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N-substituted (*E*)-4-arylidene isoquinoline-1,3-dione derivatives as potent anticancer agents – Synthesis and molecular evaluations

Manikandan A. and Sivakumar A.*

School of Bio-Science and Technology, VIT University, Vellore, Tamil Nadu, India

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ABSTRACT

This article describes a two-step synthesis of (E)-4-arylidene isoquinoline-1,3-dione derivatives and their anticancer effects. First, an intermediate N-aryl homophthalimide was synthesized using ZnO as the catalyst. Second, a new modified synthesis of (E)-4-arylidene isoquinoline-1,3-dione derivatives in the presence of oxalic acid in ethanol (EtOH). The obtained products were characterized by FT- IR, GC/MS, ^{1}H NMR, and ^{13}C NMR. Synthesized isoquinoline derivatives 5a-h possesses significant cytotoxic activity against MCF-7 cell lines. Molecular docking study results are disclosing the structure-activity similarity of the synthesized compounds with anticancer evaluations. IC_{50} ± SD values for the compounds 5g (3.57±0.54 µg/ml), 5h (3.89±0.82 µg/ml) and 5d (4.42±0.98µg/ml) were very close to the standard doxorubicin (3.36±0.38 µg/ml). The mean percentage of inhibition for doxorubicin was 70.39±7.18, for our compounds, it was 33.14±6.61 (5b) to 61.75±8.22 (5g). At the e nd of study, the necessity of in vivo animal model evaluations was understood and the same studies are progressing currently.

Keywords: Homophthalic acid; N-arylhomophthalimides; ZnO; Cytotoxicity; FT-IR; Molecular docking

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INTRODUCTION

Isoquinolines have demonstrated widespread biological activities and constitute a large number of naturally occurring alkaloids. Fused isoquinolines were used as anti-inflammatory, anti-pyretic, and anti-cancer agents. 2,3 Dihydroimidazo[2,1-a]isoquinolines have been reported to have antitumor activity.[1] Therapeutics like antimicrobial, anticancer, anti-inflammatory, antioxidant have been identified in many natural products in the isoquinoline derivatives and α-methylene-γ-butyrolactones as bioactive ingredients.[2-4] Tetrahydroisoquinolines have been observed as major structural designs within the natural products and pharmaceutical compounds which are biologically active.[5,6] In the preliminary evaluations, our compounds 5a-h were shown remarkable antioxidant and anti-inflammatory activities.[7] Due to the inductive polarization of the carbonyl group at β-position, the carbonyl compounds (α,β-unsaturated) play an vital role as intermediates in many addition reactions of nucleophiles.[8] The antimalarial,[9] anticancer,[10–13] anti-inflammatory,[14] antibacterial[15] and antifungal[16] were evidently reported for α,β-unsaturated ketone derivatives. The genotoxicity, mutagenicity, and an anticarcinogenic effect toward cultured tumor cells were also observed.[17-19] Already, quite a few modifications have been made to counter the problems like reaction circumstance, costly and poisonous reagents, poor yields and stumpy selectivity of α,β-unsaturated carbonyl compounds.[20] We expect that isoquinolines associated with α,β-unsaturated carbonyl compounds might possess remarkable biological activities. Hence, the development of simple and efficient method for the synthesis of said compounds are important for scientific community. To the best of our knowledge, we are only the second, reporting the synthesis of N-substituted *(E)*-4-arylidene isoquinoline-1,3-dione. N. Jegham *et al.* (2012), reported the *(E)*-4-arylidene isoquinoline-1,3-dione derivatives synthesized using piperidine as catalyst.[21] In this study, we designed and synthesized the isoquinoline derivatives (*(E)*-4-arylidene isoquinoline-1,3-diones) targeted as anticancer drugs. The reaction method we have developed is simple and eco-friendly. We were using -4F, -4CH_3, and -4Cl at coupled R position and -4CN, -CH_3 and -4Br coupled at R_1 position. This R, R_1combination

is totally different from the reported method [21] thus all the final compounds achieved were new and novel. Since the compounds 5a-h were synthesized for the evaluation of cytotoxicity, the MTT assay[22] was found as a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. For the *in silico* studies, the Lamarckian Genetic Algorithm is the primary and promising method for conformations searching[23] and AutoDock have been proved to be a valuable tool capable of accurate prediction of the binding conformations and binding energies of ligands with macromolecular targets.[24-26]

MATERIALS AND METHODS

Chemistry

All the chemicals employed in the synthesis were of laboratory grade. Solvents and reagents were commercially available and purchased from sigma Aldrich and Avra synthesis. The melting points were observed on an Elchem digital melting point apparatus. FT-IR spectra were issued by SHIMADZU IR affinity 1 spectrometer with anhydrous KBr pellets in the range of 4000–400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were registered in a Bruker ADVANCE III 400 spectrometer in $CDCl_3$ solution using tetramethylsilane (TMS) as an internal standard. GC-MS was analyzed in GC model Clarus 680 and Mass Spectrometer Clarus 600 (EI); Perkin Elmer, Inc., USA.

Synthesis of N-aryl homophthalimides (3a-c)

A mixture of homophthalic acid (1) and substituted anilines (2) (1:1 ratio) in toluene, and 5 mol% ZnO were amended to the suspension. The reaction mixture was refluxed and the progress of the reaction was monitored by Thin Layer Chromatography (TLC). After the completion of the reaction, the catalyst was separated by filtration. The solvent was removed under vacuum, and then a crude reaction mixture was purified by silica gel column chromatography using ethyl acetate and n-hexane mixture as an eluant. The obtained pale yellow solid compounds (3a-c) were characterized by FT- IR, GC-MS, ^{1}H NMR and ^{13}C NMR.

Synthesis of (E)-4-arylidene isoquinoline-1,3-diones derivatives (5a-h)

The general synthesis of compound 5a-h was achieved as showed in the scheme 2. N-Substituted homophthalimide (3a-c) (0.001 mol) was dissolved in ethanol (10 mL) and the aromatic aldehyde (4a-c) (0.001 mol) followed by oxalic acid (5 mmol) was added and the reaction mixture refluxed for 5 hours. After cooling to room temperature, the solid, settled on the bottom was filtered and washed with 10 ml of ethanol and the solvent was evaporated in vacuum. The obtained product was crystallized in ethanol and the purity was tested by TLC. The obtained pale yellow solid compounds (5a-h) were characterized by FT-IR, GC-MS, ^{1}H NMR,^{13}C NMR.

In vitro Cytotoxicity studies:

Cell line Culture

Anticancer study was carried out using MCF-7 (Michigan Cancer Foundation-7), a human breast cancer cell line. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), (100U) 20 µg/ml penicillin, and 100 µg/ml streptomycin and incubated at 37 °C in an atmosphere of 5% CO_2. Normal breast (MCF-7) cells were cultured in 1:1 mixture of DMEM and Ham's F12 medium with 20 mg/ml of epidermal growth factor (EGF), 100 µg/ml cholera toxins, 0.01 mg/ml insulin and 500 µg/ml hydrocortisone, and 5% chelex treated horse serum. 1 ml of homogenized cell suspension was allowed in microtiter plate wells and kept in desiccator under 5% CO_2 atmosphere for bio-assay. Cells were observed in an inverted microscope after 48 hours of incubation. 0.05 ml of the drug was dissolved in 4.95 ml of DMSO to get a working concentration of 1 mg/ml. Drug concentrations were filtered using a 0.45 micron filter before bioassay.

MTT Assay

The anticancer activity of compounds 5a-h on MCF-7 cells was determined by the MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay the cytotoxicity. Approximately 5000 cells were seeded in 96-well, flat-bottom titer plates and incubated for 24, 48, and 72 hours at 37 ° C in 5% CO_2 atmosphere. Different concentrations of compounds 5a-h (50 – 500 µg/mL) were added and incubated further for various time periods. After completion of incubation, the medium was removed. The wells were washed with Phosphate Buffer Solution (PBS). To this 100 µL of the working MTT dye in DMEM media was added and incubated for 2 hours. MTT lysis buffer (100 µL) was added and incubation continued for 4 hrs more. The absorbance was measured at 570 nm and the cell viability was calculated using the following farmula,

Cell viability (%) = Mean OD/Control OD x 100%

Molecular docking studies

Docking studies were performed by using Autodock version 4.2.6 and Autodock Tools (ADT) version 1.5.6. and the Arguslab version 4.0.1. The structures of compounds 5(a-h) and the standard Doxorubicin were generated as ligands

using Chemdraw ultra 10.0 version of Cambridge University. Their 3D atomic coordinates were created utilizing the ACD/Labs – Chemsketch 12.0 software. Compound geometries were cleaned and generated as the corresponding *pdb*. files using the Argus lab software. The three dimensional crystal structure of the BRCT (BRCA1 carboxyl-terminal) repeat region from the breast cancer associated protein BRCA1 (PDB id: 1JNX) was retrieved from the protein data bank (PDB) (Source: www.rcsb.org/pdb/). The receptor protein and ligands in the docking studies were treated using the united-atom approximation and only polar hydrogens were added to the protein, and Kollman united-atom partial charges were assigned. Unless otherwise specified, all waters were removed. The *pdbqt* files for protein and ligands preparation and grid box creation were completed using Graphical User Interface program AutoDock Tools (ADT). AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 60 × 60 × 60 xyz points with grid spacing of 0.365 Å and grid center was designated at dimensions (x, y, and z): 1.185, 0.964 and 2.865.

Ligands 5(a-h) were docked into the active sites of the cancer associated protein BRCA1. Crystal structure of the BRCT repeat region from the breast cancer associated protein BRCA1 PDB ID: 1JNX was determined at 2.5 Å resolutions. The structure provides a basis to predict the structural consequences of uncharacteristic BRCA1 mutations. The results less than 2.0 Å in positional root-mean-square deviation (RMSD) was clustered together and represented by the result of the most favorable free energy of binding. Statistical mechanical analysis for the ligands 5(a-h) was analysed and the lowest binding energy, ligand efficiency, RMSD and the inhibitory constant (*ki*) values were extracted (Table **3**). Molecular interactions like hydrogen bonding, π-π interaction and π-cation interaction results were analysed and validated for structure activity.

Statistical analysis

All results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA with Dunnett's post test was performed. GraphPad Prism version 6.07 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The 50% inhibitory concentration (IC_{50}) was calculated from the dose response curve obtained by plotting percentage inhibition verses concentrations.

RESULTS AND DISCUSSION

Chemistry

Synthesis of *E*-4-arylidene isoquinoline-1,3-dione derivatives

The (*E*-4-arylidene isoquinoline-1,3-dione derivatives 5(a–h) were obtained in good yields, as we reported earlier[27], by condensation of aromatic aldehydes (4a-c) with N-phenylhomophthalimide (3a-c) in ethanol using oxalic acid as catalyst. Compounds 3a-c was achieved by heating a mixture of homophthalic acid (1) and substituted anilines (2) (1:1 ratio) in toluene and 5 mol% of ZnO. In order to confirm the structure of the compounds (5a-h) we have recorded NMR spectra for all the compounds in deuterated chloroform using Bruker Ascent 400 MHz spectrometer. Chemical shift values are reported in δ notation of parts per million using tetramethylsilane (TMS) as the standard. In ^{1}H-NMR spectra of all protons in the range of 7.20-8.30 ppm. The anisotropic effect of the carbonyl resulted in even larger downfield shift on the H-8 signal. All protons are aromatic in nature. The number of protons is matching with our expected products structure. In ^{13}C-NMR spectra, we have got two different carbonyl peaks. One is enone carbonyl group and another one is the amide carbonyl group.

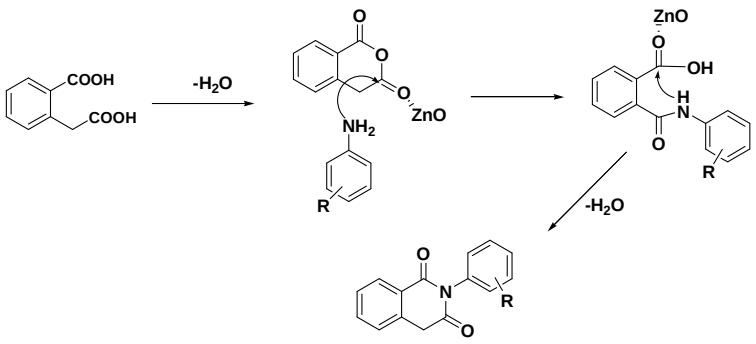

Figure 1: Proposed mechanism for formation of side-product 3a-c

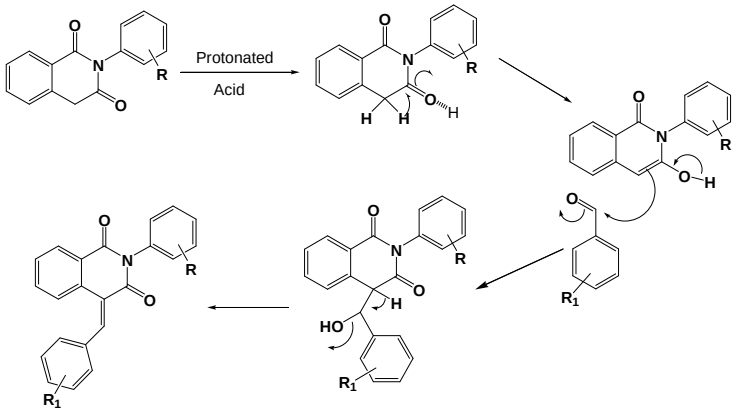

Figure 2: Proposed mechanism for formation of side-product 5a-h

Carbonyl groups in all the compounds appeared in the range of 162-165ppm. Since, the enone carbonyl group is slightly shielded compared to amide carbonyl due to conjugation, the peak appeared in the downfield. Remaining all aromatic carbons appeared in the range of 112-145ppm. The number of carbonyl peaks was matching with our expected products structure. Based on the infrared (IR) and NMR studies, it was understood that the 4-arylidene isoquinoline-1,3-dione derivatives 5(a-h) were formed in a trans configuration in all cases. Proposed mechanisms of compound 3a-c and 5a-h formation are shown in figure 1 and figure 2.

Anticancer studies

Determination of Cytotoxicity by MTT Assay

The human breast carcinoma cells (MCF-7) were incubated with different doses (50 to 500 µg/ml) of compounds 5a-h to evaluate the anticancer activity. MCF-7 cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and grown for another 24 hours. After 24 hours of incubation, cell viability was determined by the MTT assay and the inhibitory percentage were calculated (Table 1). Compounds 5a-h was able to inhibit the proliferation of the cancer cells (MCF-7). IC_{50} values indicate that some of the tested compounds were as active as doxorubicin. Compounds 5g (3.57±0.54 µg/ml), 5h (3.89±0.82 µg/ml) and 5d (4.42±0.98 µg/ml) were showed almost close activity when compare with doxorubicin (3.36±0.38). While considering percentage inhibition, it was 70.39±7.18 for doxorubicin. Among all the tested compounds, 5g showed (61.75±8.22) comparable % inhibition to doxorubicin. Rest of the compounds were showed a moderate activity. The least activity was found in 5b. None of the compounds showed activity as like as or more to the standard doxorubicin. *P-value* was remarkably best for the compound 5g (<0.0001) when comparing to the standard doxorubicin (0.0126).

Table 1. Inhibitory percentage results of MCF7 cell lines*

Entry	% Inhibition Mean±SD	R^2 value	p-value	**IC_{50} (µg/ml)
5a	45.56±7.28	0.9324	0.0076	8.82±1.24
5b	33.14±6.61	0.9171	0.0104	10.32±2.54
5c	36.41±6.27	0.9251	0.0089	9.84±2.78
5d	49.81±9.25	0.9203	0.0098	4.42±0.98
5e	46.46±9.43	0.9551	0.0041	10.01±2.81
5f	39.81±7.23	0.9235	0.0092	8.06±1.85
5g	61.75±8.22	0.9978	< 0.0001	3.57±0.54
5h	52.71±9.81	0.9725	0.0019	3.89±0.82
Std[#]	70.39±7.18	0.9062	0.0126	3.36±0.38

*[#]Standard-Doxorubicin, *Data were expressed as mean ±SD (n = 4), statistically significant differences are at P < 0.05, ** Data were expressed as mean ±SD (n = 4).*

Molecular docking analysis

Molecular docking studies of (E)-4-arylidene isoquinoline-1,3-diones derivatives were carried out to predict the anticancer activity. Dogsite [28] web server was employed to detect the binding pocket of 1JNX which is the crystal structure of the BRCT repeat region from the human breast cancer associated protein BRCA1.[29] Crystal structure of doxorubicin was used as the standard for docking analysis. The binding free energy for 1JNX was found between -23.94 (5g) and -5.54 kcal/mol (5b) (Table 2). These free energy values indicating that the newly synthesized compounds had shown a fortunate selectivity towards BRCA1. The 2D view of protein–ligand interactions of the best poses generated by 1JNX studied routines are shown in Figure **4-6**. All the top docked poses generated by each docking routine exhibited well-established bonds with one or more amino acids in the binding pocket of 1JNX. Different sets of hydrogen bonding interactions with the polar side chain residues Asn1774, Gln1811, Leu1701 and

Thr1773 were observed at distances within 2.6 Å. Ligands 5a-h were shown π-π interactions (Non-covalent interaction) whereas 5e, 5g and 5h did not show any π-π interactions. π-effects have an important contribution to biological systems since they provide a significant amount of binding enthalpy. π-π interactions involving π systems are pivotal to protein-ligand recognition.[30]

Table 3: Results of statistical mechanical analysis for 5(a-h)

Compounds	Binding Energy (kcal/mol)	Ligand Efficiency	RMSD Å	Inhibitory Constant (*ki*)
5a	-6.19	-0.23	0.95	28.96 uM
5b	-5.54	-0.21	2.41	86.28 uM
5c	-5.87	-0.21	8.03	49.59 uM
5d	-18.64	-0.69	0.89	20.05 uM
5e	-12.8	-0.47	6.60	21.60 fM
5f	-17.9	-0.66	18.7	601.3 pM
5g	-23.36	-0.87	1.70	7.480 aM
5h	-19.78	-0.78	3.30	12.47 nM
Doxorubicin	-20.01	-0.78	14.4	19.95 uM

Report says that ionic interactions (Cation-π interactions) can tune the pKa of nitrogenous side-chains by increasing the abundance of the protonated form which has implications for protein structure and function[31] hence, DNA bases are able to participate in cation–π interactions.[32, 33] It was reported that approximately 25% of nitrogen containing side chains Lys, Asn, Gln, and His were within van der waals contact with aromatics and 50% of Arg in contact with multiple aromatic residues.[34] Cation-π interactions were found between side chain residues ARG1835, LYS1702 and Ar-1, Ar-2, Ar-3 as they are within van der Waals contact. Except 5b and 5e, all compounds were found with cation-π interactions.

In this study, all ligand interactions with BRCA1 were exactly found in the predicted binding pocket (Fig. **3**). π-π interaction, π-cation interaction and three H-Bonds (Fig. **4**) was found for the standard doxorubicin which is the best among all docked compounds. Ligand efficiency showing the action potential of the compounds 5g (-0.87) and 5h (-0.78) when compared with the standard (-0.78) (Fig. **5** and **6**).

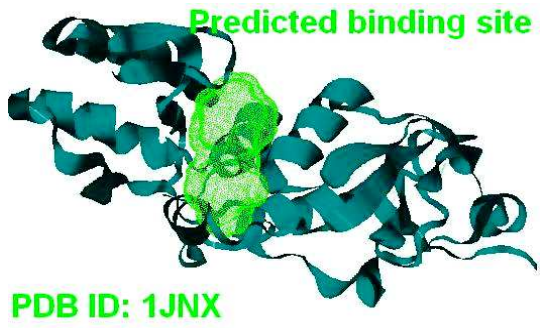

Figure 3: Predicted binding pocket for 1JNX

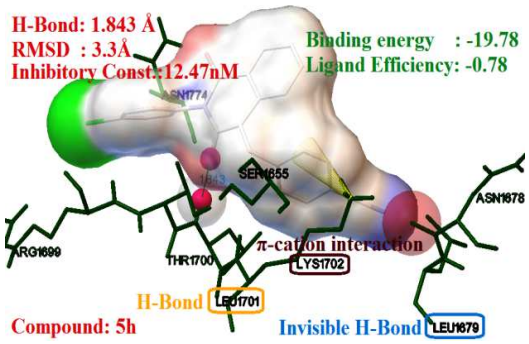

Figure 4: 1JNX-Doxorubicin interaction

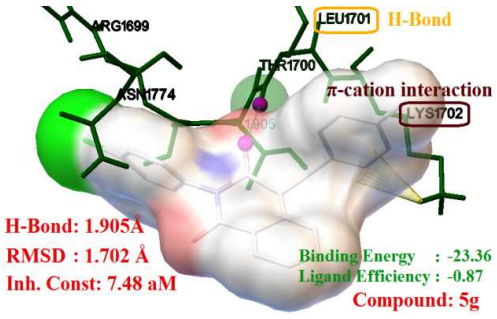

Figure 5: 1JNX-Compound 5g interaction

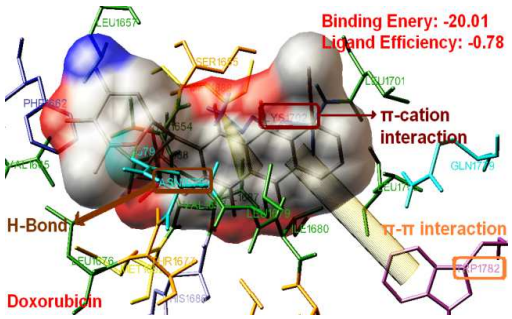

Figure 6: 1JNX-compound 5h interaction

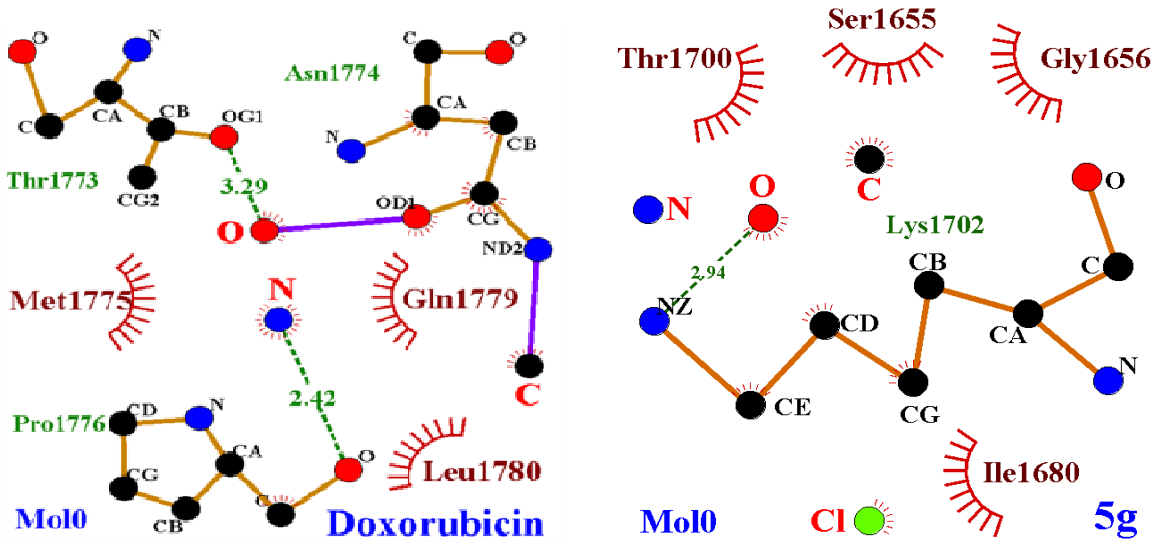

Figure 7a and 7b: Doxorubicin and compound 5h showing the interaction with the crucial residues in the active site cleft. (Orange= Non-ligand bond, Green line = H-Bond, Purple line = External bonds, Brick red rays around amino acid residues and all atoms = Hydrophobic interactions; Olive Green = H-bond lable, Brown = Hydrophobic residue, Red atom name = Ligand atom names, Black atom names = Non-ligand atoms, Blue atom names = Ligand residue names; Atoms – Red = Oxygen, Blue = Nitrogen, Black = Carbon, Lime green = Chlorine)

The estimated inhibition constant – (*ki)* value of 7.480 aM was found for the compound 5g with RMSD (Root-Mean-Square Deviation) tolerance 1.7 Å, but for doxorubicin the *ki* value is 19.95 µM with RMSD 14.4 Å. Doxorubicin shows perfect interaction with the crucial residues in the active site cleft of 1JNX (Fig. **7a**). H-Bonds to Asn1774, Thr1773 and Pro1776 were found with the ligand atoms N and O. Hydrophobic interactions between these two atoms were found with the residues Met1775, Gln1779 and Leu1780. Gly1656, Ile1680, Ser1655 and Thr1700 foud a hydrophobic interaction while Lys1702 found a H-bond to the top ranked compound 5g (Fig. **7b**).

Structure – activity similarity comparison

A structure activity similarity comparison with commercially available drugs was done in order to understand the drug efficiency of our compounds. The structure of the proposed compounds 5a-h (Sup Fig. **1-8**) has three aryle groups (Ar-1, Ar-2 and Ar-3), two carbonyl group and an enone (Fig. **8** and **9**). Docking results showed that all H-bond were generated only from carbonyl groups predominantly with enone carbonyl group. H-bonds could help with the water solubility of the ligands. Facilitating the halogen atoms results in more lipophilic and less water-soluble of a principle drug molecule.[35] So that, the halogen atoms are utilized to improve penetration through lipid membranes and tissues. The compounds 5c and 5h were substituted with –CN functional group at the C4 position.

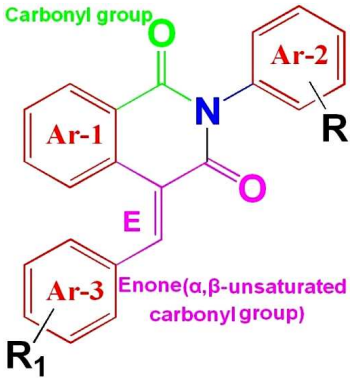

Figure 8: Functional groups of 5a-h

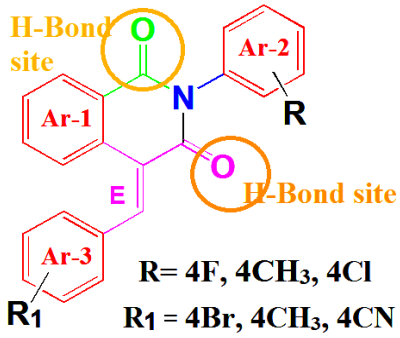

Figure 9: H-bonding site-Carbonyl groups

Fleming *et al.*, 2010, reported that the presence of a nitrile group has structural advances such as increased binding efficiency.[36] Also, the occurrence of nitrile-included pharmaceuticals proves the biocompatibility of the nitrile functionality.[37] Alogliptin (an anti-diabetic drug) having a cyanoaryl ring of a substituted quinazolinone (Fig. **10**) into a hydrophobic pocket augmented with hydrogen bonding between the nitrile and an arginine residue.[38] We found that the aforesaid point exactly matching with the compounds 5c and 5h, since their cyanoaryl group was found with π-π interaction and H-bond.

Erlotinib (Fig. **11**), a lung cancer drug, contains cyano group, has showed to be effective in patients with or without EGFR (Epidermal Growth Factor Receptor) mutations, but appears to be more effective in patients with EGFR mutations [39, 40]. Haloaryls are present in the compounds as functional units except 5e. All compounds were found with π-π interaction, π-cation interaction and H-Bonds. For example, Lapatinib (Fig. **12**) is a drug for breast cancer and other solid tumours having haloaryls.[41] When comparing the structure and function, compounds 5a-c, 5g and 5h are resembling with Lapatinip, a breast cancer drug. Which shows the need for *in vivo* evaluations of these compounds on breast cancer. In addition to that, Ciprofloxacin (Fig. **13**), contains C-F is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.

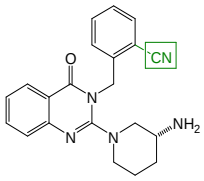

Figure 10: Alogliptin substituted quinazoline

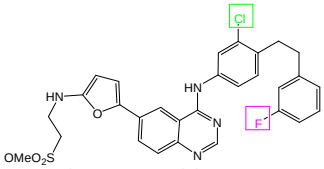

Figure 12: Lapatinip

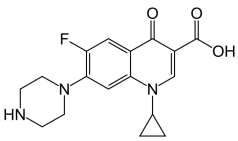

Figure 13: Ciproflaxacin

Compounds 5a, 5b and 5c also having the Fluorine as the functional unit in the Ar-2 ring system which may have same activity. Several drugs produced as C-Br has no biological significance.[42] Aryl groups Ar-1, Ar-2 and Ar-3 are mostly involving in all types of interactions (Table **4**) like non-covalent cation-π and hydrogen bonding (Fig. **4**). π-interactions are a type of non-covalent interaction that involves π systems. Cation–π interaction is also a noncovalent molecular interaction between the face of an electron-rich π system (e.g. benzene, ethylene, acetylene) and an adjacent cation (e.g. Li^+, Na^+).[43] From electrostatics (Coulomb's law), smaller and more positively charged cations lead to larger electrostatic attraction. Since cation- π interactions are predicted by electrostatics, it follows that cations with larger charge density interact more strongly with π systems. The major cation- π interaction were found with the Arg1699 and Lys1702 amino acid residues. Except compounds 5b, 5d and 5e, all compounds were shown cation- π interactions. Except 5e, 5g and 5h, all compounds were found with π-π interactions. We found that the interactions of molecules and activity efficiency of *in silico* and *in vitro* were almost resembling each other and the inevitability of *in vivo* study for the compounds 5a-h was realized.

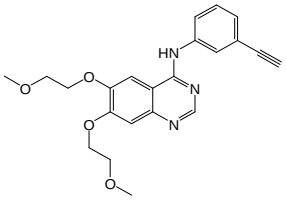

Figure 11: Erlotinip

Structure Activity Relationship

In order to explore Structure Activity Relationship (SAR) preliminarily, the compounds with different electron withdrawing and electron releasing groups –Cl (electron withdrawing), -F (electron withdrawing) and -CH_3 (electron donating) at R position (C16), -Br (electron withdrawing), -CH_3 (electron donating) and –CN (electron withdrawing) groups at R_1 position (C21) of isoquinoline ring were synthesized and evaluated for antiproliferative activity in MCF-7 cell lines. In the antiproliferative activity, SAR study demonstrated a significant increase in the potency of (E)-4-arylidene isoquinoline-1,3-diones derivatives that were substituted at C16-position (R) with functional groups. Compounds 5g IC_{50}±SD = 3.57±0.54 µM with -Br and -CH_3 groups, 5h IC_{50}±SD = 3.89±0.82 µM with –Cl and –CN groups and 5d IC_{50}±SD = 4.42±0.98 µM with –CH_3 and -Br groups against MCF-7 cell lines were found the most potent compounds. Compound 5g and 5h exhibited potent antiproliferative activities against MCF-7 cell line, which was almost equally active when compared with doxorubicin (IC_{50}±SD = 3.36±0.38 µM). Among all (C16) substituted compounds, 5b (IC_{50}±SD = 10.32±2.54 µM) and 5e (IC_{50}±SD = 10.01±2.81 µM) with R -F and -CH_3 showed less activity as compared to 5d. SAR studies based on IC_{50} values (Table **2**) showed that compounds 5g and 5h were the most potent compounds (with -Br and -CH_3 groups in 5g and with –Cl and –CN groups in 5h). Biological activity data suggested that a proper degree of electron density on isoquinoline ring was necessary to retain the activity of synthesized compounds.

CONCLUSION

In conclusion, (E)-4-arylidene isoquinoline-1,3-dione derivatives (5a-h) were successfully synthesized in good yield. Compounds 5a-h was emerged as the anticancer agents in this study. Compounds 5d, 5g and 5h were displayed the best antiproliferative effect against the MCF-7, a human breast cancer cell line. A structure activity similarity

comparison with commercially available drugs was done in order to understand the drug efficiency of our compounds. SAR study demonstrated a significant increase in the potency of (E)-4-arylidene isoquinoline-1,3-dione derivatives that were substituted at C16 and C-21 position (R and R1) with different groups. With these positive results, we are currently proceeding *in vivo* studies in animal models.

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