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Synthesis of benzimidazole based thiadiazole and carbohydrazide conjugates as Glycogen Synthase Kinase-3β inhibitors with anti-depressant activity

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Abstract

A series of benzimidazole based thiadiazole and carbohydrazide conjugates have been synthesized and evaluated for inhibition of glycogen synthase kinase-3β and anti-depressant effect. Compounds **4f, 4j, 5b, 5g** and **5i** were found to be the most potent inhibitors of GSK-3β *in vitro* amongst the twenty-five benzimidazole based thiadiazole and carbohydrazide conjugates synthesized. Compound **5i** was also found to exhibit significant antidepressant activity *in vivo* at 50 mg/Kg, when compared to fluoxetine, a known antidepressant drug. The molecular docking studies revealed multiple hydrogen bond interactions by the synthesized compounds with various amino acid residues, viz, ASP-133, LYS-183, PRO-136, VAL-135, TYR-134, or LYS-60 at the GSK-3β receptor site.

Key words: Benzimidazole; thiadiazole; molecular docking; glycogen synthase kinase; carbohydrazide.

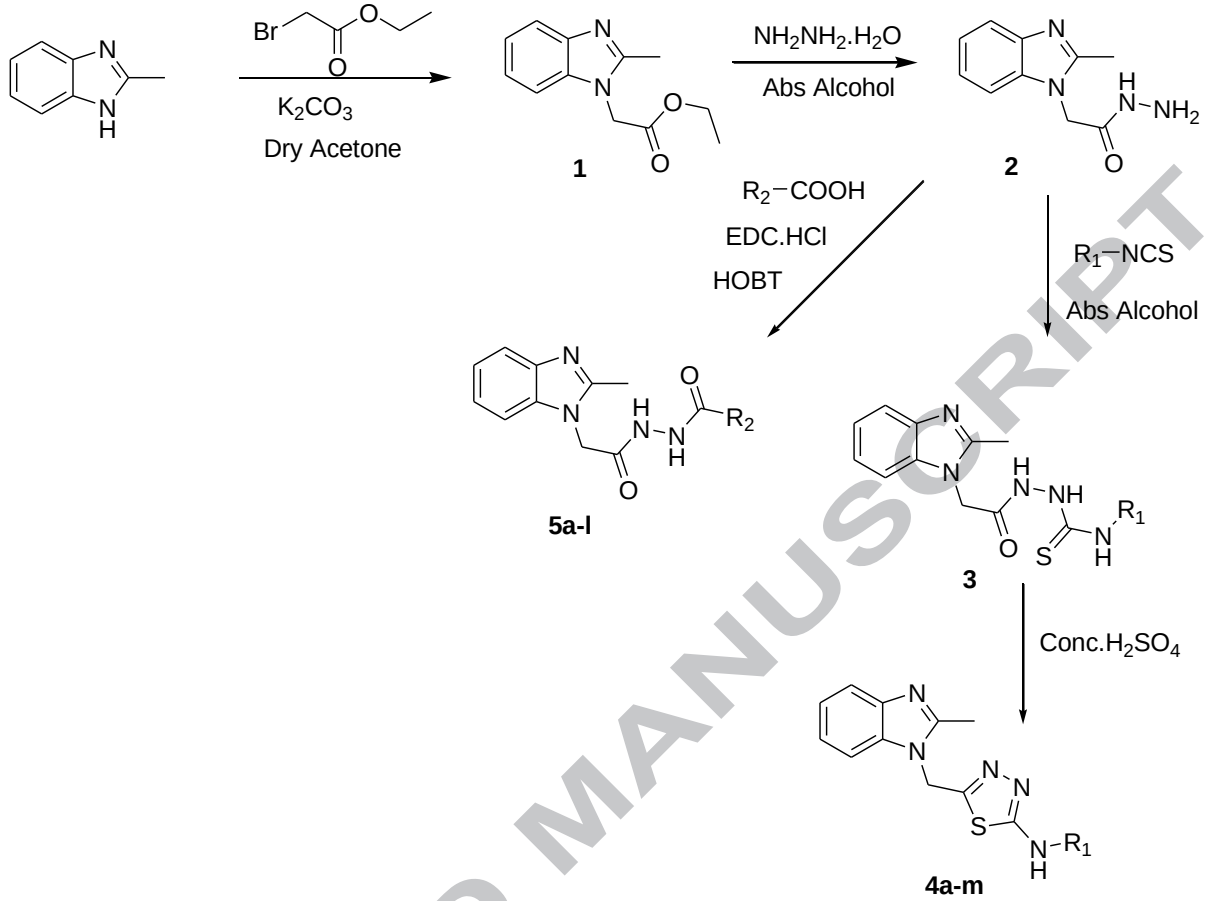
Glycogen synthase kinase 3 (GSK-3) a serine/threonine kinase of the CMGC kinase group (cyclin dependent kinases, mitogen activated protein kinases, glycogen synthase kinase and cell kinase-2), has been originally described as a key regulator of glycogen synthesis and glucose metabolism.[1] Glycogen synthase kinase 3β (GSK-3β) acts as a downstream regulatory switch that determines the output of numerous signaling pathways initiated by diverse stimuli.[2] GSK-3β targets a wide range of substrates, namely metabolic proteins (e.g. cyclin D1, APP (amyloid precursor protein) and presenilin), structural proteins (e.g. tau and other microtubule associated proteins) and transcription factors (e.g. NF-κB, p53 and Notch).[2] This plethora of biological substrates places GSK-3 as an important feature in cell cycle and development, bioenergetics and apoptosis, unveiling its role as a master regulator of key physiological processes.[3] Consequently the abnormal regulation of GSK-3β results in the development of psychiatric disorders like bipolar disorder, mood disorders, depression and schizophrenia[4-6], besides other non-psychiatric diseases such as cancer, aging immune disorders and metabolic disorders.[7, 8]

Depression is a common, debilitating, life-threatning illness with a significant incidence in the population. GSK-3β has been implicated in the mechanism of action of the mood stabilizers lithium and valproate[9, 10], largely used in the treatment of bipolar disorder.[11] Deficient serotonergic neurotransmission observed in depression[12], considering that serotonergic activity contributes to the inhibitory control of GSK-3β in mammalian brain *in vivo*.[13, 14] Serotonin (5-HT) itself, as well as fluoxetine (a selective serotonin re-uptake inhibitor) and 5-HT_{1A} agonists, augment serine 9 phosphorylation with the consequent inhibition of GSK-3β.[13] In addition, classical antidepressants, as well as atypical antipsychotics involve inhibition of GSK3β, either directly or following its phosphorylation on Ser9 by AKT in their mechanism of action.[15, 16] There have been several reports demonstrating that pharmacologic inhibition of GSK-3 activity produces antidepressant-like effects in rodents.[17-20]

Benzimidazole constitutes an indispensible construction motif for the development of compounds of medicinal interest due to its diverse pharmaceutical applications and its derivatives have also been identified as strong GSK-3β inhibitors.[21-23] Suitable structural combinations of benzimidazole core with various pharmacophores in a single molecular framework have resulted in improved GSK-3β inhibitory potential and

alleviation of associated disorders.[22] The thiadiazole as well as its bioisosteric oxadiazole heterocyclic frameworks have been regarded as the privileged scaffolds for GSK-3 inhibitory activity.[24, 25] Also, the carbohydrazide motif possesses favourable H-bond donors as well as acceptors for it to interact strongly with the GSK-3β target site and hence could give higher GSK-3β inhibition (and consequently better antidepressant activity). Keeping this in view, we attempted conjugation of benzimidazole with 2-(alkyl/aryl)amino-1,3,4-thiadiazoles and aryl/heteroaryl carbohydrazides under a single construct through methylene linkage. A library of twenty-five benzimidazole based 1,3,4-thiadiazoles and carbohydrazide conjugates (4a-m and 5a-l) has been synthesized as shown in **Table S.1 (Supplementary data)**. Various substitutions have been made to explore their effect on the GSK-3β inhibition and generate the structure-activity relationship. Among these four compounds (**4a, 4k, 4l** and **5a**), which are already known in the literature [26] and structurally similar to the benzimidazole based 1,3,4-thiadiazoles and carbohydrazides conjugates were also taken up for synthesis and evaluated for GSK-3β inhibition and anti-depressant activity.

The designed inhibitors were synthesized using the methods in **Scheme 1.** The key intermediate used for the synthesis of both series of the final compounds was 2-(2-methyl-1*H*-benzimidazol-1-yl)acetohydrazide (**2**)[27], which in turn was prepared by the reaction of 2-methyl-1*H*-benzimidazole (**1**) with ethyl bromoacetate in the presence of anhydrous potassium carbonate as a base, followed by the reaction with hydrazine hydrate. The benzimidazole thiosemicarbazides (**3**) were prepared by condensing compound **2** with appropriate isothiocyanates. Cyclization of compounds **3** in Conc. H_2SO_4 under room temperature gave the benzimidazole-1,3,4-thiadiazoles **4a-m**.[28] Further, another series of benzimidazole carbohydrazides (**5a-l**) was synthesized by using EDC coupling.[17]



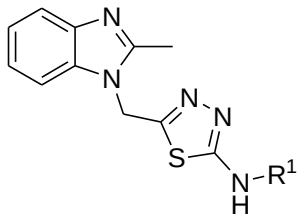
Scheme 1: Synthesis of benzimidazole based 1,3,4-thiadiazoles and carbohydrazide conjugates.

The attachment of the benzimidazole ring with the 1,3,4-thiadiazole nucleus (**4a-m**) was confirmed by the appearance of methylene protons at δ 5.65-6.06 as singlet's in the ^{1}HNMR spectra. Further structural confirmation of the final compounds was provided by the presence of singlet's for amino protons attached to the 1,3,4-thiadiazole ring at δ 9.71-11.10 (**4a-m**). The formation of benzimidazole carbohydrazide compounds (**5a-l)** was confirmed by the apperance of singlet of the methylene proton attached to aromatic ring at δ 3.38-3.55 and 4.86-5.04 for methylene protons attached to benzimidazole rings. The structure of all the compounds was further confirmed by the IR, ^{1}H-NMR, ^{13}C-NMR spectra and ESI-MS mass spectral analysis.

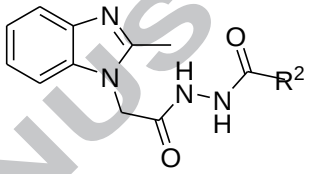
All the synthesized compounds were evaluated for GSK-3β inhibitory activity in a non-radioactive assay using Kinase-Glo method and the results are shown in **Table 1**. Staurosporine, a well known kinase inhibitor was used as the reference compound. The

test compounds were first screened at a primary concentration of 10 µM. The compounds showing considerable inhibition at 10 µM were next tested over a wide range of concentrations and IC_{50} values were determined from the dose response curves.[29] The results of kinase inhibitory assays demonstrated that compounds **4f, 4j, 5b, 5g** and **5i** were the more potent amongst benzimidazole based 1,3,4-thiadiazoles/carbohydrazides with IC_{50} value of **76, 107, 92, 80** and **72 nM** respectively.

Table 1: GSK-3β inhibitory activity of benzimidazole based 1,3,4-thiadiazoles and carbohydrazides.



4a-m



5a-l

R^1	Compound	GSK-3β IC_{50} (nM)	R^2	Compound	GSK-3β IC_{50} (nM)
Phenyl	4a	157	4-Chloro Benzyl	5a	131
4-Chloro Phenyl	4b	145	2-Methyl Benzyl	**5b**	**92**
2-Chloro Phenyl	4c	122	4-Methoxy Benzyl	5c	379
4-Flouro Phenyl	4d	134	4-Nitro Benzyl	5d	205
4-Bromo Phenyl	4e	173	3-Indole	5e	148
2-Flouro Phenyl	**4f**	**76**	2-Pyrrole	5f	124
4-Methoxy Phenyl	4g	151	4-Phenoxyphenyl-	**5g**	**80**

			4-oxobutanoyl		
2-Methoxy Phenyl	4h	184	Benzyl	5h	105
4-Ethoxy Phenyl	4i	293	3-indole glyoxalyl	**5i**	**72**
4-Nitro Phenyl	**4j**	**107**	2-acetyl-Indole	5j	135
Cyclohexyl	4k	406	2-Naphthyl	5k	157
Butyl	4l	582	4-Methoxyphenyl-4-oxobutanoyl	5l	561
2-Bromo Phenyl	4m	190	Staurosporine	-	54

Evaluation of antidepressant activity involves rodent behavioural tests and two behavioural models i.e., forced swim test (FST) and tail suspension test (TST) are widely used. Both models are based on the application of stress to the animal and measurement of the duration of immobility when rodents are exposed to an inescapable situation. These tests have good predictive validity and allow rapid and economical detection of substances with potential antidepressant activity. The results as depicted in **Fig. 1** show the effect on immobility in the tail suspension test. The mean duration of immobility was significantly reduced with the standard drug fluoxetine, which exhibited an immobility time of **153** sec. The test compounds **4f, 4j, 5b, 5g** and **5i** resulted in immobility times of **120, 174, 168, 135** and **105** sec respectively as compared to the normal saline, which showed an immobility time of **183** sec ($P < 0.001$). Decrease in immobility shown by **5i** and **4f** was found to be significant in comparison with the standard drug fluoxetine, furthermore decrease in immobility time on administration of **4f, 5g** and **5i** was also statistically significant in comparison to normal saline. The results indicate that the

compounds **4f** and **5i** showing a potent GSK-3β inhibition also possessed a significant antidepressant potential.

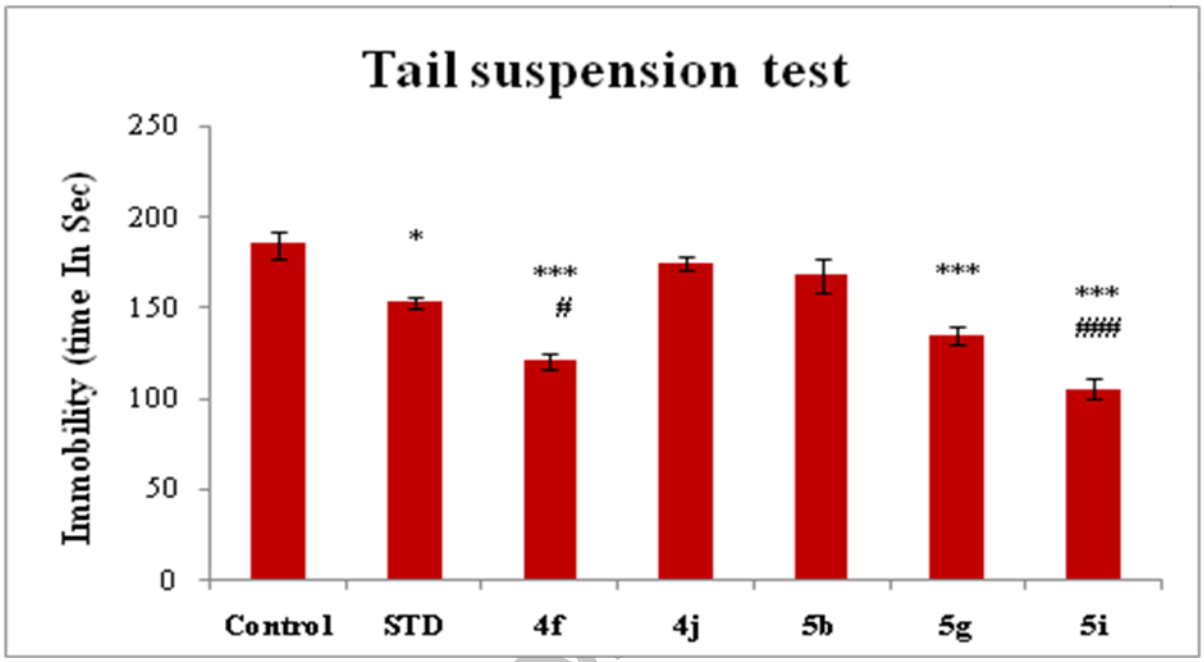


Figure 1: Tail suspension test. Data is represented as Mean± SEM and analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test, *P < 0.05, ** P < 0.01, ***P < 0.001, $^{\#}$ P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 when compared with normal control and standard (fluoxetine) respectively.

The forced swim test (FST) or despair swim test has been proposed as model to test for antidepressant activity by Vincent Castagne and co-workers's method.[30] The effect of immobility in the forced swim test results are shows in the **Fig. 2**. Significant decreases in immobility time were observed on administration of the compounds **4f, 5g** and **5i** in comparison to normal saline (P < 0.01). Furthermore the compounds **4f, 5g** and **5i** were also found to exhibit significant activity in comparison to the standard drug fluoxetine. These observations are in agreement with the results of TST, corroborating that compounds **4f** and **5i** possess significant antidepressant potential.

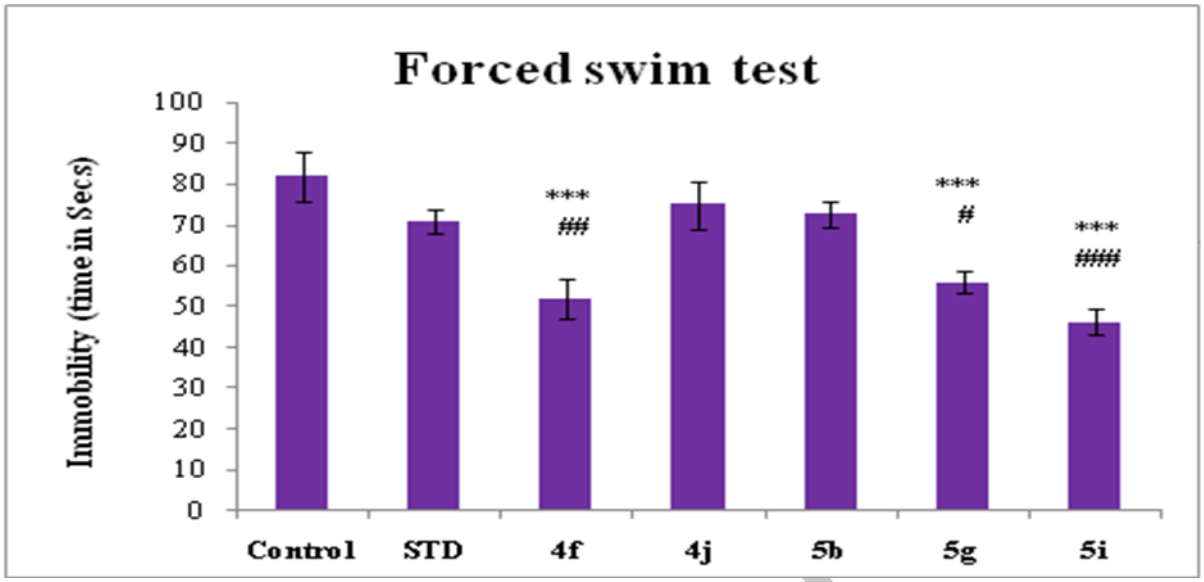


Figure 2: Forced swim test. Data is represented as Mean± SEM and analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test, *P < 0.05, ** P < 0.01, ***P < 0.001, ^{n} P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 when compared with normal control and standard (fluoxetine) respectively.

Reported *in-silico* studies for GSK-3β protein shows that the key residues in the ATP binding pocket namely ASP133 & VAL133 form hydrogen bonds with Staurosporine (PDB: 1Q3D). Furthermore within the ATP binding site, hydrophobic interaction with residues like CYS-199, LEU-132, VAL-110, ALA-83, LEU-188, TYR-134, VAL-135 & ILE-62 contributes to overall efficacy of binding drug molecule. The binding interactions of Staurosporine within the ATP binding pocket of GSK-3β protein is as shown in **Fig. 3**. The synthesised molecules were docked against the GSK-3β target protein. The Glide score of the molecules ranged from -7.69 to -4.50 in comparison to staurosporine with Glide score of -10.01**,** as shown in **Table S.2 & S.3**. The highly ranked molecule **5i** exhibiting a Glide score of **-7.69** was found to show Hydrogen bond interaction with key residues ASP-133 and LYS-183. The other high scoring molecule **5g** with Glide score **-7.64** formed Hydrogen bonds with PRO-136 & VAL-135 residues and side chains of TYR-134 & LYS-60 residues. The binding interactions of these molecules (**5i, 5g & 4f**) are shown in **Fig. 4-6**. The results from the *in silico* studies were found to be

in agreement with the *in vitro* GSK-3β assay as the top-ranked ligands (**5i** & **5g**) which showed better binding affinity *in silico* studies also exhibited highest *in vitro* activity.

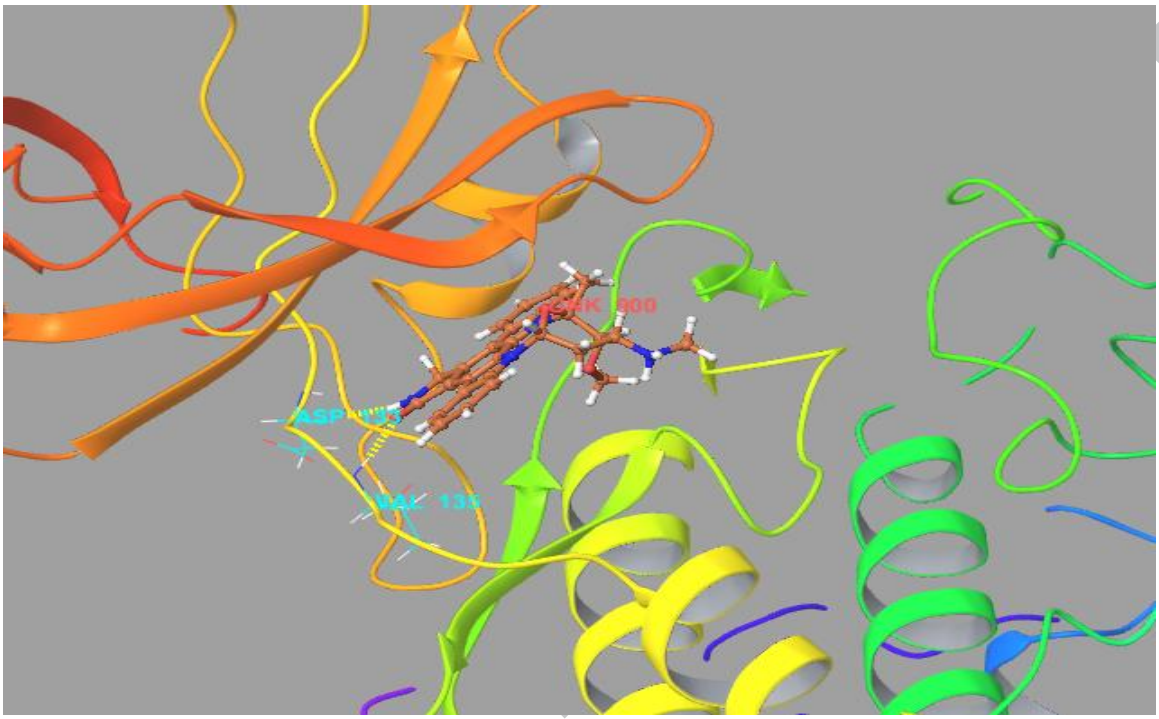


Figure 3: *In silico* molecular docking of standard (staurosporine) against GSK-3β. Staurosporine is reported to show H-bond formation with Val-135 & Asp-133 residues.

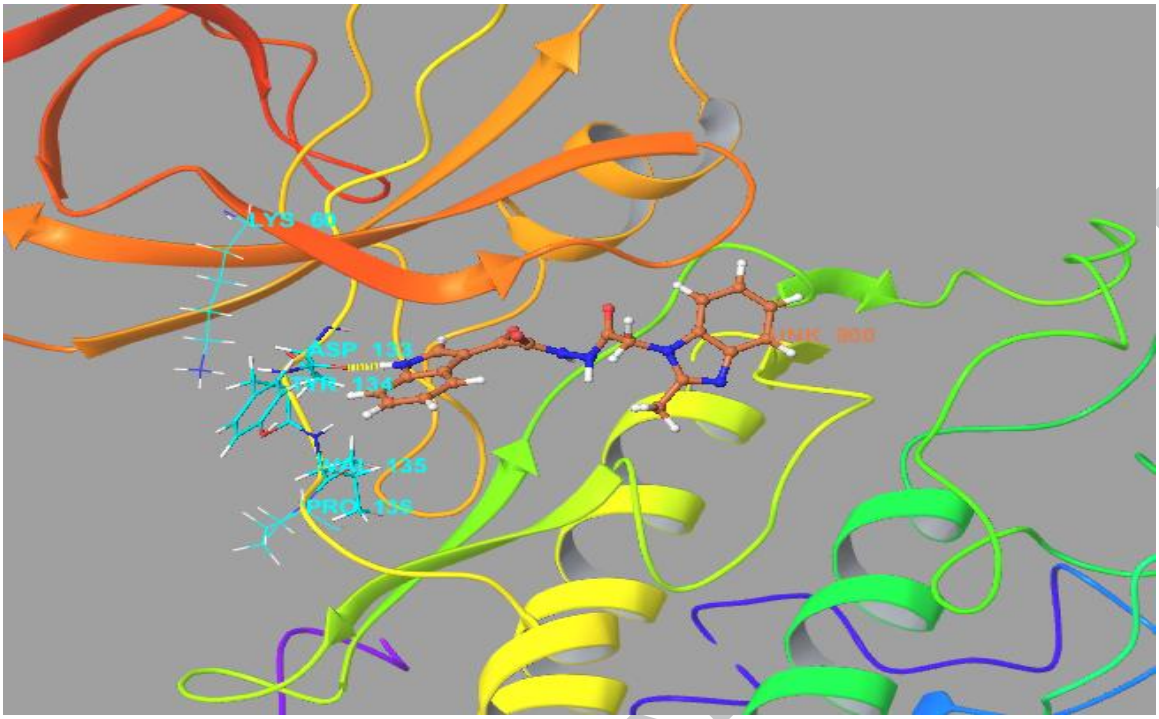


Figure 4: *In silico* molecular docking of ligand **5i** against GSK-3β

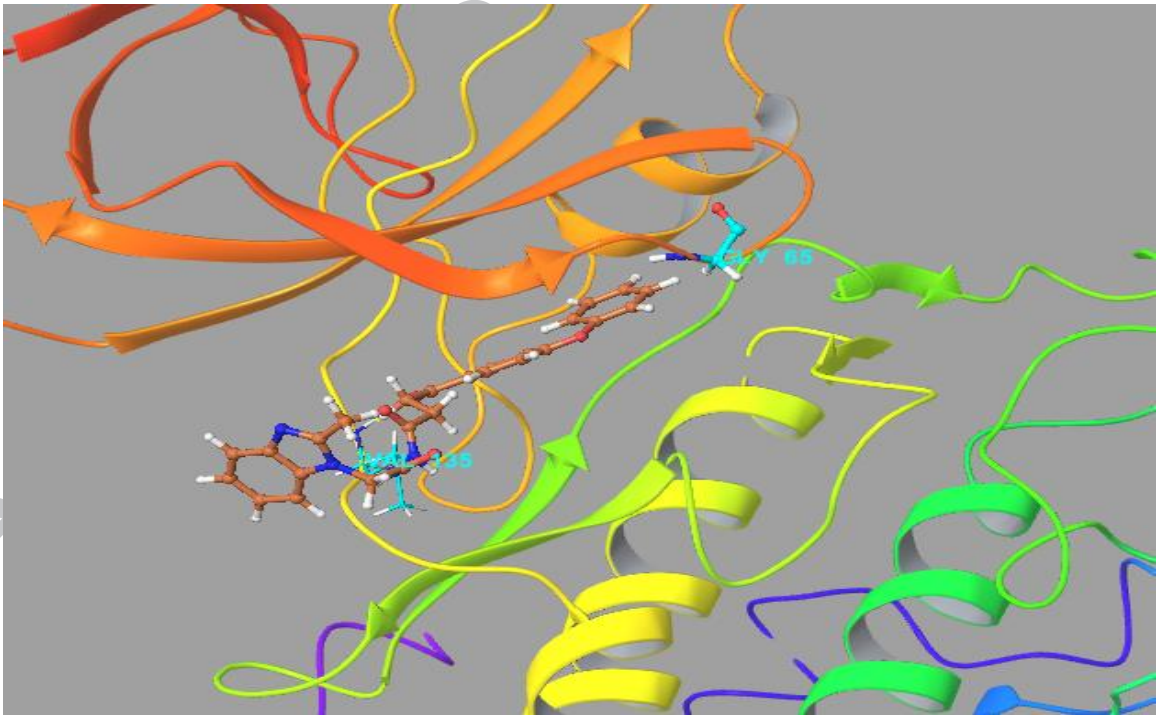


Figure 5: *In silico* molecular docking of ligand **5g** against GSK-3β

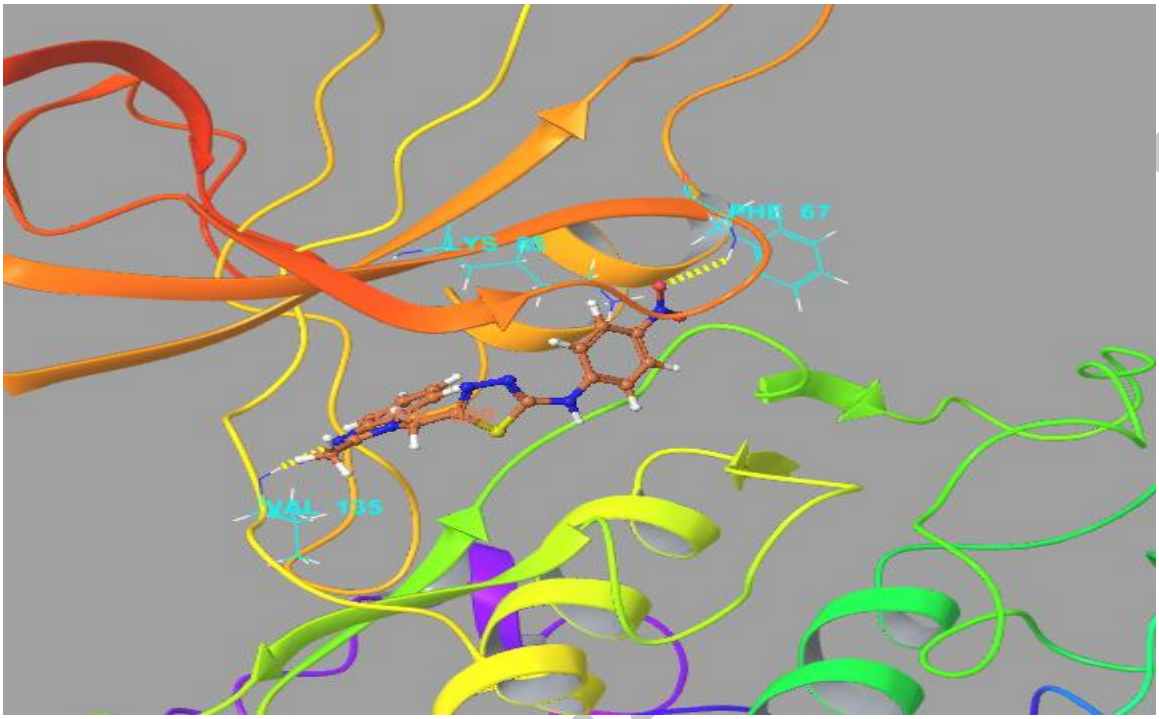


Figure 6: *In silico* molecular docking of ligand **4j** against GSK-3β

General approaches were used to achieve diversity of the synthesised compounds. From the results, the following structural activity relationship could be drawn. Among the two classes of compounds, carbohydrazides conjugates of benzimidazole were found to be more active when compared with thiadiazole conjugates of benzimidazole. Furthermore, nature and position of the substitution on the aromatic ring of carbohydrazide conjugates of benzimidazole greatly influenced the GSK-3 inhibitory activity. Glyoxalic acid conjugates were found to be most active among the carbohydrazide conjugates of benzimidazole. Hetero aromatic/aromatic carboxylic acid conjugates were found to be more active when compared to aromatic/hetero aromatic acetic acid conjugates. Amongst the hetero aromatic and aromatic carboxylic acid conjugates, hetero aromatic rings led to greater activity over the aromatic ring. In the thiadiazole conjugates of benzimidazole series, the activity of the compounds is influenced by the nature of the substituents on aromatic ring. Electron donating groups

were found to confer better activity on compounds as compared to the electron withdrawing group.

In summary, this work focused on the development of new potentially active GSK-3β and anti-depressant benzimidazole based thiadiazole and carbohydrazide conjugates. Among the tested compounds, compound **5i** was found to be the most active (**IC_{50} = 72 nM**), while compounds **4f, 4j, 5b, 5g** and **5i** were also found to exhibit significant inhibition of GSK-3β in comparison to staurosporine, used as standard. The structure activity relationships and molecular modelling study provided considerable insight into interactions between the enzyme and these ligands. The compounds **4f, 4j, 5b, 5g** and **5i** were further evaluated for their antidepressant potential and compound **5i** was found to significantly reduce the duration of immobility at 50 mg/Kg, when compared with fluoxetine, a known antidepressant drug. Phosphorylation of GSK3β predominantly mediates the acute antidepressant-like effect of fluoxetine. It could be thus concluded that molecule **5i** can act as a potential lead candidate for development of potent GSK-3β inhibitors with antidepressant potential.

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Graphical Abstract