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Original Research Article

Molecular modelling studies and synthesis of novel quinoxaline derivatives with potential inhibitory effect on GSK-3β

Lubna Swellmeen[1]*, Amal Uzrail[2], Rand Shaheen[3], Yusuf AL-Hiari[4]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Zarqa University, [2]Department of Medical Analysis, Faculty of Sciences, Al-Albayt University, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Hashemite University, [4]Faculty of Pharmacy, The University of Jordan, Amman, Jordan

For correspondence: **Email: lswellmeen@zu.edu.jo; **Tel:** +962 778004500*

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Abstract

Purpose: *To synthesize quinoxaline derivatives and investigate their inhibitory effects on glycogen synthase kinase (GSK)-3β in vitro.*
Methods: *Quinoxaline derivatives were synthesized via reaction between synthon 1 and DL- 2-amino succinic acid, and subsequent lactamization reaction. The new compounds were tested against GSK-3β in vitro to select the most potent compound which was then used for molecular modelling.*
Results: *Novel quinoxaline derivatives with quinolone nucleus were successfully synthesized via simple chemical reactions. The compounds markedly inhibited GSK-3β, with compound 45 [3-(carboxymethyl)-5-fluoro-10-(4-fluorophenyl)-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido [2,3-f] quinoxaline-8-carboxylic acid] achieving the best effect (IC_{50} = 0.18 μM). The half maximal inhibitory concentrations (IC_{50}) of the compounds were in micromolar range. Molecular modelling revealed several interactions between compound 45 and the binding site of GSK-3β.*
Conclusion: *These results indicate that 3-(carboxymethyl)-5-fluoro-10-(4-fluorophenyl)-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido [2,3-f] quinoxaline-8-carboxylic acid is a potent inhibitor of GSK-3β and is thus a promising scaffold for the development of novel drugs that can effectively inhibit GSK-3β signaling pathway.*

Keywords: *Quinoxaline derivatives, Glycogen synthase kinase (GSK)-3β, Molecular docking, Quinoline nucleus*

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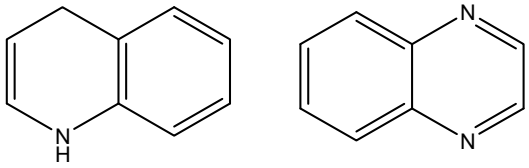
INTRODUCTION

Glycogen synthase kinase 3 (GSK-3), a highly ubiquitous serine/threonine kinase, has two isoforms: GSK-3α and GSK-3β [1]. Glycogen synthase is a key enzyme in biological processes such as apoptosis, intracellular communication, regulation of glucose metabolism and gene transcription [2]. The pathogeneses of type-2 diabetes mellitus (T2DM), Alzheimer's disease (AD) and some cancers are thought to involve GSK-3β signaling pathway [3-6]. Overexpression of GSK-3β has been implicated in pancreatic, breast, and skin cancers [7,8].

Molecular modelling is used to unravel binding interactions between newly synthesized compounds and potential target enzymes/proteins [9,10]. The technique has been successfully employed for the elucidation of the crystal lattice structure of GSK-3β.

Heterocyclic compounds are a class of substances, which play critical roles in drug discovery through their incorporation into the structures of a large variety of drugs used for the treatment of diverse diseases. Quinoxaline is an important heterocyclic nucleus with a wide spectrum of biological activity. Quinoxaline scaffold possesses promising therapeutic properties such as anticancer, antimalarial, anti-inflammatory, antimicrobial and anti-HIV effects [11]. It has been used as scaffold in drugs that function as protein kinase inhibitors [11]. Quinoline pharmacophore possesses antibacterial, anticancer and kinase inhibitory activities [12,13]. The aim of this study was to synthesize quinoxaline derivatives and investigate their inhibitory effects on GSK-3β *in vitro*.



A. B.

Figure 1: Diagram of heterocyclic nucleus. **(A):** Quinoline nucleus; and (**B):** Quinoxaline nucleus

EXPERIMENTAL

Chemicals and reagents

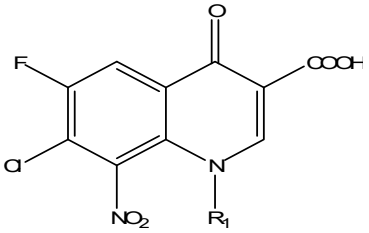
All reagents and chemicals used in this study were of analytical grade, and they were products of Sigma-Aldrich (USA). Glycogen synthase kinase (GSK)-3*β assay kit* was obtained from Thermo Fisher Scientific Co. Ltd (USA).

Technique

Melting point (mp) was measured with Stuart scientific electrothermal heating apparatus (EA3000 A). Infra-red (IR) spectrum was recorded using Shimadzu FT-IR spectrophotometer (8400F). Proton and carbon nuclear magnetic resonance (NMR) spectra were analyzed with Bruker Avance spectrometer (DPX-300), while molecular mass was measured with high resolution mass spectrophotometer (Bruker APEX-4).

Synthesis of 7-chloro-1-alkyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues

The synthons (**a, b** and **c**) were synthesized via simple chemical reactions as previously described [14,15].

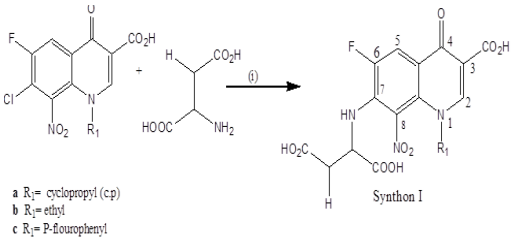


1- R_1:cyclopropyl **Synthon a**
2-R_1:ethyl **Synthon b**
3-R_1:p-fluorophenyl **Synthon c**

Figure 2: General formulae of synthons a, b and c

Synthesis of synthon I derivatives

Synthon I was produced as a product of the reaction between the synthons (**a, b** and **c)** and DL- 2-amino succinic acid **(**Scheme 1) [13]. Exactly 3.2 g of DL- 2-amino succinic acid (24.0 mmol) was mixed with 2.0 g of synthon **a, b** or **c** in 65 % ethanol (250 mL) under conditions illustrated in scheme 1. The mixing was done under reflux at 75 - 85 °C, and 3.5 M HCl was used to adjust pH of the reaction mixture to 7. The reaction lasted 10 days.



(1) $NaHCO_3$, 50 – 60 % ethanol, 70 – 80 ºC

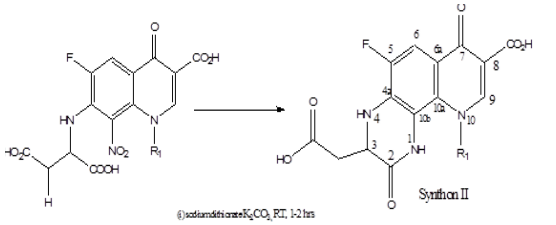
Scheme 1: Synthesis of synthon I

Synthesis of synthon II

Exactly 6.0 g of sodium dithionite powder (43.5 mmol) was dissolved in 0.33 L of distilled water and then gradually added to 1.0 g of synthon I for 45 min at room temperature (Scheme 2). Reduction of nitro group on position 8 in synthon I to amino group was done via addition of aqueous sodium dithionite in potassium carbonate. Spontaneous lactamization led to the

formation of synthon II. The reductive cyclization process was fast and direct, lasting 60 - 120 min. [17,18].

Spectrum analysis was carried out to confirm that the synthesized compound was synthon II.



Scheme 2: Synthesis of synthon II

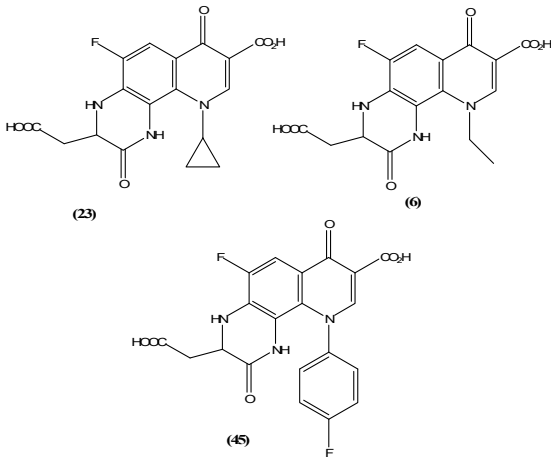


Figure 3: Chemical structures of synthesized compounds used as GSK-3β inhibitors (compounds 23, 6 and 45)

Preparation of synthon II derivatives for *in vitro* assay

Exactly 10 mg of each synthon II derivative (compounds 6, 23 and 45) was dissolved in dimethyl sulfoxide (DMSO) to obtain stock solutions of required concentrations, which were sent to Thermo Fisher Scientific Co. Ltd. (USA) where activity of GSK-3*β* was assayed.

Hits profiling against GSK-3β

To determine IC_{50} values of synthon II derivatives, inhibition of GSK-3β using Z'-LYTE GSK-3β assay was performed with varied concentrations of each compound (Z'-LYTETM Screening Protocol and Assay Conditions, 2016). The inhibition and concentration data were used to determine IC_{50} for each compound, and the most active compound was then selected. Solution of 10 mM concentration of each synthetic compound (6, 23 and 45) was prepared in DMSO and sent for analysis at Thermo Fisher Scientific Co. Ltd. (USA) [16].

Molecular modelling studies

Docking settings

LibDock is a site-feature docking algorithm that *docks ligands* into active sites under guidance by binding hotspots. The most potent of synthesized quinoxaline derivatives (based on IC_{50} values) were docked into the binding pocket of GSK-3β (PDB code: 3Q3B; resolution of 2.7 Á) using LibDock **(**Discovery Studio version 4.5). Apart from ensuring the fitting of hypothesized molecule into the binding pocket, the procedure also aided the visualization of bonds formed between the compound and amino acids in the binding pocket of GSK-3β. Molecular modelling provided clues to binding forces and inhibitory activity of the compound. The LibDock tool consisted of two major parts: allocation of binding site in the receptor, and running of docking procedure. Docking was performed via allocation of conformations of the ligand to polar receptor interaction sites and apolar ones (hotspots). A catalyst was added to ensure that conformations formed on the fly. CHARMM-based algorithm was used to analyze the interaction between the complexes formed.

RESULTS

Properties of synthesized compounds

Novel quinoxaline derivatives with quinolone nucleus were successfully synthesized via simple chemical reactions. The properties of the synthesized synthons are shown below:

Synthon I

2-[(3-Carboxy-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-7-yl) amino] succinic acid

Bright yellow crystals: yield =75 %; mp = 211 – 214 °C; ^{1}H-NMR (300 MHz, DMSO- d_6): δ 0.85,1.15 (2 m, 4 H, H2-2'/H2-3'), 1.80 (2 H, C*H*$_2$-COOH), 3.42 (m, 1 H, H-1′), 4.34 (d, *J* = 7.8 Hz, 1 H, CH-NH), 7.48 (d, J = 11.7 Hz, 1 H, H-5), 8.39 (d, $^{3}J_{H\text{-}F}$ = 5 Hz, 1 H, N*H*-CH),8.59 (s, 1 H, H-2), 13.31 - 15.5 (br m, 3 H, 3CO_2H);^{13}C-NMR (300 MHz, DMSO- d6): δ 10.34 (C-2′/ C-3′) , 37.19 (*C*H$_2$-CO_2H), 40.76 (C-1′), 70.23 (*C*H-NH), 106.53 (C-3), 108.48 (C-5), 117.75 (C-4a), 129.65 (d,$^{3}J_{C\text{-}F}$ = 5.1 Hz, C-8), 137.07 (d, $^{2}J_{C\text{-}F}$ = 14.25 Hz, C-7), 148.43 (C-2), 155.95 (C-6), 166.78 (C$_{(3)}$-CO_2H), 174.60 (C-4); IR (NaCl): v

3410, 2924, 2854, 2646, 2430, 2360, 2268, 2106, 1982, 1628, 1296, 1195, 1072 cm^{-1}.

2-[(3-carboxy-1-ethyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-7-yl) amino] succinic acid

Yellow solid; yield = 75 %; mp = 209 – 210 °C [13].

2-[(3-carboxy-1-(4-fluorophenyl)--6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-7-yl) amino] succinic acid

Yellow solid; yield = 1.5 g (82.5 %); mp = 224 - 225 °C; ^{1}H- NMR (300 MHz, DMSO- d_6): δ 2.88 (d, *J* = 17.1 Hz, 2 H, C*H*$_2$-COOH), 4.80 (d, *J* = 4.2 Hz, 1 H, CH-NH), 7.15 (d, J = 8.4 Hz, 1 H, N*H*-CH), 7.35 (2 H, H-3'/ H-5'),7.67 (d, *J* = 19.5 Hz, 2 H, H-2'/ H-6'), 8.14 (d, $^3J_{H-F}$ = 13.5 Hz, 1 H, H-5), 8.53 (s, 1 H, H-2), 13.31-15.5 (br m, 3 H, 3CO_2H); IR (NaCl): υ 3788, 3417, 2924, 2854, 2731, 2584, 2337, 2083, 1813, 1751, 1643, 1481, 1381, 1273, 1195, 1072 cm^{-1}.

Synthon II derivatives

***3-(carboxymethyl)-10-cyclopropyl-5-fluoro-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido [2,3-f]quinoxaline-8-carboxylic acid* (compound 23)**

Faint yellow crystals; yield =54 %; mp = 285 ºC; ^{1}H- NMR (300 MHz, DMSO- d_6): δ 0.97 - 1.22 (m, 4 H, H2-2′/H2-3′), 2.72 (t, J = 6.0 Hz, 2 H, C*H*$_2$-CO_2H),4.26 (m, 1 H, H-1′), 4.50 (s, 1 H, N*H*-C(3)-H), 7.34 (d, $^3J_{H-F}$ = 10.5 Hz, 1 H, H-6), 8.56 (s, 1 H, H-9), 10.39 (br s, 1 H, N(1)-H), 12.28 (br s, 1 H, CH2-CO2H),15.15 (br s, 1 H, CH2-CO_2H); ^{13}C-NMR (300 MHz, DMSO- d_6): δ 9.95 (C-2′), 10.60 (C-3′), 35.34 (CH_2-CO_2H), 38.93 (C-1′), 51.71 (CH-NH), 105.33 (d, C-6), 107.23 (C-8), 116.06 (d, $^3J_{C-F}$ = 6.3 Hz, C-6a), 117.23 (C-10b), 129.29 (C-10a), 131.59 (C-4a), 150.2 (C-6), 151.04 (C-9), 164.78 (C(8)-CO_2H), 166.14 (CH_2CO_2H), 171.75 (C-2),176.77 (C-7); IR (NaCl): v 3575, 3550, 3514, 3000, 2850, 2375, 2325, 1730, 1677, 1334, 1250, 1030 cm^{-1}.

***3-(carboxymethyl)-10-ethyl-5-fluoro-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido[2,3-f]quinoxaline-8-carboxylic acid* (compound 6)**

Yellow crystals: yield = 0.37 g (97 %); mp = 299 - 301; ^{1}HNMR (300 MHz, DMSO- d_6): δ 1.14 (br t, *J* = 6.6 Hz, 3 H, CH_2C*H*$_3$), 2.92 (brs, 2 H,C*H*$_2$-CO_2H), 4.30 (m, 1 H, NH-CH-3), 4.80 (m, 2 H, CH_2CH_3), 7.52 (NH-4-), 7.73 (d,$^3J_{C-F}$ = 10.5 Hz, 1 H, H-6), 8.86 (s, 1 H, H-9), 10.68 (br s, 1 H, N(1)-H), 12.51 (br s, 1 H,CH_2-COOH), 15.25 (s, 1 H, C(8)-COOH), 14.7 (brs, 1 H, C-2"COOH); ^{13}C-NMR (300 MHz, DMSO- d_6): δ 15.09 (CH_3), 34.95 (*C*H_2-CO_2H), 51.44 (*C*H_2CH_3), 52.18 (*C*H-NH), 105.61 (d, $^2J_{C-F}$ = 18.7 Hz, C-6),107.58 (C-8), 116.36 (d, $^3J_{C-F}$ = 6 Hz, C-10b),118.16 (d, $^3J_{C-F}$ = 7.5 Hz, C-6a), 127.7 (C-10a), 132.2 (C-4a), 149.45 (C-6), 150.94 (C-9), 165.36 (C(8)-CO_2H), 166.46 (CH_2CO_2H), 171.92 (C-2), 176.76 (C-7); IR (NaCl): v 3417, 3373, 3333, 2917, 2724, 2662, 1730,1643, 1435, 1265, 1448, 1074 cm-1; Anal. Calcd. for $C_{16}H_{14}FN_3O_6$ (363.08): C, 52.90; H, 3.88; N, 11.57. Found: C, 52.45; H, 3.64; N, 11.18.

***3-(Carboxymethyl)-5-fluoro-10-(4-fluorophenyl)-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido [2,3-f] quinoxaline-8-carboxylic acid* (compound 45)**

Yellow solid: yield = 0.37 g (38 %); mp =257 – 262 °C; ^{1}H- NMR (300 MHz, DMSO- d_6): δ 2.72 (br s ,2 H, CH_2-CO_2H), 4.12 (m, 1 H, NH-CH-3), 7.40 - 7.46 (m, 2 H, H-3'/H-5'), 7.53 (NH(4)),7.75 (m, 2 H, H-2'/H-6'), 7.80 (d, $^3J_{H-F}$ = 10.8 Hz, 1 H, H-6), 8.54 (s, 1 H, H-9), 10.68 (brs, 1 H, N(1)–H),12.51 (brs, 1 H, CH_2-COOH), 15.25 (s, 1 H, C(8)-COOH); ^{13}C-NMR (300 MHz, DMSO- d6): δ 37.23 (CH_2-CO_2H), 54.83 (CH-NH), 109.65 (C-8), 114.60 (C-6), 116.36 (C-4a), 117.16 (d, $^2J_{C-F}$ = 22.2 Hz,C-3'/ C-5'), 118.16 (d, $^3J_{C-F}$ = 7.5 Hz, C-6a), 127.7 (C-10a), 128.62 (C-2'), 128.62 (C-6'), 138.28 (C-1'), 150.98 (d, $^1J_{C-F}$ = 248.32 Hz, C-5), 152.72 (C-9), 162.38 (d, $^1J_{C-F}$ = 245.4 Hz, C-4'), 156.25 (C-2), 172.46 (C(8)-CO_2H), 172.51 (CH_2CO_2H), 175.80 (C-7); IR (NaCl): v3417, 3373, 3333, 2917, 2750, 2350, 2300, 1730, 1650, 1448, 1074 cm^{-1}.

Inhibition of GSK-3β activity *in vitro*

In vitro inhibitory activities of synthon II derivatives (compounds **6, 23** and **45**) were tested against human recombinant GSK-3β. Each compound was screened at an initial concentration of 10 nM. The results showed that inhibitory effect of compound **45** was better than those of compounds **6** and **23** (Table 1; Figure 4).

Molecular modelling results

The binding interactions of compound 45 with GSK-3β were determined using LibDock tool. The procedure revealed many interactions between compound **45** and the binding site of the enzyme (Figures 5 and 6).

Table 1: Inhibition of GSK-3β by synthon II derivatives

Compound	1X Test compound concentration (µM)	[ATP] (µM)	Inhibition (%)	IC_{50} (µM)
23	10	Km app	31	NC
6	10	Km app	94	2.01
45	10	Km app	97	0.18

NC: not calculated

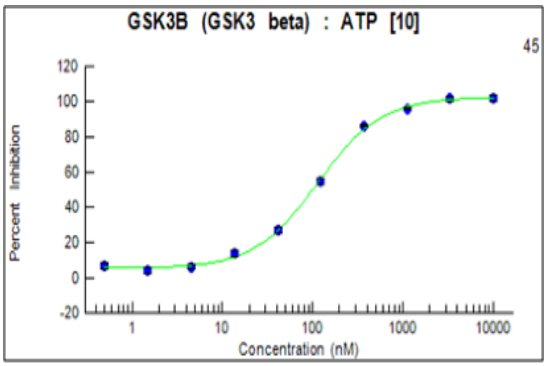


Figure 4: Inhibition of GSK-3β by compound 45

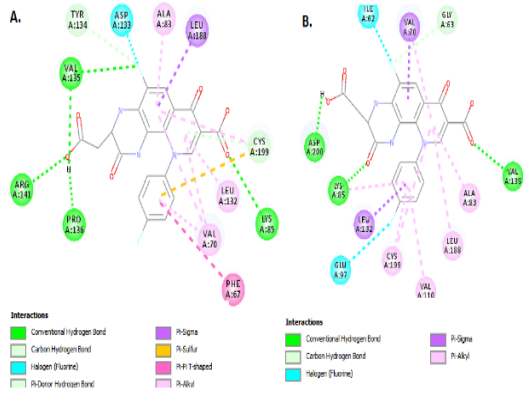


Figure 5: Two-dimensional representation of the interaction between compound 45 and GSK-3β, showing two different poses (A and B)

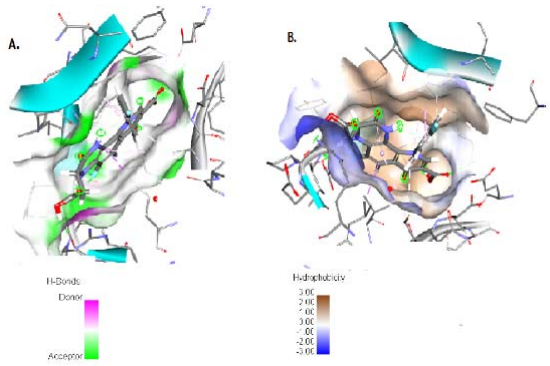


Figure 6: Interaction of compound 45 with binding site of GSK-3β. **(A):** Hydrogen bonding between compound **45** and GSK-3β; and **(B):** Hydrophobic interaction between compound **45** and GSK-3β.

DISCUSSION

The need to find novel GSK-3β inhibitors is of great importance, since the enzyme is involved in many biological processes. In this study, many compounds were synthesized and tested against GSK-3***β***. The results of *in vitro* activity assay showed that compound **45** exhibited potent inhibitory activity against GSK-3β. Molecular modelling revealed that compound **45** assumed different conformations inside the binding pocket of GSK-3β. The quinoline nucleus exhibited remarkable interactions. It interacted with Leu 188 through sigma bond formation, and with Leu 132, Val 70 and Ala 83 via *pi*-alkyl interaction.

In other poses, the quinoline nucleus of compound **45** formed sigma bond with Val 70, and pi-alkyl interaction with Ala 83 and Leu 188. In addition, the 8-carboxylic acid group formed hydrogen bond with Lys 85 and Val 135 in pose B. Fluorine atom at position 5 of compound **45** formed fluorine-hydrogen bond with Asp 133 and Ile 62, and with Val 135 in the other pose. The 10-(4-fluorophenyl) group in compound **45** formed sulfur-hydrogen bond with Val 70 and *pi*-alkyl (*pi-pi*) interaction with Phe 67, but it formed sigma bond with Leu 132. The fluorine atom formed *pi*-alkyl bond with Cys 199 and Val 110, but interacted with Glu 97 via hydrogen bonding. The quinoxoline nucleus of compound 45 had 2-oxo substitutions that formed hydrogen bond with Lys 85. The 3-(carboxymethyl) group of the compound was also involved in hydrogen bond formation with Arg 141, Pro 136, Val 135 and Asp200. Hydrogen bonds and hydrophobic interactions were also observed on surfaces of docked compound 45 and GSK-3β. These results indicate that compound **45** may have interacted with the binding site of GSK-3β via hydrogen and hydrophobic bonds.

CONCLUSION

The results obtained in this study show that 3-(carboxymethyl)-5-fluoro-10-(4-fluorophenyl)-2,7-dioxo-1,2,3,4,7,10-hexahydropyrido [2,3-f] quinoxaline-8-carboxylic acid is a potent inhibitor of GSK-3β and is thus, a promising scaffold for the development of novel drugs that can effectively inhibit GSK-3β signaling pathway.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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