Research in Pharmaceutical Sciences, April 2024; 19(2): 238-250
Received: 25-12-2022
Peer Reviewed: 24-01-2023
Revised: 06-06-2023
Accepted: 17-03-2024
Published: 01-04-2024

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Molecular docking and synthesis of N-alkyl-isatin-3-imino aromatic amine derivatives and their antileishmanial and cytotoxic activities

Farshid Hassanzadeh[1], Seyed Hossein Hejazi[2], Elham Jafari[1,3,*], Atefeh Mohammadi fard[1], and Hojjat Sadeghi-aliabadi[1]

[1]Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[2]Skin Disease and Leishmaniasis Research Center, Department of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
[3]Bioinformatics Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: Isatin derivatives have excited attention due to their biological attractions, especially, anticancer properties. Isatin analogs such as semaxanib and sunitinib were exposed to tyrosine kinase inhibitory properties. N-substituted isatins were reported to show cytotoxic activity. On the other, the extension of impressive and cost-effective agents against leishmaniasis is necessary in third-world countries. The capability of isatin derivatives to create novel anticancer and anti-leishmanial compounds has been identified in medicinal chemistry research. The current study aimed to synthesize N-alkyl-isatin-3-imino aromatic amine compounds and evaluate their biological effects.

Experimental approach: Synthesis started with the formation of 2-chloro-N-phenylacetamide derivatives by the reaction of aniline derivatives with chloroacetyl chloride. N-alkylation of isatin was performed in the presence of K2CO3 in N, N-dimethylformamide. Final products were prepared via the condensation of N-alkyl isatin derivatives with aromatic amines. Cell viability was checked out by using the MTT assay against cancer cells. Final compounds were screened for anti-leishmanial activity. The molecules were docked in the active sites of the epidermal growth factor receptor tyrosine kinase to define the possible interactions.

Findings/Results: Compounds 5c and 4d with IC_{50} value of 50 µM showed cytotoxic activity on the MCF-7 cell line. Compound 5b presented anti-leishmanial activity against promastigote form after 48 h (IC_{50}:59 µM) and 72 h (IC_{50}: 41 µM) incubations. The highest docking score was -7.33 kcal/mol for compound 4d.

Conclusions and implications: The nature of substitution in the N1 region of isatin seems to be able to influence the cytotoxic activity. Based on the obtained results of docking and cytotoxic tests, compound 4d seems to be a good compound for further investigations.

Keywords: Anti-leishmanial activity; Cytotoxicity; Isatin; Schiff base.

INTRODUCTION

Cervical and breast cancers are two types of malignancy in women. Primary screening is one of the most effective methods for decreasing the mortality attributable to these two cancers by far (1). However, cancer treatment comprises surgery, radiotherapy, and chemotherapy (2). Due to drug resistance, it could be beneficial to develop new anticancer agents (1). Isatin backbone has an active biological profile in diseases such as inflammation (3,4), cancer (5-7), and leishmaniasis (8,9).

*Corresponding author: E. Jafari
Tel: +98-3137927106, Fax: +98-3136680011
E-mail: jafari@pharm.mui.ac.ir

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/RPS.RPS_244_22

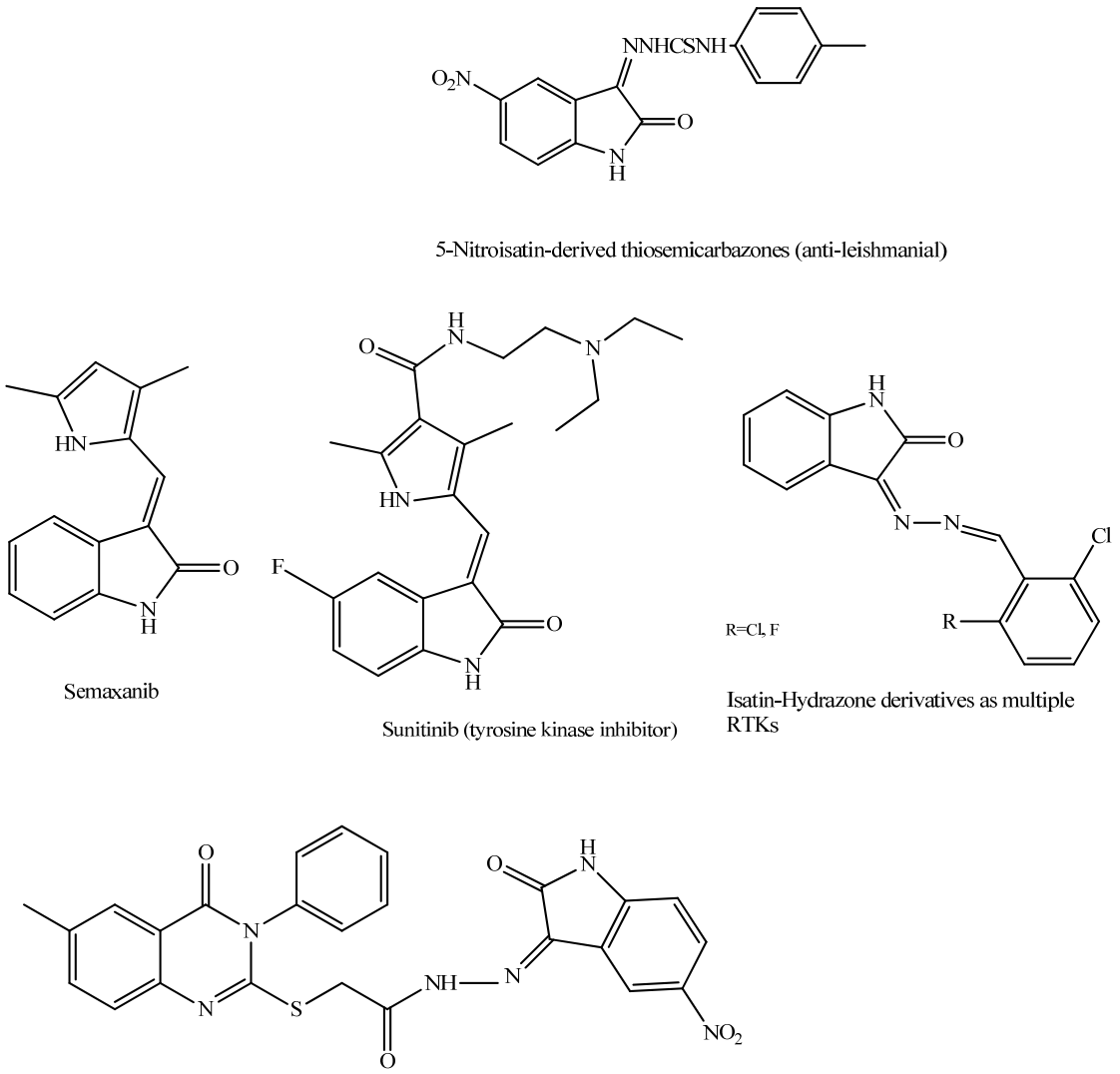


Fig. 1. Isatin analogues with tyrosine kinase inhibitory activity and anti-leishmanial activity.

Literature surveys have shown isatin scaffold as an anticancer agent against various human tumor cell lines, especially the tyrosine kinase inhibitor (10-13). The analogs of isatin such as sunitinib were exposed to tyrosine kinase inhibitory properties (10,14) (Fig. 1). Ibrahim *et al.* synthesized isatin-pyridine derivatives with tyrosine kinase inhibitory activity (12). Al-Salem *et al*. reported isatin-hydrazones as multiple receptor tyrosine kinase inhibitors (13) (Fig. 1).

Literature has reported the anti-leukemia activity of N-substituted isatins. Increased cytotoxic activity was observed with the introduction of an N-benzyl group containing electron-withdrawing groups at the meta or para position of the phenyl. Halogenated isatin derivatives such as N-alkyl isatins and 5, 7-dibromo isatin have been reported to show anticancer activity (15-18). The ketonic carbonyl of isatin can participate in condensation and addition reactions because of its electrophilic property resulted in the generation of 3-substituted isatin derivatives (19). DNA binding is a possible mechanism for the anticancer activity of isatin (19). Other mechanisms involved in the anticancer activity of isatin include the inhibitors of kinases

(11-13), cysteine and serine proteases (20), tubulin polymerization (21), and apoptosis (10,22). Interaction with extracellular signal-related protein kinases is attributed to isatin derivatives (17). 3-Phenyl hydrazone derivatives of isatin showed cytotoxic activity via the inhibition of cyclin-dependent kinases (23). Isatin hybrids were identified as promising antitumor agents (24). On the other, leishmaniasis is a neglected protozoan disease for drug development. This disease has a global prevalence and causes considerable mortality and morbidity (8). *Leishmania* parasites have two stages in their life cycle including extracellular and intracellular stages (25). The synthesis of new compounds offers an opportunity to design effective anti-leishmanial agents such as 5-Nitroisatin-derived thiosemicarbazones, which showed anti-leishmanial activity during random screening (Fig. 1) (9). Schiff-base derivatives of isatin have been found as potential anti-leishmanial agents (8,9). According to the potential of isatin for structural modifications, the present study attempted to synthesize N-alkyl-isatin-3-imino aromatic amine derivatives along with their cytotoxicity and anti-leishmanial assays. Moreover, the molecular docking of compounds on binding sites of the_epidermal growth factor receptor (EGFR) tyrosine kinase domain (Protein Data Bank; PDB: 1M17) crystal structure was also performed and their binding energies were determined.

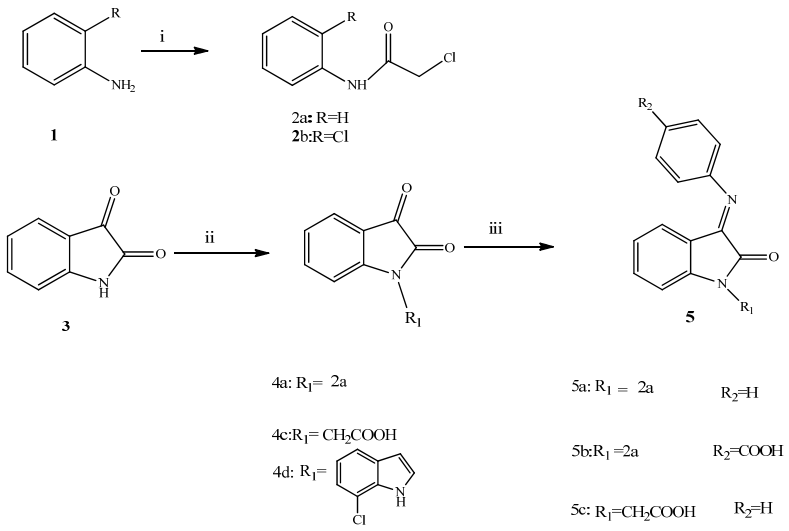
MATERIALS AND METHODS

All chemical materials were obtained from Merck (Germany) and Samchun (Korea) companies. Silica gel 60 F254 plates (Merck, Germany) were used for monitoring the reactions. An Electrothermal IA9200 apparatus (England) was used to measure melting points (mp) and are uncorrected. WQF-510 Fourier transform spectrometer (China) recorded infrared spectra using potassium bromide discs. Proton nuclear magnetic resonance (^{1}H NMR) was obtained by a 400 MHz spectrometer (Bruker, Germany). Mass spectra were run on Agilent Technologies 5975C mass spectrometer (USA).

Synthesis

Synthesis of 2-chloro-N-phenylacetamide derivatives (2a and 2b)

Aromatic amines (aniline and 2-chloroaniline) (0.04 mol) were dissolved in a mixture of saturated sodium acetate (25 mL)/glacial acetic acid (25 mL). Chloroacetyl chloride (0.06 mol) was added dropwise and stirred for 1 h. The precipitated product (2a or 2b) was filtered and washed by water, and finally crystallized from methanol (Scheme 1) (18).



Scheme 1. Synthesis of target compounds (5a-5c and 4d). (i) Glacial acetic acid /saturated sodium acetate, chloroacetyl chloride, room temperature. (ii) Dimethylformamide, potassium carbonate, 2a, 2b, or chloroacetic acid; (iii) suitable solvent, aniline, or 4-aminobenzoic acid.

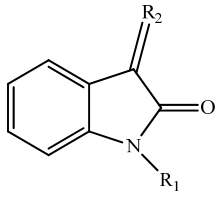
Synthesis of N-Alkyl-isatin (4a, 4c and 4d)

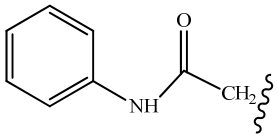
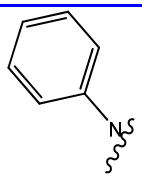
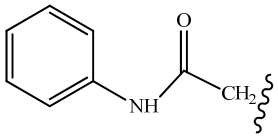
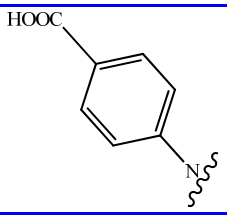
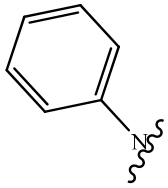
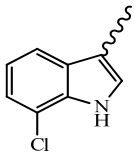
The isatin 3 (2.9 g, 0.02 mol) was dissolved in anhydrous dimethylformamide (DMF) (30 mL) at 0 °C. Potassium carbonate (0.03 mol) was added and the suspension was stirred for 1 h (16). 2-Chloro-N-phenylacetamide derivatives (2a, 2b or chloroacetic acid) (0.02 mol) and potassium iodide (0.02 mol) were added and stirred at 80 ˚C for 24-72 h. The mixture was poured into water, and the pH was adjusted between 3 and 4 by diluted hydrochloric acid. The product was filtered. After washing with water, it was crystallized from methanol (Scheme 1 and Table 1).

2-(2-Oxo-3-(phenyl-imino) indolin-1-yl)-N-phenylacetamide (5a)

The mixture of 4a (0.01 mol) and aniline (0.01 mol) was refluxed in methanol (30 mL) in the presence of acetic acid for 6 h. The solvent was removed under reduced pressure, and the residue was recrystallized from ethanol to obtain yellow product 5a (Scheme 1 and Table 1).

Table 1. The structural backbone and the substitution groups of the compounds were investigated.



Compounds	R_1	R_2
5a		
5b		
5c	-CH_2COOH	
4d		O

Synthesis of 4-(2-oxo-1-(2-oxo-2-(phenyl-amino) ethyl) indolin-3-ylideneamino) benzoic acid (5b)

A mixture of 4a (0.01 mol) and 4-aminobenzoic acid (0.01 mol) was refluxed in the mixture of chloroform/ethanol (30 mL: 3/1) (26) and acetic acid for 48 h. The obtained precipitate was filtrated off and recrystallized from methanol to acquire orange product 5b (Scheme 1 and Table 1).

Synthesis of 2-(2-oxo-3-(phenylimino) indolin-1-yl) acetic acid (5c)

A mixture of 4c (0.01 mol) and aniline (0.01 mol) was refluxed in ethanol (30 mL) in the presence of acetic acid for 48 h. The obtained precipitate was collected and recrystallized from ethanol to acquire orange product 5c (Scheme 1, Table 1).

MTT assay

The synthesized compounds (5a-5c and 4d) were evaluated for cytotoxic activity against Michigan Cancer Foundation-7 (MCF-7), Henrietta Lacks (HeLa) and human umbilical vein endothelial cells (HUVEC). Roswell Park Memorial Institute (RPMI) 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, and 5% v/v fetal bovine serum was used for growing cell lines. 96-Well plates containing the viable cells (a concentration of 5×10^4 cells/mL) per well were incubated for 24 h to allow cell attachment. Then, the cells were treated with the synthesized compounds at the concentrations of 1, 10, and 100 µM. Doxorubicin (20 µM) and dimethyl sulfoxide (DMSO, 1%) were selected as the positive and negative controls, respectively. Incubation was performed in an incubator at 37 °C for 48 h. After that, 20 µL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide solution (MTT dye, 5 mg/mL) was added to wells and incubated for an additional 3 h. Finally, after adding Formasan dissolved in DMSO (150 µL per well), the absorbance was read at 570 nm using an ELISA plate reader. The experiments were done three times, and cell viability was calculated using the following equation (27,28):

$$Cell\ survival\ (\%) = \frac{\text{MA of treated wells} - \text{MA of blank}}{\text{MA of negative control} - \text{MA of blank}} \times 100$$

where, MA was put instead of the mean of absorbance.

Assay for anti-leishmanial activity

The final compounds were evaluated for their anti-leishmanial activity according to the literature (25,29). A separate stock was provided for each of the four compounds. These compounds were diluted with DMSO and transferred to tubes to reach final concentrations of 500, 250, 125, 62, 31, and 15 µg/mL. The inhibitory effect of compounds against *L. major* promastigotes was determined using the MTT test. Living promastigotes had a direct relationship with the amount of produced purple formazan. 100 µL of the promastigotes (10^6 cells/mL) harvested from the logarithmic growth phase were added into a 96-well microtiter plate (29). Then, 100 µL of various concentrations of compounds were added to wells and incubated at 37 °C and evaluated after 24, 48, and 72 h. Glucantime was used as positive control. After incubation, 10 µL of MTT solution (5 mg/mL) was added into each well and incubated at 25 °C for 4 h. The resulting formazan crystals were solubilized by 100 µL of DMSO, and the absorbance was read by an ELISA reader at 490 nm. The assays were performed in triplicate.

Molecular docking

The three-dimensional (3D) structures of ligands were drawn by HyperChem 7.0 software (Hypercube, Inc) and then optimized using the MM^+ molecular mechanical force field and 3D geometry optimization calculations. The ultimate conformations were calculated by Austin model 1 (AM1) as a semi-empirical method, and the molecular structures were optimized using the Polak-Ribiere-Polyak conjugate gradient algorithm. These optimized structures were used by AutoDock Tools for the preparation of PDB, partial charge (Q), and atom type (T) (PDBQT) files as follows: polar and non-polar hydrogen atoms were added and merged, respectively. Then, Gasteiger partial atomic

charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, were allowed to be rotated during the automated docking process. The crystal structure of EGFR tyrosine kinase (PDB ID: 1M17) with 2.6 Å resolution was obtained from the PDB (www.rcsb.org) [27]. The PDBQT file of protein was prepared by AutoDock Tools as follows: water and ligand molecules were removed from the protein file. All missing hydrogens were added, and after determining the Kollman united atom charges, non-polar hydrogens were merged into their corresponding carbons (27,30). The grid box dimensions were set in 60 × 60 × 60 Å points with a distance of 0.375 Å between the grid points. The grid box was centered with the coordinates x = 22.00 Å, y = 0.204 Å, and z = 52.81 Å for EGFR tyrosine kinase. Docking was performed using the routine procedure and the default parameters of molecular docking AutoDock 4.2 software. After 100 independent docking runs for each ligand, conformations were clustered according to the root mean square deviation tolerance of 2.0 Å and ranked according to the binding free energy. Erlotinib as a reference ligand was relocked into the active site of EGFR tyrosine kinase for the validation of the docking procedure.

Statistical analysis

Data were presented as mean ± SD, and analyzed by software of statistical package for the social science (SPSS, version 18). One-way analysis of variance (ANOVA) followed by Tukey post-test was used to analyze cell viability among groups. The *P*-value < 0.05 was considered a significant difference.

RESULTS

Chemistry

(7-Chloro-1H-indol-3-yl) indoline-2, 3-dione (4d)

Orange solid; yield: 46%; mp: 200-203 °C, IR (ν_{max} cm^{-1}): 3308 (NH), 1746, 1678 (C=O), 1617 (C=C); ^{1}HNMR: (400 MHz; DMSO-d_6) δ 8.49 (1H, d, *J* = 8 Hz, H-isatin), 8.33 (1H, d, *J* = 8 Hz, H-isatin), 7.96-7.95 (2H, m, CH: isatin and H-isatin), 7.90-7.86 (2H, m, H-isatin), 7.77-7.73 (1H, m, H-isatin),7.49 (1H, t, *J* = 8 Hz, H-isatin); MS (m/z, %): 296 (M^+), for $C_{16}H_9ClN_2O_2$, MW 296 g/mol.

2-(2-Oxo-3-(phenylimino) indolin-1-yl)-N-phenylacetamide (5a)

Yellow solid: yield: 80%; mp: 170-173 °C, IR (ν_{max} cm^{-1}): 3304 (NH), 2989 (C-H, Aliphatic), 1730, 1671 (C=O), 1697 (C=N), 1603 (C=C); ^{1}HNMR: (400 MHz; DMSO-d_6) δ 10.38 (1H, s, NH), 7.59 (2H, d, *J* = 8 Hz, H-Ar), 7.51 (2H, t, *J* = 8 Hz, H-Ar), 7.43 (1H, t, *J* = 8 Hz, H-Ar), 7.34 (2H, t, *J* = 8 Hz, H-Ar), 7.30 (1H, t, *J* = 8 Hz, H-Ar), 7.12 (1H, d, *J* = 8 Hz, H-isatin), 7.09 (1H, t, *J* = 8 Hz, H-isatin), 7.03 (2H, d, *J* = 8 Hz, H-Ar), 6.82 (1H, t, *J* = 8 Hz, H-isatin), 6.44 (1H, d, *J* = 8 Hz, H-isatin), 4.66 (2H, s, $\underline{CH_2}$); MS (m/z, %): 355 (M^+), for $C_{22}H_{17}N_3O_2$, MW 355 g/mol.

4-(2-Oxo-1-(2-oxo-2-(phenylamino) ethyl) indolin-3-ylideneamino) benzoicacid (5b)

Orange solid: yield: 71%; mp: 272-274 °C, IR (ν_{max} cm^{-1}), 3475 (OH, acid), 3377 (NH), 1630, 1666 (C=O), 1603 (C=C); ^{1}HNMR: (400 MHz; DMSO-d_6) δ 10.52 (1H, s, OH), 8.11 (2H, d, *J* = 8 Hz, H-Ar), 7.67 (2H, d, *J* = 8 Hz, H-Ar), 7.51 (1H, t, *J* = 8 Hz, H-Ar), 7.40 (2H, t, *J* = 8 Hz, H-Ar), 7.20 (1H, d, *J* = 8 Hz, H-isatin), 7.17-7.12 (3H, m, H-isatin, H-Ar), 6.91 (1H, t, *J* = 8 Hz, H-isatin), 6.49 (1H, d, *J* = 8 Hz, H-isatin), 4.74 (2H, s, $\underline{CH_2}$); MS (m/z, %): 399 (M^+), for $C_{23}H_{17}N_3O4$, MW 399 g/mol.

2-(2-Oxo-3-(phenylimino)indolin-1-yl) acetic acid (5c)

Orange solid: yield: 80%; mp: 225-228 °C, IR (ν_{max} cm^{-1}), 3426 (OH), 1685 (C=O), 1661 (C=N); ^{1}HNMR: (400 MHz; DMSO-d_6) δ 9.97 (1H, s, OH), 7.70 (1H, t, *J* = 8 Hz, H-Ar), 7.61 (2H, t, *J* = 8 Hz, H-Ar), 7.52 (1H, d, *J* = 8 Hz, H-isatin), 7.34 (1H, t, *J* = 8 Hz, H-isatin), 7.25-7.19 (2H, m, H-isatin), 7.17 (2H, d, *J* = 8 Hz, H-Ar), 4.64 (2H, s, $\underline{CH_2}$); MS (m/z, %): 280 (M^+), for $C_{16}H_{12}N_2O_3$, MW 280 g/mol.

Cytotoxic activity

The cytotoxicity of compounds was evaluated against HeLa, MCF-7, and HUVEC cell lines at the concentrations of 1, 10, and 100 µM using MTT assay. The results were summarized in Fig. 2 (A, B and C) and Table 2.

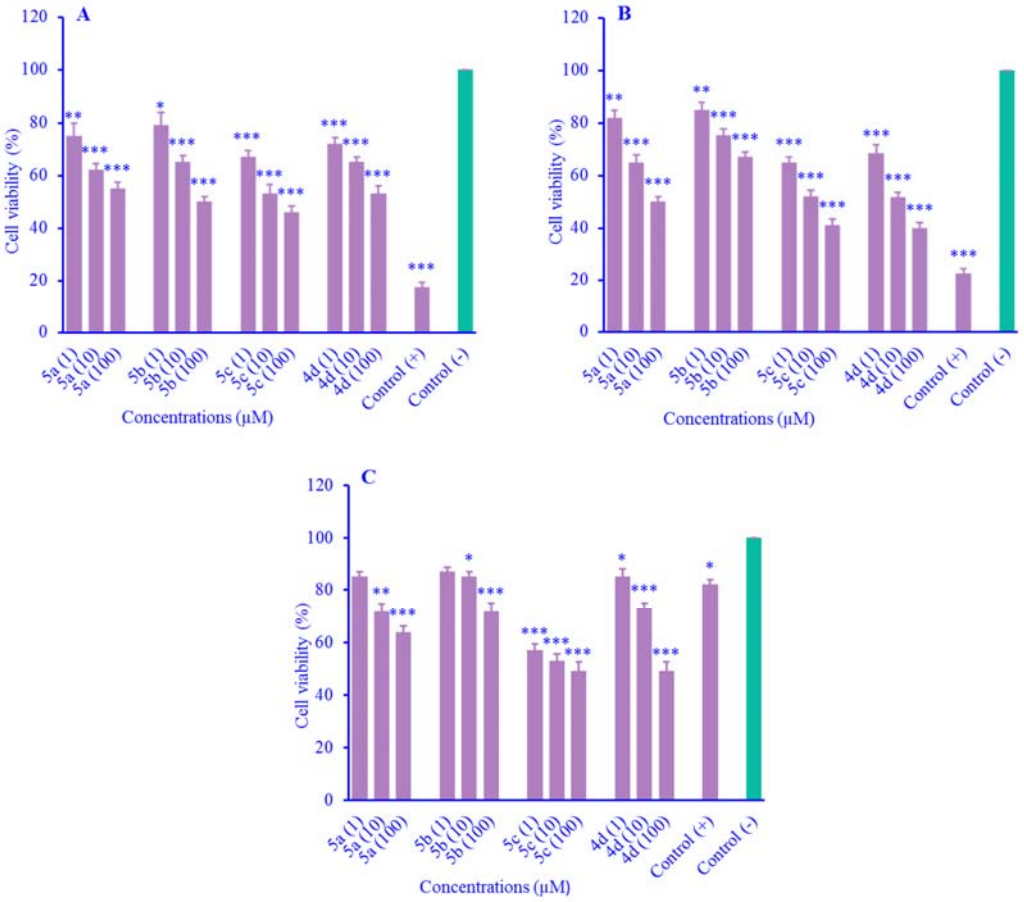


Fig. 2. Cytotoxic effects of different concentrations (1, 10, and 100 µM) of compounds 5a,5b, 5c, 4d against (A) HeLa cells, (B) MCF-7 and (C) HUVEC. Data were presented as mean ± SD, n = 3. *P < 0.05, **P < 0.01, and ***P < 0.001 indicate significant differences in comparison with negative control group; doxorubicin was used as a positive control.

Table 2. The IC_{50} of final compounds against cell lines.

Compounds	IC_{50} (µM)		
	MCF-7	HeLa	HUVEC
5a	97 ± 2	> 100	> 100
5b	> 100	98 ± 3	> 100
5c	50 ± 2	74 ± 3	82 ± 2
4d	51 ± 2	> 100	90 ± 3

The results of the cytotoxic assay revealed that compounds 5c and 4d had the highest cytotoxic activity on the MCF-7 cell line with IC_{50} values of 50 and 51 µM, respectively (Table 2). Also, all compounds at the concentrations of 1, 10, and 100 µM decreased cell viability in both HeLa and MCF-7 cell lines in comparison to negative control (Fig. 2A and 2B). Moreover, all the concentrations of compounds 5a, 5b, 5c, and 4d reduced cell viability, except the compounds 5a and 5b at the concentration of 1 µM (Fig. 2C). The cytotoxicity of final compounds was relatively weak in normal cell line (Fig. 2C).

Anti-leishmanial activity

IC_{50} values for anti-leishmanial activity against promastigote were presented in Table 3. Compound 5b was found to be active against promastigote form after 48 h (IC_{50}: 59 µM) and 72 h (IC_{50}: 41 µM) incubation.

Table 3. IC_{50} values for anti-leishmanial activity against promastigote.

Compounds	IC_{50} (µM)		
	24 h	**48 h**	**72 h**
5a	> 100	> 100	84 ± 1
5b	> 100	59 ± 1	41 ± 2
5c	> 100	> 100	> 100
4d	> 100	96 ± 2	85 ± 1
Glucantime	> 100	62.01 ± 1	14.87 ± 1

Table 4. Energy-based interactions and hydrogen bonds of the final compounds with amino acid residues.

Compounds	G Bind Δ (Kcal/mol)	Hydrogen bond (Distance, Å)	Hydrophobic	Ki (µM)
5a	-6.83	Met 769 (1.82)	Ala 719, Thr 766, Leu 768	9.83
5b	-6.96	-	-	7.95
5c	-6.73	Cys 773 (1.92), Met 769 (1.83)	Gly 772, Thr 766	11.65
4d	-7.33	Met 769 (1.87), Thr 766 (2.19)	Ala 719, Gly 772	4.26

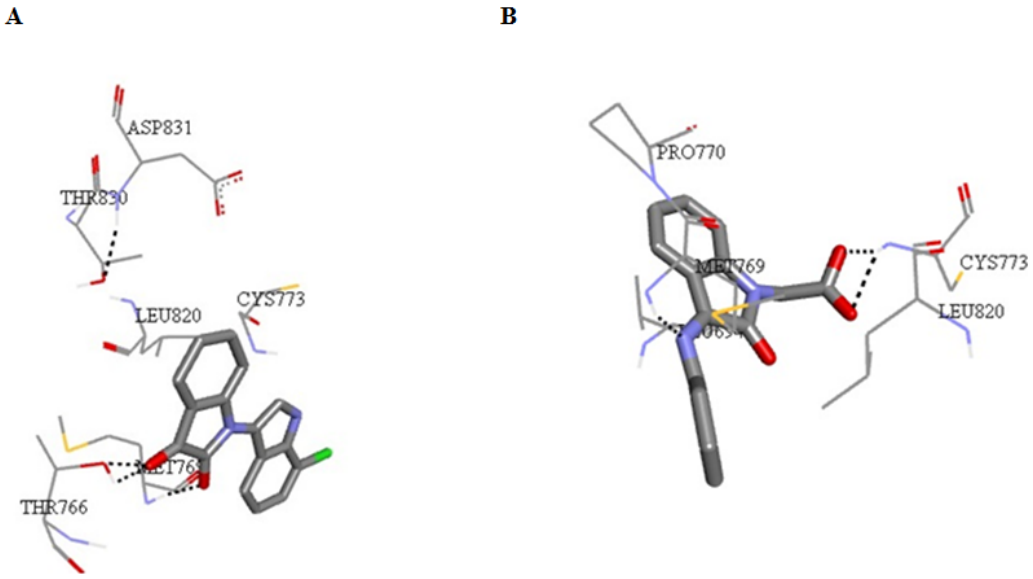


Fig. 3. Docked conformation of compounds (A) 4d and (B) 5c in the binding site of EGFR tyrosine kinase.

Docking

The docking results of compounds including the estimated free binding energy values of the docked positions and the favorable interactions with key amino acid residues at the active site of enzymes exhibited in Table 4 and Fig. 3. Compound 4d had the highest docking score and the lowest Ki (inhibition constant) in which Met 769 and Thr 766 have been detected for the formation of hydrogen bonds with carbonyl groups 2 and 3 of the isatin ring, respectively. Moreover, the amino acids of Ala 719 and Gly 772 participated in hydrophobic interactions with compound 4d (Table 4). The re-docking result of erlotinib in the active site of EGFR tyrosine kinase with acceptable root mean square deviation (RMSD) of 1.3 Å was presented in Fig. 4.

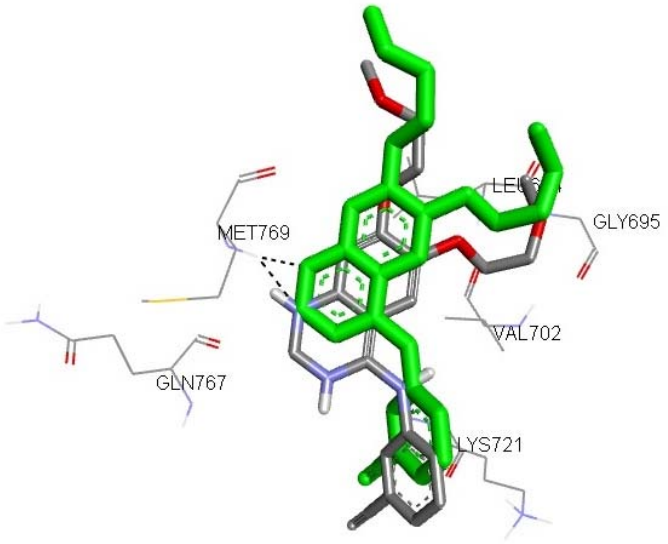


Fig. 4. Redocking results of erlotinib in the active site of EGFR tyrosine kinase (RMSD: 1.3 Å). EGFR, Epidermal growth factor receptor; RMSD, root mean square deviation.

DISCUSSION

Literature surveys indicated that isatin pharmacophore and N-alkyl/benzyl isatins correlate with diverse biological properties (19). One study has reported that the activity of isatin derivatives is significantly dependent on the presence and the nature of the substituent at 1-position of the structure (31). N-alkylation reaction was carried out with aniline or chloroacetic acid to give 4a and 4c compounds. N-alkylated isatin derivatives 4a and 4c were transferred to the corresponding imine compounds 5a, 5b, and 5c by condensation reaction. The ^{1}HNMR spectra showed the singlet signal of CH_2 in the region of 4.64-4.74 ppm related to the alkylation reaction of isatin in compounds 5a, 5b, and 5c. The hydrogens of the isatin ring were observed in the range of 6.44-7.52 ppm in these compounds.

Regarding the synthesis of compound 4d, isatin was reacted with 2b to form intermediate 1 which was in resonance with the keto-enol form. Cyclization was performed by transferring the electron pair of nitrogen in the form of an imine bond with a ring and remising the ᴨ electrons to carbon. Upon being aromatized the ring containing chlorine and dehydrated in intermediate 3, the final compound 4d was formed (Fig. 5). According to the cytotoxic results (Table 2 and Fig. 2), compounds 5c and 4d were relatively active against the MCF-7 cell line with IC_{50} values of 50 and 51 µM, respectively. According to Table 2, none of the compounds showed any cytotoxic activities on the HeLa cell line. The cytotoxicity of final compounds was relatively weak in normal cell line. It seems that the presence of electron-withdrawing groups (COOH and Cl) in compounds 5c and 4d can improve the cytotoxic activity against the MCF-7 cell line. Vine *et al.* demonstrated that the presence of benzene with electron-withdrawing groups in N-alkyl-substituted isatin could increase biological properties (32) as seen for compound 4d.

A similar study showed that *N*-alkyl isatin-3-iminobenzoic acid derivatives had cytotoxic activity on the MCF-7 cell line in the range of 23-66 µM (16). Structure-Activity Relationship (SAR) study revealed that the lipophilicity at the 1-position of the isatin presents a positive allotment to the cytotoxic activity (16). The comparison of cytotoxic activity in compounds 5a and 5c with different substitutions in N1 of isatin can confirm the importance of the nature of substitution in this area. However, the evaluation of cytotoxicity in extended isatin derivatives should be performed to identify more rational SAR. According to the reported anti-leishmanial activity of isatin-based derivatives (8,9), the final compounds were evaluated for their anti-leishmanial properties (Table 3).

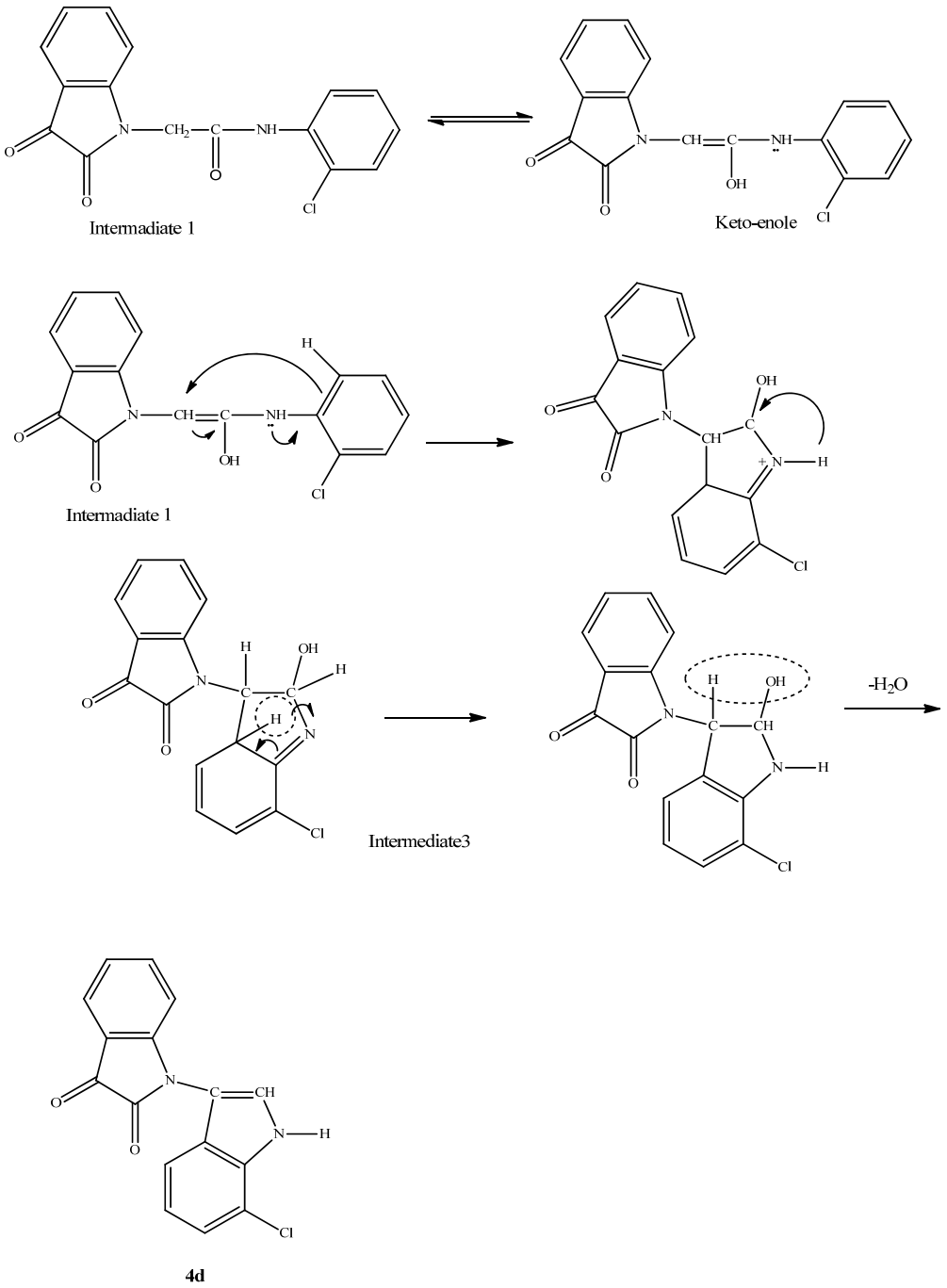


Fig. 5. Proposed mechanism for the synthesis of compound 4d.

Only compound 5b was found to be active against promastigote form after 48 h (IC_{50} 59 µM) and 72 h (IC_{50}: 41 µM) incubation. This compound can act as the metal chelator in parasites and show anti-leishmanial activity. It can be considered for further evaluation of anti-leishmanial activity and determined the exact mechanisms. A piece of evidence revealed that 5-nitroisatin-derived thiosemicarbazones synthesized as potential anti-leishmanial agents could inhibit iron-dependent enzymes catalyzing DNA synthesis in the parasite through metal chelate (9). The estimated free binding energy values and the interactions with key amino acid residues at the active site of enzymes were presented in Table 4 and Fig. 3.

The docking results revealed that compound 4d has the highest docking score and the lowest Ki (inhibition constant) in which Met 769 and Thr 766 have been detected for the formation of hydrogen bonds with carbonyl groups 2 and 3 of the isatin ring, respectively. Moreover, the amino acids of Ala 719 and Gly 772 participated in hydrophobic interactions with compound 4d. A similar study showed that isatin moiety interacted with the amino acids of Met 769 and Thr 766 (33). In the validation step of the docking protocol, the redocking of erlotinib into the active site of EGFR tyrosine kinase was performed and Met 769 has been detected for hydrogen bond formation (Fig. 4).

CONCLUSION

N-alkyl-isatin-3-imino aromatic amine derivatives were synthesized through alkylation and condensation reactions. The cytotoxic evaluation of final compounds revealed that the nature of substitution in the N1 region of isatin seems to be able to influence the cytotoxic activity. Substitutions with electron-withdrawing groups seem necessary factors in providing cytotoxicity properties. The IC_{50} values of tested compounds revealed that the MCF-7 is more susceptible to the compounds 5c and 4d. The anti-leishmanial activity of compound 5b can probably be due to chelation. To determine the exact mechanism, more evaluations should be performed. The highest docking score was -7.33 kcal/mol for compound 4d. The obtained results of docking and cytotoxic studies showed that compound 4d could be a suitable structure for the inhibition of tyrosine kinase. However, further experimental tests such as *in vitro* inhibitory studies against tyrosine kinases are essential to confirm these findings.

Acknowledgments

This work has been performed in the Department of Medicinal Chemistry and is financially supported by the Vice-Chancellor of Research of Isfahan University of Medical Sciences (Grant No. 399053).

Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

F. Hassanzadeh and E. Jafari contributed to the conception of the work, the conduction of the study, the revision of the draft, the approval of the final version of the manuscript, and agreed on all aspects of the work. S.H. Hejazi contributed to the conception of the work, and the conduction of the study, and agreed to all aspects of the work. A. Mohammadi Fard performed the experiments and analyzed the data. H. Sadeghi-Aliabadi contributed to the conception of the work, the conduction of the study, and the revision of the draft.

REFERENCES

1. Banerjee D, Mittal S, Mandal R, Basu P. Screening technologies for cervical cancer: overview. Cytojournal. 2022;19:23. DOI: 10.25259/CMAS_03_04_2021.
2. Fisusi FA, Akala EO. Drug combinations in breast cancer therapy. Pharm Nanotechnol. 2019;7(1):3-23. DOI: 10.2174/2211738507666190122111224.
3. Dantas LLSFR, Fonseca AG, Pereira JR, Furtado AA, Gomes PATM, Fernandes-Pedrosa MF, *et al*. Anti-inflammatory and antinociceptive effects of the isatin derivative (Z)-2-(5-chloro-2-oxoindolin-3-ylidene)-N-phenyl-hydrazinecarbothioamide in mice. Braz J Med Biol Res. 2020;53(10):e10204. DOI: 10.1590/1414-431X202010204.
4. Hassanzadeh F, Jafari E, Khayambashi N, Hajhashemi V. Synthesis and anti-inflammatory effects evaluation of 1, 3 substituted isatin derivatives. Thai J Pharm Sci. 2021;45(4):248-252.
5. Sabet R, Mohammadpour M, Sadeghi A, Fassihi A. QSAR study of isatin analogues as *in vitro* anti-cancer agents. Eur J Med Chem. 2010; 45(3):1113-1118. DOI: 10.1016/j.ejmech.2009.12.010.
6. Matesic L, Locke JM, Bremner JB, Pyne SG, Skropeta D, Ranson M, *et al*. N-phenethyl and N-naphthylmethyl isatins and analogues as *in vitro* cytotoxic agents. Bioorg Med Chem. 2008;16(6):3118-3124. DOI: 10.1016/j.bmc.2007.12.026.
7. Taher AT, Khalil NA, Ahmed EM. Synthesis of novel isatin-thiazoline and isatin-benzimidazole conjugates as anti-breast cancer agents. Arch Pharm Res. 2011;34(10):1615-1621. DOI: 10.1007/s12272-011-1005-3.
8. Khan KM, Mughal UR, Samreen S, Perveen S, Choudhary MI. Schiff bases of istain: Potential anti-leishmanial agents. Lett Drug Des Discov. 2008;5(4):243-249. DOI: 10.2174/157018008784619915.
9. Pervez H, Manzoor N, Yaqub M, Khan KM. 5-Nitroisatin-derived thiosemicarbazones: potential

antileishmanial agents. J Enzyme Inhib Med Chem. 2014;29(5):628-632.
DOI: 10.3109/14756366.2013.836641.

10. Alkahtani HM, Alanazi MM, Aleanizy FS, Alqahtani FY, Alhoshani A, Alanazi FE, *et al*. Synthesis, anticancer, apoptosis-inducing activities and EGFR and VEGFR2 assay mechanistic studies of 5, 5-diphenylimidazolidine-2, 4-dione derivatives: molecular docking studies. Saudi Pharm J. 2019;27(5):682-693.
DOI: 10.1016/j.jsps.2019.04.003.
11. Khaldoun K, Safer A, Boukabcha N, Dege N, Ruchaud S, Souab M, *et al*. Synthesis and evaluation of new isatin-aminorhodanine hybrids as PIM1 and CLK1 kinase inhibitors. J Mol Struct. 2019;1192: 82-90.
DOI: 10.1016/j.molstruc.2019.04.122.
12. Ibrahim NW, Mahdi M, Raauf AMR. Design, molecular docking, synthesis and evaluation of new isatin derivatives bearing pyridine moiety as potential tyrosine kinase inhibitors. Egypt J Chem. 2022;65(2):9-18.
DOI: 10.21608/EJCHEM.2021.72747.3607.
13. Al-Salem HS, Arifuzzaman M, Issa IS, Rahman AFMM. Isatin-hydrazones with multiple receptor tyrosine kinases (RTKs) inhibitory activity and in-silico binding mechanism. Appl Sci (Basel). 2021;11(9):3746,1-12.
DOI: 10.3390/app11093746.
14. Sun L, Liang C, Shirazian S, Zhou Y, Miller T, Cui J, *et al*. Discovery of 5-[5-fluoro-2-oxo-1, 2-dihydroindol-(3Z)-ylidenemethyl]-2, 4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl) amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. J Med Chem. 2003;46(7):1116-1119.
DOI: 10.1021/jm0204183.
15. Eldehna WM, Abo-Ashour MF, Nocentini A, El-Haggar RS, Bua S, Bonardi A, *et al*. Enhancement of the tail hydrophobic interactions within the carbonic anhydrase IX active site via structural extension: design and synthesis of novel N-substituted isatins-SLC-0111 hybrids as carbonic anhydrase inhibitors and antitumor agents. Eur J Med Chem. 2019;162:147-160.
DOI: 10.1016/j.ejmech.2018.10.068.
16. Farooq M, Al Marhoon ZM, Taha NA, Baabbad AA, Al-Wadaan MA, El-Faham A. Synthesis of novel class of N-alkyl-isatin-3-iminobenzoic acid derivatives and their biological activity in zebrafish embryos and human cancer cell lines. Biol Pharm Bull. 2018;41(3):350-359.
DOI: 10.1248/bpb.b17-00674.
17. Vine KL, Matesic L, Locke JM, Ranson M, Skropeta D. Cytotoxic and anticancer activities of isatin and its derivatives: a comprehensive review from 2000-2008. Anticancer Agents Med Chem. 2009;9(4):397-414.
DOI: 10.2174/1871520610909040397.
18. Modi NR, Shah RJ, Patel MJ, Suthar M, Chauhan BF, Patel LJ. Design, synthesis, and QSAR study of novel 2-(2, 3-dioxo-2, 3-dihydro-1H-indol-1-yl)-N-phenylacetamide derivatives as cytotoxic agents. Med Chem Res. 2011;20:615-625.
DOI: 10.1007/s00044-010-9361-y.
19. Pakravan P, Kashanian S, Khodaei MM, Harding FJ. Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity. Pharmacol Rep. 2013;65(2):313-335.
DOI: 10.1016/s1734-1140(13)71007-7.
20. Medvedev A, Buneeva O, Glover V. Biological targets for isatin and its analogues: implications for therapy. Biologics. 2007;1(2):151-162.
PMID: 19707325.
21. Krishnegowda G, Prakasha Gowda AS, Tagaram HRS, Staveley-O'Carroll KF, Irby RB, Sharma AK, *et al*. Synthesis and biological evaluation of a novel class of isatin analogs as dual inhibitors of tubulin polymerization and Akt pathway. Bioorg Med Chem. 2011;19(20):6006-6014.
DOI: 10.1016/j.bmc.2011.08.044.
22. Igosheva N, Lorz C, O'conner E, Glover V, Mehmet H. Isatin, an endogenous monoamine oxidase inhibitor, triggers a dose-and time-dependent switch from apoptosis to necrosis in human neuroblastoma cells. Neurochem Int. 2005;47(3):216-224.
DOI: 10.1016/j.neuint.2005.02.011.
23. Al-Salem HS, Arifuzzaman M, Alkahtani HM, Abdalla AN, Issa IS, Alqathama A, et al. A series of isatin-hydrazones with cytotoxic activity and CDK2 kinase inhibitory activity: a potential type II ATP competitive inhibitor. Molecules. 2020;25(19):4400,1-16.
DOI: 10.3390/molecules25194400.
24. Gangarapu K, Thumma G, Manda S, Jallapally A, Jarapula R, Rekulapally S. Design, synthesis and molecular docking of novel structural hybrids of substituted isatin based pyrazoline and thiadiazoline as antitumor agents. Med Chem Res. 2017;26:819-829.
DOI: 10.1007/s00044-017-1781-5.
25. Hassanzadeh F, Jafari E, Saeedi M, Saberi S. Synthesis and evaluation of thiadiazole-based antileishmanial agents. J Rep Pharma Sci. 2020;9:189-195.
DOI: 10.4103/jrptps.JRPTPS_3_20.
26. Mohammadpour M, Sadeghi A, Fassihi A, Saghaei L, Movahedian A, Rostami M. Synthesis and antioxidant evaluation of some novel *ortho*-hydroxypyridine-4-one iron chelators. Res Pharm Sci. 2012;7(3):171-179.
PMID: 23181095.
27. Hassanzadeh F, Jafari E, Zarabi M, Khodarahmi G, Vaseghi G. Synthesis, cytotoxic evaluation, and molecular docking studies of some new 1, 3, 4-oxadiazole-based compounds. Res Pharm Sci. 2020;15(5):454-462.
DOI: 10.4103/1735-5362.297848.
28. Hassanzadeh F, Sadeghi-Aliabadi H, Jafari E, Sharifzadeh A, Dana N. Synthesis and cytotoxic evaluation of some quinazolinone-5-(4-chlorophenyl) 1, 3, 4-oxadiazole conjugates. Res Pharm Sci. 2019;14(5):408-413.
DOI: 10.4103/1735-5362.268201.

29. Ramezani P, Hejazi SH, Narimani M, Soleimanifard S. *In vitro* antileishmanial activity and apoptosis induction of *Pleurotus ostreatus* alcoholic extract on *Leishmania major*. Res J Pharmacogn. 2017;4(3):51-58.
30. Hassanzadeh F, Jafari E, Zarei S, Sadeghi-Aliabadi H. Synthesis, cytotoxic effect assessment and molecular docking studies of disubstituted thiadiazole including oxadiazole as hybrid component. Hacet Univ J Fac Pharm. 2022;42(4):228-237.
DOI: 10.52794/hujpharm.1069664.
31. Orlova DD, Novikova DS, Garabadzhiu AV, Tribulovich VG. A study on hydrolytic stability of isatin N-Mannich bases. Russ J Gen Chem. 2018; 88(1):52-60.
DOI: 10.1134/S1070363218010085.
32. Vine KL, Locke JM, Ranson M, Pyne SG, Bremner JB. An investigation into the cytotoxicity and mode of action of some novel N-alky l-substituted isatins. J Med Chem. 2007;50(21): 5109-5117.
DOI: 10.1021/jm0704189.
33. Ganguly S, Debnath B. Molecular docking studies and ADME prediction of novel isatin analogs with potent anti-EGFR activity. Med Chem (Los Angeles). 2014;4(8):558-568.
DOI: 10.4172/2161-0444.1000194.