ISSN 2618-1584 Vol. 4, Issue 9: 23-29, DOI: 10.25236/FMSR.2022.040905

Design, synthesis and study of novel indoxine derivatives

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Abstract: *Keeping in view the pharmacological properties of indolinones as promising scaffold as kinase inhibitors, herein, a novel series of N- benzyl-4-phenyl-5-cyano-indoxine moiety were synthesized, studied by molecular docking, and fully characterized by spectroscopic techniques. All the prepared compounds were evaluated for their cytotoxicity attributes against a panel of tumor cell lines, including cardiovascular disease (ALA-564). They displayed moderate to promising antiproliferative effects toward ALA-564. The molecular docking studies demonstrated that our prepared compounds were potentially bound to 4UWC active site through essential H-bond and other vital interactions with critical binding residues.*

Keywords: *Cardiovascular disease, indolizine, 4UWC*

1. Introduction

Cancer is a complex disease caused by a variety of factors and has become a major cause of death together with cardiovascular diseases [1, 2]. Cancer incidence is expected to increase to more than 20 million and 13 million cancer deaths per year by 2030 [3]. Despite tremendous advances in the technology of cancer treatment over the past few decades, the rapid development of drug resistance and the limitations of current cancer drugs remain significant challenges. Therefore, finding new methods to inhibit the biomolecules involved in the proliferation, metastasis and invasion of cancer cells is the focus of cancer research [4, 5].

As tumor inhibitors, indoline derivatives have wide application value and prospect. Indolezine is a thick ring consisting of a five-membered ring and another six-membered ring and heterocyclic compounds with bridgehead nitrogen atoms. The system of 10πelectrons with conjugation, including four pairs of electrons from four double bonds and one pair of electrons from nitrogen, is isomer with indoles and isoindoles [6]. The following figure shows its structural formula:

Figure 1: Indoxine, indoles and isoindoles

After Boekelheide et al. synthesized indolezine in 1959, the study of indolezine was gradually paid attention to. Recent studies have shown that indolezine framework compounds and their derivatives show excellent expression in many biological activities. It can be used as pigment [7], herbicide [8], phospholipase inhibitor [9] and antiviral [10] drugs, etc. Indolazine skeleton compounds also showed activity against Mycobacterium [11]. In addition, indolizine skeleton compounds can also be used as key intermediates in the synthesis of alkaloids with physiological activity [12]. In addition to the excellent biological activity of indolezine skeleton compounds and their derivatives, many indolezine skeleton compounds can also be found in natural molecules isolated from plants, Marine organisms, insects and microorganisms [13].

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In recent years, in addition to the traditional synthetic methods of indolezine, scientists are also trying to find some novel and effective synthetic methods. Throughout these approaches, advantages and disadvantages coexist. In terms of advantages, some yield is high, some reaction rate is fast, some substrate is simple to operate, some reaction is novel and so on. When considering their disadvantages, some reactions are difficult to control, some reactions are complex to operate, and some reactions have low yields. When considering the preparation of target compounds, researchers should fully study, consider and compare the raw material sources, reaction conditions, reaction operations and yield of various methods so as to select the most suitable method for the preparation of target compounds. With the rapid development of combinatorial chemistry, photochemistry, microwave reaction and other novel chemical hotspots, more and more synthetic methods will be developed for the synthesis of indoline skeleton and its derivatives.

We innovatively designed and synthesized a series of indoline derivatives by simple and green means such as salt-forming, nucleophilic substitution and light irradiation, and screened the structures with anticancer activity by molecular docking and in vitro cell experiments. It can not only reduce or stop the use and generation of raw materials, catalysts, solvents and reagents, products and by-products that are harmful to human health, community safety and ecological environment, but also provide theoretical basis and basis for their potential pharmacological research.

2. Chemistry

2.1. Experimental Process

The design of this topic is divided into two parts. In the first part, the method of synthesis of light reaction raw material N-benzyl-3-cyano-1, 4-dihydropyridine is described in the reference. N-benzyl-3-cyano-pyridine bromide (white powder) is obtained by dissolving 3-cyano-pyridine in acetonitrile and reacting with benzyl bromide, and the white powder is added into anhydrous tetrahydrofuran. N-benzyl-3-cyano-1, 4-dihydropyridine (asymmetric dihydropyridine) is obtained by reaction with phenyl magnesium bromide, route 1[14,15] as follows:

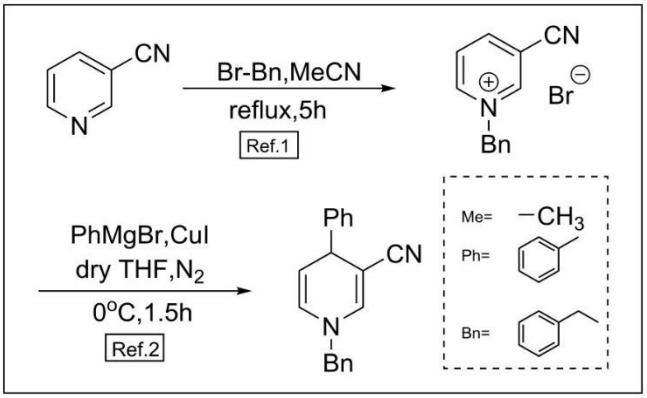

Figure 2: Route 1

In the second part, the photoreaction raw material (5mmol) and twice equivalent TEMPO (2,2,6, 6-tetramethylpiperidine oxide) (10mmol) were dissolved in dried tetrahydrofuran (30mL) under agitation and placed in a quartz reactor for degaging. The reaction endpoint was monitored by TLC (thin layer chromatography). After 48h, the reaction materials almost disappeared, the target derivatives were generated, and the reaction solvent was removed by rotation evaporation. N-benzyl-4-phenyl-5-cyano-indoxine was obtained by silica gel column chromatography with high purity and medium yield.

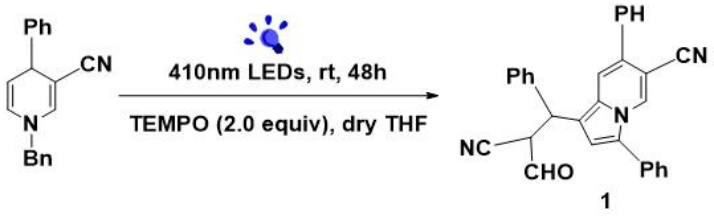

Figure 3: Route 2

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2.2. Molecular Docking

2.2.1. With The Preparation

The 2d structure of the ligand was exported from ChemDraw software in.sdF format and uploaded to the workspace. Then use LigPrep module (https://www.schrodinger.com/products/ligprep) to generate 3 d structure, and use OPLS2004 force field to generate different conformation of each ligand. The stable conformation isomers of each ligand with minimal energy are further processed for molecular docking.

2.2.2. Preparation of Macromolecules

Protein (PDB ID: 4UWC) retrieved from the protein database (https://www.rcsb.org) was simulated by docking with bioactive substances. Schrodinger Maestro's Protein preparation Wizard (Schrodinger, LLC, New York, NY, 2021) is used to prepare structures by adding hydrogen, removing water molecules, and distributing partial charges using an OPLS2004 force field, The protonated state is then assigned and conformation is limited, and part of the energy is further minimized to 0.3 A RMSD. The binding site was defined after the ligand was removed, and the mesh was generated using A mesh box volume of 10_10_10A.

2.2.3. Ligand-protein Docking

The Glide MODULE of the Schrodinger Suite is used to pair individual bioactive compounds to identified binding sites in the grid and select the lowest binding position for each docking run to visualize the Ligand-protein interaction Glide XP visualizer. The important active site interactions and scoring functions are analyzed.

3. Results and Discussion

3.1. Design of Indolizine Derivatives

Indolezine and its derivatives have a wide range of application value and prospects in medicine, biology and other research fields because of their various biological activities. Since Scholtz reaction appeared, the study on its synthesis method has been widely concerned and studied. First of all, there are many deficiencies in the above various synthetic methods of indoline and its derivatives, such as poor reaction selectivity, complex substrates, harsh reaction conditions, poor reaction activity or the participation of transition metals. These deficiencies, especially metal residues, seriously limit their application in drug synthesis. Therefore, it is still an important research direction for the synthesis of indolizines and their derivatives to explore a multi-substituted indolizine framework compound with high selectivity, simple substrate, high yield, mild reaction conditions and no transition metal involvement.

In the past decade, visible light driven organic chemical reaction, as a new research field full of charm and vitality, has attracted the attention of many chemists. Its green and mild reaction conditions provide novel and efficient synthesis strategies for the construction of complex organic molecules. With the improvement of photochemical theory and the emergence of new photoreactions, visible light catalysis plays an increasingly important role in organic synthesis. Through literature review, we can find that visible light driven organic chemical reaction has been widely used in organic synthesis as a new and efficient synthesis strategy. This synthesis method has the advantages of mild conditions, low cost, simple operation and so on.

In summary, we intend to design and synthesize a series of important indoline compounds by salting, nucleophilic substitution and light reaction, starting with 3-cyanopyridine compounds.

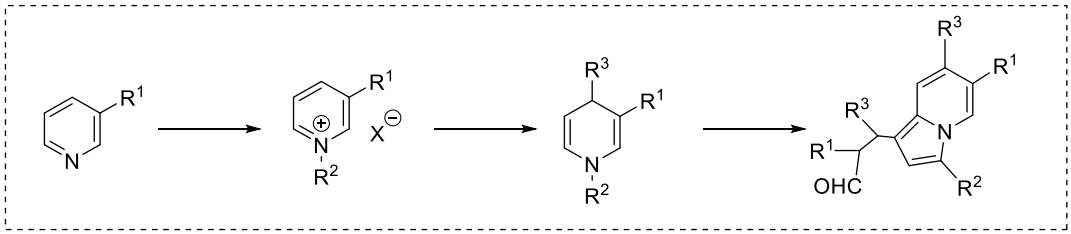

Figure 4: Synthesis method

The structures with potential antitumor activity were screened by reverse molecular docking technique. The potential antitumor derivatives of indoline were tested with related tumor cells in vitro,

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and then the compounds with antitumor activity were screened.

3.2. Molecular Modeling Studies

Discovery Studio 4.5 Client software was used for molecular docking calculations. B-fgf basic fibroblast growth factor (PDB ID:4UWC) was selected as the target protein for molecular docking. It is available from the RCSB Protein Database (RCSB PDB, http://www.rcsb.org (accessed November 7, 2021). The active site was defined according to the eutectic structure of the protein complex. The docking results of our study were visualized using PyMOL molecular graphics system (version 2.5.2).

Some important components, such as binding mode, molecular interaction with the active site, binding energy and docking fraction, are considered as criteria for selecting the best position of the docking compound. The docking results showed that n-benzyl-4-phenyl-5-cyano-indolizine was bound to the cavity of the original ligand of 4UWC, and two hydrogen bond interactions were formed, namely, nitrogen on the cyanide group and AlA-564, and carbonyl oxygen and Glu-486, which were the same as the amino acid residue bound by the original ligand. It is suggested that N- benzyl-4-phenyl-5-cyanoindoxine may have antitumor activity.

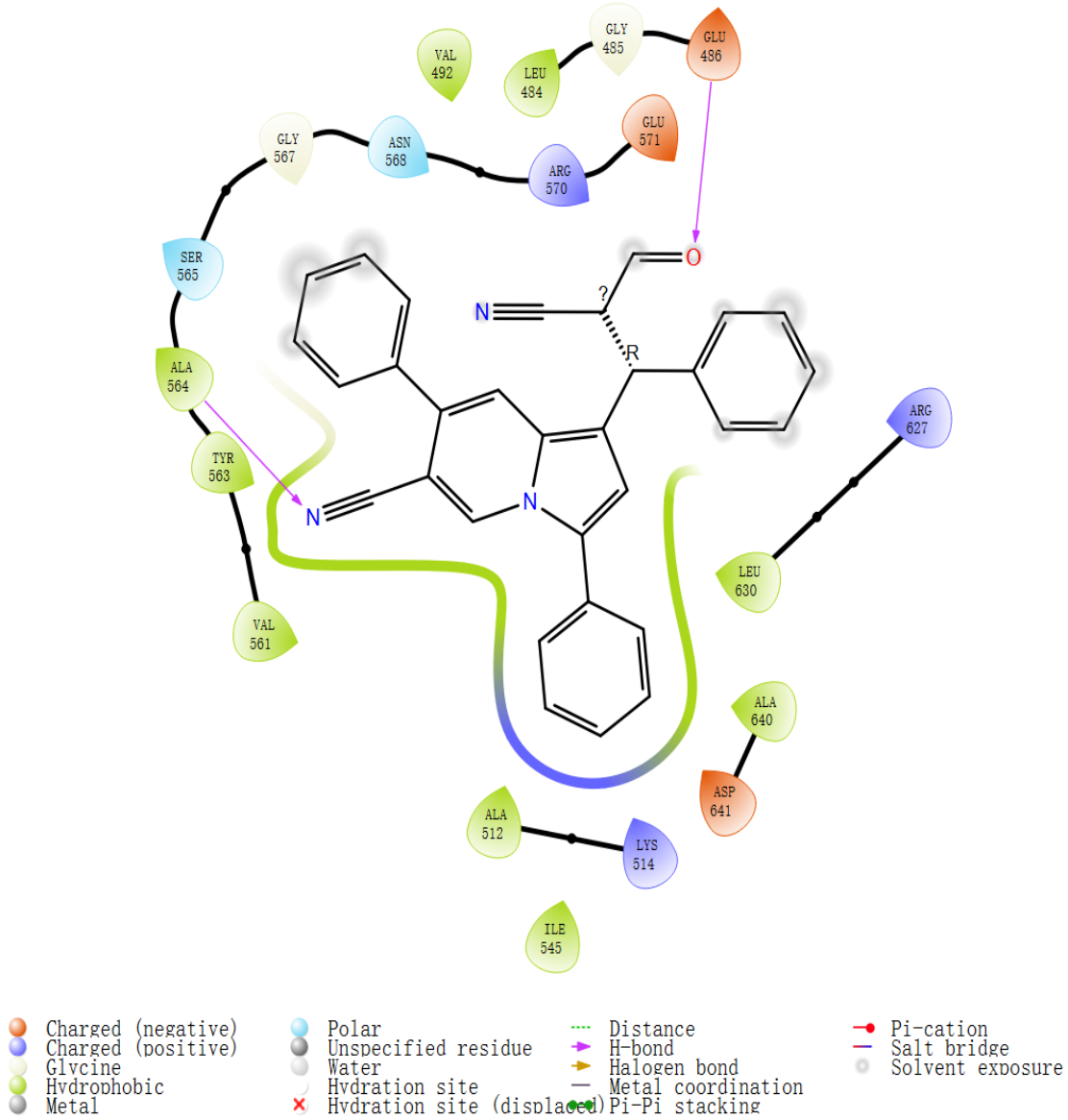

Figure 5: Molecular docking model

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3.3. Nuclear Magnetic Data

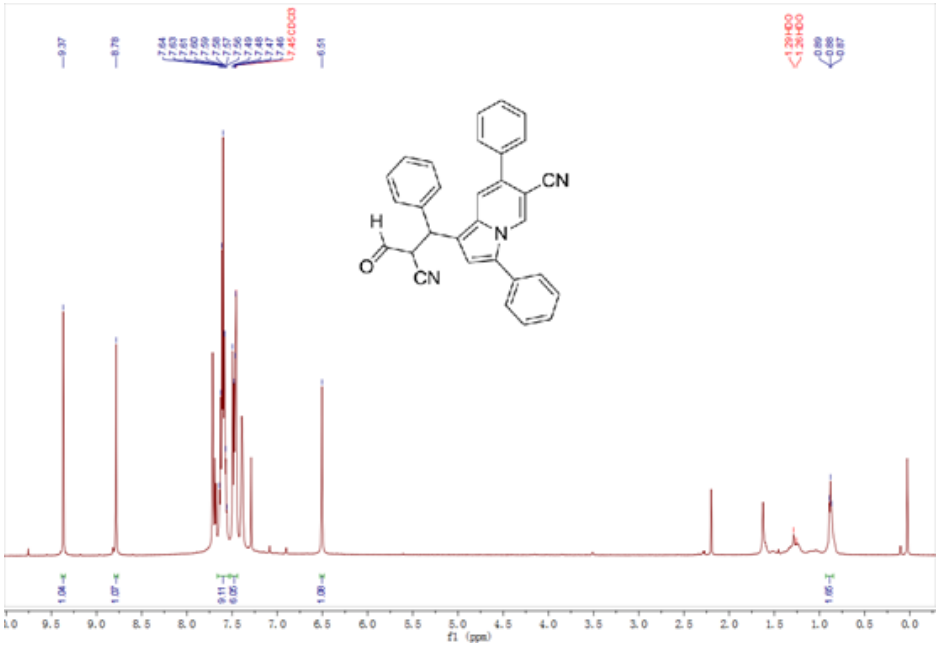

Figure 6: ^{1}H NMR spectra

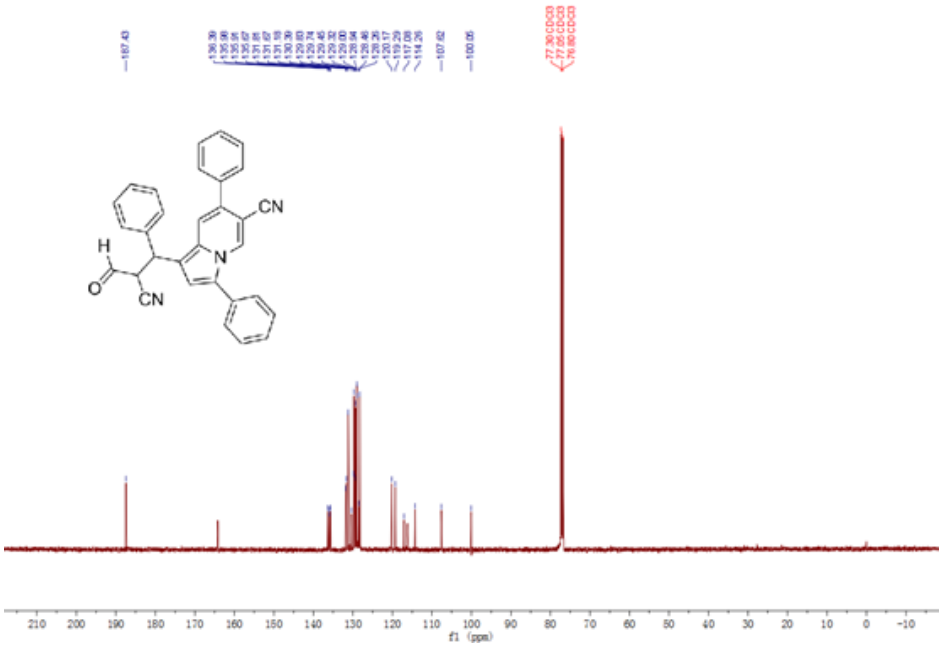

Figure 7: ^{13}C NMR spectra

The 1H NMR and 13C NMR data of N-benzyl-4-phenyl-5-cyano-indolezine are as follows:

1H NMR (500 MHz, CDCl3) δ 9.37 (s, 1H), 8.78 (s, 1H), 7.66-7.43 (m, 15H), 6.51 (s, 1H), 1.26 (s, 1H), 0.93-0.80 (m, 2H);

13C {1H} NMR (125 MHz, CDCl3) δ 187.4, 164.2, 136.4, 136.0, 135.9, 135.7, 131.8, 131.7, 131.2, 130.8, 130.4, 129.8, 129.7, 129.5, 129.3, 129.0, 128.9, 128.5, 128.3, 120.2, 119.3, 116.2, 114.3, 107.6, 100.1. HRMS (+ESI) m/z calcd for C31H21N3O3 (M+H)+: 452.1757, found 452.1780.

4. Conclusions

In recent years, in addition to the traditional synthetic methods of indolezine, scientists are also trying to find some novel and effective synthetic methods. Throughout these approaches, advantages and

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In this study, based on a large number of literatures and the previous research foundation of the research group, the designed compound has sufficient theoretical basis: The structural design of indoline derivatives is based on literature reports, and has special spatial structure and good lipophile. Other research data show that such compounds not only have anti-tumor activity, but also have antibacterial, antiviral, mite killing, anti-inflammatory, anti-arrhythmia, anti-hypertension and other activities.

Compared with the traditional synthetic methods of indoline derivatives, the synthetic method adopted in this paper is green and environmentally friendly, and can reduce the use of raw materials, catalysts, solvents and reagents that are harmful to human health, community safety and ecological environment. The photochemical reaction does not require the participation of transition metal and photocatalyst. Through molecular docking and in vitro cell test screening, indoline derivatives with anti-tumor activity can lay a foundation for the development of anti-tumor drugs and accelerate the development of anti-tumor targeted drugs.

Acknowledgements

This study was supported by the Special Program for Cultivating Scientific and Technological Innovation Ability of College and High School Students, grant number: 2021H020904. And college Students innovation and Entrepreneurship Training Program, grant number: X2021189.

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