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Synthesis, Molecular Docking and Cytotoxicity Evaluation of 2-(4-Substituted-Benzyl) Isoindoline-1, 3-Dione Derivatives as Anticancer Agents

Alireza Aliabadi[a]*, Alireza Foroumadi[b], Maliheh Safavi[c], Sussan K. Ardestani[c]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran
[c]Institute of Biochemistry and Biophysics, Department of Biochemistry, University of Tehran, Tehran, Iran

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The development and discovery of new anticancer agents is one of the main goals in medicinal chemistry. The conventional anticancer drugs are concomitant with high incidence of unpleasant side effects like severe gastrointestinal side effects and bone marrow suppression. In recent years, various selective anticancer agents have been emerged like dasatinib. The exact mechanism of dasatinib is the inhibition of c-Src tyrosine kinase. In fact, over-expression of some types of tyrosine kinases such as c-Src have been proved in some neoplastic disorders like breast cancer. As mentioned above, unwanted side effects and also the emergence of resistant tumors are encouraging agents for discovery of new anticancer drugs. Synthesis and *in vitro* cytotoxicity evaluation of 2-(4-Substituted-benzyl) isoindoline-1,3-dione derivatives (**3-7**) in T47D breast cancer cell line, proved the acceptable cytotoxic potency of this series. Compound **7** with IC_{50} = 1 µg/mL was the most active derivative. This compound showed higher activity in comparison with doxorubicin as reference drug. Molecular docking of these compounds as ligand into the active site of c-Src tyrosine kinase demonstrated the high potency for inhibition of the related enzyme. Compound **7** with binding free energy equal to -10.19 KCal/mol and five hydrogen bonds was the most potent inhibitor in comparison with other ligands.

***Corresponding author: Alireza Aliabadi, E-mail:** aliabadi.alireza@gmail.com

Introduction

Cancer is one the global health problem and the most frightening and fatal disease of human [1]. Development and discovery of new anticancer agents is one of the main goals in medicinal chemistry. The conventional anticancer drugs administering in the clinic like alkylating agents, antimetabolites and intercalators have high incidence of unpleasant side effects like severe gastrointestinal side effects (nausea and vomiting) and bone marrow suppression. In recent years, medicinal chemists have focused on the development of novel selective anticancer agents to be devoid of the adverse reactions of conventional anticancer agents. Protein tyrosine kinases are one of the interesting targets in the recent years. Protein tyrosine kinases have pivotal role in many cellular processes such as cell proliferation, metabolism, survival and apoptosis [2,3]. Several protein tyrosine kinases are known to be activated in neoplastic cells and to drive tumor growth and progression. Totally protein tyrosine kinases classify in two main categories as receptor tyrosine kinases such as EGFR and non-receptor tyrosine kinases. Inappropriate or uncontrolled activation of many of these kinases by over-expression have been resulted in uncontrolled cell growth. Overexpre- ssion of these receptors has been proved in a number of cancers like breast cancer. SRC is a tyrosine kinase that plays an important role in oncogenic, invasive and bone-metastatic process- es. It is a candidate therapeutic target for cancer in patients with solid tumors such as breast cancer. Several SRC inhibitors are in the market like dasatinib (Fig. 1). Dasatinib acts as a cytostatic agent and also inhibits the processes of cell proliferation, invasion and metastasis [4-6]. In the present study, we synthesized and investigated the cytotoxic activity of these compounds against T47D breast cancer cell line by MTT assay. Molecular modeling studies of synthesized compounds by docking method demonstrated that one the probable mechanism of these compounds could be the inhibition of c-Src tyrosine kinase.

Materials and Methods

Chemistry

All starter materials, reagents and solvents were purchased from diverse commercial companies such as merck and sigma-aldrich. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel $60F_{254}$ plates were applied for analytical TLC.

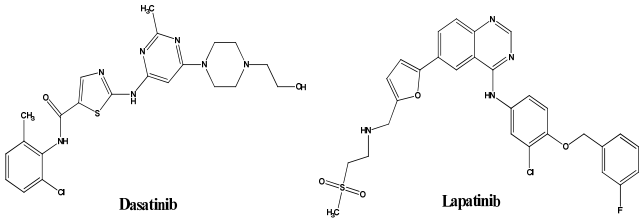
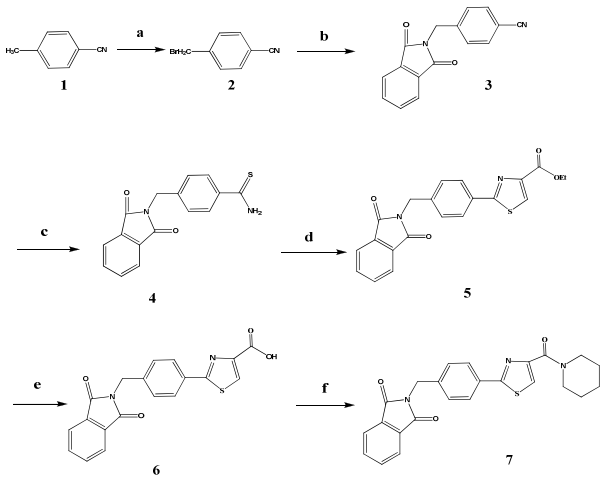


Fig. 1. Structures of two tyrosine kinase inhibitor in the clinic for treatment of breast cancer.

Column chromatography was performed on Merck silica gel (70-230 mesh) for purification of intermediate and final compounds. ^{1}H-NMR spectra were measured using a Varian 400 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV.



Scheme 1. a) NBS, $CHCl_3$, reflux, 24 h, b) Potassium phthalimide, CH_3CN, reflux, 24 h, c) Ammonium sulfide, DMF, rt, 5 h, d) Ethyl bromopyrovate, EtOH, 3 h, e) NaOH, rt, 20 h, f) EDC, HOBt, CH_3CN, rt, 24 h.

According to the scheme 1 and also literature all compounds were synthesized with high yield and

characterized by ^{13}C NMR, ^{1}H NMR, IR and Mass spectroscopy [7].

Docking studies

Molecular docking studies were performed using ArgusLab 4.0 software [8, 9]. All intended ligands were constructed in arguslab workspace and energy minimization was performed for all ligands by AM1 as semiemperical method. The pdb file of c-Src protein tyrosine kinase in complex with dasatinib was downloaded from brookhaven protein databank with 3G5D pdb code [10]. The geometry optimization of protein structure was performed using universal force field (UFF) as a molecular mechanic method. The docking process was done for all ligands in the workspace of ArgusLab software after defining the related groups for each ligand and also for protein. The binding location of dasatinib was defined as binding site for finding the best pose and conformation for all ligands. Binding free energies were calculated and listed in **Table 1.** Binding mode and related interactions of ligands were explored in Molegro molecular viewer software [11].

Table 1. Cytotoxicity results against T47D cell line and binding free energies after docking studies.

	3	**4**	**5**	**6**	**7**	**Doxorubicin**
IC_{50} (µg/mL)	2.5	4	>5	2.5	1	2.5
Free energy (Kcal/mol)	- 9.10	- 9.88	- 8.43	- 9.85	- 10.19	-

Cytotoxicity assay

Cytotoxiciy evaluation were done using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The cytotoxic activity of compounds 3-7 were evaluated against T47D (Breast cancer). Cells were seeded in 96-well plates and incubated for 48 h to allow cell attachment. The cells were then incubated with various concentrations of compounds 3-7. After washing of cells with PBS, MTT solution was added to each well. The amount of produced purple formazan is proportional to the number of viable cells. The absorbance of each well was measured by plate reader. Two independent experiments in triplicate were performed for determination of sensitivity to each compound, the IC_{50} were calculated. Doxorubicin was used as a reference drug [7].

Results and Discussion

Cytotoxicity

According to the Table 1, *in vitro* cytotoxicity results deduced from compounds 3 and 6, showed equal potency (2.5 µg/mL) of these derivatives to doxorubicin as standard drug. Compound 4 with thioamide moiety showed lower activity (4 µg/mL) than doxorubicin. Compound 5 with esteric substitution have the lowest activity in this series against T47D cell line and its potency is not acceptable in comparison with doxorubicin. Amidic residue of compound 7 enhanced the *in vitro* cytotoxicity activity. Cytotoxic effect of compound 7 is acceptable and is higher (1 µg/mL) than doxorubicin. Compound 7 could be a lead compound for discovery of new anticancer agents (Fig.3).

Docking studies

All compounds 3-7 were defined as ligand and molecular docking were performed using arguslab software. After molecular docking, calculated binding free energies (Kcal/mol) were extracted for each ligand and listed in Table 1. According to the Table 1, Comparison of binding free energies shows that compound 7 is the best *in silico* inhibitor of c-Src tyrosine kinase (Fig. 2). Five hydrogen bonds were detected between the compound 7 and protein. Cys 345 and Gly 344, as represented in ball-stick, each of them binds through two hydrogen bonds (red lines). The carbonyl moiety of amidic bond also participates in a water H-bond (yellow line). The predicted binding free energy for compound 7 was -10.19 Kcal/mol and is proportional to the *in vitro* cytotoxicity result. Compound 4 with thioamide moiety had the lowest potency for inhibition of c-Src tyrosine kinase and its binding free energy was -8.43 Kcal/mol only. The related amino acids in the active site for each ligand were detected and also the binding modes were compared with dasatinib as reference molecule.

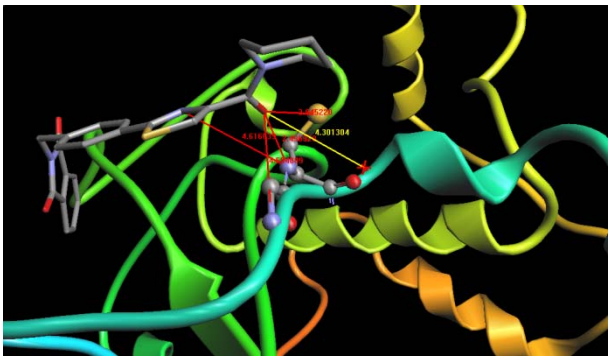


Fig. 2. 3D view of compound **7** in the active site of c-Src tyrosine kinase (PDB code: 3G5D) after docking. The ligand has been represented in cylinder and protein represented in cartoon ribbon. There are five hydrogen bindings between ligand and protein. Cys 345 and Gly 344 residues, as represented in ball-stick, each of them forms two hydrogen bonds and also there is a water H-bond that has been illustrated in yellow line (totally five hydrogen bonds).

The current project proved the *in silico* efficacy of compound 7 as potent inhibitor of c-Src tyrosine kinase. This compound showed also a great *in vitro* cytotoxic activity against T47D breast cancer cell line. According to the obtained results, 2-(4-Substituted-benzyl)isoindoline-1,3-dione derivatives especially compound 7 could be suggested as potential anticancer lead compound (Fig. 3).

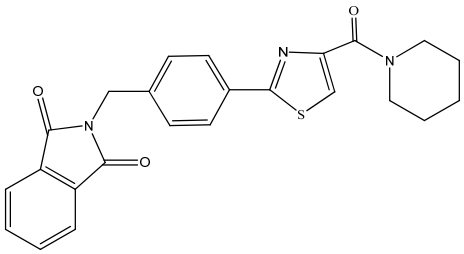


Fig. 3. Structure of compound **7** with higher *in vitro* cytotoxic activity (1 µg/mL) than doxorubicin. These structure also showed a great *in silico* inhibition of c-Src tyrosine kinase (-10.19 Kcal/mol). Therefore, this compound could be proposed as new lead compound for discovery of novel anticancer agents.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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