

Article

Synthesis, In Vitro Cytotoxicity Evaluation and GSK-3 β Binding Study of Some Indole–Triazole-Linked Pyrazolone Derivatives

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Abstract

Glycogen synthase kinase-3 beta (GSK-3 β) is a multifunctional serine/threonine kinase mediating multiple cellular functions, such as differentiation, apoptosis, and cell proliferation. Because of their ability to alter carcinogenic pathways, GSK-3 β inhibitors are being explored for the development of anticancer molecules. In the present study, we synthesized and evaluated the cytotoxic properties of a series of twenty indole–triazole-linked pyrazolone derivatives, **10Aa–Ed**. All derivatives were characterized by FTIR, ¹H/¹³C NMR, and high-resolution mass spectrometry (HRMS) methods. All compounds and standards, sunitinib and 5-Fluorouracil (5-FU), were screened against four adherent cell lines, including pancreatic adenocarcinoma (Capan-1), colorectal carcinoma (HCT-116), glioblastoma (LN229), and lung carcinoma (NCI-4460), and four non-adherent cell lines, including acute myeloid leukemia (HL-60), chronic myeloid leukemia (K562), T lymphoblast (MOLT4), and non-Hodgkin lymphoma (Z138). Among the screened derivatives, molecule **10Aa** showed cytotoxicity against MOLT 4, Z138, and HL60 with CC₅₀ values of 14.45 μ M, 15.34 μ M, and 17.56 μ M, respectively. GSK-3 β kinase inhibition was evaluated with the **10Aa**, which is capable of inhibiting GSK-3 β in a dose-dependent manner. Additionally, molecular docking was performed to estimate the correlation between invitro data and GSK-3 β binding affinity. The outcomes of the invitro experiments demonstrated strong concordance with the insilico data. The discovery yielded compounds **10Aa** and **10Cd**, which can be modified to create effective anticancer agents that target GSK-3 β .

Keywords: indole; 1,2,3-triazole; pyrazolone; cytotoxicity; GSK-3 β ; insilico study



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1. Introduction

Cancer is a major global challenge, affecting individuals across all segments of society. It significantly contributes to global morbidity and mortality. The National Cancer Registry Programme (NCRP), India, has reported approximately 1.46 million cancer cases in 2022, with nearly 395,400 deaths [1]. Among different types of cancer, breast cancer leads the list with an estimated 98,300 deaths, while cervical, colorectal, and lung cancer accounted for approximately 79,900, 41,000, and 75,000 deaths, respectively, in 2022 across India [2]. These statistics are scary and need immediate attention. The existing anticancer regime is not only toxic but also costly to afford for the common human being. Hence, there is an unmet need for an effective lead that can be translated into a clinical drug to treat cancer.

In this context, the development of small nitrogen-containing heterocyclic scaffolds, such as indole and triazole hybrids, can be promising in cancer research. The nitrogen-containing heterocyclic compounds are an integral part of medicinal chemistry by virtue of low toxicity, efficient receptor binding in biological systems, and improved aqueous solubility [3]. The literature reports indole as a synthetically versatile scaffold with promising activities against cancer, human immunodeficiency virus (HIV), tuberculosis, hypertension, diabetes, and microbial infections [4]. Bajad et al. had reviewed indole as a promising scaffold for the discovery and development of potential anti-tubercular agents targeting cell-wall synthesis and membrane depolarization in mycobacterial cells [5]. Hasan et al. have reviewed indole derivatives as a potential anticancer lead acting via kinase inhibition, apoptosis, DNA intercalation, and disruption of cell signaling pathways [6]. The antidiabetic property of indole-linked thiazolidine-2,4-dione inhibiting α -glucosidase was reported by Hu et al. [7] and Mo et al. [8]. Recently, Madarakhandi et al. have reported some hydrazide-2-oxindole derivatives as potent GSK-3 β kinase inhibitors against cancer [9]. Likewise, indole [10], pyrazole [11], and triazole [12] show remarkable binding capabilities with receptors and enzymes in biological systems and elicit promising pharmacological responses against microbes, free radicals, inflammatory responses, cancer, and viruses. Sucheta et al. [13] reviewed the multifaceted biological potential of pyrazole scaffolds, while Mallisetty et al., in a separate study, reported the pyrazole-linked 1,2,4-triazole hybrid as a topoisomerase-IIa inhibitor and evaluated its antibacterial and anticancer properties against *Staphylococcus aureus* and MCF7 cell lines, respectively [14]. Further, the study by Glomb et al. [15], Li et al. [16], and Matta et al. [17] underscores the biological significance of pyrazole- and triazole-based scaffolds. Figure 1 depicts some clinically used indole, pyrazole, and 1,2,3-triazole hybrids.

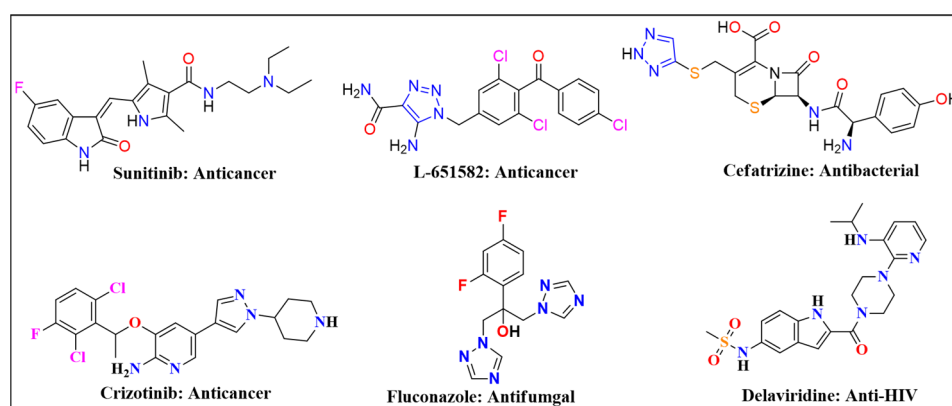


Figure 1. Clinically used indole, pyrazole, and 1,2,3-triazole derivatives.

Glycogen synthase kinase-3 beta (GSK-3 β) is a multifunctional serine/threonine kinase involved in several cellular functions, such as differentiation, apoptosis, and pro-

liferation [18]. GSK-3 β plays dual roles in cancer, depending on the tumor types and biochemical pathways involved. It not only suppresses tumors by blocking β -catenin in the Wnt pathway but also promotes the growth of new tumors by promoting survival signaling mediated by NF- κ B and epithelial-to-mesenchymal transition [19]. GSK-3 β is a possible therapeutic target because it has been shown to exhibit aberrant activation in malignancies such as glioblastoma, colorectal, and pancreatic tumors. Because of GSK-3 β 's ability to alter several carcinogenic pathways, its inhibitors are being researched for anticancer treatment [20]. Considering the diverse pharmacological relevance of the indole, pyrazole, and triazole nuclei, along with the significance of GSK-3 β inhibition in cancer research, we synthesized and evaluated the cytotoxicity of a series of twenty indole–triazole-linked pyrazolone derivatives in the present study. The indole-substituted triazoles were synthesized via a Cu(I)-catalyzed click reaction between indole-substituted alkynes and benzyl azides in the presence of CuSO₄·5H₂O and sodium ascorbate. Subsequently, substituted pyrazolones bearing an active methylene (–CH₂) group were condensed with the triazole-linked 3-formyl indole derivatives in methanol using piperidine as a base catalyst to get **10Aa–Ed** [21–25]. Furthermore, the molecular docking of compound **10Aa** was carried out against GSK-3 β (PDB ID: 1Q41) to elucidate its receptor binding affinity. Figure 2 illustrates selected nitrogen-containing GSK-3 β inhibitors that are clinically approved or currently under investigation.

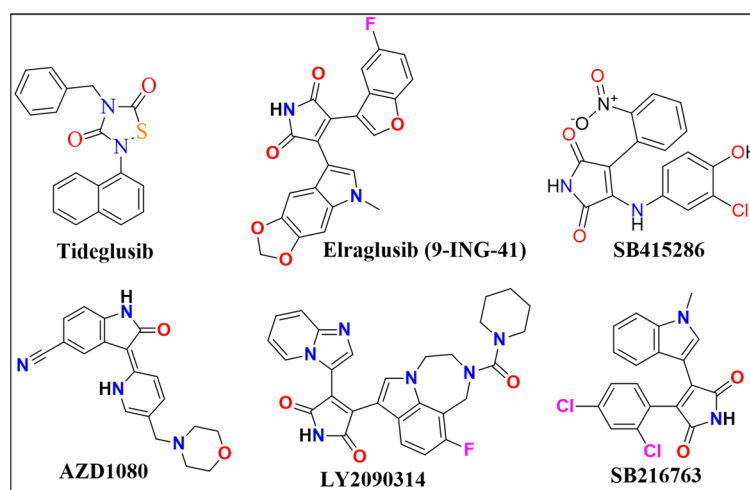


Figure 2. Structures of potential GSK-3 β inhibitors.

2. Materials and Methods

2.1. Chemicals and Reagents

Spectrochem Pvt. Ltd., Bengaluru, India and BLD Pharma Hyderabad provided all chemicals and reagents. On pre-coated silica gel plates 60 F254 (Merck, Darmstadt, Germany), the progress of the reaction was monitored. A digital melting point instrument (5067/2, DBK, Mumbai, India) was used to measure melting points (m.p), which were then presented uncorrected. The ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were acquired on 400 Mega Hertz (MHz) Avance-III NMR spectrometer (Bruker, MA, USA) in deuterated dimethyl sulfoxide (DMSO-*d*₆, 99.8%, Eurisotop, Saint-Aubin, France). The peaks' positions (Chemical shift, J) were reported in hertz (Hz). A Fourier transform infrared (FTIR, JASCO 460+, Tokyo, Japan) spectrophotometer was used to get FT-IR spectra. SynaptG2 QTOF high-resolution mass spectrometer (HRMS, Waters, MA, USA) was used to determine the molecular weight (M.W) of compounds **10Aa–Ed** by the electrospray ionization (ESI) method. Intermediate 1-(prop-2-yn-1-yl)-1H-indole-3-carbaldehyde (**3**) [21],

aryl azides (**5a–e**) [22], 1-((1-(4-substituted-benzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indole-3-carbaldehyde (**6a–e**) [23] and 1-substituted-3-methyl-1H-pyrazol-5(4H)-one (**9a–d**) [24,25] were obtained as per literature. Compounds **1** (98%, BLD Pharm, Hyderabad, India), **2** (80%, Spectrochem, Mumbai, India), **4** (97%, BLD Pharm, Hyderabad, India), **7** (98%, SD Fine Chemical Limited, Mumbai, India), and **8a–d** (95%, BLD Pharm, Hyderabad, India) were procured commercially.

2.2. General Procedure for Synthesis of 4-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**10Aa–Ed**)

An equimolar quantity of different 1-((1-(4-substituted-benzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indole-3-carbaldehyde (**6a–e**) and 1-substituted-3-methyl-1H-pyrazol-5(4H)-one (**9a–d**) were refluxed using a catalytic amount of piperidine (98%, SD Fine Chemical Limited, Mumbai, India) in methanol (99.5%, Loba Chemie, Mumbai, India) to get respective 4-((1-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**10Aa–Ed**). Recrystallized the precipitated product from appropriate solvents.

2.2.1. 4-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**10Aa**)

Yellow crystals, m.p: 244.0–245.1 °C, % yield: 73, IR (KBr) ν : 3145, 3121, 1670, 1585 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 11.07 (1H, s, -NH), 9.78 (1H, s), 8.26 (1H, s, ar-H), 8.11–8.09 (1H, d J = 6.8 Hz, ar-H), 7.90 (1H, s, ar-H), 7.74–7.72 (1H, d J = 7.6 Hz, ar-H), 7.34–7.26 (7H, m, ar-H), 5.62 (2H, s, -N-CH₂), 5.55 (2H, s, -N-CH₂), 2.23 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.43, 31.15, 42.39, 53.31, 111.88, 112.05, 119.21, 119.73, 122.65, 123.83, 124.55, 128.33, 128.62, 128.62, 129.24, 129.24, 134.90, 136.31, 136.57, 139.45, 142.81, 150.00, 166.70. M.F: C₂₃H₂₀N₆O, HRMS m/z (ESI): [M + H]⁺ 397.1753 (397.4445).

2.2.2. 4-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one, (**10Ab**)

Orange crystals, m.p: 205.7–206.5 °C, % yield: 68, IR (KBr) ν : 3126, 3080, 1671, 1600, 1469 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.97 (1H, s), 8.02–8.00 (2H, d J = 10.0 Hz, ar-H), 7.91–7.89 (1H, m, ar-H), 7.83 (1H, s, ar-H), 7.65–7.63 (1H, m, ar-H), 7.47–7.43 (2H, t J = 17.6 Hz, ar-H), 7.40–7.33 (6H, m, ar-H), 7.23–7.19 (3H, m, ar-H), 5.60 (2H, s, -N-CH₂), 5.47 (2H, s, -N-CH₂), 2.45 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.48, 31.16, 42.39, 53.33, 112.12, 112.31, 118.60, 118.60, 119.17, 119.41, 123.10, 124.20, 124.59, 124.59, 128.36, 128.36, 128.64, 129.26, 129.26, 129.26, 134.90, 136.30, 136.72, 137.08, 139.24, 140.69, 142.72, 151.53, 163.21. M.F: C₂₉H₂₄N₆O, HRMS m/z (ESI): [M + H]⁺ 473.2061 (473.5405).

2.2.3. 4-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(p-tolyl)-1H-pyrazol-5(4H)-one, (**10Ac**)

Yellow powder, m.p: 228.6–229.1 °C, % yield: 71, IR (KBr) ν : 3100, 3064, 1668, 1602, 1469 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.97 (1H, s), 7.91–7.85 (3H, m, ar-H), 7.82 (1H, s, ar-H), 7.64–7.62 (1H, m, ar-H), 7.54 (1H, s, ar-H), 7.40–7.34 (6H, m, ar-H), 7.26–7.21 (3H, m, ar-H), 5.60 (2H, s, -N-CH₂), 5.47 (2H, s, -N-CH₂), 2.44 (3H, s, -CH₃), 2.39 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.45, 20.96, 31.15, 42.53, 53.33, 112.12, 112.31, 118.60, 118.60, 119.17, 119.41, 123.10, 124.20, 124.59, 124.59, 128.36, 128.36, 128.64, 129.26, 129.26, 129.26, 134.90, 136.30, 136.72, 137.08, 139.24, 140.69, 142.72, 151.53, 163.21. M.F: C₃₀H₂₆N₆O, HRMS m/z (ESI): [M + H]⁺ 487.2153 (487.5670).

2.2.4. 4-((1-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one, (**10Ad**)

Green powder, m.p: 242.6–244.1 °C, % yield: 78, IR (KBr) ν : 3110, 3052, 1681, 1469, 1351 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz): δ = 9.91 (1H, s), 8.33 (4H, s, ar-H), 7.91–7.87 (2H, m, ar-H), 7.67–7.65 (1H, m, ar-H), 7.41–7.35 (6H, m, ar-H), 7.25–7.23 (2H, m, ar-H), 5.63 (2H, s, -N-CH₂), 5.50 (2H, s, -N-CH₂), 2.47 (3H, s, -CH₃). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz): δ = 13.15, 31.15, 42.65, 53.35, 112.45, 112.45, 117.62, 117.62, 117.89, 123.39, 124.62, 124.62, 125.51, 125.51, 128.39, 128.39, 128.39, 128.66, 129.30, 129.30, 129.30, 129.30, 136.81, 138.34, 142.61, 153.75. M.F: C₂₉H₂₃N₇O₃, HRMS m/z (ESI): [M + H]⁺518.1940 (518.5380).

2.2.5. 4-((1-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one, (**10Ba**)

Orange powder, m.p: 260.1–261.5 °C, % yield: 63, IR (KBr) ν : 3118, 2980, 1670, 1578 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz): δ = 11.06 (1H, s, -NH), 9.81 (1H, s), 8.29 (1H, s, ar-H), 8.13–8.11 (1H, d J = 8.8 Hz, ar-H), 7.89 (1H, s, ar-H), 7.76–7.74 (1H, d J = 8.8 Hz, ar-H), 7.43–7.41 (2H, d J = 8.4 Hz, ar-H), 7.35–7.30 (4H, m, ar-H), 5.65 (2H, s, -N-CH₂), 5.58 (2H, s, -N-CH₂), 2.25 (3H, s, -CH₃). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz): δ = 13.43, 31.15, 42.36, 52.52, 111.89, 112.03, 119.22, 119.73, 122.66, 123.85, 124.65, 129.15, 129.15, 129.15, 130.31, 130.31, 130.31, 133.35, 134.91, 136.59, 139.41, 142.82, 150.01, 166.71. M.F: C₂₃H₁₉ClN₆O, HRMS m/z (ESI): [M + H]⁺431.1393 (431.8896).

2.2.6. 4-((1-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one, (**10Bb**)

Orange crystals, m.p: 203.2–204.1 °C, % yield: 70, IR (KBr) ν : 3104, 3068, 1675, 1597, 1437 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz): δ = 9.97 (1H, s), 8.01–7.99 (2H, d J = 8.8 Hz, ar-H), 7.91–7.89 (1H, m, ar-H), 7.83 (1H, s, ar-H), 7.63–7.61 (1H, m, ar-H), 7.48–7.44 (2H, t J = 16.0 Hz, ar-H), 7.39–7.37 (3H, m, ar-H), 7.33–7.30 (2H, m, ar-H), 7.23–7.20 (1H, t J = 15.8 Hz, ar-H), 7.17–7.15 (2H, d J = 8.4 Hz, ar-H), 5.60 (2H, s, -N-CH₂), 5.44 (2H, s, -N-CH₂), 2.45 (3H, s, -CH₃). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz): δ = 13.48, 31.15, 42.51, 52.53, 112.12, 112.30, 118.62, 118.62, 119.18, 119.41, 123.10, 124.21, 124.60, 124.66, 129.21, 129.21, 129.21, 129.26, 130.32, 130.32, 130.32, 133.35, 136.28, 136.73, 137.07, 139.23, 140.66, 142.76, 151.52, 163.21. M.F: C₂₉H₂₃ClN₆O, HRMS m/z (ESI): [M] 506.1718 (506.9855).

2.2.7. 4-((1-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(p-tolyl)-1H-pyrazol-5(4H)-one, (**10Bc**)

Orange crystals, m.p: 216.3–217.1 °C, % yield: 74, IR (KBr) ν : 3108, 3055, 1668, 1600, 1469 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz): δ = 9.97 (1H, s), 7.91–7.82 (4H, m, ar-H), 7.63–7.61 (1H, m, ar-H), 7.39–7.36 (3H, m, ar-H), 7.32–7.29 (2H, m, ar-H), 7.26–7.24 (2H, m, ar-H), 7.17–7.15 (2H, d J = 8.0 Hz, ar-H), 5.60 (2H, s, -N-CH₂), 5.44 (2H, s, -N-CH₂), 2.44 (3H, s, -CH₃), 2.39 (3H, s, -CH₃). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz): δ = 13.45, 20.96, 31.15, 42.48, 52.52, 112.08, 112.28, 118.66, 118.66, 119.32, 119.40, 123.07, 124.18, 124.68, 129.21, 129.21, 129.21, 129.28, 129.65, 129.65, 130.30, 130.30, 133.34, 133.65, 135.29, 136.72, 136.89, 140.58, 142.76, 151.24, 163.00. M.F: C₃₀H₂₅ClN₆O, HRMS m/z (ESI): [M] 521.1871 (521.0121).

2.2.8. 4-((1-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one, (**10Bd**)

Green powder, m.p: 251.3–252.9 °C, % yield: 68, IR (KBr) ν : 3130, 1672, 1590, 1321 cm^{-1} . $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 400 MHz): δ = 9.91 (1H, s), 8.35 (4H, s, ar-H), 7.92–7.87 (1H, m, ar-H), 7.66–7.63 (1H, m, ar-H), 7.42–7.40 (3H, m, ar-H), 7.34–7.32 (3H, m, ar-H), 7.19–7.17 (2H, d J = 8.4 Hz, ar-H), 5.63 (2H, s, -N-CH₂), 5.47 (2H, s, -N-CH₂), 2.47 (3H, s, -CH₃). $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 100 MHz): δ = 13.62, 42.64, 52.52, 112.46, 112.46, 117.60, 117.95, 119.54, 123.29,

124.33, 124.70, 125.52, 129.21, 129.21, 129.21, 129.21, 129.21, 130.36, 130.36, 130.36, 130.36, 133.34, 135.28, 136.83, 141.20, 142.70, 144.36, 148.27, 159.33. M.F: C₂₉H₂₂ClN₇O₃, HRMS *m/z* (ESI): [M + H]⁺ 552.1574 (552.9831).

2.2.9. 3-Methyl-4-((1-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one, (10Ca)

Yellow crystals, m.p: 237.8–239.2 °C, % yield: 67, IR (KBr) ν : 3111, 3040, 1671, 1585, 1468 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 11.06 (1H, s, -NH), 9.82 (1H, s), 8.22 (1H, s, ar-H), 8.13–8.11 (1H, m, ar-H), 7.89 (1H, s, ar-H), 7.77–7.74 (1H, m, ar-H), 7.35–7.29 (2H, m, ar-H), 7.20–7.18 (4H, m, ar-H), 5.64 (2H, s, -N-CH₂), 5.51 (2H, s, -N-CH₂), 2.27 (3H, s, -CH₃), 2.25 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.43, 21.13, 31.35, 53.13, 111.87, 119.26, 119.79, 122.65, 123.84, 124.35, 128.41, 128.41, 128.41, 129.15, 129.77, 129.77, 129.77, 133.28, 134.97, 136.64, 138.09, 139.46, 142.78, 150.01. M.F: C₂₄H₂₂N₆O, HRMS *m/z* (ESI): [M + H]⁺ 411.1936 (411.4711).

2.2.10. 3-Methyl-4-((1-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one, (10Cb)

Yellow powder, m.p: 204.8–206.1 °C, % yield: 60, IR (KBr) ν : 3114, 3061, 1671, 1597, 1488 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.96 (1H, s), 8.02–7.99 (2H, d J = 9.6 Hz, ar-H), 7.91–7.88 (1H, m, ar-H), 7.83 (1H, s, ar-H), 7.65–7.63 (1H, m, ar-H), 7.47–7.43 (2H, t J = 16.0 Hz, ar-H), 7.39–7.36 (2H, m, ar-H), 7.34 (1H, s, ar-H), 7.23–7.19 (2H, t J = 15.4 Hz, ar-H), 7.16–7.11 (3H, m, ar-H), 5.59 (2H, s, -N-CH₂), 5.42 (2H, s, -N-CH₂), 2.45 (3H, s, -CH₃), 2.33 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.48, 21.11, 31.15, 42.51, 53.13, 112.11, 112.31, 118.61, 118.61, 119.17, 119.41, 123.10, 124.20, 124.43, 124.59, 128.40, 128.40, 129.26, 129.26, 129.26, 129.76, 129.76, 129.76, 133.27, 136.75, 137.09, 138.00, 139.24, 140.68, 142.68, 151.53. M.F: C₃₀H₂₆N₆O, HRMS *m/z* (ESI): [M + H]⁺ 487.2221 (487.5670).

2.2.11. 3-Methyl-4-((1-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-(p-tolyl)-1H-pyrazol-5(4H)-one, (10Cc)

Yellow powder, m.p: 228.9–230.1 °C, % yield: 62, IR (KBr) ν : 3108, 3061, 1669, 1600, 1469 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.96 (1H, s), 7.90–7.82 (4H, m, ar-H), 7.65–7.63 (1H, m, ar-H), 7.38–7.33 (4H, m, ar-H), 7.24 (3H, s, ar-H), 7.17–7.11 (4H, m, ar-H), 5.59 (2H, s, -N-CH₂), 5.42 (2H, s, -N-CH₂), 2.44 (3H, s, -CH₃), 2.39 (3H, s, -CH₃), 2.33 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.44, 21.10, 21.10, 31.15, 53.13, 112.42, 118.04, 118.04, 118.65, 118.65, 119.31, 119.39, 123.04, 124.43, 128.39, 128.39, 128.39, 129.27, 129.65, 129.65, 129.76, 129.76, 129.76, 133.27, 133.62, 134.10, 136.88, 137.99, 140.60, 142.69, 151.25. M.F: C₃₁H₂₈N₆O, HRMS *m/z* (ESI): [M + H]⁺ 501.2451 (501.5936).

2.2.12. 3-Methyl-4-((1-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one, (10Cd)

Yellow powder, m.p: 266.4–268.1 °C, % yield: 69, IR (KBr) ν : 3119, 1670, 1590, 1468, 1324 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.90 (1H, s), 8.35 (4H, s, ar-H), 7.91–7.87 (2H, m, ar-H), 7.67–7.65 (1H, m, ar-H), 7.41–7.38 (3H, m, ar-H), 7.17–7.13 (4H, m, ar-H), 5.61 (2H, s, -N-CH₂), 5.45 (2H, s, -N-CH₂), 2.47 (3H, s, -CH₃), 2.34 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.63, 21.13, 53.13, 112.51, 112.51, 117.62, 117.62, 117.90, 119.56, 123.30, 124.44, 124.44, 125.54, 125.54, 128.45, 128.45, 128.45, 129.75, 129.75, 129.75, 129.75, 133.31, 136.85, 138.40, 142.59, 143.09, 149.32, 149.32, 151.47, 153.75. M.F: C₃₀H₂₅N₇O₃, HRMS *m/z* (ESI): [M + H]⁺ 532.2106 (532.5646).

2.2.13. 4-((1-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1H-pyrazol-5(4H)-one, (**10Da**)

Orange crystals, m.p: 223.8–225.1 °C, % yield: 64, IR (KBr) ν : 3132, 2947, 1678, 1590 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.87 (1H, s), 8.59 (1H, s, ar-H), 7.88–7.86 (1H, d J = 8.8 Hz, ar-H), 7.89 (1H, s, ar-H), 7.63–7.61 (1H, d J = 8.8 Hz, ar-H), 7.37–7.33 (3H, m, ar-H), 7.20–7.18 (2H, d J = 8.8 Hz, ar-H), 5.57 (2H, s, -N-CH₂), 5.41 (2H, s, -N-CH₂), 3.81 (3H, s, -OCH₃), 2.34 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.43, 31.15, 42.38, 52.88, 55.58, 111.87, 112.06, 114.59, 114.59, 119.21, 119.73, 122.64, 123.84, 124.16, 128.20, 129.14, 130.05, 130.05, 134.90, 136.57, 139.45, 142.77, 150.01, 159.59, 166.70. M.F: C₂₄H₂₂N₆O₂, HRMS m/z (ESI): [M + H]⁺ 427.1900 (427.4705).

2.2.14. 4-((1-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one, (**10Db**)

Yellow powder, m.p: 201.1–202.3 °C, % yield: 66, IR (KBr) ν : 3110, 3064, 1670, 1595, 1438 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.95 (1H, s), 8.02–8.00 (2H, d J = 9.6 Hz, ar-H), 7.91–7.88 (1H, m, ar-H), 7.83 (1H, s, ar-H), 7.65–7.63 (1H, m, ar-H), 7.47–7.43 (2H, t J = 16.0 Hz, ar-H), 7.39–7.36 (2H, m, ar-H), 7.33 (1H, s, ar-H), 7.23–7.17 (3H, m, ar-H), 6.87–6.85 (2H, d J = 8.8 Hz, ar-H), 5.58 (2H, s, -N-CH₂), 5.40 (2H, s, -N-CH₂), 3.78 (3H, s, -OCH₃), 2.45 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.47, 31.15, 42.52, 52.89, 55.54, 112.11, 112.31, 114.57, 114.57, 114.57, 118.57, 118.57, 119.17, 119.40, 123.09, 124.23, 123.57, 128.18, 129.26, 129.26, 130.06, 130.06, 130.06, 136.72, 137.06, 139.25, 140.68, 142.67, 151.52, 159.58, 163.21. M.F: C₃₀H₂₆N₆O₂, HRMS m/z (ESI): [M + H]⁺ 503.2213 (503.5664).

2.2.15. 4-((1-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(p-tolyl)-1H-pyrazol-5(4H)-one, (**10Dc**)

Yellow powder, m.p: 224.6–226.1 °C, % yield: 65, IR (KBr) ν : 3107, 3055, 1670, 1602, 1469 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.96 (1H, s), 7.89–7.86 (3H, m, ar-H), 7.82 (1H, s, ar-H), 7.65–7.63 (1H, m, ar-H), 7.38–7.36 (2H, s, ar-H), 7.32 (1H, s, ar-H), 7.26–7.24 (2H, m, ar-H), 7.19–7.17 (2H, d J = 8.4 Hz, ar-H), 6.87–6.85 (2H, d J = 8.8 Hz, ar-H), 5.58 (2H, s, -N-CH₂), 5.40 (2H, s, -N-CH₂), 3.79 (3H, s, -OCH₃), 2.44 (3H, s, -CH₃), 2.39 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.45, 20.96, 31.15, 42.50, 52.89, 55.54, 112.07, 112.30, 114.57, 114.57, 118.59, 118.59, 119.31, 119.38, 123.05, 124.14, 124.22, 128.18, 129.27, 129.65, 129.65, 130.06, 130.06, 130.06, 133.61, 136.71, 136.92, 140.61, 142.69, 151.25, 159.58, 162.99. M.F: C₃₁H₂₈N₆O₂, HRMS m/z (ESI): [M + H]⁺ 517.2357 (517.5930).

2.2.16. 4-((1-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-3-methyl-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one, (**10Dd**)

Yellow powder, m.p: 223.8–225.2 °C, % yield: 67, IR (KBr) ν : 3145, 3105, 1685, 1591, 1320 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 9.90 (1H, s), 8.33 (4H, s, ar-H), 7.91–7.87 (2H, m, ar-H), 7.68–7.65 (1H, m, ar-H), 7.41–7.37 (3H, m, ar-H), 7.21–7.19 (2H, d J = 8.4 Hz, ar-H), 6.89–6.86 (2H, d J = 8.8 Hz, ar-H), 5.61 (2H, s, -N-CH₂), 5.43 (2H, s, -N-CH₂), 3.79 (3H, s, -OCH₃), 2.47 (3H, s, -CH₃). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ = 13.58, 31.15, 42.61, 52.91, 55.54, 112.44, 114.56, 114.56, 117.58, 117.58, 117.86, 119.50, 123.37, 124.28, 124.46, 125.49, 128.16, 129.26, 130.09, 130.09, 130.09, 136.80, 138.28, 141.30, 142.55, 142.95, 144.35, 153.04, 153.73, 159.58, 163.97. M.F: C₃₀H₂₆N₇O₄, HRMS m/z (ESI): [M + H]⁺ 548.2011 (548.5640).

2.2.17. 3-Methyl-4-((1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one, (**10Ea**)

Yellow powder, m.p: 279.7–280.9 °C, % yield: 65, IR (KBr) ν : 3172, 3118, 1666, 1440, 1348 cm^{-1} . $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ = 11.08 (1H, s, -NH), 9.79 (1H, s), 8.33 (1H, s, ar-H), 8.21–8.18 (2H, d J = 8.8 Hz, ar-H), 8.12–8.10 (1H, d J = 8.8 Hz, ar-H), 7.90 (1H, s,

ar-H), 7.75–7.73 (1H, d J = 8.8 Hz, ar-H), 7.51–7.49 (2H, d J = 8.8 Hz, ar-H), 7.35–7.32 (2H, m, ar-H), 5.74 (2H, s, -N-CH₂), 5.65 (2H, s, -N-CH₂), 2.23 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.43, 31.15, 42.35, 52.44, 111.90, 112.03, 119.23, 119.75, 122.67, 123.87, 124.36, 124.36, 125.01, 129.15, 129.49, 129.49, 134.91, 136.59, 139.40, 142.96, 143.70, 147.71, 150.02, 166.71. M.F: C₂₃H₁₉N₇O₃, HRMS *m/z* (ESI): [M + H]⁺ 442.1632 (442.4421).

2.2.18. 3-Methyl-4-((1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one, (**10Eb**)

Orange crystals, m.p: 232.6–234.7 °C, % yield: 65, IR (KBr)*ν*: 3107, 3073, 1664, 1595, 1334 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.97 (1H, s), 8.20–8.17 (2H, d J = 13.6 Hz, ar-H), 8.00–7.97 (2H, d J = 9.6 Hz, ar-H), 7.92–7.90 (1H, m, ar-H), 7.83 (1H, s, ar-H), 7.62–7.60 (1H, m, ar-H), 7.47–7.43 (3H, t J = 16.0 Hz, ar-H), 7.40–7.35 (4H, m, ar-H), 7.23–7.19 (1H, t J = 16.0 Hz, ar-H), 5.63 (2H, s, -N-CH₂), 5.58 (2H, s, -N-CH₂), 2.45 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.48, 31.15, 42.33, 52.44, 112.14, 112.30, 118.60, 118.60, 119.21, 119.43, 123.12, 124.24, 124.35, 124.35, 124.59, 125.05, 129.25, 129.25, 129.48, 129.48, 136.74, 137.08, 139.21, 140.66, 140.66, 142.89, 143.69, 146.79, 147.68, 151.53, 163.22. M.F: C₂₉H₂₃N₇O₃, HRMS *m/z* (ESI): [M + H]⁺ 518.1981 (518.5380).

2.2.19. 3-Methyl-4-((1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-(*p*-tolyl)-1H-pyrazol-5(4H)-one, (**10Ec**)

Orange crystals, m.p: 208.3–209.5 °C, % yield: 60, IR (KBr)*ν*: 3146, 3110, 1671, 1467, 1351 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.98 (1H, s), 8.20–8.18 (2H, d J = 8.8 Hz, ar-H), 7.92–7.90 (1H, m, ar-H), 7.85–7.82 (3H, m, ar-H), 7.62–7.60 (1H, m, ar-H), 7.43 (1H, s, ar-H), 7.40–7.36 (4H, m, ar-H), 7.26–7.24 (2H, d J = 8.0 Hz, ar-H), 5.63 (2H, s, -N-CH₂), 5.58 (2H, s, -N-CH₂), 2.44 (3H, s, -CH₃), 2.39 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.44, 20.95, 31.15, 42.48, 52.44, 112.10, 112.27, 118.62, 118.62, 119.35, 119.40, 123.08, 124.20, 124.20, 124.35, 125.06, 129.28, 129.28, 129.46, 129.46, 129.46, 129.63, 133.64, 136.73, 136.88, 140.56, 142.89, 143.68, 147.68, 151.25, 163.00. M.F: C₃₀H₂₅N₇O₃, HRMS *m/z* (ESI): [M + H]⁺ 532.2106 (532.5646).

2.2.20. 3-Methyl-4-((1-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)methylene)-1-(4-nitrophenyl)-1H-pyrazol-5(4H)-one, (**10Ed**)

Orange powder, m.p: 279.8–281.2 °C, % yield: 60, IR (KBr)*ν*: 3136, 3112, 1682, 1589 cm^{−1}. ¹H-NMR (DMSO-*d*₆, 400 MHz): δ = 9.83 (1H, s), 8.39 (1H, s, ar-H), 8.32–8.25 (4H, m, ar-H), 8.19–8.13 (4H, m, ar-H), 7.82–7.79 (1H, d J = 8.8 Hz, ar-H), 7.50–7.48 (2H, d J = 8.8 Hz, ar-H), 7.41–7.35 (2H, m, ar-H), 5.76 (2H, s, -N-CH₂), 5.74 (2H, s, -N-CH₂), 2.43 (3H, s, -CH₃). ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ = 13.61, 42.62, 52.43, 112.48, 117.54, 117.93, 119.55, 123.33, 124.36, 124.36, 124.36, 125.11, 125.48, 125.48, 129.30, 129.30, 129.30, 129.49, 129.49, 136.85, 138.29, 141.29, 142.83, 142.94, 143.75, 144.36, 147.67, 153.69, 163.96.

2.3. Biological Investigations

2.3.1. Cancer Cell Lines

The American Type Culture Collection (ATCC, Manassas, VA, USA) provided the human cancer cell lines Capan-1, HCT-116, NCI-H460, LN-229, HL-60, K-562, MOLT4, and Z-138. All cell lines were stored in accordance with the suppliers' instructions. Gibco (Gibco Life Technologies, Merelbeke, Belgium) provided the culture media, which were supplemented with 10% fetal bovine serum (HyClone, Cytiva, Marlborough, MA, USA). The reference and test molecules were dissolved in DMSO (99.5%, Sigma, Hoeilaart, Belgium) to a concentration of 100 micromolar (μM).

2.3.2. Cytotoxicity Assays

Adherent cells were seeded at densities between 500 and 1500 cells/well in 384-well plates (Greiner Bio-One, Vilvoorde, Belgium). After 24 h of incubation, cells were exposed to seven varying concentrations of the test compounds (100–0.006 μM). The untreated cells served as negative controls. Suspension cell lines (non-adherent) were placed at densities between 2500 and 5000 cells/well in 384-well culture plates with the test compounds of the same concentration (100 to 0.006 μM). All cell lines were incubated for 72 h with test compounds and were subsequently assessed using the Cell Titer 96[®] AQueous One Solution Cell Proliferation Assay (MTS) reagent (Promega, Leiden, Netherlands) following the supplier's guidelines. The absorbance was recorded at 490 nanometers (nm) with a SpectraMaxPlus 384 (Molecular Devices, San Jose, CA, USA), and optical density (OD) was measured to determine the 50% cytotoxic concentration (CC50) of test compounds. All experiments were performed in triplicate [26], Table 2.

2.3.3. GSK-3 β Inhibition Assay

This assay was performed using the GSK-3 β Kinase Enzyme System (Promega V1991, Madison, WI, USA) and ADP-Glo Kinase Assay (Promega V6930, Madison, USA) kits. A reaction mix was prepared using 1X reaction buffer, 50 μM ATP, 50 μM DTT, 1 $\mu\text{g}/\mu\text{L}$ GSK3 β substrate, and 0.5 ng/ μL GSK3 β enzyme with increasing concentrations of **10Aa** drug dissolved in DMSO. A reaction mix containing DMSO served as the vehicle control, and 12 nM Laduviglusib (MedChemExpress, New Jersey, USA), a GSK-3 β inhibitor, was used as a positive control for the experiment. This reaction mix was incubated for 60 min at room temperature. Following incubation, 5 μL of ADP-Glo Reagent was added and incubated additionally for 40 min at room temperature. Following this, 10 μL of kinase detection reagent was added, and the mixture was incubated for 30 min at room temperature. Luminescence was measured using a microplate reader with an integration time of 0.5 s [9] (Figure 3).

2.3.4. InSilico Study [27–29]

Drug-Likeness and InSilico Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Prediction

The physicochemical and pharmacokinetic parameters, such as molecular weight, hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), lipophilicity (Log P), solubility, and compliance with Lipinski's rule of five (RO5), along with bioavailability, gastrointestinal (GI) absorption, and toxicity profiles of the synthesized derivatives **10Aa–Ed**, were predicted using Swiss-ADME (version-23, University of Lausanne and the SIB Swiss Institute of Bioinformatics, Switzerland) [27]. The blood–brain barrier (BBB) permeability and unbound fraction were also evaluated, together with CYP1A2 substrate binding affinity and half-lives. Toxicity indicators, including overall safety and skin sensitization potential, were predicted and summarized in Table 3 and in the Supplementary (S80–S99).

Protein Preparation

The crystal structure of the protein was retrieved from the PDB and prepared by removing co-crystallized ligands, buffer ions, and non-essential water molecules. The catalytic or structural waters known to mediate conserved H-bonding with the reference ligand were retained with the structures. Missing side-chain atoms were rebuilt and locally optimized. The polar hydrogens were added, and receptor protonation was set for pH 7.4. Gasteiger charges were assigned for docking compatibility [28,29].

Docking Protocol

The ligand molecules **10Aa–Ed** and bioacetoxime were sketched using Chem Draw-Version 20.1 and subjected to energy minimization in Chem3D (v20.0, PerkinElmer Inc., Waltham, MA, USA). Molecular docking was performed utilizing PyRx v.0.8 software (Source Forge, San Diego, CA, USA; AutoDockVina engine). The binding orientations and interaction profiles were analyzed in BIOVIA Discovery Studio Visualizer 2021 (Dassault Systèmes Biovia Corp., San Diego, CA, USA). The docking protocol was validated by re-docking with native ligands into their specific binding sites, with an acceptance criterion of $\text{RMSD} \leq 2.0 \text{ \AA}$. The MM-GBSA binding free-energy computations were carried out on snapshots taken from molecular dynamics trajectories utilizing the g_mmpbsa package (a GROMACS-compatible version of MM-PBSA/MM-GBSA) to obtain refined ΔG_{bind} estimates. The pharmacokinetic and drug-likeness evaluations were conducted through Swiss-ADME (Swiss Institute of Bioinformatics, Lausanne, Switzerland) and pkCSM (Bioinformatics & Computational Biology Biosig Lab, The University of Queensland, Brisbane, Australia), facilitating the prediction of physicochemical characteristics, ADMET profiles, and overall drug-likeness potential (Figures 4–7, Table 4).

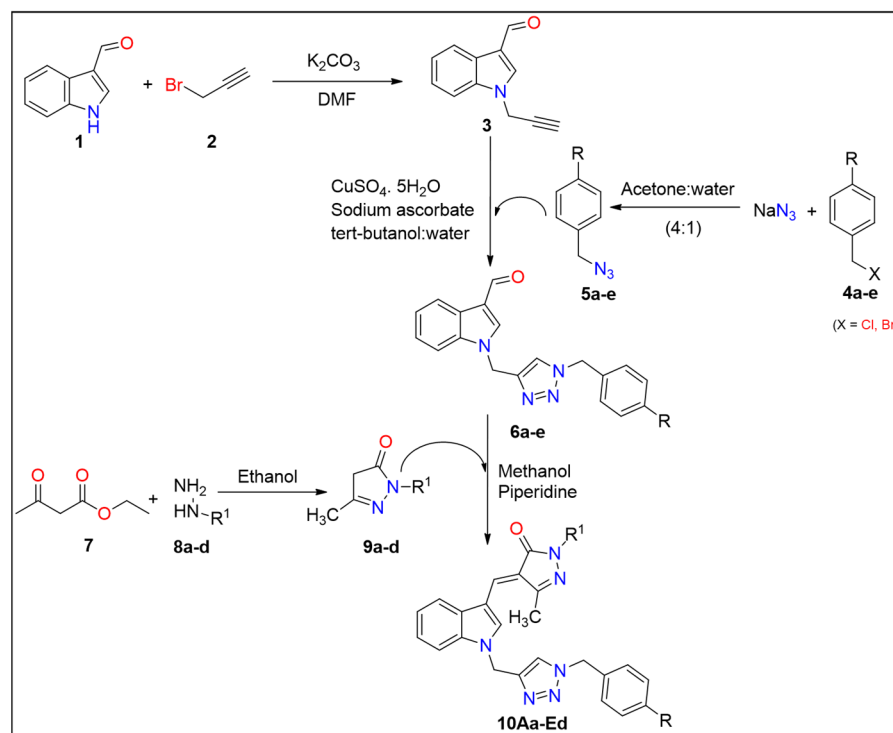
MM-GBSA Binding Free Energy Calculations

Refined binding free energies (ΔG_{bind}) were calculated using the MM-GBSA method implemented in Schrödinger Prime (release 2023-1, Schrodinger, Mangaluru, India). Snapshots from the MD trajectories were used to derive ligand-protein binding free-energy estimates. Calculations were performed on 200 snapshots per replica (last 40 ns, stride = 0.2 ns) using the VSGB 2.1 solvation model, with ligand- and receptor-reparametrized partial charges consistent with CHARMM36 mapping. Results were reported as mean $\pm$ SD. MM-PBSA calculations were performed on snapshots extracted every 0.2 ns from the last 40 ns of each replica (≥ 200 frames/replica), using a Poisson-Boltzmann polar solvation term and a SASA-based non-polar term. For each complex, the per-frame $\Delta G_{\text{bind}}(t)$ traces and the time-averaged $\Delta G_{\text{bind}} \pm \text{SD}$ across replicas convergence were checked via block-averaging (Table 5).

3. Results and Discussion

3.1. Chemistry

The compound 2-(3-formyl-1*H*-indol-1-yl)acetonitrile (**3**) was synthesized by reacting 3-formylindole (**1**) with propargyl bromide (**2**) in dimethylformamide (DMF, 99%, SD Fine Chemical, Bengaluru, India) using potassium carbonate (100%, SD Fine Chemical, Bengaluru, India) as a base. The respective intermediates 1-((1-aralkyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indole-3-carbaldehyde (**6a–d**) were subsequently obtained via a click reaction between 2-(3-formyl-1*H*-indol-1-yl)acetonitrile (**3**) and various aralkylazides (**5a–d**). Separately, 5-methyl-2-substituted or unsubstituted-2,4-dihydro-3*H*-pyrazol-3-ones (**9a–e**) were prepared by condensing ethyl acetoacetate (**7**) with hydrazine or arylhydrazines (**8a–d**) in ethanol (100%, Bio Liqua Research, Bengaluru, India). Finally, the target molecules, 4-(((1-aralkyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)methylene)-5-methyl-2-aryl-2,4-dihydro-3*H*-pyrazol-3-ones (**10Aa–Ed**), were synthesized by coupling indole-3-carbaldehyde derivatives (**6**) with pyrazol-3-one derivatives (**9**) in methanol, using piperidine as a catalytic (Scheme 1, Table 1).



Scheme 1. Synthesis of 10Aa–Ed.

Table 1. Details of the compounds 10Aa–Ed.

Code	R	R'	Code	R	R'	Code	R	R'
10Aa	H	H	10Bd	Cl	4-NO ₂ -C ₆ H ₄	10Dc	OCH ₃	4-CH ₃ -C ₆ H ₄
10Ab	H	C ₆ H ₅	10Ca	CH ₃	H	10Dd	OCH ₃	4-NO ₂ -C ₆ H ₄
10Ac	H	4-CH ₃ -C ₆ H ₄	10Cb	CH ₃	C ₆ H ₅	10Ea	NO ₂	H
10Ad	H	4-NO ₂ -C ₆ H ₄	10Cc	CH ₃	4-CH ₃ -C ₆ H ₄	10Eb	NO ₂	C ₆ H ₅
10Ba	Cl	H	10Cd	CH ₃	4-NO ₂ -C ₆ H ₄	10Ec	NO ₂	4-CH ₃ -C ₆ H ₄
10Bb	Cl	C ₆ H ₅	10Da	OCH ₃	H	10Ed	NO ₂	4-NO ₂ -C ₆ H ₄
10Bc	Cl	4-CH ₃ -C ₆ H ₄	10Db	OCH ₃	C ₆ H ₅			

All the compounds were confirmed for their structure using FTIR, ¹H/¹³C NMR, and HRMS data. The FT-IR spectra of compounds 10Aa–Ed displayed characteristic absorption bands. The indole -NH stretching vibrations were observed in the region of 3172–3100 cm^{−1}, while signals corresponding to aromatic and aliphatic -CH groups appeared between 3121 and 2947 cm^{−1}. A strong absorption due to carbonyl (>C=O) stretching was evident in the range of 1685 and 1664 cm^{−1}. In the ¹H-NMR spectra, the -NH proton resonances were detected at 11.08–11.06 δ parts per million (ppm). Aromatic protons resonated between 8.35 and 6.85 δ ppm, whereas the -N-CH₂ protons appeared in the 5.76–5.40 δ ppm region. Signals for methoxy (-OCH₃) groups were found at 3.81–3.78 δ ppm, and methyl (-CH₃) protons resonated between 2.47 and 2.23 δ ppm. In the ¹³C-NMR spectra, the carbonyl carbons resonated between 166.71 and 163.00 ppm, while aromatic carbons were distributed across 159.59–111.87 δ ppm. The methoxy carbon appeared at 55.58–55.54 δ ppm, and the -N-CH₂ methylene carbons were observed between 53.33 and 31.15 δ ppm. Methyl carbons (-CH₃) resonated in the 21.00–13.15 δ ppm range. Finally, the mass spectrometric values (*m/z*) of all compounds closely matched their calculated molecular weights, confirming the synthesized structures.

3.2. Biology

3.2.1. Cytotoxicity Evaluation

The cytotoxic activities of compounds **10Aa–Ed** were assessed across a panel of eight cancer cell lines (adherent and non-adherent types). The adherent cell lines included pancreatic adenocarcinoma (Capan-1), colorectal carcinoma (HCT-116), glioblastoma (LN229), and lung carcinoma (NCI-H460), while the non-adherent group consisted of myeloid leukemia (HL-60), chronic myeloid leukemia (K562), T-lymphoblasts (MOLT4), and non-Hodgkin lymphoma (Z138) cells. Sunitinib and 5-Fluorouracil (5-FU) were employed as standard reference drugs. Compound **10Cd** exhibited notable cytotoxic activity against glioblastoma (LN229) cells (CC_{50} : 13 μ M), which is comparable to Sunitinib (CC_{50} : 12 μ M, BLD Pharm, Hyderabad, India) but less potent than 5-Fluorouracil (CC_{50} : 7 μ M, Sun Pharmaceuticals, Baroda, India). Among the tested cell lines, LN229 glioblastoma cells showed increased sensitivity to **10Bd**, **10Cd**, and **10Dd**. In addition, compound **10Aa** demonstrated cytotoxic effects across multiple cell lines, including Capan-1, NCI-H460, MOLT4, HL-60, and Z138 (CC_{50} : 14 to 34 μ M). Based on these findings, molecule **10Aa** was identified as moderately active against six different cell lines. All experiments were performed in triplicate (Table 2).

Table 2. The cytotoxic activity of compounds **10Aa–Ed** at a concentration of 50 μ M against Capan-1, HCT-116, LN229, NCI-H460, MOLT-4, HL-60, K562, and Z138 cell lines.

Compound	Capan-1	HCT-116	LN229	NCI-H460	Molt-4	HL-60	K562	Z138
	Adherent Cells (CC_{50})				Suspension Cells (CC_{50})			
10Aa	23.87 $\pm$ 6.98	>50	>50	34.06 $\pm$ 22.43	14.45 $\pm$ 1.46	17.56 $\pm$ 7.04	>50	15.34 $\pm$ 0.72
10Ab	>50	>50	>50	>50	>50	>50	>50	>50
10Ac	>50	>50	>50	>50	>50	>50	>50	>50
10Ad	>50	>50	>50	>50	>50	>50	>50	>50
10Ba	>50	>50	>50	>50	>50	>50	>50	>50
10Bb	>50	>50	>50	>50	>50	>50	>50	>50
10Bc	>50	>50	>50	>50	>50	>50	>50	>50
10Bd	>50	>50	35.78 $\pm$ 17.81	>50	>50	>50	>50	>50
10Ca	>50	>50	>50	>50	>50	>50	>50	>50
10Cb	>50	>50	>50	>50	>50	>50	>50	>50
10Cc	>50	>50	>50	>50	>50	>50	>50	>50
10Cd	>50	>50	13.41 $\pm$ 1.75	>50	>50	>50	>50	>50
10Da	>50	>50	>50	>50	>50	>50	>50	>50
10Db	>50	>50	>50	>50	>50	>50	>50	>50
10Dc	>50	>50	>50	>50	>50	>50	>50	>50
10Dd	>50	>50	41.22 $\pm$ 6.65	>50	>50	>50	>50	>50
10Ea	>50	>50	>50	>50	>50	>50	>50	>50
10Eb	>50	>50	>50	>50	>50	>50	>50	>50
10Ec	>50	>50	>50	>50	>50	>50	>50	>50
10Ed	>50	>50	>50	>50	>50	>50	>50	>50
Sunitinib (100 μ M)	1.5 $\pm$ 0.01	4.25 $\pm$ 0.71	11.80 $\pm$ 1.41	8.85 $\pm$ 3.04	NT	5.00 $\pm$ 1.27	8.4 $\pm$ 2.12	8.15 $\pm$ 1.49
5-Fluorouracil (100 μ M)	2.7 $\pm$ 0.01	2.3 $\pm$ 0.71	7.35 $\pm$ 0.35	5.0 $\pm$ 1.70	NT	5.90 $\pm$ 0.99	12.90 $\pm$ 0.42	7.85 $\pm$ 0.71

Note: All data are expressed as mean $\pm$ standard deviations (SD) from three independent experiments. CC_{50} : 50% cytotoxic concentration.

3.2.2. Assessment of GSK-3 β inhibition by Compound **10Aa**

GSK-3 β kinase inhibition was evaluated using a luminescence-based kinase assay with the **10Aa**. Increasing concentrations of **10Aa** produced a dose-dependent reduction in GSK-3 β kinase activity, indicating that **10Aa** is capable of inhibiting GSK-3 β , with 100 μ M **10Aa** showing a significant 25% inhibition (Figure 3). This dose-dependent inhibition further supports the binding affinity of **10Aa** toward GSK-3 β .

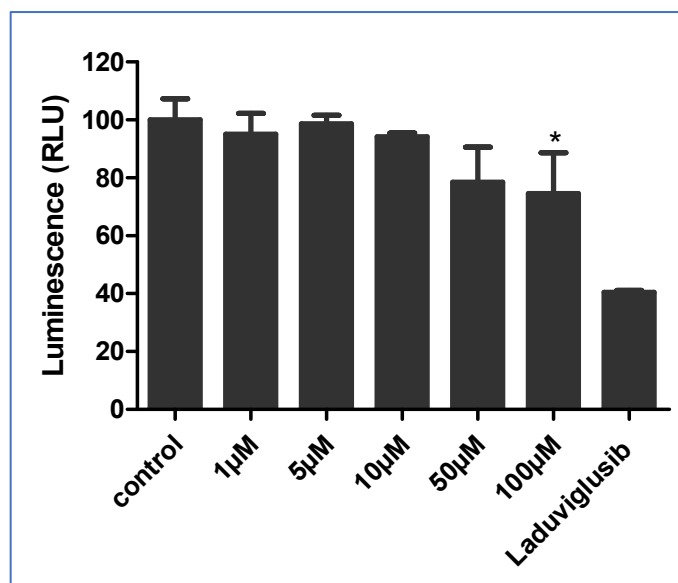


Figure 3. Kinase assay with **10Aa** depicting dose-dependent decrease in the inhibition of GSK-3 β . Laduviglusib was used as a positive control for this assay as it is a known GSK-3 β inhibitor. Data are presented as mean $\pm$ SDM. Significance was plotted based on p values and denoted as * (p -value ≤ 0.05).

3.2.3. InSilico Study

Drug Likeness and ADMET Prediction

In silico ADMET evaluation indicated that the synthesized compounds possess acceptable drug-like physicochemical properties. Molecular weights ranged from 396.44 to 562.54 Da, with hydrogen bond acceptors (HBA, 4–8), hydrogen bond donors (HBD, 0–1), and rotatable bonds (5–8) largely within acceptable limits. The calculated LogP values (1.99–5.00) suggest moderate to high lipophilicity, supporting membrane permeability, while ESOL-predicted aqueous solubility (LogS -4.18 to -6.62) indicates low to moderate solubility, typical of hetero-aromatic scaffolds. Compounds from the **10A** and **10C** series showed blood–brain barrier (BBB) permeability. Skin permeability (log Kp -5.60 to -7.18) values indicated moderate transdermal penetration. Drug-likeness assessment using Lipinski, Ghose, Veber, Egan, and Muegge filters revealed minimal violations across the series, suggesting favorable oral bioavailability. All compounds were predicted to act as P-glycoprotein (P-gp) inhibitors and to exhibit high plasma protein binding. Compounds **10Ab**, **10Ac**, **10Ba**, **10Bb**, **10Bc**, **10Cb**, **10Cc**, **10Db**, and **10Dc** were identified as CYP1A2 substrates, indicating potential phase-I metabolic clearance via this enzyme. The predicted short half-life (<3 h) suggests moderate systemic clearance. Overall, the ADMET analysis confirms that the majority of the compounds exhibit excellent drug-likeness, high oral absorption, acceptable metabolic behavior, and promising safety characteristics (Table 3).

Table 3. Predicted physicochemical, pharmacokinetic, and toxicity profile of **10Aa–Ed**.

Compounds	MW	HBA	RB	HBD	LogP	LogS (ESOL)	LogK _p	BBB	LV	GV	VV	EV	MV	BA
10Aa	396.44	4	5	1	2.82	-4.18	-6.78	Yes	0	0	0	0	0	0.55
10Ab	472.54	4	6	0	4.22	-5.72	-6.01	Yes	0	1	0	0	0	0.55
10Ac	486.57	4	6	0	4.44	-6.02	-5.84	Yes	0	2	0	0	0	0.55
10Ad	517.54	6	7	0	3.42	-5.78	-6.41	Yes	1	2	0	0	0	0.55
10Ba	430.89	4	5	1	3.3	-4.77	-6.55	No	0	0	0	0	0	0.55
10Bb	506.99	4	6	0	4.67	-6.31	-5.78	Yes	2	2	0	0	1	0.17
10Bc	521.01	4	6	0	5.0	-6.62	-5.60	No	2	2	0	0	1	0.17

Table 3. Cont.

Compounds	MW	HBA	RB	HBD	LogP	LogS (ESOL)	LogK _p	BBB	LV	GV	VV	EV	MV	BA
10Bd	551.98	6	7	0	4.02	−6.38	−6.17	No	1	2	0	0	0	0.55
10Ca	410.47	4	5	1	3.09	−4.48	−6.61	Yes	0	0	0	0	0	0.55
10Cb	486.57	4	6	0	4.55	−6.09	−6.23	Yes	1	2	0	0	0	0.55
10Cc	500.59	4	6	0	4.80	−6.32	−5.67	Yes	2	2	0	0	1	0.17
10Cd	531.56	6	7	0	3.83	−6.09	−6.32	No	1	2	0	0	0	0.55
10Da	426.47	5	8	1	2.76	−4.25	−6.98	No	0	0	0	0	0	0.55
10Db	502.57	5	7	0	4.18	−5.80	−6.21	No	1	2	0	0	0	0.55
10Dc	516.59	5	7	0	4.56	−6.10	−6.04	No	1	2	0	0	0	0.55
10Dd	547.56	7	8	0	3.49	−5.87	−6.61	No	2	2	0	0	0	0.17
10Ea	441.44	6	6	1	1.99	−4.24	−7.18	No	0	1	0	0	0	0.55
10Eb	517.54	6	7	0	3.5	−5.78	−6.41	No	1	2	0	0	0	0.55
10Ec	531.56	6	7	0	3.71	−6.02	−5.84	No	0	2	0	0	0	0.55
10Ed	562.54	8	8	0	2.6	−5.85	−6.81	No	2	2	1	1	1	0.17

MW: molecular weight, HBA: hydrogen bond acceptor(s), RB: rotatable bonds, HBD: number of hydrogen bond donor(s). LogP: log of octanol/water partition coefficient, LogS: aqueous solubility, LogK_p: skin permeability partition coefficient, BBB: blood–brain barrier, LV: Lipinski violation(s), GV: Ghose violation(s), VV: Veber violation(s), EV: Egan violation(s), MV: Muegge violation(s), BA: bioavailability.

Molecular Docking Analysis

The information derived from the molecular docking analysis, detailed in Table 4 and graphically represented in Figures 4 and 5, indicated that molecules spanning from **10Aa–10Ed** exhibited substantial binding affinities towards GSK-3 β (identified by PDB ID: 1Q41). Despite the observation of intermediate-level interactions with compounds **10Ac** and **10Da**, compounds **10Cc**, **10Dd**, and **10Bd** displayed the most pronounced binding energies, quantified as −11.7 kcal/mol, −11.2 kcal/mol, and −11.1 kcal/mol, respectively. The binding affinity of the reference ligand bioacetoxime, as shown in Figure 6, is −8.6 kcal/mol, which is lower than the binding strength of Molecule **10Dc**, which is −10.7 kcal/mol. In short, these findings lend credence to the notion that the chosen molecules improved interactions with the designated protein, implying that they ought to continue to be developed into molecular dynamics models and possibly confirmed through laboratory testing. Figure 7 illustrates the binding mode of **10Cc**, demonstrating its extensive penetration into the ATP-binding site of GSK-3 β and the generation of several contacts that enhance stability. A typical hydrogen bond in the hinge region secures the ligand, and numerous van der Waals interactions with amino acids such as VAL, ALA, and GLY reinforce the pocket fit. An additional π -cation interaction enhances electrostatic compatibility, while the aromatic components interact with hydrophobic residues like ILE, VAL, and LEU through π - σ and π -alkyl interactions. These combined interactions lead to a stable and energetically favorable binding arrangement. Figure 4 shows that compound **10Dd** also takes on a very stable orientation when bound to ATP. A multitude of van der Waals interactions involving GLY, VAL, ALA, and ASN contribute to the close-knit hydrophobic arrangement, and the ligand forms essential hydrogen bonds, securing its location in proximity to the flexible joint area. The binding of ASP133 to π -anion, ILE, LEU, and VAL, coupled with π -alkyl and π - σ activities, fosters greater stabilization. Additionally, the π -sulfur interaction reinforces the binding at the functionally important site. These interactions reveal that **10Dd** forms a robust connection with GSK-3 β , suggesting its capacity for broad biological effects. Furthermore, the docking score for the compound **10Aa** reached −10.6 kcal/mol. The indole fragment interacted with Val70, Ala83, Lys85, Leu132, and Cys199, whereas the pyrazole segment of **10Aa** produced a hydrogen bond with Ile62. In addition, the triazolyl ring created hydrogen bonds with Asn186 and Val135, showing the positive agreement between the in vitro and in silico results of **10Aa** (Figure 6), along with the stabilization

of the ligand-protein complex by multiple van der Waals interactions involving Gly63, Asn64, Val119, Tyr134, Tyr140, Arg141, Val135, Val135, Pro136, Thr138, and Gln185 residues, making it an attractive candidate for future studies.

Table 4. Docking scores of 10Aa–Ed against the target protein, highlighting top binding molecules.

Compounds	Binding Affinity	rmsd/ub	rmsd/lb	Compounds	Binding Affinity	rmsd/ub	rmsd/lb
10Aa	−10.6	6.437	3.808	10Cc	−11.7	8.518	1.639
10Ab	−10.4	6.673	2.496	10Cd	−09.9	9.642	2.876
10Ac	−10.7	6.349	2.258	10Da	−10.7	9.394	2.712
10Ad	−09.6	6.568	3.911	10Db	−09.6	2.042	1.370
10Ba	−10.3	6.817	3.421	10Dc	−10.7	9.394	2.712
10Bb	−10.6	6.482	2.682	10Dd	−11.2	4.604	2.928
10Bc	−10.0	7.911	3.339	10Ea	−10.4	3.729	2.422
10Bd	−11.1	8.926	4.480	10Eb	−10.3	5.205	2.570
10Ca	−10.6	5.214	2.575	10Ec	−09.7	9.843	3.199
10Cb	−10.4	6.590	1.641	10Ed	−10.4	8.226	3.539

rmsd/ub-root mean square deviation upper bound; rmsd/lb-root mean square deviation lower bound.

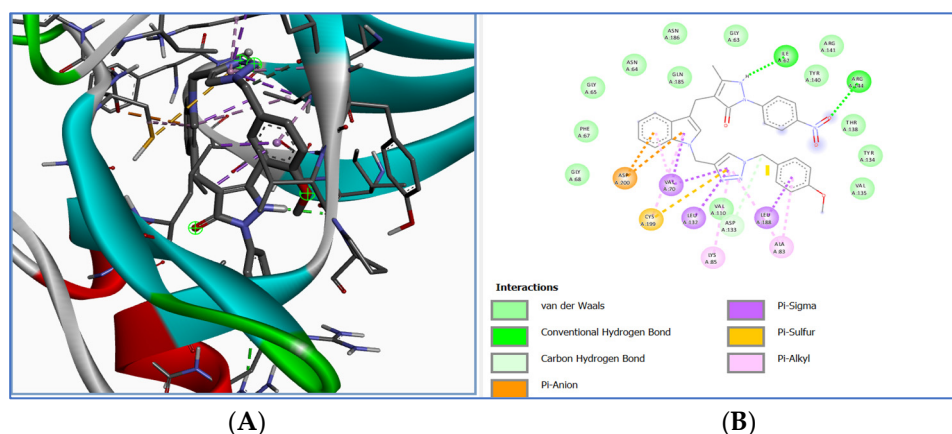


Figure 4. Binding interactions of compound 10Dd with GSK-3 β (PDB ID: 1Q41). (A) 3D image (B) 2D image; show hydrogen bonding, van der Waals contacts, and π -based interactions that collectively support the stable accommodation of 10Dd in the active site.

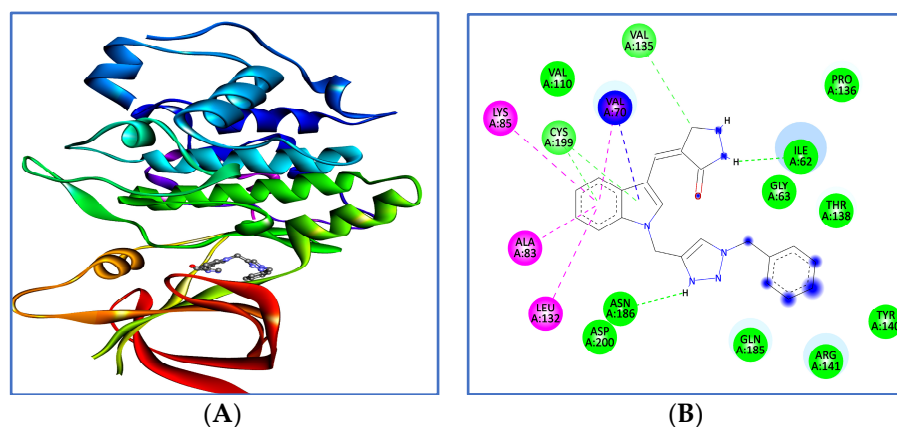


Figure 5. Docking interaction of 10Aa with GSK-3 β (PDB ID: 1Q41). (A) 3D image (B) 2D image; green line indicates H-bond, light green represented as van der Waals force; and lipophilic contact denoted by pink arrow.

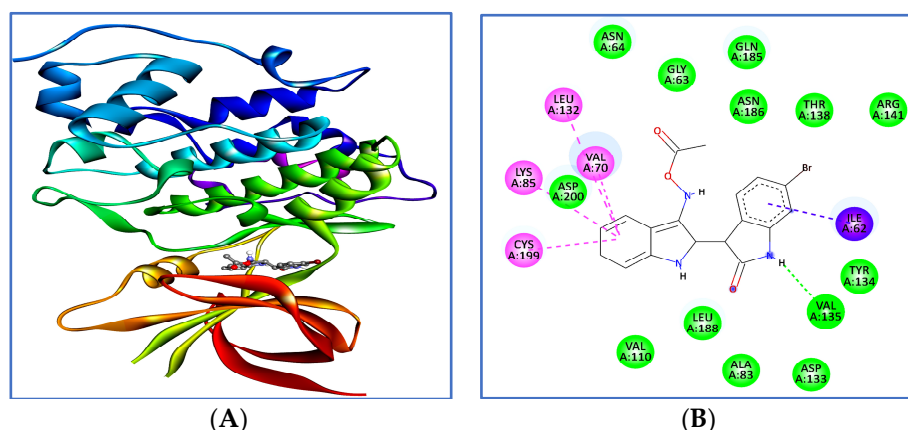


Figure 6. Docking interaction of **bioacetoxime** with GSK-3 β (PDB ID: 1Q41). (A) 3D image (B) 2D image; green line indicates- H-bond, light green represented as van der Waals force; and lipophilic contact denoted by pink arrow.

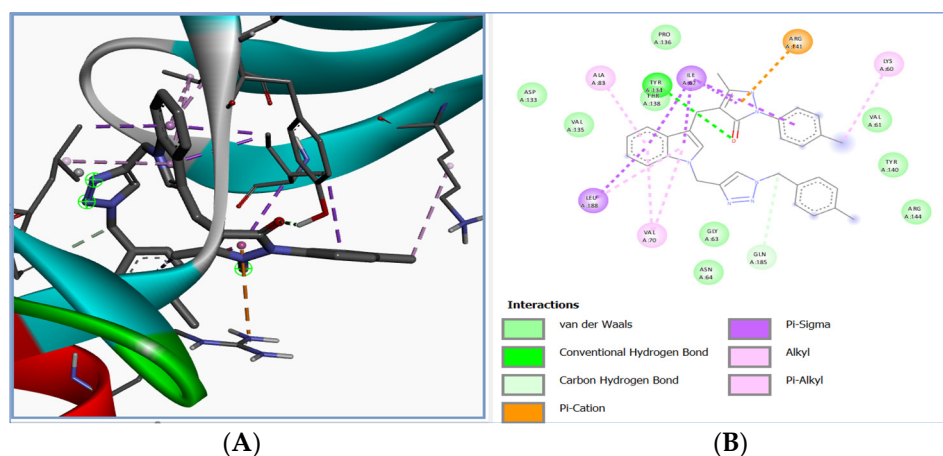


Figure 7. Docking interactions of compound **10Cc** with GSK-3 β (PDB ID: 1Q41). (A) 3D image (B) 2D image; interaction map illustrates hydrogen bonds, van der Waals contacts, and key π -interactions stabilizing the ligand within the ATP-binding pocket.

MM-GBSA Binding Free Energy Analysis

The synthetic compounds exhibit varied degrees of binding stability within the target protein, as indicated by the wide range of MM-GBSA binding free energy (ΔG_{bind}) values, which range from -25.05 to -41.98 kcal/mol. The most favorable binding energies were observed in **10Cc** (-41.98 kcal/mol), **10Ec** (-41.05 kcal/mol), **10Db** (-37.14 kcal/mol), and **10Dd** (-40.70 kcal/mol). These molecules were found to form strong and thermodynamically stable ligand-protein complexes, as shown in Table 2. The binding energies were mainly contributed to by van der Waals interactions (ΔG_{vdw} , -38 to -52 kcal/mol) and lipophilic interactions (ΔG_{Lipo} , -16 to -22 kcal/mol), indicating that hydrophobic contacts were crucial in stabilizing these ligands within the active site. Electrostatic (coulombic) contributions were also considerable for numerous high-affinity compounds, particularly **10Ab**, **10Db**, **10Bd**, **10Ca**, and **10Eb**, indicating further stabilization through charged or polar contacts. On the other hand, the solvation energy (ΔG_{solvGB}) had a negative impact on the total binding in all compounds because of the anticipated desolvation penalties when the ligand entered the hydrophobic pocket. While hydrogen bonds and covalent bonds did play a role in binding, they were not the main factors and did vary across molecules. The docking results were supported by the MM-GBSA findings, which showed that compounds **10Cc**, **10Db**, **10Ec**, and **10Dd** were the most promising. These compounds exhibited strong

binding affinity and stability within the protein active site, as well as highly favorable van der Waals, lipophilic, and electrostatic interactions (Table 5). Therefore, these compounds were identified as promising leads for further biological evaluation.

Table 5. MM-GBSA binding free energy (ΔG_{bind}) and energy decomposition parameters for compounds **10Aa–Ed** against the target protein.

Compounds	ΔG_{Bind}	$\Delta G_{\text{Bind Coulomb}}$	$\Delta G_{\text{Bind Covalent}}$	$\Delta G_{\text{Bind Hbond}}$	$\Delta G_{\text{Bind Lipo}}$	$\Delta G_{\text{Bind solvGB}}$	$\Delta G_{\text{Bind vdw}}$
10Aa	−30.92	−04.85	03.77	−2.94	−18.53	34.92	−40.52
10Ab	−35.98	−13.61	02.18	−2.28	−16.73	37.22	−46.72
10Ac	−37.20	−06.25	00.82	−2.08	−18.25	38.89	−46.27
10Ad	−30.87	−17.94	05.33	−3.15	−18.38	45.90	−38.69
10Ba	−27.08	−08.93	11.23	−1.01	−18.47	43.29	−49.60
10Bb	−25.38	−02.08	04.31	−2.27	−19.12	44.28	−47.18
10Bc	−29.31	−11.02	04.77	−2.44	−16.25	38.30	−40.12
10Bd	−41.55	−19.74	08.64	−2.24	−16.80	39.09	35.09
10Ca	−26.66	−12.57	03.83	−3.39	−20.27	58.92	−50.59
10Cb	−29.50	−10.26	02.59	−2.56	−21.13	46.78	−42.46
10Cc	−41.98	−11.40	03.92	−0.83	−20.31	46.78	−49.59
10Cd	−27.92	−13.95	03.13	−3.28	−20.84	52.96	−43.57
10Da	−32.18	−11.79	03.35	−2.56	−22.42	48.62	−44.91
10Db	−38.70	−13.22	06.34	−1.02	−19.72	40.35	−49.09
10Dc	−26.13	−08.56	07.66	−0.98	−19.19	46.62	−47.28
10Dd	−40.73	−09.63	04.25	−0.98	−19.29	43.92	−50.71
10Ea	−25.05	−06.66	10.39	−0.98	−16.96	44.93	−52.09
10Eb	−28.37	−11.16	06.46	−2.68	−20.25	46.23	−44.73
10Ec	−39.05	00.53	04.10	−1.21	−17.11	28.99	−51.73
10Ed	−40.23	−03.82	03.58	−2.66	−20.28	39.87	−47.93

ΔG_{Bind} —binding free energy; $\Delta G_{\text{Bind coulomb}}$ —change in Gibbs free energy of binding that is specially attributed to electrostatic interactions (coulomb interactions) between a ligand and a protein or receptor; $\Delta G_{\text{Bind covalent}}$ —binding free energy of covalent bond; $\Delta G_{\text{Bind Hbond}}$ —binding free energy of hydrogen bonds; $\Delta G_{\text{Bind Lipo}}$ —lipophilic contribution to the overall binding free energy of a ligand–protein complex; $\Delta G_{\text{Bind solvGB}}$ —binding free energy of solvation; $\Delta G_{\text{Bind vdw}}$ —van der Waals force contribution to the overall binding free energy of a ligand–protein complexes.

Correlation Between Docking and MM-GBSA Results

The predicted ligand–protein interactions were found to be reliable, as evidenced by the strong agreement between the molecular docking scores and the MM-GBSA binding free energy values. In the MM-GBSA analysis, compounds with better docking scores, especially **10Cc**, **10Bd**, **10Dd**, **10Ed**, and **10Ec**, confirmed their great thermodynamic stability at the active site by showing very favorable ΔG_{bind} values. The fact that the structural properties that lead to high docking scores, like hydrophobic contact, van der Waals packing, and polar interactions, are faithfully reflected in the free energy decomposition is supported by this consistency. The association was further supported by the fact that molecules with moderate docking scores also showed lower MM-GBSA energies. Generally, the consistent patterns observed in the two computational approaches support the reliability of the binding predictions and further solidify the selection of these top-ranked compounds as potential leads for additional biological investigation.

4. Conclusions

In this study, twenty compounds were synthesized and characterized using various spectral techniques. Their cytotoxic effects were assessed against a variety of cancer cell lines, including pancreas, lung, blood, colon, and brain. The cytotoxicity data indicate that compound **10Aa** exhibits greater sensitivity toward hematological cancer cell lines,

including Molt-4, Z138, and HL-60, whereas the brain cancer cell line LN226 was more susceptible to compound **10Cd**. Increasing concentrations of **10Aa** produced a dose-dependent reduction in GSK-3 β kinase activity, indicating that **10Aa** was capable of inhibiting GSK-3 β . Additionally, the higher docking score and significant stability in the MD simulation indicate that the synthesized compounds acted via GSK-3 β inhibition. Further, mechanism-based investigation is required to determine the pharmacological potential of **10Aa** as a therapeutic candidate.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/chemistry8020017/s1>, Figures S1–S79: Spectra of FTIR, $^1\text{H}/^{13}\text{C}$ NMR and HRMS; Figures S80–S99: Details of Swiss ADME.

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Abbreviations

The following abbreviations are used in this manuscript:

μM	Micro-Molar
ADMET	Absorption, Distribution, Metabolism, Excretion
ALA	Alanine
ASN	Asparagine
ASP	Aspartic acid
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
BBB	Blood–Brain Barrier
CC	Cytotoxic Concentration
CDCl_3	Deuterated Chloroform
DMF	Dimethyl Formamide
DMSO-d_6	Deuterated Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infra-Red
GI	Gastrointestinal
GLY	Glycine
GSK	Glycogen Synthase Kinase
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HIV	Human Immunodeficiency Virus
HRMS	High Resolution Mass Spectra
IC	Inhibitory Concentration
ILE	Isoleucine
LEU	Leucine
m.p	Melting Point
M.W	Molecular Weight

MD	Molecular Dynamics
MHz	Mega Hertz
MM-GBSA	Molecular Mechanism-General Born Surface Area
NCRP	National Cancer Registry Program
nm	Nano Meter
NMR	Nuclear Magnetic Resonance
NT	Not Tested
OD	Optical Density
PDB	Protein Data Bank
P-gp	P-Glycoprotein
ppm	Part Per Million
RO5	Rule of Five
SD	Standard Deviation
USA	United States of America
VAL	Valine

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