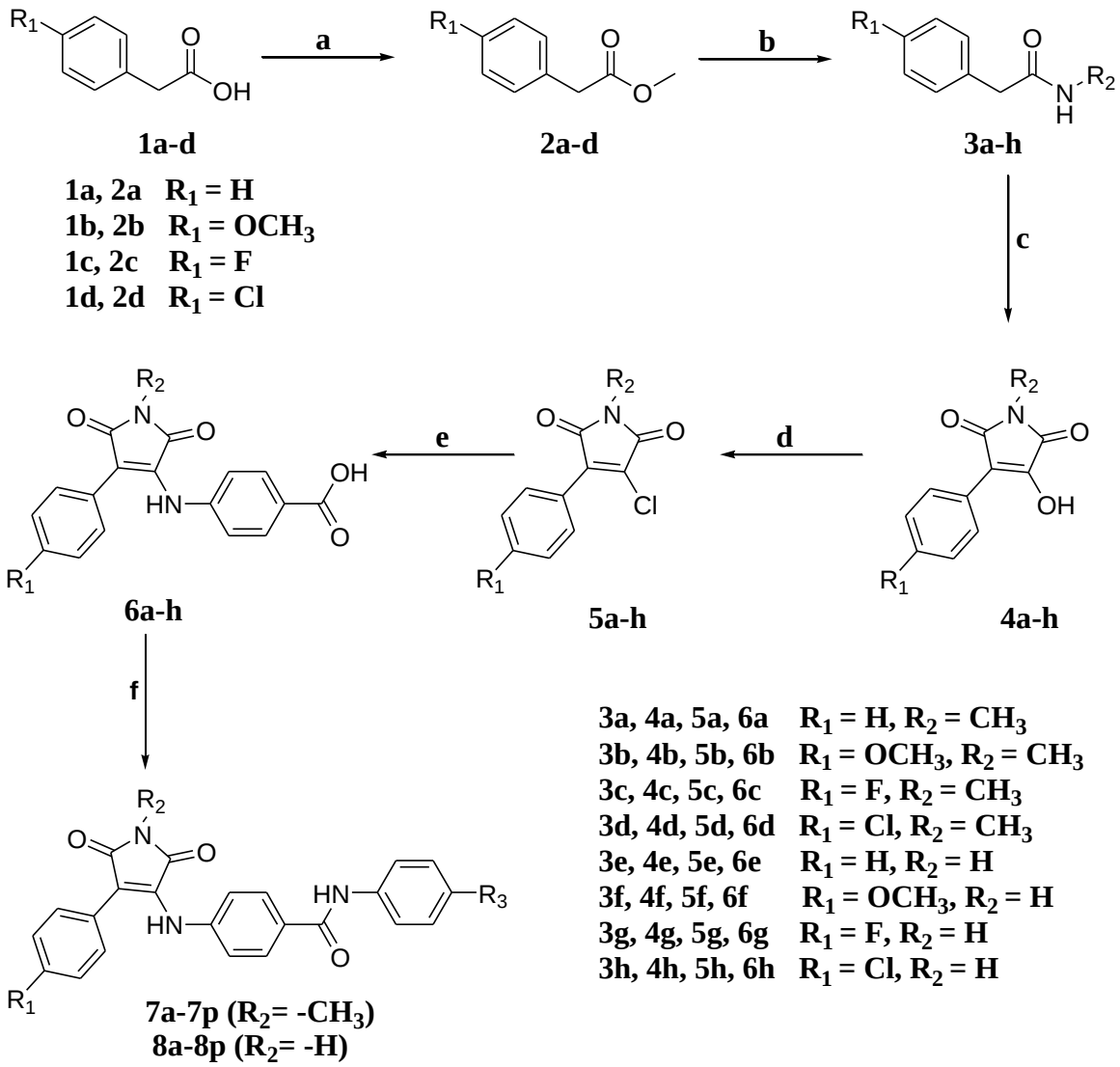
inhibitor, is in clinical trial stages for the treatment of cancer[21]. Anilinomaleimides have been reported as potent and selective GSK-3 inhibitors[18, 20, 22] and are amenable for further structural modifications in order to search for the development of effective chemotherapeutics.

Encouraged by the need to develop new GSK-3β inhibitors with manifold therapeutic applications, we thought it worthwhile to synthesize new small molecules based on aryl anilinomaleimide scaffold as GSK-3β inhibitors, focusing in particular on their application in depression. A library of thirty-two compounds has been synthesized and evaluated for *in vitro* GSK-3β inhibition. The compounds which exhibited significant GSK-3β inhibition profile were further subjected for *in vivo* evaluation of antidepressant activity using forced swim test and tail swim test models. *In silico* molecular docking studies of all the synthesized compounds have also been performed against GSK-3β to understand the binding interactions of these compounds with its active site.

Results and Discussion

Chemistry

The compounds were synthesized as illustrated in **Scheme 1**. Aryl acetamides **3a-h** were synthesized from corresponding acids **1a-d** by esterification followed by treatment with ammonium hydroxide or methyl amine. The aryl acetamides **3a-f** on condensation with diethyl oxalate in the presence of potassium tert-butoxide formed 3-hydroxymaleimide derivatives **4a-h** which were then treated with oxalyl chloride to form 3-chloromaleimide derivatives **5a-h**. The 3-chloromaleimide derivatives were subsequently treated with 4-aminobenzoic acid in N-methyl pyrrolidine (NMP) to obtain 4-carboxyanilinomaleimide derivatives **6a-h** which on treatment with appropriate anilines in presence of EDC.HCl and HOBT afforded the target compounds **7a-7p** and **8a-8p (Table 1)**.

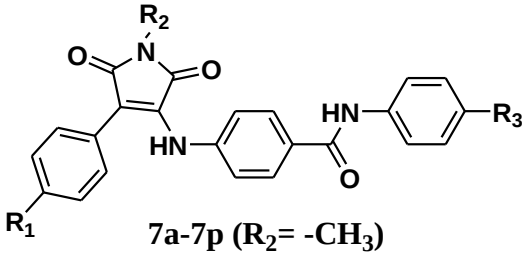


Reagents and conditions: (a) Methanol, H_2SO_4, Reflux, 12 h (b) R-NH_2, Methanol, RT, 15 h (c) Diethyl oxalate, t-BuOK, THF/DMF, 0 ^{0}C-RT, 6 h (d) Oxalyl chloride, THF/DMF, 0 ^{0}C-RT, 4 h (e) 4-Aminobenzoic acid, NMP, 90 ^{0}C, 24 h (f) Anilines, EDC.HCl, HOBT, DMF, RT, 8 h.

Scheme 1 - Synthesis of aryl anilinomaleimide based derivatives.

Table 1

Structural data and GSK-3β inhibitory activity of the synthesized compounds.



7a-7p (R_2= -CH_3)
8a-8p (R_2= -H)

Compd. (R_2=CH_3)	R_1	R_3	IC_{50} (μM) ± SEM[a]	Compd. (R_2=H)	R_1	R_3	IC_{50} (μM) ± SEM[a]
7a	-H	-H	16.49±0.57	**8a**	-H	-H	**0.21±0.03**
7b	-H	-OCH_3	25.70±2.16	**8b**	-H	-OCH_3	**0.12±0.01**
7c	-H	-F	23.82±1.97	**8c**	-H	-F	0.51±0.06
7d	-H	-Cl	19.05±2.34	**8d**	-H	-Cl	0.34±0.03
7e	-Cl	-H	20.71±0.92	**8e**	-Cl	-H	0.56±0.03
7f	-Cl	-OCH_3	18.24±1.24	**8f**	-Cl	-OCH_3	0.62±0.05
7g	-Cl	-F	>50	**8g**	-Cl	-F	2.57±0.10
7h	-Cl	-Cl	>50	**8h**	-Cl	-Cl	3.78±0.46
7i	-OCH_3	-H	14.73±0.83	**8i**	-OCH_3	-H	**0.17±0.02**
7j	-OCH_3	-OCH_3	15.37±1.05	**8j**	-OCH_3	-OCH_3	**0.09±0.01**
7k	-OCH_3	-F	16.81±1.01	**8k**	-OCH_3	-F	0.76±0.04
7l	-OCH_3	-Cl	16.34±1.13	**8l**	-OCH_3	-Cl	**0.19±0.01**
7m	-NO_2	-H	>50	**8m**	-NO_2	-H	0.32±0.02
7n	-NO_2	-OCH_3	23.72±1.72	**8n**	-NO_2	-OCH_3	**0.23±0.02**
7o	-NO_2	-F	>50	**8o**	-NO_2	-F	1.82±0.12
7p	-NO_2	-Cl	14.40±0.78	**8p**	-NO_2	-Cl	6.86±0.32
Staurosporine			**0.04±01**				

[a] SEM: Standard Error Mean.

GSK-3β inhibitory activity

All the 32 synthesized aryl anilinomaleimide based derivatives were evaluated for GSK-3β inhibitory activity by non-radioactive based Kinase-Glo™ luminescence assay[23]. Staurosporine, a known kinase inhibitor was taken as standard. The *in vitro* assay results are summarized in **Table 1**.

As illustrated in Table 1, many compounds from the series exhibited moderate to potent inhibitory activity against GSK-3β, with more than one-third of the compounds showing inhibition with IC_{50} < 1 μM. Compound **8j** (R_1 = R_3 = OCH_3) displayed highest GSK-3β inhibition of 0.09 μM. The inhibitory potency was found to be influenced both by the substitutions on the maleimides nitrogen (R_2) as well as on the aryl rings (R_1 and R_3). For compound **8a,** with NH-maleimide and both aryl rings unsubstituted (R_1= R_2= R_3= H), significant GSK-3β inhibition was observed with IC_{50} of 0.21 μM. Replacement of hydrogen on the maleimide core by methyl group led to drastic loss of activity, as observed in **7a** (R_2= CH_3; R_1 = R_3= H) exhibiting IC_{50} of 16.49 μM. Similar trend was displayed by all the other compounds with R_2 = H (**8b-8p**) in comparison to their respective counterparts with R_2 = CH_3 (**7b-7p**), signifying the importance of NH of the imide motif (R_2 = H) as an essential requirement of the pharmacophore for exhibiting GSK-3β inhibitory activity.

A preliminary structure-activity relationship has also been generated around aryl rings by introducing different substitutions (R_1 and R_3), to have an understanding of the resulting impact on the efficiency of GSK-3β inhibition. The presence of methoxy or hydrogen at either or both R_1 and R_3 positions were found to be more favourable for exhibiting GSK-3β inhibitory activity than either chloro or nitro at R_1 and chloro or fluoro at R_3. For example, replacement of H at R_3 in **8a** by methoxy resulted in increase in GSK-3β inhibitory activity to 0.12 μM in **8b**, while as substitution by chloro (**8d**) or fluoro (**8c**) at R_3 resulted in decrease in activity (**8d**, IC_{50} = 0.34 μM; **8c**, IC_{50} = 0.51 μM) in comparison to **8a**. Similarly, replacing H at R_1 in **8a** by methoxy caused an increase in GSK-3β inhibition in **8i** (IC_{50} = 0.17 μM) while presence of nitro (**8m**) or chloro (**8e**) resulted in lowering of GSK-3β inhibition (**8m**, IC_{50} = 0.32 μM; **8e**, IC_{50} = 0.56 μM) in comparison to **8a**. Similar trend was observed by introducing hydrogen, methoxy, chloro or fluoro at R_3 while substituting either methoxy, nitro or chloro group at R_1. The correlation can be summarized as shown in **Figure 1**.

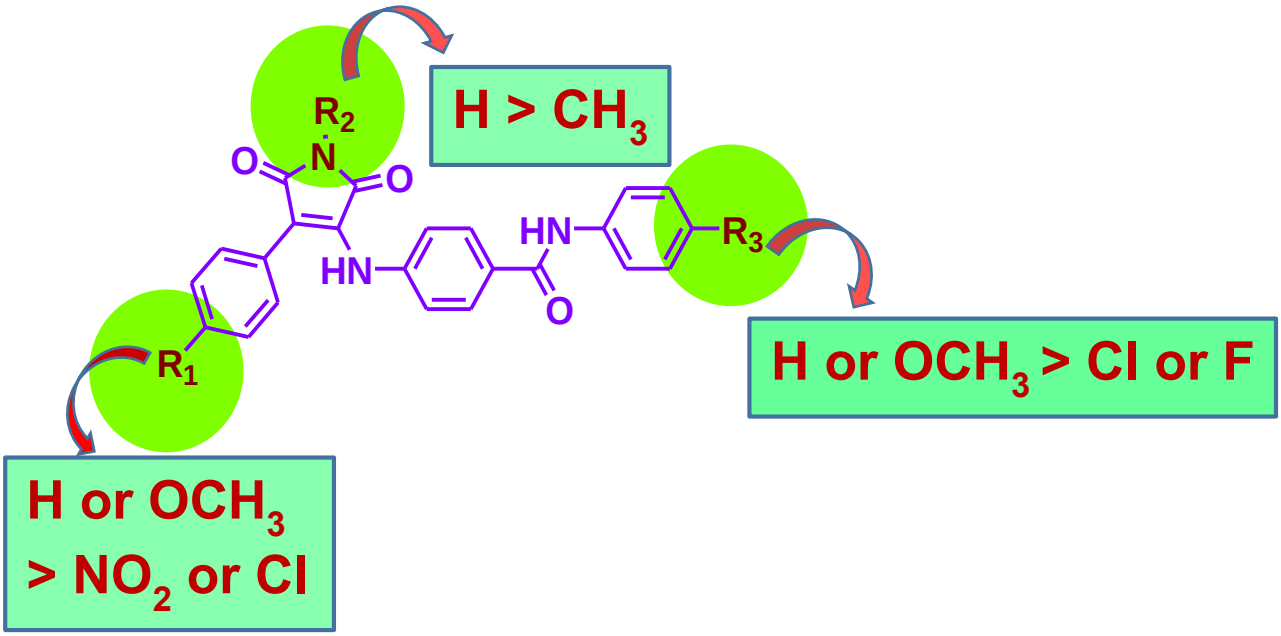


Figure 1: Structure-Activity Relationship against GSK-3β

Docking studies:

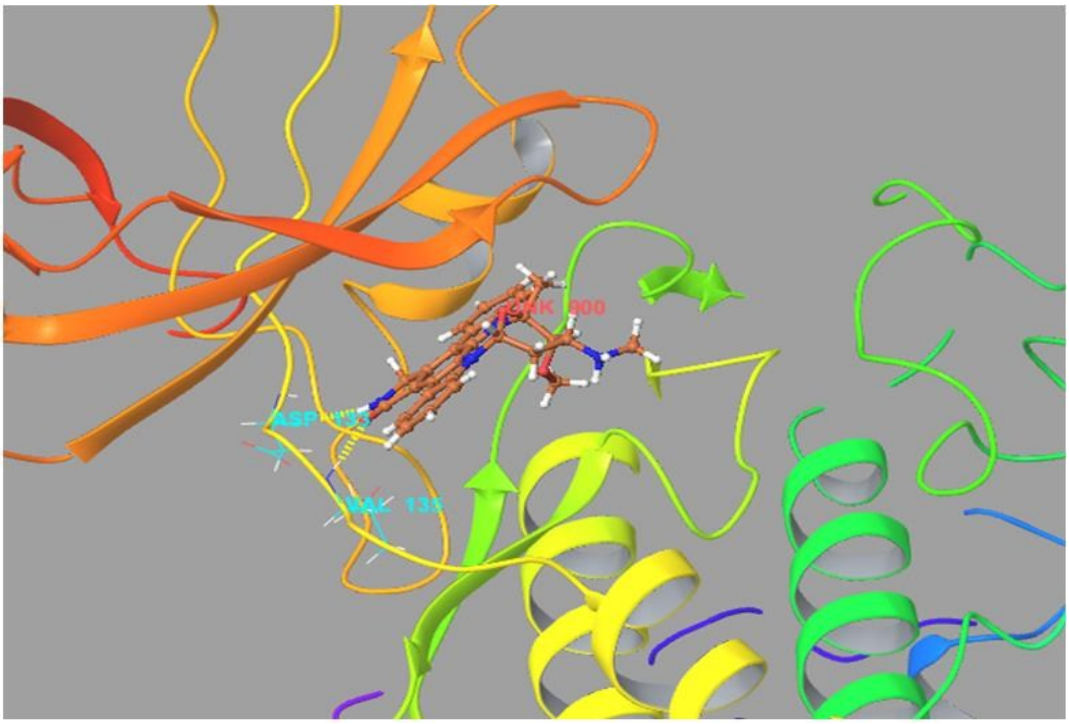
In order to gain a better insight of the binding interactions of synthesized maleimide derivatives against GSK-3β, molecular docking studies were performed. Compounds were docked individually against GSK-3β protein target (PDB: 1Q3D) containing Staurosporine as binding ligand. Staurosporine displays key interactions by forming two hydrogen bonds with carbonyl oxygen of ASP-133 and backbone nitrogen of VAL-135 residues respectively, besides showing hydrophobic interactions with ILE-62, VAL-70 and PHE-67 residues within the ATP binding site of GSK-3β protein contributing to enhance inhibitory activity of Staurosporine ligand. The glide score along with the glide energies of all the synthesized compounds and the standard Staurosporine are shown in **Table 2**. The docking results revealed the synthesized molecules exhibited multiple H-bonding interactions with important amino acid residues on the receptor site in addition to H-bond with water residue and π-cation interactions by some compounds. The N-H of maleimide core (R_2= H, compounds **8a-8p**) shows H-bonding interaction with ASP-133 residue in the hinge region. Substitution of H by -CH_3 (R_2= CH_3, compounds **7a-7p**) at this position prevents the H-bond formation with ASP-133 which probably results in lower dock scores displayed by these molecules and lower activity in *in vitro* assays. Compound **8j** was found to be the most active molecule with significant binding affinity towards GSK-3β (Glide score -8.77) closest to reference Staurosporine with Glide score -10.01, forming hydrogen bond between the nitrogen atom (maleimide ring) and carbonyl oxygen of ASP-133 residue and a similar interaction between oxygen atom (maleimide ring) and backbone nitrogen

of VAL-135 residue. Additionally, one amide nitrogen and one of the methoxy group show H-bond interactions with the side chain residue of ASN-186 and water molecule in the system respectively. Furthermore, π-cation interaction between the terminal benzene ring and LYS-183 residue is also observed. This finding justifies the *in vitro* study results, wherein compound **8j** has been found to exhibit least IC_{50} value of 0.09 μM (maximum GSK-3β inhibition). Likewise, multiple hydrogen bond interactions of **8b** with VAL-135, ASP-133 and ASP-200 residues and the π-cation interaction between the terminal benzene ring and LYS-183 residue resulted in high binding efficiency with glide score of -8.32, thereby supporting its significant *in vitro* GSK-3β inhibitory activity (IC_{50} = 0.12 μM). The snapshots of Staurosporine and top inhibitory molecules **8j** and **8b,** with their binding mode and molecular interactions are shown in **Figure 2a-f**.

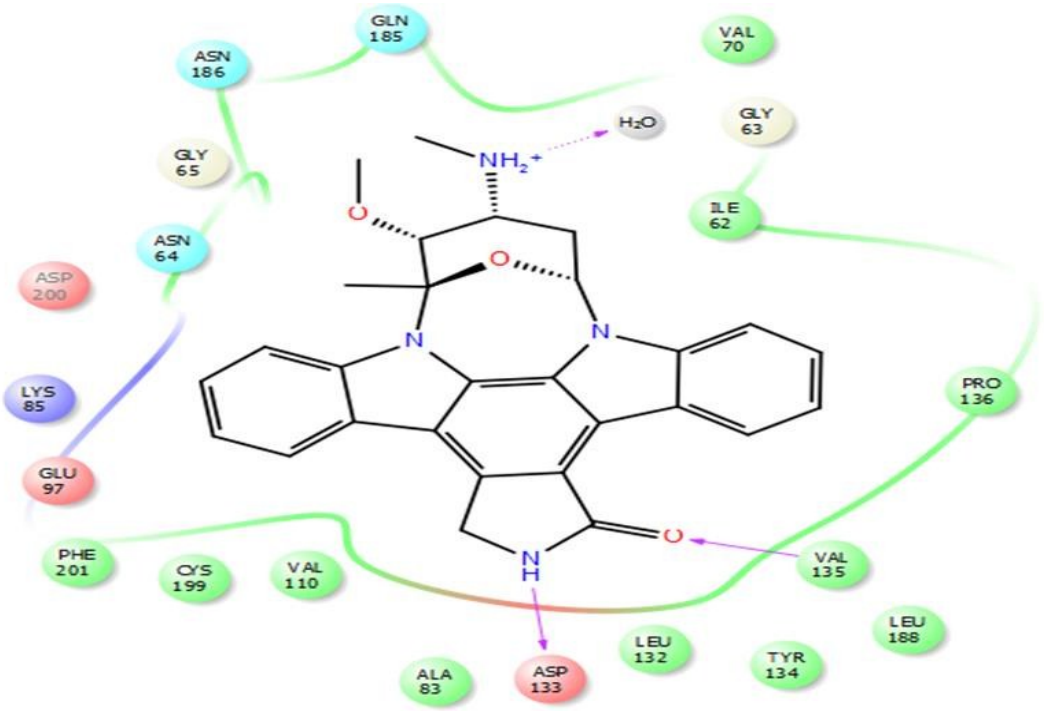
Table-2

Molecular docking study results of the synthesized compounds against GSK-3β.

Compd.	Glide Score	Glide Energy	Compd.	Glide Score	Glide Energy
7a	-5.61	-48.23	**8a**	-7.93	-50.77
7b	-6.16	-40.88	**8b**	**-8.32**	-51.03
7c	-5.57	-45.88	**8c**	-7.86	-50.36
7d	-5.03	-45.8	**8d**	-7.85	-50.89
7e	-6.11	-45.92	**8e**	-7.84	-50.5
7f	-6.12	-47.99	**8f**	-7.77	-51.63
7g	-6.05	-46.79	**8g**	-7.75	-50.74
7h	-5.95	-46.8	**8h**	-7.72	-51.11
7i	-6.38	-48.61	**8i**	-7.96	-51.93
7j	-4.36	-47.39	**8j**	**-8.77**	-53.65
7k	-6.44	-49.06	**8k**	-7.79	-52.84
7l	-6.33	-49.87	**8l**	-8.19	-47.72
7m	-2.4	-46.01	**8m**	-7.87	-53.65
7n	-4.52	-41.44	**8n**	-7.91	-53.33
7o	-6.18	-48.53	**8o**	-7.69	-52.12
7p	-6.46	-49.74	**8p**	-7.05	-53.43
Staurosporine	-10.01	-59.09			



2a



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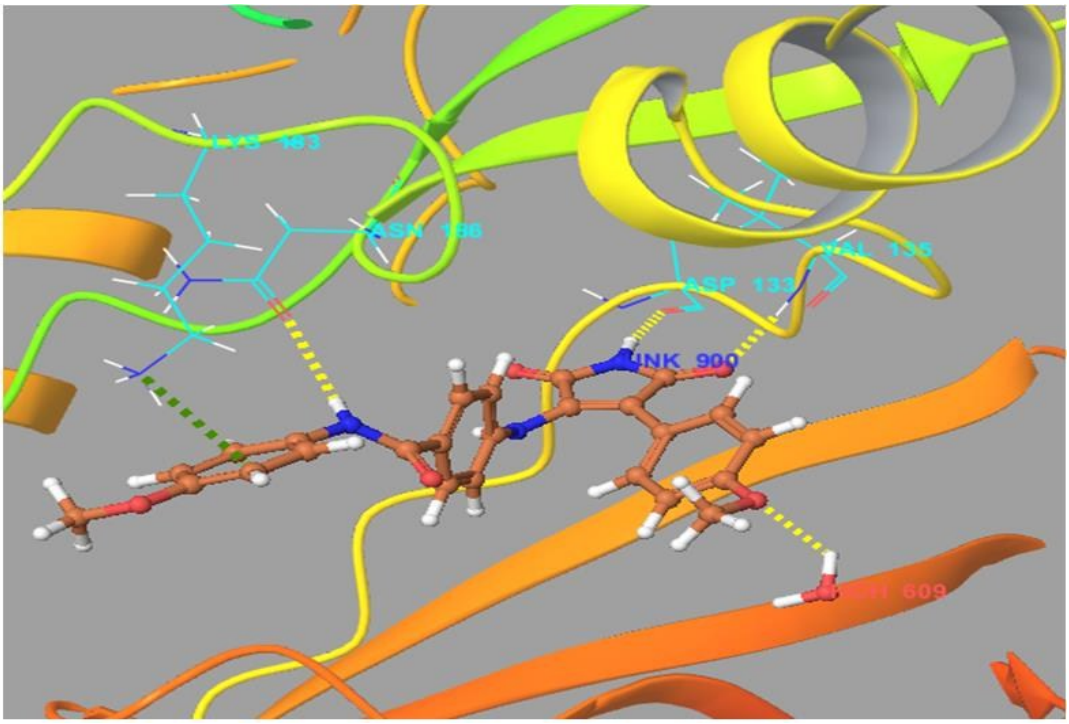
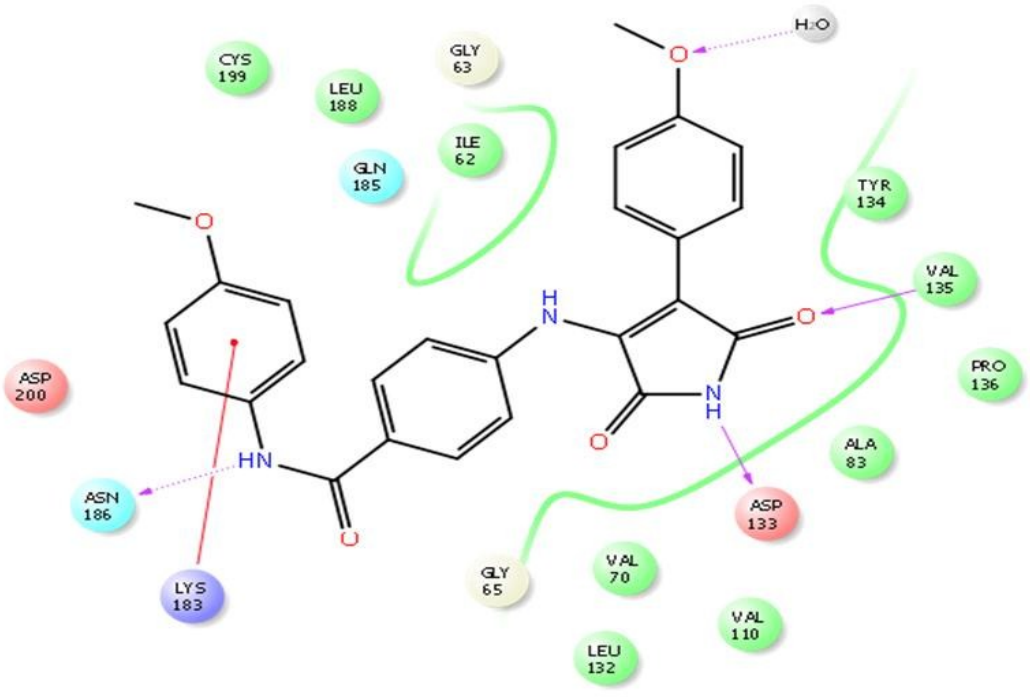
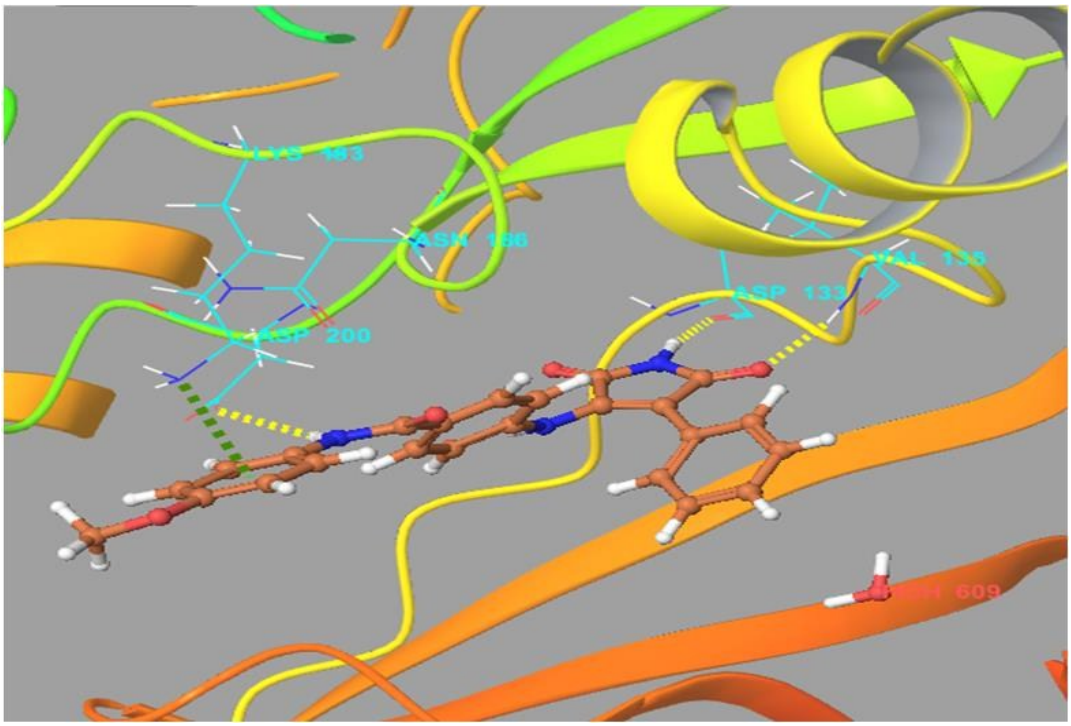


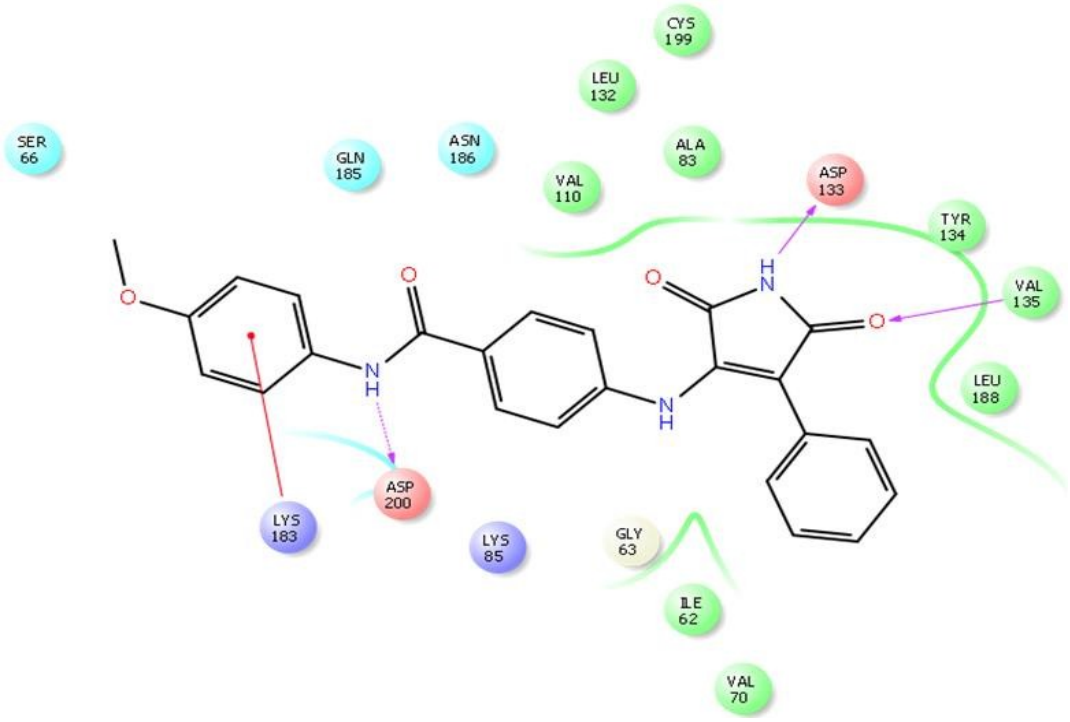
2c



2d



2e



2f

Figure 2: Docking poses and interaction of ligands with respect to GSK-3β protein.

(a) Docking pose of Staurosporine **(b)** Ligplot of Staurosporine **(c)** Docking pose of **8j**

(d) Ligplot of **8j** **(e)** Docking pose of **8b** **(f)** Ligplot of **8b**.

Antidepressant activity

Considering the close association of GSK-3β inhibition with antidepressant activity, the compounds **8j**, **8b**, **8i**, **8l**, **8a** and **8n** exhibiting potent GSK-3β inhibitory activity were further investigated for antidepressant activity by the widely accepted forced swim test and tail suspension test (TST and FST) models.

Forced swim test

The results of the effect of test compounds **8j**, **8b**, **8i**, **8l**, **8a** and **8n** on immobility in the forced swim test are depicted in **Figure 3**. Except **8n** and **8a**, all the other test compounds significantly reduced the immobility time in experimental rats compared to normal saline and fluoxetine treated groups ($P < 0.05$). Administration of compound **8j** or **8b** registered an almost 1.6-fold decrease in immobility time in comparison to normal saline group and 1.4-fold decrease in comparison to standard drug fluoxetine treated group.

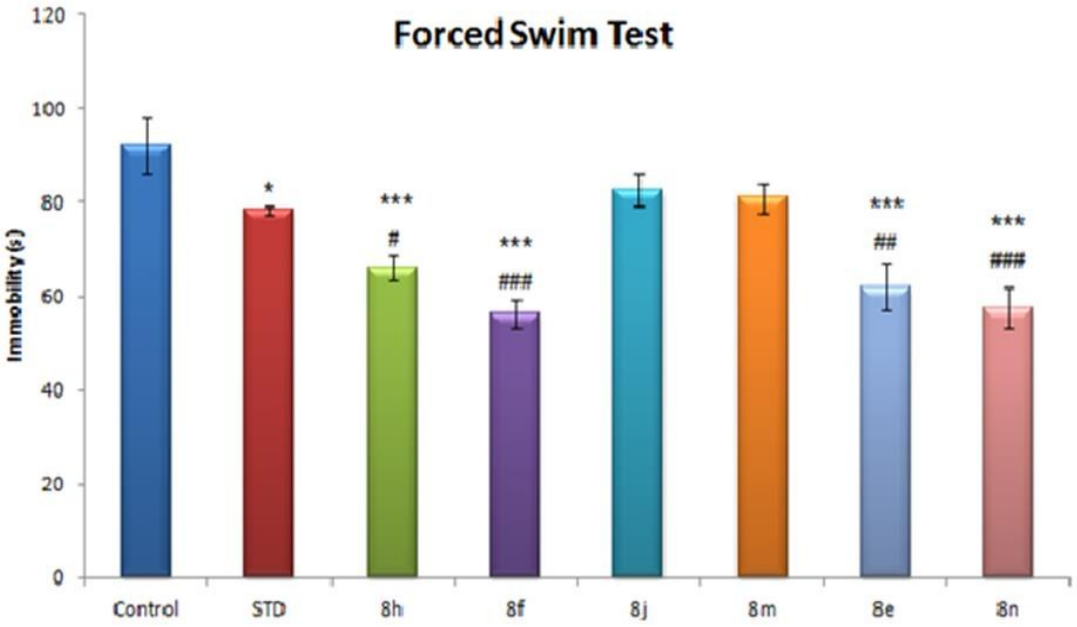


Figure 3: Forced swim test. Data is represented as Mean±SEM and analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test, *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$ when compared with normal control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared with standard fluoxetine.

Tail suspension test

The results from tail suspension test illustrating the effect of test compounds **8j**, **8b**, **8i**, **8l**, **8a** and **8n** on immobility are shown in **Figure 4**.Compounds **8j, 8b** and **8i** significantly

shortened the immobility time periods in experimental rats compared to both normal saline and fluoxetine treated groups ($P < 0.05$), while as compound **8l** showed significant effect with respect to normal saline treated group only. Compounds **8j** and **8b** were particularly most active. Pre-treatment with compound **8j** or **8b** resulted in a considerable decrease in mean duration of immobility by 1.75 to 1.8-fold relative to normal control group and by about 1.4 to 1.5-fold relative to standard drug fluoxetine.

Among the compounds tested, compounds **8j** and **8b** thus exhibited the maximum antidepressant-like effects in both forced swim test and tail suspension test (TST and FST) models. Furthermore, the antidepressant activity of the tested compounds was observed consistent with respect to their GSK-3β inhibitory profile. These results reinforce the notion that GSK-3β inhibitors hold a significant potential for treatment of depression and related disorders.

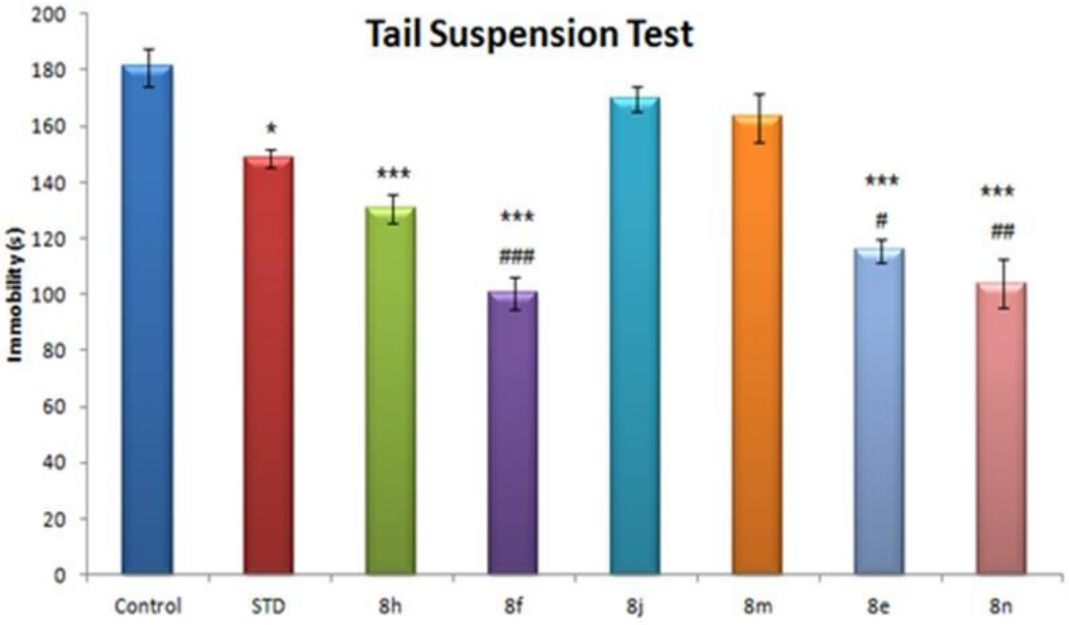


Figure 4: Tail suspension test. Data is represented as Mean±SEM and analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test, *$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$ when compared with normal control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared with standard fluoxetine.

Conclusion

A novel series of aryl anilinomaleimide based derivatives has been synthesized and tested for *in vitro* GSK-3β inhibition potential. Large number of compounds displayed moderate to potent inhibitory activity against GSK-3β, with more than one-third showing inhibition with $IC_{50} < 1$

μM. The molecular docking studies showed multiple H-bonding interactions formed by the synthesized molecules with important amino acid residues on the receptor site in addition to H-bond with water residue and π-cation interactions by some compounds. Compound **8j** and **8b** were found to have the closest binding efficiency (G score of -8.77 and -8.32 respectively) relative to Staurosporine (G score of -10.01). Compounds **8j**, **8b**, **8i**, **8l**, **8a** and **8n** exhibiting potent GSK-3β inhibition were further assessed for *in vivo* antidepressant activity by the widely accepted forced swim test and tail suspension test models. Among the tested compounds, **8j** and **8b** exhibited significant antidepressant activity in comparison to normal control group as well as fluoxetine, a standard antidepressant drug, treated group in both forced swim test and tail suspension test models. Furthermore, the antidepressant activity of the tested compounds correlated well with respect to their GSK-3β inhibitory profile signifying the role of GSK-3β inhibitors for the treatment of depression and related disorders. The results of our study suggest that compounds **8j** and **8b** may serve as valuable candidates for further exploration of pathological implications of GSK-3β inhibition on depression and related abnormalities.

Experimental

Materials and Methods

The chemicals used as starting materials and reagents were of analytical grade and purchased from Spectrochem and Sigma Aldrich. Human recombinant GSK-3β and prephosphorylated polypeptide substrate GS-2 were purchased from Merck-Millipore Corporation (India). Kinase-Glo Luminescent Kinase Assay (catalog number V6713) was obtained from Promega Corporation (Madison, WI). Staurosporine and ATP were purchased from Sigma-Aldrich. Luminescence was recorded on Infinite F200® PRO (Tecan) instrument. Fluoxetine hydrochloride was purchased from the local market.

All the melting points have been measured using Veego VMP-DS apparatus and are uncorrected. IR spectra were recorded on Perkin Elmer 1650 spectrophotometer (USA). NMR spectra (^{1}H and ^{13}C) were determined on a Bruker (300 MHz or 400 MHz) spectrometer and chemical shifts are expressed as ppm against TMS as internal reference. Mass spectra were recorded on 70 eV (EI Ms-QP 1000EX, Shimadzu, Japan). Elemental analysis was carried out using Elementar Vario EL III elemental analyzer. Elemental analysis data is reported in % standard.

Synthesis

Procedure for the synthesis of intermediates (**1a-d** to **6a-h)**

Intermediates **1a-d** to **6a-h** have been synthesized as per the procedure reported in the literature[18, 24].

General procedure for the synthesis of target compounds **(7a-7p** and **8a-8p)**

Appropriate acid (6a-h**)** was dissolved in dry DMF. EDC.HCl and HOBT were added and stirring was continued for 30 min at room temperature followed by addition of appropriate anilines. The reaction mixture was allowed to stir for 8-10 h with regular monitoring by TLC. After completion of the reaction, the reaction mixture was poured onto crushed ice and the solid obtained was filtered and air dried. The compounds were then crystallized in ethyl acetate to afford purified target compounds (7a-7p and 8a-8p).

4-(2,5-dihydro-1-methyl-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**7a**)

Yellow-orange powder; yield: 80%; m. p. 259-261 ºC; IR (KBr): ν (cm^{-1}) 3390, 3285, 1754, 1703, 1632, 1596, 1523, 1503, 1440, 1385, 1314, 1262, 1241, 1162, 1062, 986, 825; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H), 6.72 (d, 2H, *J* = 8.8 Hz), 6.98-7.01 (m, 2H), 7.03-7.09 (m, 4H), 7.22-7.26 (m, 2H), 7.50-7.54 (m, 4H), 9.76 (s, 1H), 10.11 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.77, 104.95, 120.05, 120.28, 123.45, 127.29, 127.49, 128.41, 128.50, 129.11, 129.81, 136.68, 139.13, 140.81, 164.41, 167.89, 171.61; ESI-MS: 398 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{19}N_3O_3$: C, 72.53; H, 4.82; N, 10.57; O, 12.08%; Found: C, 72.57; H, 4.81; N, 10.53%.

4-(2,5-dihydro-1-methyl-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**7b**)

Yellow-orange powder; yield: 81%; m. p. 240-241 ºC; IR (KBr): ν (cm^{-1}) 3383, 3278, 1755, 1695, 1642, 1600, 1513, 1453, 1381, 1312, 1270, 1243, 1168, 1106, 991, 824; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.02 (s, 3H), 3.72 (s, 3H), 6.81 (d, 2H, *J* = 8.8 Hz), 6.89-6.91 (m, 2H), 7.03-7.06 (m, 2H), 7.12-7.15 (m, 3H), 7.52-7.57 (m, 4H), 9.85 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.27, 55.61, 105.31, 114.12, 120.56, 122.39, 127.77, 127.85, 129.06, 129.61, 130.32, 132.68, 137.21, 141.12, 155.88, 164.48, 168.38, 172.12; ESI-MS: 428 $(M+1)^+$; Anal. Calcd. for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83; O, 14.97%; Found: C, 70.29; H, 4.92; N, 9.85%.

4-(2,5-dihydro-1-methyl-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**7c**)

Yellow-orange powder; yield: 83%; m. p. 233-234 ^{o}C; IR (KBr): ν (cm^{-1}) 3386, 3299, 1758, 1703, 1634, 1600, 1515, 1453, 1390, 1309, 1263, 1246, 1181, 1126, 994, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.00 (s, 3H), 6.82 (d, 2H, *J* = 7.6 Hz), 7.05-7.06 (m, 2H), 7.14-7.17 (m, 5H), 7.62 (d, 2H, *J* = 7.6 Hz), 7.74 (dd, 2H, *J* = 7.6 Hz and 5.2 Hz), 9.87 (s, 1H), 10.06 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.76, 105.00, 115.06 (d, *J* = 22.0 Hz), 120.05, 122.08 (d, *J* = 8.0 Hz), 127.28, 127.47, 128.22, 129.11, 129.81, 135.48, 135.50, 136.66, 140.87, 158.14 (d, *J* = 239.0 Hz), 164.34, 167.88, 171.60; ESI-MS: 416 $(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{18}FN_3O_3$: C, 69.39; H, 4.37; F, 4.57; N, 10.12; O, 11.55%; Found: C, 69.44; H, 4.39; N, 10.11%.

4-(2,5-dihydro-1-methyl-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**7d**)

Yellow-orange powder; yield: 84%; m. p. 242-243^{o}C; IR (KBr): ν (cm^{-1}) 3386, 3301, 1756, 1702, 1639, 1599, 1524, 1494, 1444, 1389, 1305, 1284, 1239, 1179, 1090, 993, 817;^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H), 6.71 (d, 2H, *J* = 8.0 Hz), 6.98-7.07 (m, 5H), 7.22 (d, 2H, *J* = 9.2 Hz), 7.50-7.55 (m, 4H), 9.87 (s, 1H), 10.06 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.26, 105.64, 120.54, 122.26, 127.54, 127.79, 128.04, 128.59, 128.90, 129.61, 130.29, 137.13, 138.63, 141.48, 165.01, 168.37, 172.08; ESI-MS: 432 $(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{18}ClN_3O_3$: C, 66.75; H, 4.20; Cl, 8.21; N, 9.73; O, 11.11%; Found: C, 66.69; H, 4.23; N, 9.72%.

4-(4-(4-chlorophenyl)-2,5-dihydro-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**7e**)

Yellow-orange powder; yield: 86%; m. p. 216-217 ^{o}C; IR (KBr): ν (cm^{-1}) 3385, 3301, 1757, 1701, 1639, 1599, 1524, 1494, 1444, 1389, 1305, 1285, 1239, 1175, 1090, 993, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.04 (s, 3H), 6.86 (d, 2H, *J* = 8.4 Hz), 7.05-7.10 (m, 3H), 7.23 (d, 2H, *J* = 8.8 Hz), 7.31-7.35 (m, 2H), 7.69-7.74 (m, 4H), 9.93 (s, 1H), 9.99 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.61, 102.90, 120.13, 120.33, 123.27, 127.06, 127.53, 128.18, 128.37, 128.89, 130.47, 132.00, 136.76, 138.98, 140.34, 164.36, 167.49, 171.22; ESI-MS: 432 $(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{18}ClN_3O_3$: C, 66.75; H, 4.20; Cl, 8.21; N, 9.73; O, 11.11%; Found: C, 66.70; H, 4.21; N, 9.72%.

4-(4-(4-chlorophenyl)-2,5-dihydro-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**7f**)

Yellow-orange powder; yield: 79%; m. p. 212-213 ºC; IR (KBr): ν (cm^{-1}) 3392, 3261, 1750, 1671, 1649, 1610, 1590, 1506, 1454, 1392, 1307, 1290, 1234, 1175, 1093, 961, 820; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.04 (s, 3H), 3.70 (s, 3H), 6.60 (d, 2H, *J* = 8.4 Hz), 6.79 (d, 2H, *J* = 9.0 Hz), 6.95 (d, 2H, *J* = 8.4 Hz), 7.07 (d, 2H, *J* = 8.7 Hz), 7.39 (d, 2H, *J* = 9.0 Hz), 7.51 (d, 2H, *J* = 8.4 Hz), 9.93 (s, 1H), 9.99 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.28, 60.23, 103.74, 114.13, 120.82, 122.45, 127.79, 127.97, 129.23, 129.36, 131.17, 132.17, 132.65, 137.68, 141.07, 155.92, 164.43, 168.15, 171.93; ESI-MS: 462 $(M+1)^+$; Anal. Calcd. for $C_{25}H_{20}ClN_3O_4$: C, 65.01; H, 4.36; Cl, 7.68; N, 9.10; O, 13.86%; Found: C, 65.06; H, 4.34; N, 9.07%.

4-(4-(4-chlorophenyl)-2,5-dihydro-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**7g**)

Yellow-orange powder; yield: 82%; m. p. 206-207 ºC; IR (KBr): ν (cm^{-1}) 3382, 3291, 1753, 1697, 1644, 1612, 1587, 1505, 1454, 1387, 1319, 1176, 1084, 993, 828; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.98 (s, 3H); 6.84 (d, 2H, *J* = 8.4 Hz); 7.04 (d, 2H, *J* = 8.4 Hz); 7.13-7.18 (m, 2H); 7.22 (d, 2H, *J* = 8.4 Hz); 7.67 (d, 2H, *J* = 8.4 Hz); 7.75-7.71 (m, 2H); 9.96 (s, 1H); 10.06 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.27, 103.97, 115.56 (d, *J* = 22.0 Hz), 120.80, 122.64 (d, *J* = 8.0 Hz), 127.80, 128.09, 129.04, 129.16, 131.18, 132.24, 135.96, 137.55, 141.21, 158.68 (d, *J* = 239.0 Hz), 164.78, 168.15, 171.91; ESI-MS: 450 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{17}ClFN_3O_3$: C, 64.08; H, 3.81; Cl, 7.88; F, 4.22; N, 9.34; O, 10.67%; Found: C, 64.13; H, 3.83; N, 9.31%.

4-(4-(4-chlorophenyl)-2,5-dihydro-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**7h**)

Orange powder; yield: 85%; m. p. 257-259 ºC; IR (KBr): ν (cm^{-1}) 3396, 3298, 1753, 1689, 1632, 1603, 1587, 1495, 1434, 1387, 1306, 1291, 1229, 1178, 1083, 990, 825; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.00 (s, 3H), 6.86 (d, 2H, *J* = 8.4 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 2H, *J* = 8.4 Hz), 7.38 (d, 2H, *J* = 9.2 Hz), 7.69 (d, 2H, *J* = 8.4 Hz), 7.78 (d, 2H, *J* = 8.8 Hz), 9.95 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.80, 103.65, 120.29, 121.80, 127.08, 127.33, 127.67, 128.41, 128.66, 130.69, 131.77, 137.04, 138.10, 140.83, 164.45, 167.66, 171.41; ESI-MS: 466 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{17}Cl_2N_3O_3$: C, 61.82; H, 3.67; Cl, 15.21; N, 9.01; O, 10.29%; Found: C, 61.76; H, 3.69; N, 9.04%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**7i**)

Orange powder; yield: 86%; m. p. 211-212 ^{o}C; IR (KBr): ν (cm^{-1}) 3384, 3295, 1756, 1688, 1658, 1603, 1581, 1512, 1435, 1395, 1338, 1294, 1246, 1173, 1090, 992, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H), 3.68 (s, 3H), 6.76 (d, 2H, *J* = 8.4 Hz), 6.82 (d, 2H, *J* = 8.4 Hz), 7.02-7.09 (m, 3H), 7.32 (d, 2H, *J* = 7.6 Hz), 7.66 (d, 2H, *J* = 8.4 Hz), 7.72 (d, 2H, *J* = 7.6 Hz), 9.72 (s, 1H), 9.96 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.73, 55.05, 106.05, 112.91, 119.54, 120.31, 122.04, 123.42, 127.59, 128.01, 128.49, 130.45, 135.21, 139.17, 141.05, 158.44, 164.49, 168.13, 171.79; ESI-MS: 428 $(M+1)^{+}$; Anal. Calcd. for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83; O, 14.97%; Found: C, 70.18; H, 4.98; N, 9.81%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**7j**)

Orange powder; yield: 79%; m. p. 213-214 ^{o}C; IR (KBr): ν (cm^{-1}) 3385, 3282, 1756, 1695, 1643, 1596, 1503, 1454, 1388, 1315, 1294, 1246, 1179, 1092, 993, 825; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H), 3.68 (s, 3H), 3.73 (s, 3H), 6.75 (d, 2H, *J* = 8.8 Hz), 6.81 (d, 2H, *J* = 8.4 Hz), 6.90 (d, 2H, *J* = 9.2 Hz), 7.03 (d, 2H, *J* = 8.8 Hz), 7.60-7.66 (m, 4H), 9.70 (s, 1H), 9.86 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.73, 55.05, 55.10, 105.88, 112.89, 113.61, 119.56, 121.92, 122.05, 127.44, 128.15, 130.45, 132.21, 135.23, 140.85, 155.37, 158.42, 164.08, 168.13, 171.80; ESI-MS: 458 $(M+1)^{+}$; Anal. Calcd. for $C_{26}H_{23}N_3O_5$: C, 68.26; H, 5.07; N, 9.19; O, 17.49%; Found: C, 68.20; H, 5.09; N, 9.22%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**7k**)

Orange powder; yield: 83%; m. p. 202-203 ^{o}C; IR (KBr): ν (cm^{-1}) 3390, 3271, 1760, 1695, 1650, 1603, 1581, 1504, 1435, 1392, 1309, 1294, 1235, 1175, 1096, 990, 818; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H); 3.68 (s, 3H); 6.75 (d, 2H, *J* = 8.4 Hz); 6.82 (d, 2H, *J* = 8.4 Hz), 7.03 (d, 2H, *J* = 8.4 Hz), 7.14-7.18 (m, 2H), 7.65 (d, 2H, J = 8.4 Hz), 7.72-7.75 (m, 2H), 9.72 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): 23.73, 55.05, 106.11, 112.91, 115.05 (d, *J* = 22.0 Hz), 119.54, 122.04, 122.10 (d, *J* = 8.0 Hz), 127.57, 127.81, 130.45, 135.18, 135.51, 140.10, 158.13 (d, *J* = 239.0 Hz), 158.44, 164.43, 168.12, 171.78; ESI-MS: 446 $(M+1)^{+}$; Anal. Calcd. for $C_{25}H_{20}FN_3O_4$: C, 67.41; H, 4.53; F, 4.27; N, 9.43; O, 14.37%; Found: C, 67.38; H, 4.52; N, 9.43%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**7l**)

Orange powder; yield: 86%; m. p. 208-209 ^{o}C; IR (KBr): ν (cm^{-1}) 3389, 3263, 1750, 1672, 1650, 1612, 1591, 1506, 1455, 1394, 1307, 1290, 1236, 1176, 1093, 961, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.99 (s, 3H), 3.68 (s, 3H), 6.76 (d, 2H, *J* = 8.4 Hz), 6.83 (d, 2H, *J* = 8.4 Hz), 7.03 (d, 2H, *J* = 8.4 Hz), 7.38 (d, 2H, *J* = 8.8 Hz), 7.66 (d, 2H, *J* = 8.4 Hz), 7.77 (d, 2H, *J* = 8.4 Hz), 9.74 (s, 1H), 10.11 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.74, 55.05, 106.25, 112.92, 119.52, 121.76, 122.02, 127.00, 127.65, 128.41, 130.45, 135.14, 138.17, 140.23, 158.45, 164.59, 168.13, 171.17; ESI-MS: 462 $(M+1)^{+}$; Anal. Calcd. for $C_{25}H_{20}ClN_3O_4$: C, 65.01; H, 4.36; Cl, 7.68; N, 9.10; O, 13.86%; Found: C, 65.05; H, 4.34; N, 9.08%.

4-(2,5-dihydro-4-(4-nitrophenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**7m**)

Orange powder; yield: 87%; m. p. 231-232 ^{o}C; IR (KBr): ν (cm^{-1}) 3396, 3288, 1763, 1695, 1632, 1591, 1519, 1456, 1341, 1306, 1291, 1228, 1174, 1111, 990, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.01 (s, 3H), 6.84 (d, 2H, *J* = 8.4 Hz), 7.06-7.09 (m, 1H), 7.23 (d, 2H, *J* = 8.0 Hz), 7.58 (d, 2H, *J* = 8.4 Hz), 7.67-7.71 (m, 4H), 7.98 (d, 2H, *J* = 8.4 Hz), 9.74 (s, 1H), 10.11 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.85, 101.72, 120.93, 121.46, 122.65, 124.05, 128,15, 128.99, 129.97, 130.49, 137.42, 139.52, 139.64, 140.93, 145.91, 164.80, 168.78, 172.51; ESI-MS: 443 $(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{18}N_4O_5$: C, 65.15; H, 4.10; N, 12.66; O, 18.08%; Found: C, 65.09; H, 4.12; N, 12.63%.

4-(2,5-dihydro-4-(4-nitrophenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**7n**)

Red-orange powder; yield: 85%; m. p. 227-229 ^{o}C; IR (KBr): ν (cm^{-1}) 3396, 3307, 1763, 1685, 1648, 1598, 1511, 1434, 1410, 1340, 1320, 1236, 1175, 1093, 960, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.95 (s, 3H), 3.66 (s, 3H), 6.80-6.83 (m, 4H), 7.17 (d, 2H, *J* = 8.8 Hz), 7.52 (d, 2H, *J* = 8.8 Hz), 7.60 (d, 2H, *J* = 9.2 Hz), 7.92 (d, 2H, *J* = 8.8 Hz), 9.81 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.86, 55.09, 101.20, 113.62, 121.11, 122.12, 122.22, 127.53, 129.67, 129.79, 131.92, 136.85, 139.27, 140.19, 145.32, 155.48, 163.95, 167.15, 171.08; ESI-MS: 473 $(M+1)^{+}$; Anal. Calcd. for $C_{25}H_{20}N_4O_6$: C, 63.56; H, 4.27; N, 11.86; O, 20.32%; Found: C, 63.60; H, 4.26; N, 11.83%.

4-(2,5-dihydro-4-(4-nitrophenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**7o**)

Yellow-orange powder; yield: 86%; m. p. 265-267 ^{o}C; IR (KBr): ν (cm^{-1}) 3398, 3291, 1743, 1684, 1626, 1614, 1594, 1501, 1458, 1399, 1339, 1249, 1205, 1094, 990, 825; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.95 (s, 3H), 6.82 (d, 2H, *J* = 8.4 Hz), 7.07-7.11 (m, 2H), 7.18 (d, 2H, *J* = 8.8 Hz), 7.59-7.66 (m, 4H), 7.92 (d, 2H, *J* = 8.8 Hz), 9.98 (s, 1H); 10.20 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.88, 101.43, 115.09 (d, *J* = 21.00 Hz), 121.04, 122.21(d, *J* = 9.00 Hz), 127.67, 129.38, 129.78, 132.49, 135.34, 135.45, 136.85, 139.21, 145.35, 158.19 (d, *J* = 239.00 Hz), 164.22, 167.16, 171.05; ESI-MS: 461$(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{17}FN_4O_5$: C, 62.61; H, 3.72; F, 4.13; N, 12.17; O, 17.38%; Found: C, 62.64; H, 3.71; N, 12.18%.

4-(2,5-dihydro-4-(4-nitrophenyl)-1-methyl-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**7p**)

Orange powder; yield: 88%; m. p. 280-282^{o}C; IR (KBr): ν (cm^{-1}) 3390, 3294, 1760, 1696, 1643, 1591, 1517, 1501, 1454, 1384, 1346, 1302, 1238, 1173, 1090, 986, 848; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.02 (s, 3H), 6.90 (d, 2H, *J* = 8.8 Hz), 7.26 (d, 2H, *J* = 8.0 Hz), 7.38 (d, 2H, *J* = 8.8 Hz), 7.68 (d, 2H, *J* = 8.4 Hz), 7.74 (d, 2H, *J* = 8.8 Hz), 8.00 (d, 2H, *J* = 8.8 Hz), 10.11 (s, 1H); 10.27 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.88, 101.54, 121.02, 122.85, 127.26, 127.14, 127.75, 128.42, 129.26, 129.80, 136.81, 138.01, 139.15, 140.52, 145.37, 164.39, 167.16, 171.04; ESI-MS: 477 $(M+1)^{+}$; Anal. Calcd. for $C_{24}H_{17}ClN_4O_5$: C, 60.45; H, 3.59; Cl, 7.43; N, 11.75; O, 16.78%; Found: C, 60.41; H, 3.61; N, 11.78%.

4-(2,5-dihydro-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**8a**)

Orange powder; yield: 81%; m. p. 276-278 ^{o}C; IR (KBr): ν (cm^{-1}) 3391, 3286, 1754, 1701, 1630, 1594, 1521, 1501, 1440, 1385, 1314, 1260, 1241, 1162, 1062, 985, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.81 (d, 2H, *J* = 8.8 Hz); 7.03-7.08 (m, 3H); 7.14-7.18 (m, 3H); 7.29-7.33 (m, 2H); 7.63 (d, 2H, *J* = 8.8 Hz); 7.72 (d, 2H, *J* = 7.6 Hz); 9.70 (s, 1H); 9.76 (s, 1H); 10.88 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 106.29, 120.45, 120.81, 123.94, 127.72, 127.99, 128.82, 128.99, 129.75, 130.37, 137.15, 139.65, 141.38, 164.94, 169.54, 173.09; ESI-MS: 384 $(M+1)^{+}$; Anal. Calcd. for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96; O, 12.52%; Found: C, 72.12; H, 4.49; N, 10.94%.

4-(2,5-dihydro-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**8b**)

Yellow-orange powder; yield: 85%; m. p. 290-292 °C; IR (KBr): ν (cm^{-1}) 3385, 3336, 3280, 1758, 1697, 1669, 1634, 1595, 1504, 1475, 1410, 1349, 1301, 1249, 1237, 1175, 1103, 1027, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.72 (s, 3H), 6.80 (d, 2H, *J* = 8.8 Hz), 6.88-6.90 (m, 2H), 7.02-7.05 (m, 2H), 7.13-7.16 (m, 3H), 7.59-7.62 (m, 4H), 9.68 (s, 1H), 9.85 (s, 1H), 10.87 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.62, 106.12, 114.13, 120.46, 122.42, 127.71, 127.84, 128.95, 129.75, 130.38, 132.70, 137.17, 141.19, 155.89, 164.52, 169.54, 173.11; ESI-MS: 414 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{19}N_3O_4$: C, 69.72; H, 4.63; N, 10.16; O, 15.48%; Found: C, 69.74; H, 4.61; N, 10.15%.

4-(2,5-dihydro-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**8c**) Red-orange powder; yield: 86%; m. p. 292-293 °C; IR (KBr): ν (cm^{-1}) 3392, 3284, 1757, 1699, 1632, 1596, 1521, 1501, 1439, 1384, 1314, 1260, 1241, 1162, 1062, 985, 825; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.81 (d, 2H, *J* = 8.8 Hz), 7.03-7.06 (m, 2H), 7.12-7.19 (m, 5H), 7.62 (d, 2H, *J* = 8.8 Hz), 7.71-7.76 (m, 2H), 9.70 (s, 1H), 10.03 (s, 1H), 10.88 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 106.33, 115.55 (d, *J* = 22.0 Hz), 120.45, 122.57, 122.68 (d, *J* = 8.0 Hz), 127.96, 128.62, 129.74, 130.36, 136.01, 137.13, 141.43, 158.65 (d, *J* = 238.0 Hz), 164.87, 169.53, 173.09; ESI-MS: 402 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{16}FN_3O_3$: C, 68.82; H, 4.02; F, 4.73; N, 10.47; O, 11.96%; Found: C, 68.77; H, 3.99; N, 10.43%.

4-(2,5-dihydro-2,5-dioxo-4-phenyl-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**8d**) Red-orange powder; yield: 82%; m. p. 274-275 °C; IR (KBr): ν (cm^{-1}) 3381, 3342, 3303, 3145, 1759, 1696, 1668, 1634, 1599, 1582, 1505, 1432, 1415, 1308, 1296, 1240, 1179, 1088, 998, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.81 (d, 2H, *J* = 8.8 Hz), 7.03-7.05 (m, 2H), 7.14-7.16 (m, 3H), 7.36-7.38 (m, 2H), 7.61 (d, 2H, *J* = 8.8 Hz), 7.75-7.77 (m, 2H), 9.71 (s, 1H), 10.09 (s, 1H), 10.88 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 106.45, 120.43, 122.27, 127.53, 127.73, 128.03, 128.46, 128.90, 129.73, 130.35, 137.10, 138.64, 141.56, 165.03, 169.52, 173.07; ESI-MS: 418 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{16}ClN_3O_3$: C, 66.11; H, 3.86; Cl, 8.48; N, 10.06; O, 11.49%; Found: C, 66.17; H, 3.89; N, 10.04%.

4-(4-(4-chlorophenyl)-2,5-dihydro-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**8e**) Yellow-orange powder; yield: 85%; m. p. 258-260 °C; IR (KBr): ν (cm^{-1}) 3387, 3343, 3301, 3145, 1758, 1696, 1668, 1632, 1597, 1582, 1506, 1433, 1415, 1308, 1296, 1240, 1180, 1088, 998, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.85 (d, 2H, *J* = 8.4 Hz), 7.04-7.09 (m, 3H), 7.22 (d, 2H, *J* = 8.4 Hz), 7.31-7.34 (m, 2H), 7.68-7.74 (m, 4H), 9.81 (s, 1H), 10.00 (s, 1H), 10.94

(s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 104.25, 120.21, 120.36, 123.49, 127.24, 127.61, 128.50, 128.61, 128.74, 130.85, 131.72, 137.05, 139.11, 140.73, 164.37, 168.82, 172.43; ESI-MS: 418 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{16}ClN_3O_3$: C, 66.11; H, 3.86; Cl, 8.48; N, 10.06; O, 11.49%; Found: C, 66.05; H, 3.89; N, 10.03%.

4-(4-(4-chlorophenyl)-2,5-dihydro-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**8f**)

Yellow-orange powder; yield: 83%; m. p. 282-284 ºC; IR (KBr): ν (cm^{-1}) 3390, 3344, 3301, 3189, 1757, 1666, 1698, 1639, 1602, 1514, 1488, 1391, 1355, 1303, 1242, 1169, 1094, 1013, 826; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.74 (s, 3H), 6.83 (d, 2H, J = 8.4 Hz), 6.90 (d, 2H, J = 8.4 Hz), 7.04 (d, 2H, J = 8.0 Hz), 7.22 (d, 2H, J = 8.4 Hz), 7.61-7.69 (m, 4H), 9.79 (s, 1H), 9.90 (s, 1H), 10.94 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.11, 104.09, 113.62, 120.22, 121.98, 127.22, 127.46, 128.74, 130.85, 131.70, 132.14, 137.07, 140.53, 155.42, 163.95, 168.82, 172.44; ESI-MS: 448 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{18}ClN_3O_4$: C, 64.36; H, 4.05; Cl, 7.92; N, 9.38; O, 14.29%; Found: C, 64.31; H, 4.08; N, 9.35%.

4-(4-(4-chlorophenyl)-2,5-dihydro-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**8g**)

Red-orange powder; yield: 83%; m. p. 272-273 ºC; IR (KBr): ν (cm^{-1}) 3402, 3334, 3300, 3197, 1761, 1697, 1661, 1633, 1601, 1505, 1487, 1401, 1347, 1302, 1249, 1209, 1091, 1012, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): 6.84 (d, 2H, J = 8.4 Hz), 7.05 (d, 2H, J = 8.4 Hz), 7.12-7.17 (m, 2H), 7.23 (d, 2H, J = 8.4 Hz), 7.65 (d, 2H, J = 8.4 Hz), 7.76-7.71 (m, 2H), 9.85 (s, 1H), 10.11 (s, 1H), 10.81 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 104.30, 115.07 (d, J = 22.0 Hz), 120.21, 122.16 (d, J = 8.0 Hz), 127.25, 127.58, 128.41, 128.73, 130.84, 131.73, 135.45, 137.03, 140.78, 158.17 (d, J = 238.0 Hz), 164.30, 168.82, 172.42; ESI-MS: 436 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{15}ClFN_3O_3$: C, 63.38; H, 3.47; Cl, 8.13; F, 4.36; N, 9.64; O, 11.01%; Found: C, 63.44; H, 3.45; N, 9.62%.

4-(4-(4-chlorophenyl)-2,5-dihydro-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**8h**)

Red-orange powder; yield: 85%; m. p. 290-292 ºC; IR (KBr): ν (cm^{-1}) 3392, 3319, 3281, 3155, 1759, 1694, 1661, 1633, 1602, 1504, 1487, 1395, 1353, 1303, 1283, 1237, 1176, 1086, 1012, 829; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.85 (d, 2H, J = 7.6 Hz), 7.05 (d, 2H, J = 7.6 Hz), 7.23 (d, 2H, J = 7.2 Hz), 7.39 (d, 2H, J = 8.0 Hz), 7.68 (d, 2H, J = 7.6 Hz), 7.77 (d, 2H, J = 7.6 Hz),

9.82 (s, 1H), 10.14 (s, 1H), 10.96 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 104.40, 120.19, 121.81, 127.07, 127.26, 127.66, 128.26, 128.42, 128.71, 130.84, 131.75, 136.99, 138.11, 140.89, 164.47, 168.82, 172.42; ESI-MS: 452 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{15}Cl_2N_3O_3$: C, 61.08; H, 3.34; Cl, 15.68; N, 9.29; O, 10.61%; Found: C, 61.13; H, 3.31; N, 9.26%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**8i**)

Yellow-orange powder; yield: 86%; m. p. 268-270^{o}C; IR (KBr): ν (cm^{-1}) 3386, 3338, 3281, 1759, 1696, 1669, 1635, 1596, 1506, 1472, 1410, 1349, 1302, 1249, 1237, 1172, 1103, 1028, 831; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.68 (s, 3H), 6.74-6.76 (m, 2H), 6.81 (d, 2H, J = 8.0 Hz), 7.02-7.09 (m, 3H), 7.30-7.34 (m, 2H), 7.66 (d, 2H, J = 8.8 Hz), 7.73 (d, 2H, J = 8.8 Hz), 9.59 (s, 1H), 9.98 (s, 1H), 10.83 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.56, 107.34, 113.36, 119.94, 120.83, 122.60, 123.92, 128.08, 128.40, 128.98, 131.08, 135.75, 139.68, 141.62, 158.94, 165.01, 169.77, 173.28; ESI-MS: 414 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{19}N_3O_4$: C, 69.72; H, 4.63; N, 10.16; O, 15.48%; Found: C, 69.77; H, 4.61; N, 10.15%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**8j**)

Orange powder; yield: 84%; m. p. 274-276 ^{o}C; IR (KBr): ν (cm^{-1}) 3382, 3318, 3223, 3198, 1760, 1694, 1650, 1630, 1601, 1517, 1497, 1411, 1361, 1303, 1295, 1259, 1174, 1104, 1026, 820; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.68 (s, 3H), 3.73 (s, 3H), 6.73-6.76 (m, 2H), 6.80 (d, 2H, J = 8.8 Hz), 6.88-6.91 (m, 2H), 7.01-7.03 (m, 2H), 7.61-7.65 (m, 4H), 9.57 (s, 1H), 9.87 (s, 1H), 10.83 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.55, 55.61, 107.14, 113.34, 114.12, 119.97, 122.45, 122.62, 127.94, 128.55, 131.08, 132.72, 135.75, 141.42, 155.89, 158.92, 164.61, 169.78, 173.30; ESI-MS: 444 $(M+1)^+$; Anal. Calcd. for $C_{25}H_{21}N_3O_5$: C, 67.71; H, 4.77; N, 9.48; O, 18.04%; Found: C, 67.66; H, 4.74; N, 9.49%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**8k**)

Red-orange powder; yield: 85%; m. p. 271-272 ^{o}C; IR (KBr): ν (cm^{-1}) 3383, 3349, 3281, 3166, 1758, 1696, 1666, 1634, 1601, 1504, 1470, 1410, 1349, 1303, 1249, 1173, 1104, 1025, 830; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.68 (s, 3H), 6.74-6.76 (m, 2H), 6.81 (d, 2H, J = 8.8 Hz), 7.02-7.04 (m, 2H), 7.14-7.18 (m, 2H), 7.65 (d, 2H, J = 8.8 Hz), 7.72-7.76 (m, 2H), 9.59 (s, 1H), 10.04 (s, 1H), 10.84 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.55, 107.38, 113.36,

115.54 (d, *J* = 22.0 Hz), 119.95, 122.62 (d, *J* = 8.0 Hz), 122.87, 128.06, 128.21, 131.08, 135.70, 136.04, 141.68, 158.64 (d, *J* = 239.0 Hz), 158.94, 164.95, 169.77, 173.28; ESI-MS: 432 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{18}FN_3O_4$: C, 66.82; H, 4.21; F, 4.40; N, 9.74; O, 14.83%; Found: C, 66.86; H, 4.23; N, 9.71%.

4-(2,5-dihydro-4-(4-methoxyphenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**8l**)

Orange powder; yield: 88%; m. p. 286-288 ^oC; IR (KBr): ν (cm^{-1}) 3375, 3339, 3292, 3166, 1757, 1695, 1667, 1633, 1601, 1504, 1411, 1350, 1302, 1249, 1173, 1105, 1026, 825; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.68 (s, 3H), 6.74-6.76 (m, 2H), 6.82 (d, 2H, *J* = 8.8 Hz), 7.02-7.04 (m, 2H), 7.37-7.39 (m, 2H), 7.65 (d, 2H, *J* = 8.8 Hz), 7.76-7.78 (m, 2H), 9.59 (s, 1H), 10.10 (s, 1H), 10.84 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.56, 107.52, 113.37, 119.93, 122.28, 122.59, 127.51, 128.07, 128.14, 128.89, 131.08, 135.67, 138.68, 141.80, 158.96, 165.11, 169.76, 173.26; ESI-MS: 448 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{18}ClN_3O_4$: C, 64.36; H, 4.05; Cl, 7.92; N, 9.38; O, 14.29%; Found: C, 64.41; H, 4.07; N, 9.40%.

4-(2,5-dihydro-4-(4-nitrophenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-phenylbenzamide (**8m**)

Red-orange powder; yield: 81%; m. p. 281-282 ^oC; IR (KBr): ν (cm^{-1}) 3386, 3349, 3294, 3187, 1758, 1703, 1667, 1632, 1597, 1514, 1442, 1402, 1341, 1317, 1248, 1184, 1110, 1024, 854; ^1H NMR (DMSO-d_6, 400 MHz): δ 6.89 (d, 2H, *J* = 8.8 Hz), 7.06-7.09 (m, 1H), 7.26 (d, 2H, *J* = 8.4 Hz), 7.30-7.34 (m, 2H), 7.67-7.71 (m, 4H), 7.99 (d, 2H, *J* = 8.8 Hz), 9.98 (s, 1H), 10.11 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 102.68, 120.92, 121.46, 122.65, 124.05, 128.17, 128.99, 129.97, 130.48, 137.43, 139.52, 139.64, 140.93, 145.90, 164.80, 168.79, 172.52; ESI-MS: 429 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{16}N_4O_5$: C, 64.48; H, 3.76; N, 13.08; O, 18.67%; Found: C, 64.53; H, 3.74; N, 13.11%.

4-(2,5-dihydro-4-(4-nitrophenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-methoxyphenyl)benzamide (**8n**)

Red-orange powder; yield: 84%; m. p. 294-296 ^oC; IR (KBr): ν (cm^{-1}) 3378, 3349, 3294, 3164, 1770, 1715, 1651, 1633, 1601, 1513, 1412, 1342, 1315, 1247, 1179, 1107, 1026, 820; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.73 (s, 3H), 6.86-6.90 (m, 4H), 7.24 (d, 2H, *J* = 8.8 Hz), 7.59 (d, 2H, *J* = 8.8 Hz), 7.66 (d, 2H, *J* = 8.8 Hz), 7.98 (d, 2H, *J* = 8.8 Hz), 9.86 (s, 1H), 10.09 (s, 1H), 11.06 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.62, 102.56, 114.13, 121.49, 122.56, 122.63, 128.03, 130.13, 130.48, 132.56, 137.44, 139.68, 140.75, 145.90, 155.98, 164.40, 168.79,

172.52; ESI-MS: 459 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{18}N_4O_6$: C, 62.88; H, 3.96; N, 12.22; O, 20.94%; Found: C, 62.92; H, 3.94; N, 12.23%.

4-(2,5-dihydro-4-(4-nitrophenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-fluorophenyl)benzamide (**8o**)

Red-orange powder; yield: 77%; m. p. 292-294 ^oC; IR (KBr): ν (cm^{-1}) 3378, 3345, 3301, 3106, 1766, 1714, 1643, 1609, 1596, 1531, 1509, 1405, 1345, 1311, 1261, 1208, 1135, 1007, 832; ^1H NMR (DMSO-d_6, 400 MHz): δ 6.88 (d, 2H, *J* = 8.8 Hz), 7.14-7.18 (m, 2H), 7.26 (d, 2H, *J* = 8.4 Hz), 7.66-7.73 (m, 4H), 7.99 (d, 2H, *J* = 8.8 Hz), 10.04 (s, 1H), 10.11 (s, 1H), 11.06 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 102.69, 115.55 (d, *J* = 22.0 Hz), 121.47, 122.65, 122.74 (d, *J* = 8.0 Hz), 128.15, 129.78, 130.45, 135.89, 137.46, 139.68, 141.05, 145.88, 158.71 (d, *J* = 239.0 Hz), 164.75, 168.77, 172.51; ESI-MS: 447 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{15}FN_4O_5$: C, 61.88; H, 3.39; F, 4.26; N, 12.55; O, 17.92%; Found: C, 61.93; H, 3.42; N, 12.52%.

4-(2,5-dihydro-4-(4-nitrophenyl)-2,5-dioxo-1H-pyrrol-3-ylamino)-N-(4-chlorophenyl)benzamide (**8p**)

Red-orange powder; yield: 83%; m. p. 280-282 ^oC; IR (KBr): ν (cm^{-1}) 3343, 3266, 3222, 3116, 1769, 1709, 1646, 1621, 1589, 1538, 1496, 1398, 1336, 1307, 1255, 1188, 1112, 1012, 821; ^1H NMR (DMSO-d_6, 400 MHz): δ 6.89 (d, 2H, *J* = 8.8 Hz); 7.25 (d, 2H, *J* = 8.8 Hz); 7.36-7.39 (m, 2H); 7.67 (d, 2H, *J* = 8.8 Hz); 7.73-7.76 (m, 2H); 7.98-8.00 (m, 2H); 10.10 (s, 1H); 10.11 (s, 1H); 11.07 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 102.83, 121.45, 122.37, 122.66, 127.65, 128.22, 128.90, 129.65, 130.48, 137.41, 138.53, 139.60, 141.09, 145.92, 164.91, 168.79, 172.51; ESI-MS: 463 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{15}ClN_4O_5$: C, 59.68; H, 3.27; Cl, 7.66; N, 12.10; O, 17.28%; Found: C, 59.65; H, 3.28; N, 12.11%.

GSK-3β inhibitory activity

The synthesized compounds were screened for their GSK-3β inhibition potentialby Kinase-Glo assay luminescence assay[23]. This assay determines the kinase activity by quantifying the remaining amount of ATP in solution following a kinase reaction. In a conventional assay, 10 µL of test compound of different concentrations (dissolved in dimethyl sulfoxide [DMSO] and diluted with assay buffer) and 10µL (20 ng) of enzyme were added to

each well succeeded by 20μL of assay buffer containing substrate and ATP to obtain a concentration of 25 μM substrate and 1 μM ATP per well. The final DMSO concentration in the reaction mixture was kept below 1%. The enzymatic reaction,after half-an-hour of incubation at 30°C, was quenched with 40 μL of Kinase-Glo reagent. Luminescence was then recorded after 10 min using Infinite F200®PRO multimode reader (Tecan). The activity is proportional to the difference of the total and consumed ATP. Staurosporine, a well-known kinase inhibitor, was used as a standard GSK-3β inhibitor.

In silico molecular docking studies

To provide more insights at molecular level, the synthesized molecules are virtually prepared and docked against GSK-3β protein target (PDB: 1Q3D) using Schrodinger Maestro suite (Version 10.2). The ligands were virtually prepared by Lig-Prep module (Version 3.3). The GSK-3β protein was prepared by Protein Preparation Wizard through Maestro Interface (Version 10.2). The binding pocket was generated by selecting a grid with respect to reference ligand staurosporine. The prepared molecules were docked against the generated grid using Glide Module (Version 6.6) with Extra Precision (XP) mode. The binding pocket which is defined by important residues like ASP-133, VAL-135 residues and other residues like ILE-62, VAL70 and PHE-67 form the hydrophobic pocket. The docked molecules were ranked with respect to Glide score.

Antidepressant Activity

Antidepressant activity was evaluated by the two well-known behavioural models- Forced swim test and Tail suspension test.

Animals

Adult male wistar rats (180-200 g) were used for antidepressant studies. The rats were procured from Central Animal House, Hamdard University (Jamia Hamdard), New Delhi, kept incages at room temperature and fed with food and water *ad libitum*. The experiments were performed in compliance with Institutional Animals Ethics Committee guidelines, approved by the Institutional Animal Ethics Committee of the University, Jamia Hamdard, New Delhi, India (Registration No. 1103-CPSCEA). All the compounds were either suspended in 0.5% methylcellulose (Methocel) or dissolved in distilled water when soluble and administered orally 1 h before behavioural testing.

Forced Swim Test:

The FST is the most widely used pre-clinic behavioural test for screening of novel compounds for potential antidepressant activity[25]. The test was performed following the procedure described by Vincent Castagne et al[26]. Rats were moved from the housing colony room to the testing laboratory and kept undisturbed for at least 1 h before testing. They were provided free access to standard rodent diet and tap water, except during the test. A controlled temperature of 21±3 ^{0}C and standard light/dark cycle with illumination from 0700 to 1900 h were maintained. Each experimental group consisted of 6 rats. The rats were individually placed to swim in a plexiglas cylinders (20 cm in diameter × 40 cm high) filled with water (25°C) to a depth of 30 cm. A prior habituation session of 15-min was conducted prior to drug administration and without behavioural recording. After 24 h, each group were administered with the appropriate treatment of fluoxetine (30 mg/kg BW) or test compounds (20 mg/kg BW) or their vehicles followed 1 h later by a 6-min test session. The total immobility time, defined by lack of activity, except for necessary movements to keep their heads above water, was scored during this session. Data was compared with the data from the control and the standard group.

Tail suspension test (TST)

The tail suspension test as a model of behavioural despair is conceptually related to the forced swimming test, except that immobility is induced by suspending the rat by its tail [27, 28]. Rats were moved from the housing colony room to the testing laboratory and kept undisturbed for at least 1 h before testing. The dosage, temperature and other environmental conditions were provided same as in forced swim test. The test was performed using an automated apparatus. Rats were individually suspended upside-down using an adhesive scotch tape to fix its tail to a hook at 60 cm connected to a strain gauge. A square platform was temporarily positioned 20 cm below the apparatus, just under the rat's forepaws, in order to minimize the weight sustained by the animal's tail until the beginning of the recording session. The movements of the rat were picked up by the strain gauge and transmitted there from to a central unit that calculated the total duration of immobility during a 6-min test with the help of a maze software version 3.0. The rat was considered immobile when it was not making any movements of struggling, attempting to catch the adhesive tape, body torsions, or jerks. Data collected were expressed as a mean of immobility time (in second ± the standard error mean: S.E.M).

Statistical analysis

Results are expressed as the mean ± SEM, and different groups were compared using one way analysis of variance (ANOVA) followed by Tukey Kramer test for multiple comparisons.

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Table of Content

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