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SYNTHESIS, CHARACTERIZATION & IN-VITRO ACTIVITY OF NEW SERIES OF COUMARIN SUBSTITUTED MERCAPTO-1, 3, 4-OXADIAZOLE DERIVATIVES AS GSK-3β INHIBITORS.

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ABSTRACT

A new series of 2, 5-disubstituted-mercapto-1,3,4-oxadiazole derivatives were synthesized in good yield by reacting different coumarin hydrazide with carbon disulfide & further reacting with various aryl halides. The reactions were monitored by TLC & all derivatives were purified by recrystallization using suitable solvents. The synthesized derivatives were characterized by IR, ^{1}H-NMR, Mass spectral data. Further these derivatives were studied for their *in-vitro* GSK-3β inhibitory activity by using Kinase-Glo Plus luminescence kinase assay & percentage inhibitions were calculated. The standard compound used was CHIR-99021. Some of the derivatives have shown significant *in-vitro* activity.

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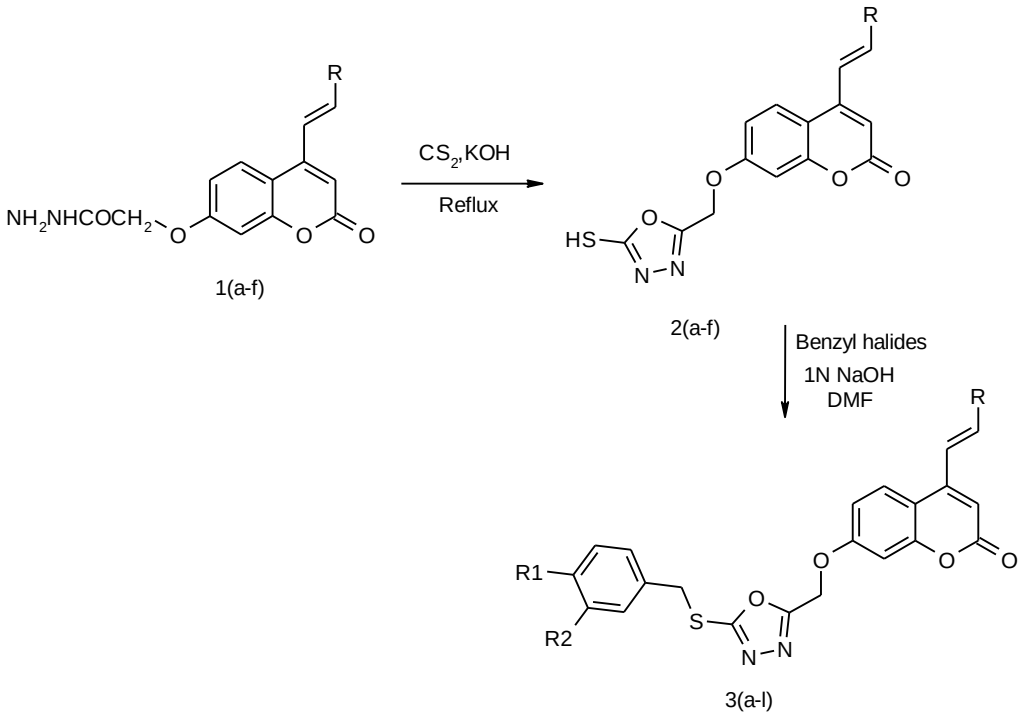
INTRODUCTION

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and threonine amino acid residues. First discovered in 1980 as a regulatory kinase for its namesake, Glycogen synthase, GSK-3 has since been identified as a kinase for over forty different proteins in a variety of different pathways.[1] GSK-3β has critical role in regulation of glycogen synthase which is implicated in development of type-2 diabetes. Diabetes is the most threatened metabolic disorder world wide affecting millions of people. Studies reported that diabetes is mainly associated with the oxidative stress & protein phosphorylation controlled by protein kinases. Glycogen synthesis a key metabolic pathway in disposing of skeletal muscle after insulin stimulation. Studies show that over expression of GSK-3β inhibits glycogen synthesis & leads to the development of type-2 diabetes.[2] In our previous article, we have reported about the design & molecular studies of 2,5-disubstituted-mercapto-1,3,4-oxadiazole ligands. In the current paper explains about the synthesis, characterization & *in-vitro* activity of new series of 2,5-disubstituted-mercapto-1,3,4-oxadiazole derivatives as GSK-3β inhibitors.

Chemistry

Scheme:



Step-1: General Procedure for synthesis of Ethyl (E)-2-((2-oxo-4-(substituted) -2H-chromen -7-yl) aceto hydrazide. [1,4] 1(a-f).

To a solution of ethyl (E)-2- -4- (2 substituted) -2-oxo-2H chromen-7-yl) oxy) acetates (6.0 mmol) in EtOH (30 mL) was added hydrazine hydrate (60 mmol) and the mixture was heated at reflux for appropriate time & the reaction was monitored by TLC. After cooling to room temperature pure crystals are formed, collected by filtration and washed several times with EtOH to give compounds.

Step-2: General Procedure for synthesis of (E)-4-(substituted styryl)-7-((5-mercapto-1, 3, 4-oxadiazol-2-yl)methoxy)- 2H-chromen-2-one.[1,4] 2(a-f)

To a solution of 1(a-f) (3.00 mmol) in EtOH (5 mL) were added carbon disulfide (6.60 mmol) and Et_3N (3.30 mmol) and the mixture was heated at reflux overnight. The reaction mixture was diluted with EtOAc and the organic layer was washed with 0.1 N HCl, brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the obtained residue was recrystallized from suitable solvents to give the compounds.

Step-3: General Procedure for synthesis of (E)-7-((2, 5-disubstituted -1,3,4-oxadiazol) -methoxy)- 4-styryl-2*H*- chromen -2-one.[1,4] 3(a-l)

To the solution compound 2(a-f) (25 mmol) in DMF (1 mL) was added the appropriate substituted biphenyl system (0.38 mmol) at room temperature, and the mixture was stirred for appropriate time & monitored by TLC. The precipitate formed was collected by filtration and washed once with less DMF and thereafter several times with EtOH to give compounds 3(a-l). **Note:** The compounds which did not precipitate from the solution were purified as follows. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM/EtOAc (20:1).

***In-vitro* GSK3 enzymatic bioassay studies:**

The *in vitro* enzyme inhibitory studies was carried out using bioassay kit to determine the effects of twelve compounds on the enzymatic activities of recombinant human GSK-3β [5].

Assay Conditions

The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity.The compounds were diluted to 10 µM in 10% DMSO and 5µl of the dilution was added to a 50µl reaction so that the final concentration of DMSO is 1% in all of reactions.All of the enzymatic reactions were conducted at 30 ^{o}C for 40 minutes. The 50µl reaction mixture contains 40 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 0.1 mg/ml GSKtide substrate, 10 mM ATP and GSK3β. After the enzymatic reaction, 50 µl of Kinase-Glo Plus Luminescence kinase assay solution was added to each reaction and incubate the plate for 5 minutes at room temperature. Luminescence signal was measured using a Biotek Synergy 2 microplate reader.

Data Analysis

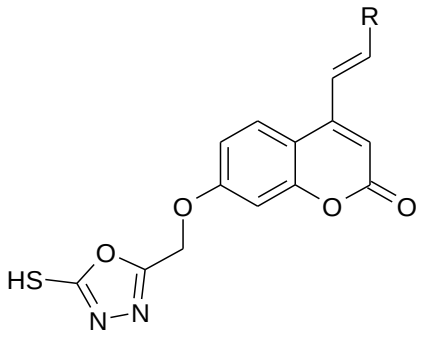
GSK-3β activity assays were performed in duplicate at each concentration. The luminescence data were analyzed using the computer software, Graphpad Prism. The difference between luminescence intensities in the absence of GSK3β (Lu_t) and in the presence of GSK3β (Lu_c) was defined as 100 % activity ($Lu_t - Lu_c$). Using luminescence signal (Lu) in the presence of the compound, % activity was calculated as:

% activity = {(Lu_t - Lu)/(Lu_t - Lu_c)}×100%, where Lu= the luminescence intensity in the presence of the compound (all percent activities below zero were set to 0 %).

% inhibition was calculated as: % inhibition = 100 (%) - % activity

Results:

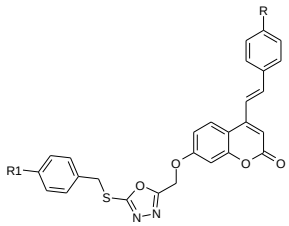
Table No. 1: Physico-chemical properties of (E)-4-(substituted styryl)-7-((5-mercapto-1, 3, 4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one 2(a-f).



Sl. No.	Compound code	R	Mol. F	Mol. wt	Melting Point	R_f value	% yield	RT(hr)
1	2a	------	$C_{13}H_{10}N_2O_4S$	290	135-38	0.63	70	12
2	2b	C_6H_5	$C_{20}H_{14}N_2O_4S$	378	140-43	0.67	75	13
3	2c	4-CH_3-C_6H_5	$C_{21}H_{16}N_2O_4S$	392	145-49	0.65	68	14
4	2d	4$OCH_3C_6H_5$	$C_{21}H_{16}N_2O_5S$	408	138-41	0.64	78	12
5	2e	2-Cl-C_6H_5	$C_{20}H_{13}ClN_2O_4S$	412	148-52	0.6	74	14
6	2f	2-Br-C_6H_5	$C_{20}H_{13}BrN_2O_4S$	455	138-42	0.59	80	15

Recrystallization solvent: Ethanol

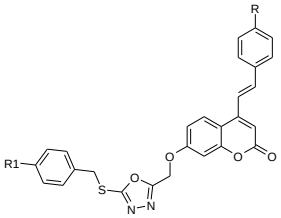
Table No.2: Physico-chemical properties of (E)-7-((2,5-disubstituted-1,3,4-oxadiazol)-methoxy)-4-styryl -2*H*-chromen-2-one.3(a-l).



Sl. No.	Compound code	R (Ar-)	R^1 (Ar-CH_2)	Mol. Formula	Mol. Wt.	Melting Point	R_f value	% yield	RT(hr)
1	3a	H	H	$C_{27}H_{20}N_2O_4S$	468.52	135-38	0.63	70	12
2	3b	H	4-OH	$C_{27}H_{20}N_2O_4S$	484.52	140-43	0.67	60	13
3	3c	H	4-OCH_3	$C_{28}H_{22}N_2O_5S$	498.55	145-49	0.65	68	14
4	3d	H	Biphenyl	$C_{33}H_{24}N_2O_4S$	544.62	138-41	0.64	60	12
5	3e	4-CH_3	H	$C_{28}H_{22}N_2O_4S$	482.55	148-52	0.6	70	14
6	3f	4-CH_3	4-OH	$C_{28}H_{22}N_2O_5S$	498.55	138-42	0.59	60	15
7	3g	4-CH_3	4-OCH_3	$C_{29}H_{24}N_2O_5S$	512.58	140-44	0.64	55	13
8	3h	4-CH_3	Biphenyl	$C_{33}H_{24}N_2O_4S$	544.62	165-68	0.7	60	15
9	3i	4-OCH_3	H	$C_{28}H_{22}N_2O_5S$	498.55	145-47	0.32	55	14
10	3j	4-OCH_3	4-OH	$C_{28}H_{24}N_2O_6S$	516.56	174-76	0.63	60	15
11	3k	4-OCH_3	4-OCH_3	$C_{29}H_{24}N_2O_6S$	528.58	165-67	0.55	55	15
12	3l	4-OCH_3	Biphenyl	$C_{34}H_{26}N_2O_5S$	574.65	182-85	0.64	55	12

Recrystallization solvent: Ethanol

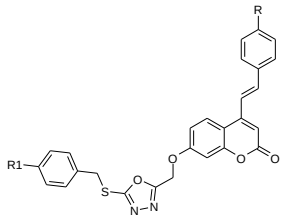
Table No.3: Spectral data of (E)-7-((2, 5-disubstituted-1, 3, 4-oxadiazol)-methoxy)-4-styryl-2*H*-chromen-2-one. 3(a-l).



Sl. No.	Compound code	R (Ar-)	R^1 (Ar-CH_2)	IR (cm^{-1})
1	3a	H	H	1685.0, 1655.9 (C=N), 3005.9 (C-H; aromatic), 1073.9 (C-O-C)
2	3b	H	4-OH	1635.5 (C=N), 2922.2,2961.5 (C-H; aromatic), 1059.8 (C-O-C)
3	3c	H	4-OCH_3	1639.62 (C=N), 3025.21 (C-H; aromatic), 1071,48 (C-O-C)
4	3d	H	Biphenyl	1684.51 (C=N), 3010.44 (C-H; aromatic), 973.52 (C-O-C)
5	3e	4-CH_3	H	1611.56 (C=N), 2938.56.0 (C-H; aromatic), 1009.08 (C-O-C)
6	3f	4-CH_3	4-OH	1691.22 (C=N), 3014.98 (C-H; aromatic), 1073.9 (C-O-C).
7	3g	4-CH_3	4-OCH_3	1613.47, 1545.68 (C=N), 3048.86 (C-H; aromatic), 1068.38 (C-O-C)
8	3h	4-CH_3	Biphenyl	1600.83 (C=N), 2831.07 (C-H; aromatic), 1084.98 (C-O-C)
9	3i	4-OCH_3	H	1618.52 (C=N), 2924.34 (C-H; aromatic), 1015.09 (C-O-C)
10	3j	4-OCH_3	4-OH	1678.19 (C=N), 3010.61 (C-H; aromatic), 940.98 (C-O-C)
11	3k	4-OCH_3	4-OCH_3	1691.06 (C=N), 2924.38 (C-H; aromatic), 1084.20 (C-O-C)
12	3l	4-OCH_3	Biphenyl	1657.51 (C=N), 2945.44 (C-H; aromatic), 979.57 (C-O-C)

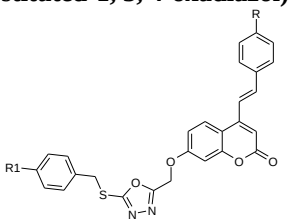
Recrystallization solvent: Ethanol

Table No.4:[1]H-NMR spectral data of (E)-7-((2, 5-disubstituted-1,3,4-oxadiazol)-methoxy)-4-styryl-2*H*-chromen-2-one. 3(a-l).



Sl. No.	Compound Code	[1]H-NMR (δ values)
1	3a	δ 7.58 – 7.52 (m, 1H), 7.53 – 7.46 (m, 2H), 7.42 (dq, *J* = 8.1, 1.1 Hz, 2H), 7.38 – 7.15 (m, 8H), 7.09 (d, *J* = 16.0 Hz, 1H), 6.96 – 6.90 (m, 2H), 6.15 (s, 1H), 5.41 (s, 2H), 4.46 (t, *J* = 1.0 Hz, 2H).
2	3b	δ 7.67 (s, 1H), 7.54 – 7.46 (m, 3H), 7.27 (td, *J* = 7.0, 0.8 Hz, 2H), 7.28 – 7.13 (m, 2H), 7.12 – 7.03 (m, 3H), 6.96 – 6.89 (m, 2H), 6.79 – 6.72 (m, 2H), 6.13 (s, 1H), 5.41 (s, 2H), 4.46 (t, *J* = 1.0 Hz, 2H).
3	3c	δ 7.54 – 7.46 (m, 3H), 7.31 – 7.23 (m, 2H), 7.20 (ddt, *J* = 7.6, 6.3, 2.4 Hz, 1H), 7.11 – 7.02 (m, 4H), 7.03 – 6.97 (m, 1H), 6.93 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.89 – 6.83 (m, 2H), 6.15 (s, 1H), 5.41 (s, 2H), 4.39 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 3H).
4	3d	δ 7.62 – 7.55 (m, 2H), 7.54 – 7.32 (m, 10H), 7.27 (td, *J* = 7.0, 0.8 Hz, 2H), 7.28 – 7.17 (m, 1H), 7.21 – 7.12 (m, 1H), 7.07 (d, *J* = 15.9 Hz, 1H), 6.96 – 6.90 (m, 2H), 5.99 (s, 1H), 5.41 (s, 2H), 4.48 (t, *J* = 0.9 Hz, 2H).
5	3e	δ 7.57 – 7.51 (m, 1H), 7.47 – 7.38 (m, 4H), 7.38 – 7.30 (m, 2H), 7.24 (ddt, *J* = 8.1, 6.7, 1.3 Hz, 1H), 7.22 – 7.14 (m, 3H), 7.11 (d, *J* = 15.9 Hz, 1H), 6.96 – 6.90 (m, 2H), 6.15 (s, 1H), 5.41 (s, 2H), 4.45 (t, *J* = 1.0 Hz, 2H), 2.38 (d, *J* = 0.6 Hz, 3H).
6	3f	δ 7.67 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.22 – 7.15 (m, 3H), 7.10 (d, *J* = 16.0 Hz, 1H), 7.02 (dt, *J* = 8.4, 1.1 Hz, 2H), 6.93 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.70 – 6.63 (m, 2H), 6.18 (s, 1H), 5.41 (s, 2H), 4.44 (t, *J* = 1.0 Hz, 2H), 2.38 (d, *J* = 0.7 Hz, 3H).
7	3g	δ 7.52 – 7.42 (m, 3H), 7.22 – 7.14 (m, 4H), 7.06 (dt, *J* = 8.4, 1.0 Hz, 2H), 6.96 – 6.90 (m, 2H), 6.89 – 6.83 (m, 2H), 6.16 (s, 1H), 5.41 (s, 2H), 4.42 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 3H), 2.38 (d, *J* = 0.7 Hz, 3H).
8	3h	δ 8.17 – 8.10 (m, 2H), 7.62 – 7.55 (m, 2H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.48 – 7.40 (m, 6H), 7.40 – 7.32 (m, 1H), 7.22 – 7.16 (m, 2H), 7.13 (d, *J* = 16.0 Hz, 1H), 7.00 (d, *J* = 16.0 Hz, 1H), 6.97 – 6.90 (m, 2H), 6.02 (s, 1H), 5.41 (s, 2H), 2.38 (d, *J* = 0.7 Hz, 3H).
9	3i	[1]H NMR (500 MHz, Chloroform-*d*) δ 7.42 (dt, *J* = 6.9, 1.3 Hz, 4H), 7.42 – 7.30 (m, 4H), 7.24 (ddt, *J* = 8.1, 6.6, 1.3 Hz, 1H), 7.15 (d, *J* = 15.9 Hz, 1H), 7.09 (d, *J* = 15.9 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.79 – 6.72 (m, 2H), 6.17 (s, 1H), 5.41 (s, 2H), 4.43 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 3H).
10	3j	δ 7.67 (s, 1H), 7.53 (dt, *J* = 8.7, 1.2 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.16 – 7.05 (m, 4H), 6.96 – 6.90 (m, 2H), 6.80 – 6.72 (m, 4H), 6.13 (s, 1H), 5.41 (s, 2H), 4.46 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 3H).
11	3k	δ 7.52 (d, *J* = 8.3 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.14 – 7.03 (m, 4H), 6.96 – 6.89 (m, 2H), 6.89 – 6.83 (m, 2H), 6.79 – 6.72 (m, 2H), 6.12 (s, 1H), 5.41 (s, 2H), 4.45 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 5H).
12	3l	δ 7.62 – 7.51 (m, 3H), 7.48 – 7.32 (m, 9H), 7.36 – 7.30 (m, 1H), 7.04 (s, 2H), 6.96 – 6.87 (m, 2H), 6.79 – 6.72 (m, 2H), 5.95 (s, 1H), 5.41 (s, 2H), 4.46 (t, *J* = 1.0 Hz, 2H), 3.78 (s, 3H).

Table No.5: Spectral data of (E)-7-((2, 5-disubstituted-1, 3, 4-oxadiazol)-methoxy)-4-styryl-2*H*-chromen-2-one. 3(a-l).



Sl. No.	Compound code	R (Ar-)	R^1 (Ar-CH_2)	Mass (m/z)
1	3a	H	H	468
2	3b	H	4-OH	484
3	3c	H	4-OCH_3	498
4	3d	H	Biphenyl	544
5	3e	4-CH_3	H	482
6	3f	4-CH_3	4-OH	498
7	3g	4-CH_3	4-OCH_3	512
8	3h	4-CH_3	Biphenyl	544
9	3i	4-OCH_3	H	498
10	3j	4-OCH_3	4-OH	516
11	3k	4-OCH_3	4-OCH_3	528
12	3l	4-OCH_3	Biphenyl	774

***In-vitro* Assay Results**

Summary of the Inhibitory Effects of the Compounds on GSK-3β Activities

The mean percentage inhibition of the compounds at the testing concentration (10 μM) shown below.

Table No.6 Inhibitory Effects of the Compounds on GSK-3β Activities.

Compound Code	% Inhibition at 10 μM
3a	85
3b	90
3c	36
3d	100
3e	85
3f	87
3g	75
3h	79
3i	64
3j	74
3k	75
3l	80
CHIR-99021	100

DISCUSSION

Final compounds 3(a-l) were synthesized by reacting 2(a-f) with different aryl halides in presence of 1N NaOH in DMF. The reaction was monitored by thin layer chromatography & their R_f values were calculated. All the compounds were characterized & identified by IR, ^{1}H-NMR, Mass spectroscopic techniques & their data were recorded. The final compounds 3(a-l) were screened for GSK-3β inhibitory activities & their percentage yield were calculated. Out of twelve compounds, compound 3d has shown promising inhibitory activity of 100% at the single point assay of 10μM concentration which is having biphenyl substitution while compound 3a,3b,3e,3f,3l have shown good inhibition for GSK-3β which are having simple aromatic substitution.

CONCLUSION

Final compounds 3(a-l) were synthesized by reacting 2(a-f) with different aryl halides in presence of 1N NaOH in DMF. The final compounds 3(a-l) were screened for GSK3-β inhibitory activities & their percentage yield were calculated. Out of twelve compounds, compound 3d has shown promising inhibitory activity of 100% at the single point assay of 10μM concentration which is having biphenyl substitution In conclusion, the compounds having biphenyl substitution can show promising inhibition for GSK3-β & can be further explored.

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Conflicts of Interest

The authors hereby declare that there is no conflicts of interest for this publication.

Abbreviations

GSK3 - Glycogen Synthase Kinase-3
GSK-3β - Glycogen Synthase Kinase-3β
DMF - Dimethyl Formamide
EtOAc - Ethyl acetate

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