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Synthesis and Molecular Docking Studies of 2-arylideneindan-1,3-diones Derivatives as an Inhibitor of 17β-hydroxysteroid Dehydrogenase Type 1

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Abstract

Due to the drawbacks of applying catalysts in the synthesis of α,β-unsaturated structure units and the importance of these materials, electrochemistry has been introduced as an efficient alternative. Therefore, herein a high-yield synthesis of 2-arylideneindan-1,3-diones is proposed. The procedure is carried out in propanol, using electrons as a green catalyst for generating propanol anion as a base, to obtain indandione anion which readily underwent Knovenagel condensation with aromatic aldehydes. The affecting parameters such as current, reagent ratio and anode type were studied and their optimized amounts were observed to be 40 mA/cm^2, benzaldehyde / indandion (3/1) and magnesium anode in an undivided cell at room temperature. The proposed method produces 2-arylideneindan-1,3-diones directly from initial compounds in a safe and mild condition. All synthesized compounds were screened by molecular docking studies using the crystal structure of 17β-hydroxysteroid dehydrogenase type 1. Among products compound 4a depicted minimum binding energy and good affinity toward the active pocket of 17β-hydroxysteroid dehydrogenase type 1 compared to Equilin as a 17β-hydroxysteroid dehydrogenase inhibitor.

Keywords:Electrosynthesis, Knoevenagel, inhibitors, 17β-hydroxysteroid dehydrogenase type 1,Molecular docking studies.

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Introduction

The Knovenagel condensation has been an important tool for constructing α,β-unsaturated structure units from a carbonyl and an active methylene component.[1] Previously, the Knoevenagel condensation was carried out using various catalysts such as amines and its corresponding ammonium salts, $TiCl_4$/base, $ZnCl_2$, $BiCl_3$, CdI_2, Al_2O_3, Ni-SiO_2, MgO, ZnO, $AlPO_4$-Al_2O_3, KF-Al_2O_3, natural phosphate and synthetic phosphate[2-5]. Although many investigations were aimed at finding new and more effective catalysts, the conditions allowing the complete elimination of catalysts are also being investigated. Considering this matter, effective alternative solutions are suggested by the electrochemical methodology. In other words, in the absence of catalysts and in mild conditions we can selectively obtain anionic intermediates by direct cathodic reduction or deprotonation of propanol which is used as a solvent in these reactions. 2-arylideneindan-1,3-diones are important intermediates for the synthesis of various compounds with biological activity such as antiphosphorylation inhibition of EGF-receptor and anti-proliferative activity[6] and thus are tremendously interesting in industries. On the other hand, they show important activities as anticoagulants, [7] antitumor [8] and cytotoxics[9].

Excess 17β-estradiol (E_2), the most potent of human estrogens, is known to act as a stimulus for the growth of tumors mainly in breast tissue. Human estrogenic 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), which catalyzes the reduction of inactive estrone (E_1) to the active 17β-estradiol in breast, is animportant enzyme responsible for elevated levels of E_2 in breast tumor tissues. Whereas 17β-HSD type 2 primarily catalyzes the oxidation of E_2 to E_1. In human breast, 17β-HSD type 1 is expressed in proliferative disease without atypia, atypical ductal hyperplasia, ductal carcinoma in situ and invasive ductal carcinoma. 17β -HSD type 2 has not been detected in any of these breast lesions. As appeared from the results, breast carcinoma can effectively convert E_1, produced as a result of in situ aromatization, to E_2, a biologically potent estrogen, which exerts estrogenic actions on tumor cells through estrogen receptor, especially the α subtype in carcinoma cells. Therefore, inhibiting intratumoral 17β-HSD type 1 is also considered to contribute to inhibition of cell proliferation by decreasing intratumoral estradiol. In a previously published work the structure of the ternary complex of 17β -HSD1 with the cofactor NADP1 and 3-hydroxyestra-1,3,5,7-tetraen-17-one (equilin), anequine estrogen was used in estrogen replacement therapy.Presented structural and kinetic data showed that 17β-HSD1- catalyzed the reduction of E_1 to E_2in vitro which is specifically inhibited by equilin [10].

Although several conventional methods were reported for the synthesis of 2-arylideneindan-1,3-diones using different solvents such as water, ethanol, glacial acetic acidand catalysts such as$ZrOCl_2 \cdot 8H_2O$, magnesium oxide, silica gel, sulfuric acid, andpiperidine but most of these

methodssuffer at least one limitation, matters such as low yields, high temperature requirement, complicated workup procedure, requiring large amounts of organic solvents, long reaction time, and technical intricacy[11-14].Therefore developing a simple and efficient method for synthesizing 2-arylideneindan-1,3-diones derivatives would be an interesting challenge.Taking all this data into account and also in continuation of our work on electro synthesis of organic compounds [15-18], herein, a simple, facile and rapid method for the synthesis andmolecular docking studies of 2-arylideneindan-1,3-diones is reported.

Experimental

Apparatus and Reagents

Controlled-current coulometry and preparative electrolysis were performed on a SAMA potentiostate/galvanostate (Isfahan, Iran). The working electrodes were an iron cathode (5 cm^2) and a magnesium anode (5 cm^2). NMR spectra were recorded using a Bruker DRX-300 Avance instrument. The IR spectra were recorded by a Bruker IFS-66 FT-IR spectrophotometer. Scanning electron microscopy (SEM) was run using an axl30 scanning electron microanalyzer (Philips, Netherlands) at 20.0 kV as an acceleration voltage. The melting point of the products was obtained using an electrothermal melting point apparatus (U. K.), model 9200. All compounds were commercially available, purchased from Merck and used without further purification.

General electro-synthesis procedure for the synthesis of nanoparticles of 2-(3-nitrobenzylidene)-1H-indene-1,3(2H)-dione

A mixture of 3- nitro-benzaldehyde (1a) (3 mmol, 0.453 g), indan-1, 3-dione (2) (1 mmol, 0.146 g), and NaBr (0.5 mmol, 0.05 g) in anhydrous propanol (25 mL) was stirred and electrolyzed in an undivided cell equipped with an iron cathode (5 cm^2) and a magnesium anode (5 cm^2) at room temperature, under constant current density of 40 mA/cm^2 (I = 200 mA). After the completion of the reaction (monitored by thin-layer chromatography; ethyl acetate/*n*-hexane 1/2), the resulting solid was separated by centrifugation. The product (4a) was collected for analysis. Yellow crystal; mp 240-242°C; yield(92%); IR (KBr) (νmax, cm^{-1}): 3094, 1731, 1686, 1591, 1528, 1350. ^{1}H NMR (300 MHz, DMSO-d_6) δ= 7.83 (1H, t, J = 7.5 Hz, Ar-H), 7.97-8.01 (5H, m, Ar-H), 8.41 (1H, d, J = 7.5 Hz, Ar-H), 8.67(d, J= 7.2Hz, Ar-H), 9.55(s, 1H, ArH).

Ligand preparation

Firstly, the structure of all the synthesized compounds was drawn using chem draw and converted for optimization by gauss view. Then fully optimized geometries and the electronic and structural

properties of all synthesized compounds were derived by means of density functional theory (DFT) method with the B3LYP functional, for all systems, a geometry optimization calculation was performed using STO-3G56 basis set. The calculations were carried out using Gaussian 03 package. The free available program Open Babel was used for generating SMILES strings from the optimized structures representation and using them for similarity study by PubChem Structure Search and Chemical Structure Search of Drug bank web service.

Target protein preparation

In this study 17β-hydroxysteroid dehydrogenase type 1 involved in apoptosis was chosen as the target of our study. The crystal structure of these molecules for docking calculation was obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home .do) by PDBID: 1equ. All water molecules, ligands and heavy atoms were removed from crystal structure of protein and then chain A of crystal structure was selected as target.

Results and discussion

Electro synthesis of 2-arylideneindan-1,3-diones

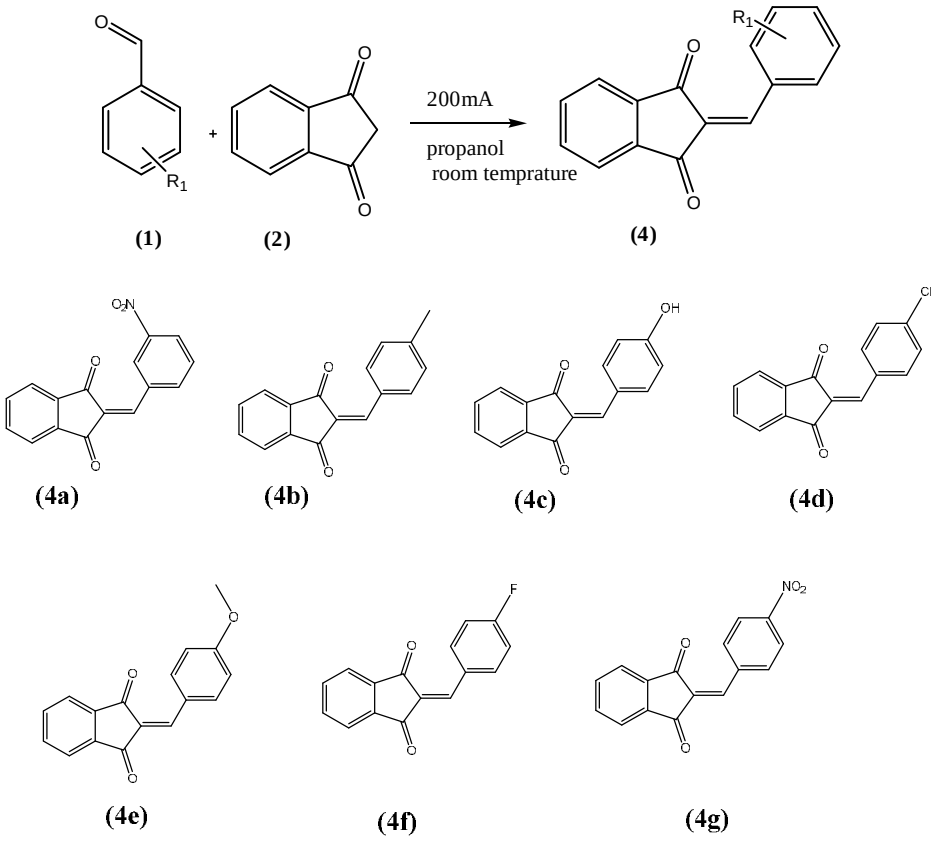
To the best of our knowledge, there are no reports about the electro synthesis of 2-arylideneindan-1,3-diones in propanol at ambient temperature using electrons as the catalyzing agent. In the model experiment, a mixture of 3-nitrobenzaldehyde (1a) and indan-1,3-dione (2) was electrolyzed and several parameters were investigated to achieve the optimum conditions, including current, stoichiometry of the reagents and anode. The results are summarized and reported in Table 1.

As it can be seen from Table 1, 1 mmol of each reagent using 50 mA and a magnesium anode at room temperature after 145 min obtained 2-(3-Nitro-benzylidene)-indan-1,3-dione (**4a**) with 80% yield (Entry1). Increasing the current amount up to 200 mA improved the yield (Entry 2-4). When graphite was used as the anode, the yield decreased while time of the reaction increased (Entriy 5) compared to the reactions performed in the presence of a magnesium anode (Entries 1-4). Increasing the amount of 3- nitro-benzaldehyde (1.5 and 3 mmol, Entries 6 and 7) improved the yield and decreased the reaction time. Reaction conditions of entry 7 afforded compound **4** with a synthetically useful yield and in a shorter period of time. Therefore, seven compounds were synthesized in the optimum conditions (Scheme 1, Table 2 providing considerable yields (78%-98%) within 28 min.

Table 1. Comparing of the effect different currents, reagent ratio and anodes on the reaction of 3- nitro-benzaldehyde (1a) and indan-1,3-dione (2) to obtain 2-(3-Nitro-benzylidene)-indan-1,3-dione (4a).

Entry[a]	Current (mA)	Reagent ratio (Aldehydes: indandion in mmol)	Time(min)	Electricity passed (F/mol)	Yield (%)
1[a]	50	1:1	145	4.5	80
2[a]	100	1:1	195	12.1	81
3[a]	200	1:1	130	16.2	87
4[a]	400	1:1	240	٥٩.٧	79
5[b]	200	1:1	240	29.9	76
6[a]	200	1.5:1	38	4.7	90
7[a]	200	3:1	28	3.5	92

For all the reactions, 0.5 mmol of NaBr, an iron cathode (5 cm^2), propanol as solvent, [a]magnesium anode (5 cm^2), [b] graphite anode (5 cm^2) were used at room temperature.



Scheme 1. Structures of 2-arylideneindan-1,3-diones 4a-4g.

Table 2. Results obtained in the reaction of a series of representative aryl aldehydes with indan-1,3-dione (2) to produce 2-arylideneindan-1,3-diones (**4a-4g**). [a]

Compound[a]	**R_1**	**Yield(٪)**[b]	**M. p(◦C)**	**Lit. M. p(◦C)**
4a	3-NO_2	92	240-242	245-247[13]
4b	4-CH_3	90	146-148	150-151[12]
4c	4-OH	85	230-232	233-234[11]
4d	4-Cl	94	170-172	173-174[12]
4e	4-OCH_3	78	154-156	156-157 [12]
4f	4-F	82	178-180	181.7[6]
4g	4-NO_2	98	231-233	229-230[13]

[a]For all the reactions, 0.5 mmol of NaBr, an iron cathode (5 cm^2), [a] magnesium anode (5 cm^2), and room temperature were applied. [b]Isolated yields based on indandion in the condensation of 3 mmol of arylaldhyde and 1 mmol of indandion. For all the products reaction time is 28 min.

The reaction with aldehydes carrying electron-donating groups such as methoxy group obtained relatively lower yields; however, those carrying aromatic electron-withdrawing groups mostly did quite well and gave almost quantitative yields. The identities of substances **4a-4g** were confirmed by IR and NMR spectroscopy. In the IR spectrum, two bands, one observed about ~1700 cm^{-1} and the other around ~1600 cm^{-1}, were attributed to the carbonyl groups. The ^1H NMR spectra contained proton characteristic signals of the vinylic hydrogen at ~ **9.0** ppm. While synthesizing these compounds, some interesting properties were observed,for instancethe products could not be sinteredusing a normal sinter or sinter glass.Centrifuge was needed for separating the products from propanol medium; then SEM micrographs of template-synthesized nanoparticles obtained from powder showed that the products are nanoparticles and their average size, DSEM, is < 100 nm (Figure 1).

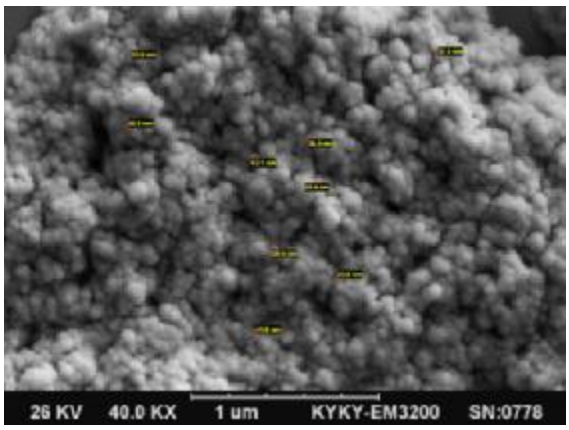
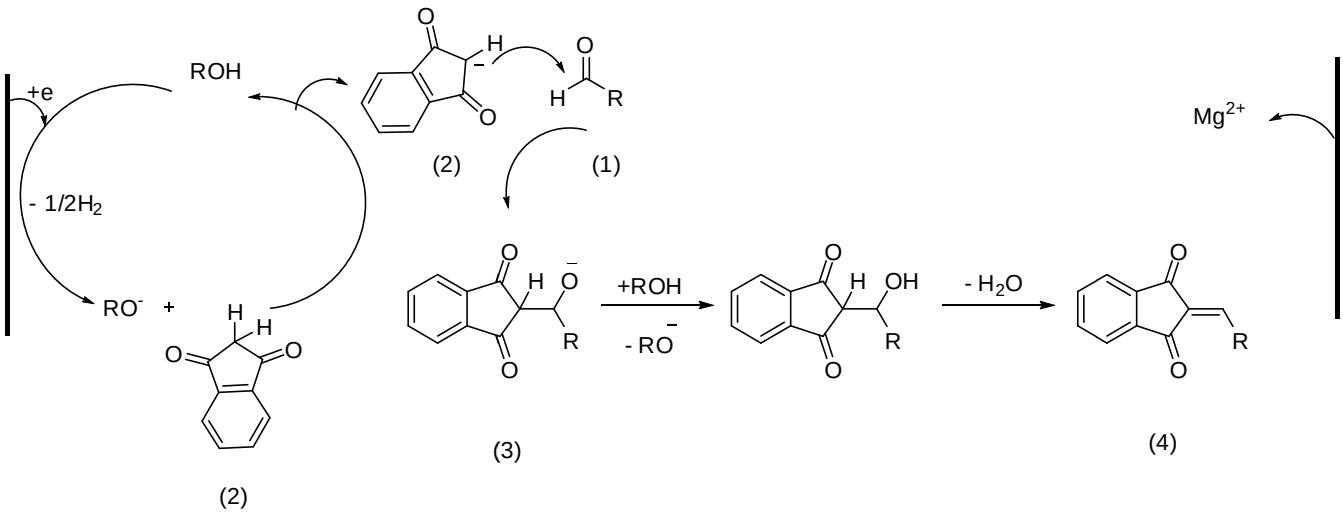


Figure 1.SEM image of nanoparticles of 2-(3-nitro-benzylidene)-indan-1,3-dione.

According to the experimental results, an acceptable mechanism was proposed for the electron-catalyzed Knoevenagel reaction. First, the deprotonation of an alcohol on the surface of the cathode leads to the formation of an alkoxide anion, and then the abstraction of a proton by the alkoxide anion yields a carbanion on the methylenic group. Subsequently the carbanion attacks the carbonyl group followed by the protonation of the negatively charged oxygen, which formed a hydroxyl group. Finally, a water elimination occurs to form a C=C bond (Scheme 2).



Scheme 2. Formation of 2-arylideneindan-1,3-diones derivatives.

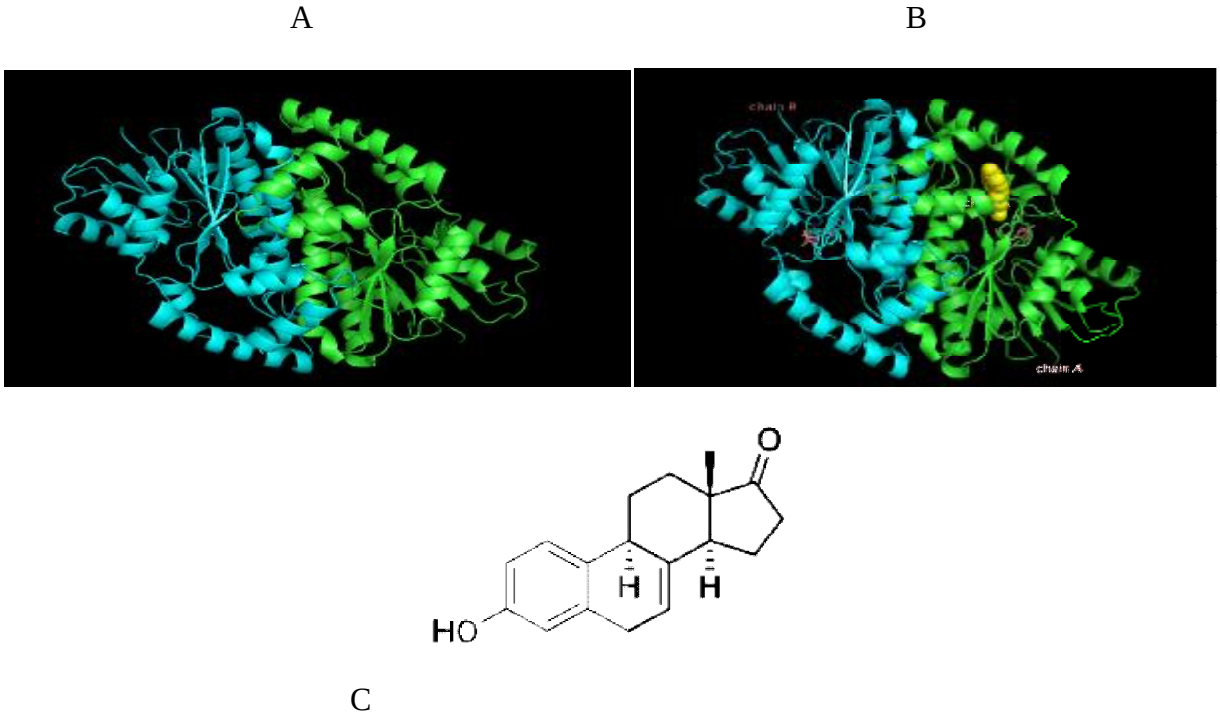


Figure 2. A: Crystal structure of 17 β -hydroxysteroid dehydrogenase. B: Crystal structure of complexed 17 β -hydroxysteroid dehydrogenase in complex with Equilin from Protein Data Bank. C : Equilin retrieved from pubchem substance database.

Figure 3 shows the results of the docking analyses depicting the best and most stable conformations plus binding site of all the synthesized molecules with the standard drug (equilin) using the program Autodock vina.

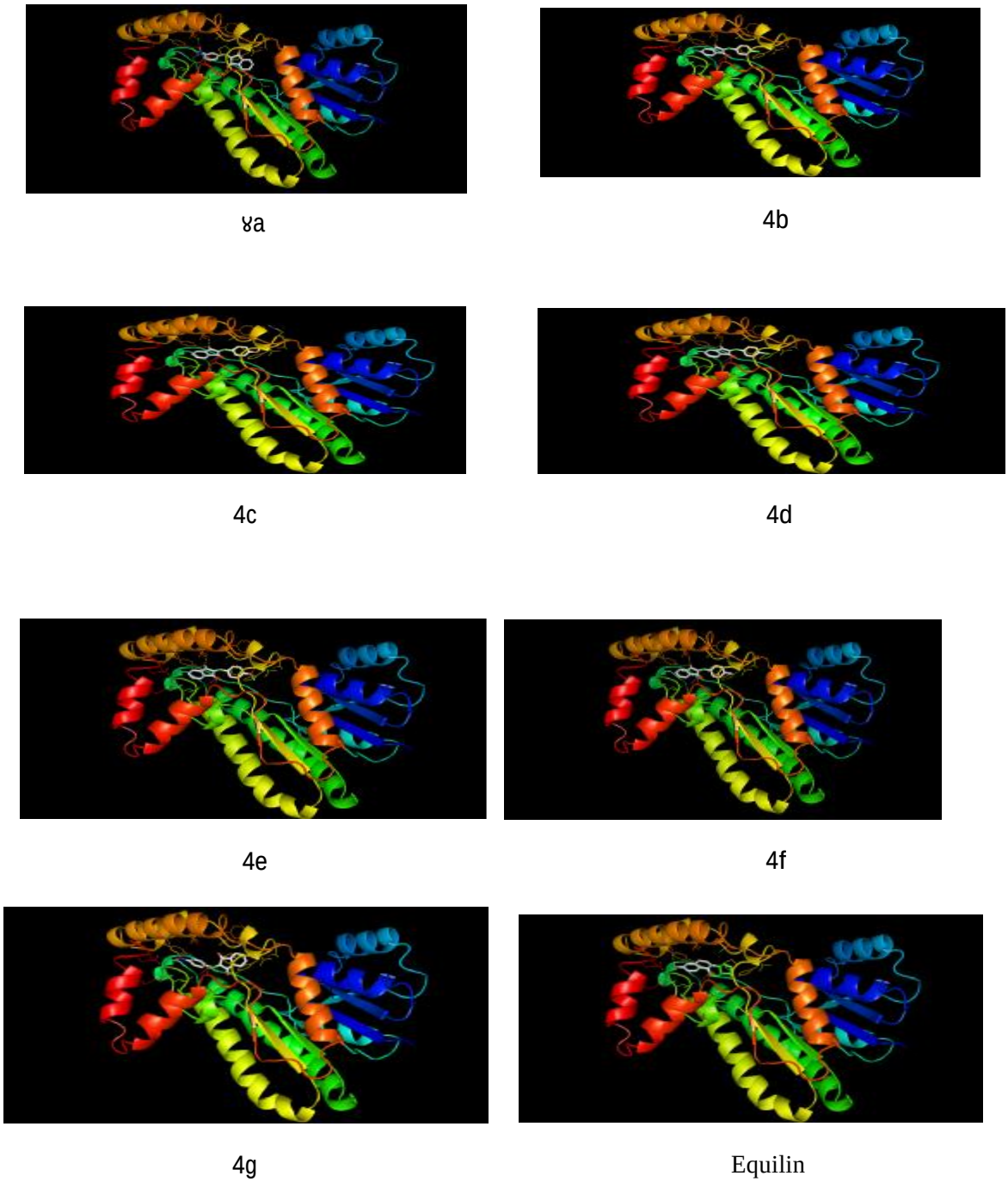
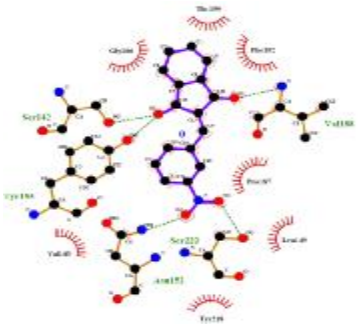
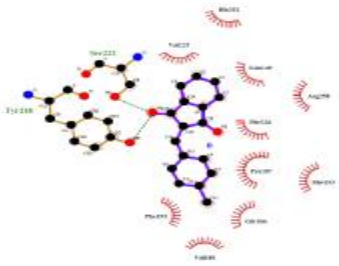


Figure 3. Binding site of chain A of 17β-hydroxysteroid dehydrogenase type 1 with all synthesized compounds and equilin (Autodock vina).

According to these results, all these compounds exhibit hydrogen bonding with one or more amino acids of 17β-hydroxysteroid dehydrogenase type 1 just similar to the standard drug (Figure 4).

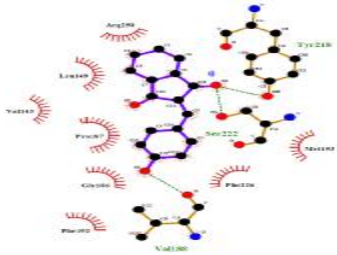


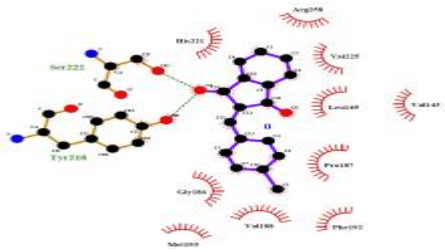


4a:4b:

Ser 142, Tyr155, Val 188, Ser 222, Asn152

Ser 222, Tyr 218

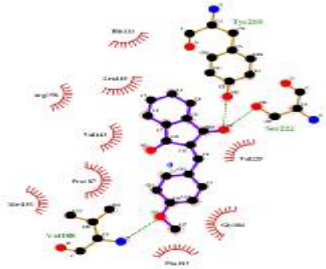


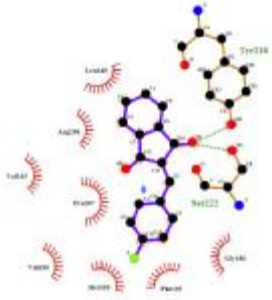


4c:4d:

Tyr 218, Ser 222, Val 188

Ser 222, Tyr 218



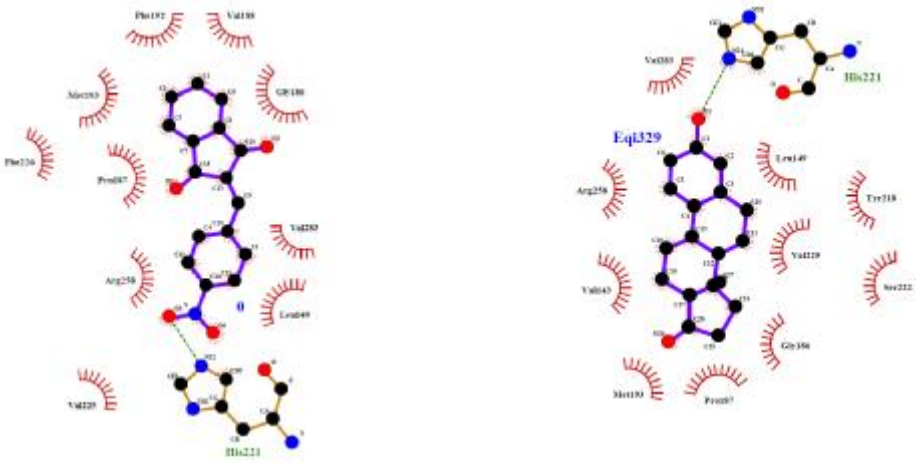


4e:

Tyr 218, Ser 222, Val 188

4f:

Tyr 218, Ser 222



4g: **Equlin:**

His 221 His 221

Figure 4. Docking of the hydrogen bonding amino acids of 17 β-hydroxysteroid dehydrogenase type 1 with synthesized compounds and Equilin in the active site of target protein.

The final docking score was calculated in terms of Kcal/mole for each docking experiment. A more negative score indicates that which of these synthesized compounds as a ligand is more likely to dock with 17β-hydroxysteroid dehydrogenase type 1 as a receptor and achieves more favorable interactions. [19] Interactions with the active site of target proteins are given in Table 3. The standard drug compound, equilin, gave a docking score of -9.4 for the structure extracted from Pubchem database.

Interestingly, although the molecular size of the synthesized compounds are very smaller to equilin but according to our in silico studies 4a derivatives reveal good binding energy toward the target protein. It was also observed that the compound containing a NO_2 group in the meta position of the phenyl ring, 2-(3-nitrobenzylidene)-1*H*-indene-1,3(2*H*)-dione 4a shows a better binding energy (-9.5 kcal/mol) compared to the other derivatives through investigating the results obtained from Autodock Vina.

Table 3. Binding energy and Inhibition Constant of the 2-arylideneindan-1,3-diones (4a-4g) and the standard drug Equilin.

Entry	Ligand	Free Energy of Binding (kcal/mol) Auto Dock Vina
١	Equilin_crystal[a]	-9.4
٣	Structure 4a	-9.5
٤	Structure 4b	-9.2
٥	Structure 4c	-9
٦	Structure 4d	-9.1
٧	Structure 4e	-8.9
٨	Structure4f	-9.1
٩	Structure4g	-9.2

[a] The native crystal structure of 17β-hydroxysteroid dehydrogenase type 1 in complex with Equilin was obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with thePDBID: 1equ.[b] Equilin was obtained from Pubchem (http://pubchem.ncbi.nlm.nih.gov/) database ID: 223368.

Conclusion

To conclude, a highly efficient and easy to apply method was presented for the production of 2-arylideneindan-1,3-diones due to the important biological properties of these compounds and complex conventional methods reported for the production of such organic compounds. We succeeded to synthesize nanoparticles of 2-arylideneindan-1,3-diones directly from its initial materials in a single step at room temperature with good to excellent yieldsusing electron as a renewable, inexpensive, easily available and environmental friendly reagent.These methods come along with several significant benefits, such as a using low concentration current, no by product isolation required, high yield, inexpensive reagents, environmental friendliness, and ease of product isolation.Molecular docking as an increasingly important tool for drug discovery was used for all compounds and their biological properties compared with equilin as a 17β-hydroxysteroid dehydrogenase inhibitor.

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