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Original Research Article

Targeting GSK-3β enzyme by diazepino-quinolone derivatives

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Abstract

Purpose: *To synthesize a heterocyclic system containing quinolone and diazepine scaffolds as GSK-3β inhibitor.*
Methods: *The diazepino-quinoline derivatives were synthesized starting from quinolone nucleus in a simple chemical reaction. The in vitro GSK-3β enzyme assay and MTT assay against cancer cell lines were carried out followed by Z´ı-LYTE GSK-3β assay. Anticancer activity was determined using U-87 glioma cell line.*
Results: *Diazepino-quinoline derivatives were obtained in a good yield, and compound 102 exhibited significant activity against in vitro GSK-3β (IC_{50}: 0.114 μM), and anticancer activity (IC_{50}: 37 μM) against U-87 glioma cell line.*
Conclusion: *The GSK-3β enzyme is a potential target to treat different diseases, and diazepines derivatives are a successful template for inhibitors design against GSK-3β enzyme with IC_{50} in a micromolar range.*

Keywords: *GSK-3β, Heterocyclic compounds, Quinolone, Benzodiazepine nucleus*

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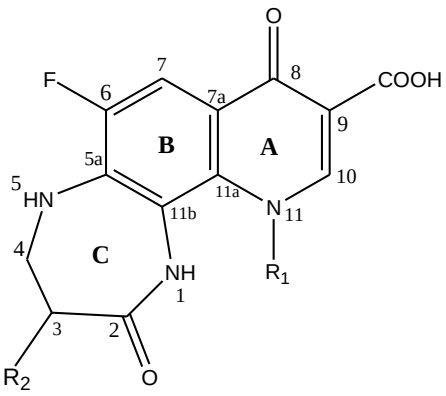
INTRODUCTION

One of the most important serine/threonine kinase enzymes is the GSK-3β enzyme that is active, pervasive, and vital for life [1]. The GSK-3β enzyme is a key signaling molecule that is involved in insulin and glycogen metabolism [2] and inflammatory responses. It plays a significant role in viral infections [3,4] and in cancer [5]. Its expression in cancer has been associated with survival, proliferation, and migration of cancer cells and their resistance to chemotherapy [6].

The GSK-3β enzyme inhibitors have gained a lot of interest as a potential treatment of neurodegenerative diseases such as Alzheimer´s disease [7], diabetes [2] and malignancies [8,9]. The enzyme inhibitors were previously synthesized from heterocyclic compounds having a quinoxaline nucleus that showed good inhibitory activity against GSK-3β enzyme [10].

Diazepine derivatives exhibit many biological activities such as anti-leukemia and anti-platelet activities [11], anti-tumor [12], anti-malarial, anti-trypanosomal, anti-leishmanial agents [13], and anti-convulsant [14]. This study introduces a new hetero-tricyclic system that contains diazepine nucleus (Figure 1) as a potential GSK-3β enzyme inhibitor.



R_1: Cyclopropyl, or p-fluorophenyl
R_2:H, or Methyl

Figure 1: General chemical structure of diazepine derivatives

EXPERIMENTAL

Equipment and reagents

Melting point were measured by Stuart melting point apparatus, IR spectrum was recorded by Shimadzu 8400F FT-IR spectrophotometer. Bruker, Advance DPX-300 Nuclear magnetic resonance spectrometer was used to record (NMR) spectra. High-resolution mass spectrophotometer was used for mass instrument. Reagents and chemicals used are of synthetic grade and were bought from Sigma-Aldrich.

Synthon I derivatives

The synthetic procedure used in scheme I is based on reported methods [10,15]. By applying three steps of reaction, synthon I was obtained: The first step was done by reacting synthon a, and b with different 3-aminopropanoic acid derivatives under conditions shown in Figure 2, and the reaction was done under reflux at 80 ^oC then solution of sodium dithionite (8.0 g, 46 mmol) was added. Finally, a reflux condition was used and polyphosphoric acid was added (10 mL) to get synthon I.

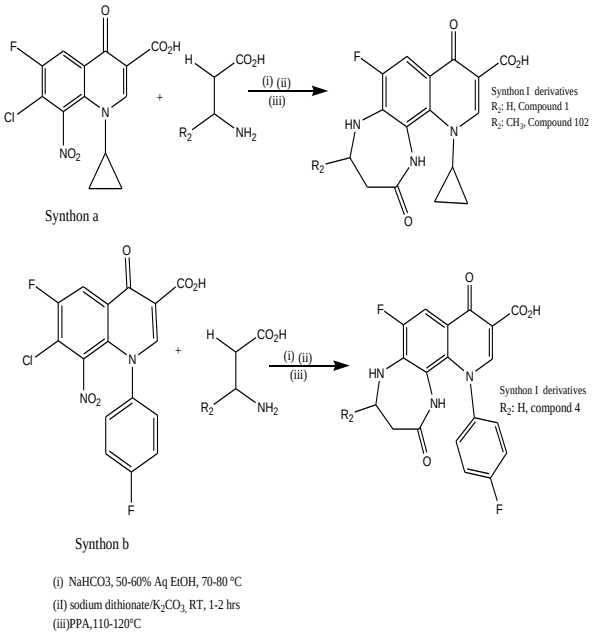


Figure 2: synthesis scheme for synthon I

GSK 3β *in vitro* assay

The Z´1-LYTE GSK-3β assay was used to make GSK-3β *in vitro* assay test (Z_-LYTETM Screening Protocol and Assay Conditions 2016); Z´ı-LYTE test is used to get the % Inhibition.

The compounds that have the highest percentage inhibition were tested for their IC_{50} values. Stock solutions of 10 mM concentration of the tested compounds (1, 4, and 102) in dimethyl sulfoxide (DMSO) was prepared. Then each was tested at 10 µM using the Select Screen Kinase Profiling Services (ThermoFisher Scientific, USA) [16].

MTT assay

The cytotoxicity of GSK-3β inhibitors was determined by MTT assay protocol based on reported procedure carried out by Al-Sha'er *et al* [17]. The U-87 glioma cell line was used for the MTT assay and was provided by Dr. Ahmad Sharab, of the American University of Madaba while the chemicals were purchased from Sigma Aldrich.

Molecular modeling studies

In order to support the *in vitro* assay results, a molecular docking procedure was carried out. The GSK-3β enzyme (PDB code: 3Q3B, resolution 2.7 Á) binding pocket were determined and used in the docking procedure and the most active compound was docked using the Dock Ligands (LibDock).

RESULTS

Spectral characteristics

Based on the synthetic approach in Figure 2, the following compounds were obtained:

11-Cyclopropyl-2,8-dioxo-6-fluoro-2,3,4,5,8,11-hexahydro-1H-(1,4)diazepino(2,3-h)quinoline-9- carboxylic acid (1)

Brownish product was obtained. Yield 0.36 g (95 %); mp 346 - 347 ° [16].

6-fluoro-11-(4-fluorophenyl)-2,8-dioxo-2,3,4,5,8,11-hexahydro-1*H*-(1,4)diazepino(2,3-*h*)quinoline-9-carboxylic acid (4)

Yellow powder, mp 299 - 302 °C**.** ^{1}H-NMR (300 MHz, DMSO, *d6*): *δ* 2.33 (m, 2H, CH2-3), 3.01 (m, 2H, CH2-4), 6.89 (t, 1H, N*H*-CH_2), 7.30 (d, *J* = 111.4 Hz, 2H, H-3'/ H-5'), 7.64(d, *J* = 8.4 Hz, 2H, H-2'/ H-6'), 7.75 (d, $^{3}J_{H\text{-}F}$ = 11.4Hz, 1H, H-7), 8.42 (s, 1H, H-10), 8.62 (s, 1H, N(1)-*H*), 14 (br s, 1H, C(9)-CO_2H); HERMS (ESI, +ve): m/z (M^{+2} + H) 387.10306 $C_{19}H_{15}F_2N_3O_4$ 387.1030.

11-cyclopropyl-6-fluoro-3-methyl-2,8-dioxo-2,3,4,5,8,11-hexahydro-1-*H*-(1,4)diazepino(2,3-*h*)quinoline-9-carboxylic acid (102)

Brownish powder, mp 309 - 315 °C; ^{1}H-NMR (300 MHz, DMSO- *d6*): *δ* 0.81 (m, 2H, H_2-2′), 1.54 (d, *J* = 5.1 Hz, 3H, CH_3), 3.24 - 3.66 (m, 3H; 1H, H-3 and 2H, H-4), 3.47 (d, d, *J* = 12, 10.5 Hz, 1H, H α- 4), 3.66 (s,1H, Hβ-4), 4.21 (m, 1H, H-1′), 6.88 (br s, 1H, N(5)-*H*), 7.71 (d, $^{3}J_{H\text{-}F}$ = 11.1 Hz, 1H, H-7), 8.66 (s, 1H, H-10), 9.62 (s, 1H, N(1)-*H*), 16 (s, 1H, CO_2H); IR (NaCl): *v* 3500, 3250, 2999, 2355, 1710, 1659, 1550, 1250 cm^{-1}.

GSK-3β inhibitors activity

The synthon I derivatives were tested by using the *in vitro* human recombinant GSK-3β kinase (human recombinant) assay kit. The GSK-3β inhibitors was screened in this assay at 10 nM. Compounds that showed good percentage inhibition, namely, (1, 4, and 102) were screened at different concentrations to find their IC_{50} values. Compounds (1, 4, and 102) activities were measured against GSK 3 enzyme and the results are shown in Table 1. As presented in the Table; compound 1 showed 79 % inhibition while 4 showed 11 % inhibition and compound 102 showed 100 % inhibition the IC_{50} was calculated for compounds 1 and 102 and it was 4.18 and 0.114 µM, respectively. Figure 3 illustrates the concentration (nM)/percent inhibition plots of the most active compound 102. Next step was to determine if these compounds have any pharmacological activities as potential anti-cancer treatments. The U-87 glioma cell line was used in the MTT assay as it is known to express GSK-3β [18]. The most active (compound **102)** and the least active (compound 4) compounds from the enzyme assay were chosen to assess their anti-proliferative activity. The MTT results are in accordance with the enzyme assay results with IC_{50} 37 µM and > 100 µM for compounds 102 and 4, respectively (Table 1). The results, confirm the importance of GSK-3β in cancer progression and open new avenues for the use of the synthon I derivatives GSK-3β inhibitors as anti-cancer drugs [18].

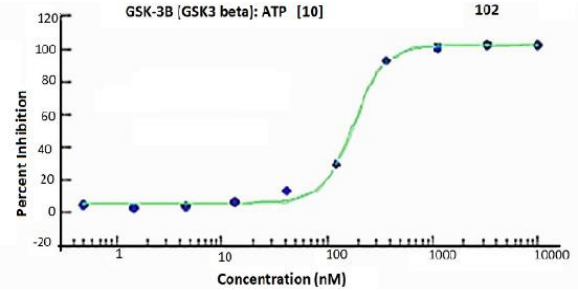


Figure 3: Plot of concentration (nM) vs. percent inhibition for compound (102)

Docking results

The software assists in identifying the binding site in the receptor of GSK-3β that binds to the synthesized compound and determined the conformations of the active compound 102 by docking procedure that allowed the types of interactions between the binding pocket and the active compound. Figure 4 shows the most active compound (102) with different poses that might be acquired inside the binding pocket of (3Q3B).

Table 1: The synthesized compounds tested against (1) GSK-3β enzyme presented with their % inhibition and IC_{50} (µM). (2) U-87 glioma cell line in MTT assay presented with their % inhibition and IC_{50} (µM)

Compound name 10 (µM)	(ATP) Tested (µM)	% Inhibition mean	IC_{50}(µM) GSK 3 enzyme	IC_{50}(µM) MTT assay
102	Km app	100	0.114	37
1	Km app	79	4.180	NC
4	Km app	11	NC	>100

NC: not calculated

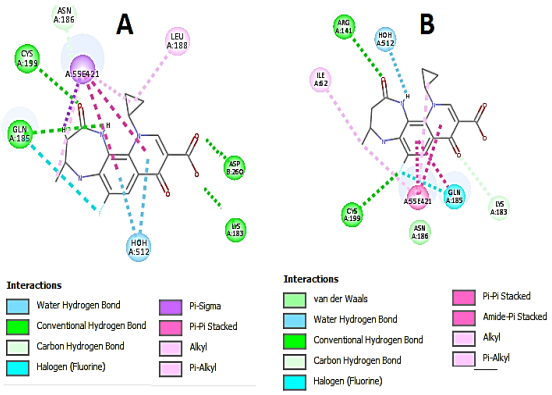


Figure 4: 2D diagram showing the interaction between docked compound 102; IC_{50} (0.114 µM) and GSK-3β enzyme showing two different poses (in A and B) for the compound 102 in the binding pocket of GSK-3β enzyme

DISCUSSION

Synthon I derivatives were obtained in a good yield and tested against GSK-3β enzyme with interesting inhibitory concentration as shown in Table 1. Compound 102 showed 100 % inhibition with IC_{50} (0.114 µM), while compound 1, had 79 % inhibition with IC_{50} (4.180 µM) while compound 4 had 11% inhibition so the IC_{50} was not measured and the IC_{50} can be explained by docking studies that revealed that there are many interactions for compound 1 GSK 3β inhibitor in the binding pocket of GSK-3β kinase (3Q3B), among these interactions, there is pi-pi stacking, pi-sigma, pi-alkyl, halogen, and H-bonding.

The results from this study, confirm the importance of GSK-3β in cancer progression and open new avenues for the use of the synthon I derivatives GSK-3β inhibitors as anti-cancer drugs [17]. The molecular docking studies also elucidated the binding modes of the compounds with the GSK-3β target

CONCLUSION

Synthesis of novel GSK-3β enzyme inhibitors has been carried out using simple chemical reactions. Diazepine derivatives could be a successful template for the design of inhibitors against GSK-3β enzyme with IC_{50} in a micromolar range. Compound 102 shows potent inhibitory activity against both GSK-3β enzyme and U-87 glioma cell line in MTT assay. With these findings, efforts are expanding for further optimization of diazepine derivatives in order to develop potent inhibitors with potential therapeutic activity against GSK-3β.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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