Original Article

Docking Studies, Synthesis and Evaluation of Anticancer Activity of 4H-Chromene Derivatives

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ABSTRACT

Aim: The present study involves to design, synthesis and evaluate the anticancer activity of 4H-chromene derivatives (PKB 1-10) on human breast cancer MCF-7 cell line. **Materials and Methods:** 4H-chromene derivatives (PKB 1-10) were designed by docking, *in silico* ADME and predicted toxicity studies. These designed compounds were then synthesized by acid catalyzed Michael Addition of phenols to benzylidene oxobutanoates. These compounds were characterized by ^{1}H NMR, ^{13}C NMR, FTIR and melting point. Then all synthesized compounds were tested for anticancer activity on MCF-7 cell line. **Results:** Compounds PKB-4 and PKB-10 showed better docking scores than standard drug, Adriamycin. *In silico* ADME and toxicity studies were also found significant for most of the compounds. The majority of the compounds displayed promising to potent anticancer activity on MCF-7 cell line. **Conclusion:** It may be concluded that most of the compounds showed significant docking and *in silico* ADME and toxicity profiles. Compounds have excellent anticancer potential and could be considered as novel anticancer agents for more investigation.

Key words: Anticancer, 4H-chromenes, Docking, MCF-7 cell line, Toxicity.

INTRODUCTION

Cancer is one of the leading causes of death in the world among all chronic diseases.[1] Breast cancer is the most prevalent form of cancer diagnosed in women worldwide and affecting 2.1 million women each year. According to 2018 report, the estimated number of female died due to breast cancer was 6.2 million. It is accounting approximately 15% of all cancer deaths among women.[2] There are many difficulties in the treatment of cancer, but the most concerning are drug resistance, toxicity and low specificity.[3] Therefore, there has been increscent interest in the field of cancer chemotherapy by discovery and development of novel agents with high efficacy, low toxicity and minimum side effects.[4] Due to the less selectivity of suitable drugs, drug resistance and complex mechanisms, the current drug treatment of breast cancer seem to be challenging.[5] Therefore, the development of potent, competent and having less adverse effect anticancer agents over the synthesis of new molecules is significant in breast cancer research.

4H-Chromenes are important heterocyclic pharmacophores with a benzene ring fused to a pyran nucleus. They are found in natural and synthetic compounds and have recently attracted much more interest due to their useful biological and pharmacological properties. A wide range of new chromene-based natural products were reported during the last two decades.[6] These heterocyclic natural and synthetic benzopyran derivatives possess important biological properties such as anticancer,[7] anti-coagulant,[8] antimicrobial,[9] anti-proliferative,[10] antioxidant,[11] antifungal,[12] antiestrogenic,[13] antiviral,[14] antitubercular,[15] anti-inflammatory[16] and anticonvulsant[17] activities. During recent years, several researchers developed

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different 4H-chromene derivatives with anti-breast cancer activity through the introduction of various heterocyclic scaffolds (Figure 1).

Recently, high-throughput screening of drug libraries results in the identification of some new 4H-chromene compounds that exhibited cytotoxic activity against breast cancer cell lines MCF-7 and MBD-MB-231. MCF-7 breast cancer cells are estrogen (E2) sensitive cells and depend on E2 in order to proliferate. They express high levels of ERα transcripts but low levels of ERβ. They are used pervasively in research for ER-positive breast cancer cell experiments and development of anti-estrogen drug. MCF-7 cells are well-suited for anti-hormone therapy studies since they are readily cultured and retain ER expression when they were treated with such targeted-therapy.[18]

The Cytochromes P450 (CYPs) constitute the major enzyme family efficient of catalyzing the oxidative biotransformation of most drugs and therefore of particular relevance for clinical pharmacology.[19] Overexpression of P-glycoprotein leads to multidrug resistance in the ovarian cancer cell line.[20] Accordingly, in continuation of our research program to find anti-breast cancer agents and considering the importance of 4H-chromenes as anti-breast cancer agents, we have synthesized a series of 4H-Chromene derivatives.

Therefore, we hereby report the design, synthesis and anticancer property of 4H-chromene derivatives (PKB 1-10). These compounds were screened for their anti-breast cancer properties on the MCF-7 cell line.

MATERIALS AND METHODS

All chemicals used were procured commercially from Spectrochem and Avra Synthesis Pvt. Ltd. Solvents were dried and distilled according to conventional procedures. Reactions were carried out under nitrogen (N_2)

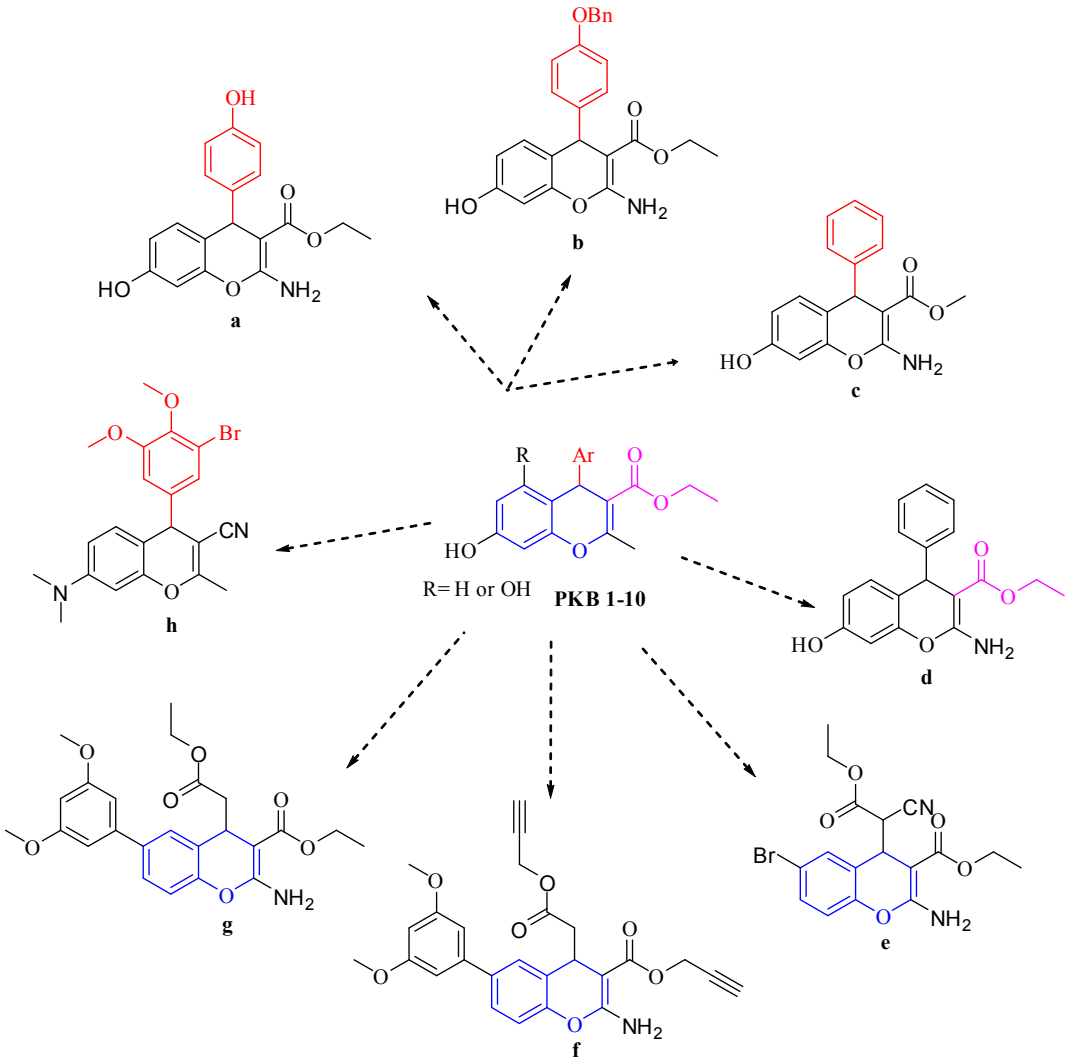


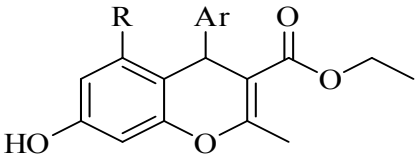
Figure 1: Pharmacophoric pattern of anticancer drugs (a-h) and model compounds (PKB 1-10).

atmosphere. The progress of the reaction was checked by Thin Layer Chromatography (TLC). Melting points were measured in open glass capillaries. NMR spectra were carried out on the JEOL ECX-400 spectrometer in DMSO-d^6 and $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts (δ) were determined in δ parts per million (ppm) relative to TMS. The mass spectra of samples were carried out on Waters Q-Tof Premier-HAB213 spectrometer. The FTIR spectra (KBr) of samples were carried out on a PerkinElmer Spectrum Version 10.03.06 spectrophotometer.

RESULTS AND DISCUSSION

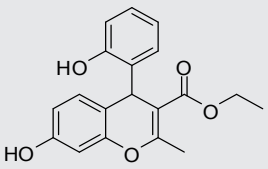
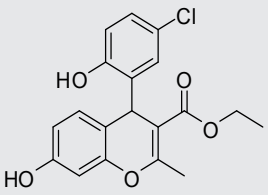
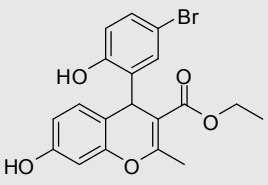
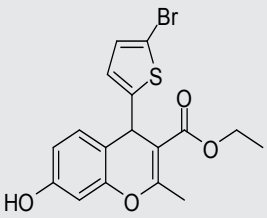
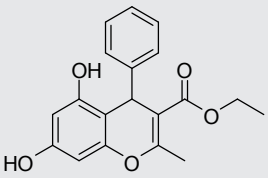
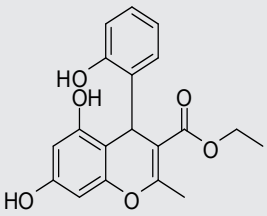
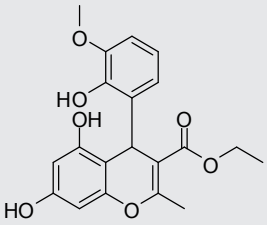
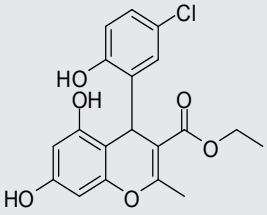
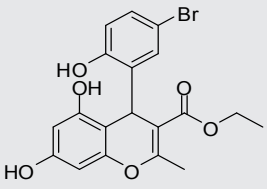
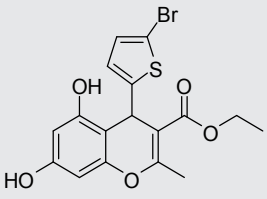
Molecular Docking

The molecular docking studies of compounds (PKB 1-10) was performed by Autodock 1.4.6 software on estrogen receptor (PDB: 2POG) for anti-breast cancer screening (Table 1). The docking score of compounds was ranging between -10.26 to -7.10. The compound PKB-10 having 5-bromothiophen moiety (-10.26) showed the highest and better docking scores than standard drug, Adriamycin (-9.90). Other compound



PKB 1-10

Table 1: Docking scores of designed compounds PKB 1-10 and standard drug (Adriamycin).

Compounds	Structures	Docking Score
PKB-1		-9.02
PKB-2		-8.51
PKB-3		-8.61
PKB-4		-9.94
PKB-5		-9.75
PKB-6		-9.04
PKB-7		-7.54
PKB-8		-7.19
PKB-9		-7.10
PKB-10		-10.26
Adriamycin	-	-9.90

PKB-4 having 5-bromothiophen moiety (-9.94) with one hydroxyl group also possessed great result. Therefore, it can be postulated that compound with 5-bromothiophen moiety displayed best results among the series. Compounds PKB-1 (-9.02), PKB-5 (-9.75) and PKB-6 (-9.04) also exhibited promising score. The

compound PKB-7 (-7.54) possess 5-methoxyphenyl group yielded less score as compared to other compounds having only hydroxyl group. Thus, presence of methoxy group yielded less activity. The results showed that the presence of electronegative groups (chlorine and bromine) were responsible for a significant docking score. The binding pose of best docked compound PKB-10 with alpha ligand-binding domain of the estrogen receptor was shown in Figure 2. The -O- of hydroxyl group in highest docking scored compound PKB-10 involved in hydrogen bonding interaction with LEU346 via -H of -NH. The visualization pose of the ligand WST (3AS,4R,9BR)-4-(4-Hydroxyphenyl)-1,2,3,3a,4,9b-hexahydrocyclopenta[c]chromen-9-ol) with ERα binding pocket of the estrogen receptor was shown Figure 3.

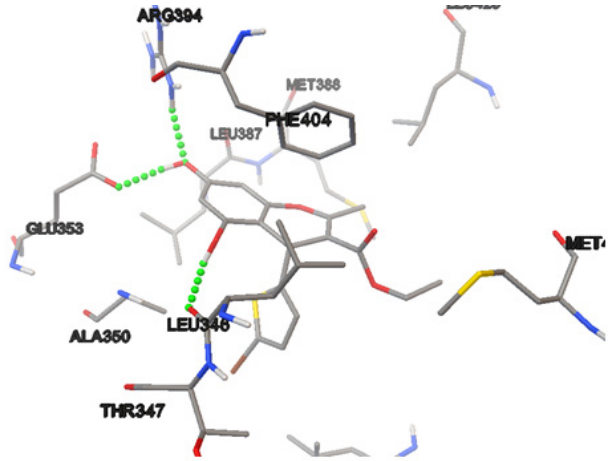


Figure 2: Docked conformation of PKB-10 showing important amino acid residues of the alpha ligand-binding domain of the estrogen receptor. H-bonds are displayed in green dotted line.

Predicted ADME Studies

All designed compounds (PKB 1-10) showed high gastrointestinal absorption (GI). Compounds PKB-1, PKB-2 and PKB-5 showed BBB permeability. Compounds PKB-2, PKB-6 and PKB-7, displayed inhibition to P-glycoprotein. All compounds showed inhibition to Cytochrome P 450 isomers (CYP1A2). Compounds PKB-1, PKB-5, PKB-6, PKB-7, PKB-8 and PKB-9 possessed inhibition for CYP2D6. All compounds followed drug-likeness prediction depending on the selected Ghose, Lipinski and Veber rule. All compounds displayed excellent bioavailability score. It could be seen that the majority of compounds among the series possessed better pharmacokinetic properties (Table 2). The ADME results of compounds (PKB 1-10)

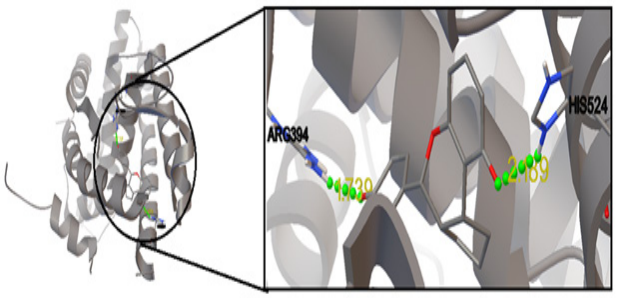


Figure 3: Autodock-predicted visualization pose of the ligand WST with ERα binding pocket of the estrogen receptor.

Table 2: Pharmacokinetic studies (ADME) of compounds (PKB 1-10).

Compounds	Pharmacokinetics						Drug-likeness			
	GI absorption	BBB permeant	P-gp	CYP1A2	CYP2D6	Log K_p (skin permeation), cm/s	Lipinski	Ghose	Veber	Bioavailability Score
PKB-1	High	Yes	No	Yes	Yes	-5.89	Yes	Yes	Yes	0.56
PKB-2	High	Yes	Yes	Yes	No	-5.65	Yes	Yes	Yes	0.56
PKB-3	High	No	No	Yes	No	-5.88	Yes	Yes	Yes	0.56
PKB-4	High	No	No	Yes	No	-5.54	Yes	Yes	Yes	0.56
PKB-5	High	Yes	No	Yes	Yes	-5.89	Yes	Yes	Yes	0.56
PKB-6	High	No	Yes	Yes	Yes	-6.24	Yes	Yes	Yes	0.56
PKB-7	High	No	Yes	Yes	Yes	-6.45	Yes	Yes	Yes	0.56
PKB-8	High	No	No	Yes	Yes	-6.24	Yes	Yes	Yes	0.56
PKB-9	High	No	No	Yes	Yes	-6.24	Yes	Yes	Yes	0.56
PKB-10	High	No	No	Yes	No	-5.88	Yes	Yes	Yes	0.56

P-gp: P-glycoprotein; GI: Gastro Intestinal; BBB: Blood Brain Barrier; CYP1A2: Cytochrome P450 family 1 subfamily A member 2 (PDB: 2HI4); CYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB: 5TFT).

were compared with adriamycin, an anti-breast cancer drug. Adriamycin displayed low oral bioavailability[21] and poor penetration into the brain.[22] The efflux of the adriamycin was mediated by P-glycoprotein[23] and it competitively inhibits cytochrome P450 enzymes in human liver microsomes.[24]

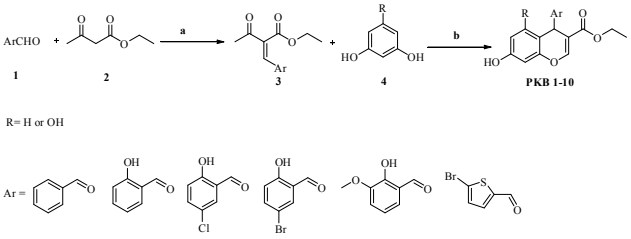
Predicted Toxicity Studies

All compounds predicted to be non-mutagenic and non-carcinogenic except for compound PKB-7 which was found to be mutagenic. All compounds had significant drug score. None of the compounds showed positive DL values. Compound PKB-6 displayed better solubility than other compounds. High cLogP (CLP) values are an estimation of low hydrophilicity and therefore cause low absorption or permeation. Compounds PKB-1, PKB-5, PKB-6 and PKB-7, displayed significant cLogP values (Table 3). The toxicity results of compounds (PKB 1-10) were compared with adriamycin. Despite the clinical effectiveness of adriamycin, its use is limited by off-target adverse effects, particularly dose-related cardiotoxicity, systemic toxicities and renal toxicity, which involves the free radical formation and tissue damage.[22]

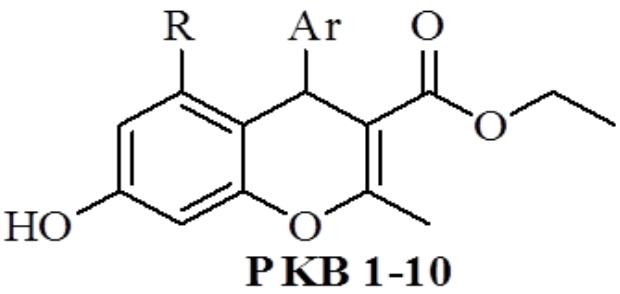
Chemistry

The 4H chromene derivatives (PKB 1-10) were synthesized by following known procedures of literature.[25] The first step involved the synthesis of substituted ethyl benzylidiene oxobutanoates 3 by the reaction between substituted aromatic aldehydes 1 and ethyl acetoacetate 2 in DMSO at 80°C using base L-proline. The synthesis of final compounds (PKB 1-10) was accomplished by trifluoroacetic acid (TFA) catalyzed Michael addition of phenols 4 to different substituted ethyl benzylidiene oxobutanoates 3. In all cases, the targeted final 4H chromene derivatives (PKB 1-10) were obtained in good yield (Scheme 1).

Compounds (PKB 1-10) displayed a characteristic broad absorption band of the hydroxyl group (-OH, stretch) at 3333-3512 cm^{-1}. In ^1H NMR, the $-CH_3$ of



Scheme 1: Synthetic pathway for compounds PKB 1-10. (a) L-Proline (30 mol%), DMSO, 80°C, 8 h; (b) TFA, CH3NO2, reflux , 4-7 h



PKB 1-10

Table 4: Anti-breast cancer activity of compounds (PKB 1-10) on MCF-7 cell line.

Compounds	GI_{50} (µg/ml)[a]	Compounds	GI_{50} (µg/ml)[a]
PKB-1	65.20	PKB-7	3.72 X10^3
PKB-2	7.20	PKB-8	15.04
PKB-3	40.40	PKB-9	21.06
PKB-4	2.37	PKB-10	4.11
PKB-5	3.26	Adriamycin	<10
PKB-6	34.20		

[a]GI_{50} = Concentration of drug causing 50% inhibition of cell growth

Table 3: Toxicity analysis results based on compounds (PKB 1-10).

Compounds	Toxicity Risks		Osiris Calculations				
	MUT	TUMO	MW	CLP	S	DL	D-S
PKB-1	Green	Green	326	2.88	-3.32	-6.46	0.41
PKB-2	Green	Green	360	3.49	-4.05	-5.73	0.36
PKB-3	Green	Green	404	3.60	-4.15	-8.04	0.34
PKB-4	Green	Green	395	4.02	-4.47	-8.37	0.31
PKB-5	Green	Green	326	2.88	-3.32	-4.43	0.42
PKB-6	Green	Green	342	2.53	-3.02	-5.08	0.42
PKB-7	Red	Green	372	2.46	-3.04	-6.11	0.20
PKB-8	Green	Green	376	3.14	-3.76	-4.24	0.38
PKB-9	Green	Green	421	3.26	-3.85	-6.54	0.35
PKB-10	Green	Green	411	3.68	-4.18	-6.99	0.33

ester group was observed as a triplet at δ 1.30-1.07 ppm and >CH_2 of ester group was observed as multiplet at δ 4.49-3.96 ppm in all compounds. The single proton at 4th position was observed as a singlet at around δ 4.16-5.31 ppm in all compounds. Three protons of the methyl group at 2nd position were found as a singlet at around δ 2.43-1.87 ppm. Other aromatic and aliphatic protons were present at their respective place. In ^{13}C NMR of compound PKB-4 and PKB-10, -CH_3 and >CH_2 of ester group were observed at δ 60.7 ppm and 14.3 ppm, respectively.

In vitro Anticancer Screening

The synthesized compounds (PKB 1-10) were tested for their *in vitro* anticancer activity against human cancer (MCF-7) cell line by using sulforhodamine B assay (SRB Assay).[26] The GI_{50} concentration of all compounds was calculated with reference to a control sample. For each compound, 50% growth inhibition (GI_{50}) was determined from Sigmoidal dose-response curves and given in Table 4. For reference purposes, adriamycin (ADR) data were included.

The resultant data showed that compounds exhibited potent cytotoxic effects on human breast cancer MCF-7 cell line (Figure 4). Among the series, compound PKB-4 showed the most potent activity (GI_{50}= 2.37 µg/ml). The compounds PKB-2 (GI_{50}= 7.20 µg/ml), PKB-5 (GI_{50}= 3.26 µg/ml) and PKB-10 (GI_{50}= 4.11 µg/ml) also displayed significant activity as compared to standard, Adriamycin (GI_{50} <10 µg/ml). It had been found that the presence of 5-bromothiophen moiety accounted for potent activity as in compound PKB-4 and PKB-10. These compounds also displayed significant docking scores. The compound having no substitution at benzene ring attached to the benzopyran ring also exhibited potent activity. Compounds PKB-8 (GI_{50}= 15.04 µg/ml) and PKB-9 (GI_{50}= 21.06 µg/ml) possessed promising activity. Other compounds also showed somewhat activity. The presence of methoxy moiety resulted in less activity as in compound PKB-7 (GI_{50}= 3.72 X10^3 µg/ml).

The results revealed that compounds (PKB 1-10) were active on the MCF-7 cell line; therefore, it can be postulated that compounds were active against ER-positive breast tumours. About 80% of breast cancers were reported as ER-positive.

The structures of synthesized compounds (PKB 1-10) were confirmed by ^1H-NMR, ^{13}C-NMR spectra, FT-IR and melting point analysis. The obtained data and melting points were matching with known data of literature.[25]

Experimental Section

Molecular Docking

The autodock tool (autodock 4.2) was used for creating PDBQT files from traditional PDB files. The sequence of WST (3AS,4R,9BR)-4-(4-Hydroxyphenyl)-1,2,3,3a,4,9b-hexahydrocyclopenta[c]chromen-9-ol) was retrieved from UniProt database.[27] The three-dimensional structure of WST was downloaded from the PDB database. The drug compound structures were drawn using ACD chem sketch and converted into PDB format using an open Babel tool. The 3D structures of above targeted proteins were docked with designed compounds (PKB 1-10) using autodock software. The docking results were analyzed using the discovery studio visualize tool. The ligands were prepared in the autodock 4.2 for docking studies. The optimized ligands were docked into targeted proteins using "Ligand fit" model in autodock 4.2. The energy interaction between protein and ligand was calculated.

Table 5: Compounds (PKB 1-10) differing in the substitution at R and Ar.

Compounds.[a,b]	R	Ar	Reaction Time (hours)	Mol. formula	Mol. weight	Yield (%)[c]	MP (°C)
PKB-1	H	2-hydroxyphenyl	5	$C_{19}H_{18}O_5$	326.34	71	151
PKB-2	H	5-chloro-2-hydroxyphenyl	6	$C_{19}H_{17}ClO_5$	360.07	65	155
PKB-3	H	5-bromo-2-hydroxyphenyl	6	$C_{19}H_{17}BrO_5$	404.23	64	170
PKB-4	H	5-bromothiophen	6	$C_{17}H_{15}BrO_4S$	395.26	65	110
PKB-5	OH	phenyl	5	$C_{19}H_{18}O_5$	326.34	78	102
PKB-6	OH	2-hydroxyphenyl	4	$C_{19}H_{18}O_6$	342.34	80	150
PKB-7	OH	2-hydroxy-5-methoxyphenyl	5	$C_{20}H_{20}O_7$	372.36	73	120
PKB-8	OH	5-chloro-2-hydroxyphenyl	4	$C_{19}H_{17}ClO_6$	376.78	75	162
PKB-9	OH	5-bromo-2-hydroxyphenyl	4	$C_{19}H_{17}BrO_6$	421.23	76	174
PKB-10	OH	5-bromothiophen	5	$C_{17}H_{15}BrO_5S$	411.26	75	130

[a]Reagents and conditions: **3** (1.2 mmol), **4** (1 mmol), TFA (1 mmol), nitromethane (5 ml), reflux 4-7 h.
[b]Final product was confirmed by the analysis of ^1H NMR, ^{13}C NMR, FT-IR and melting points.
[c]Yield refers to pure products after chromatography using 20% ethyl acetate in hexane as eluent.

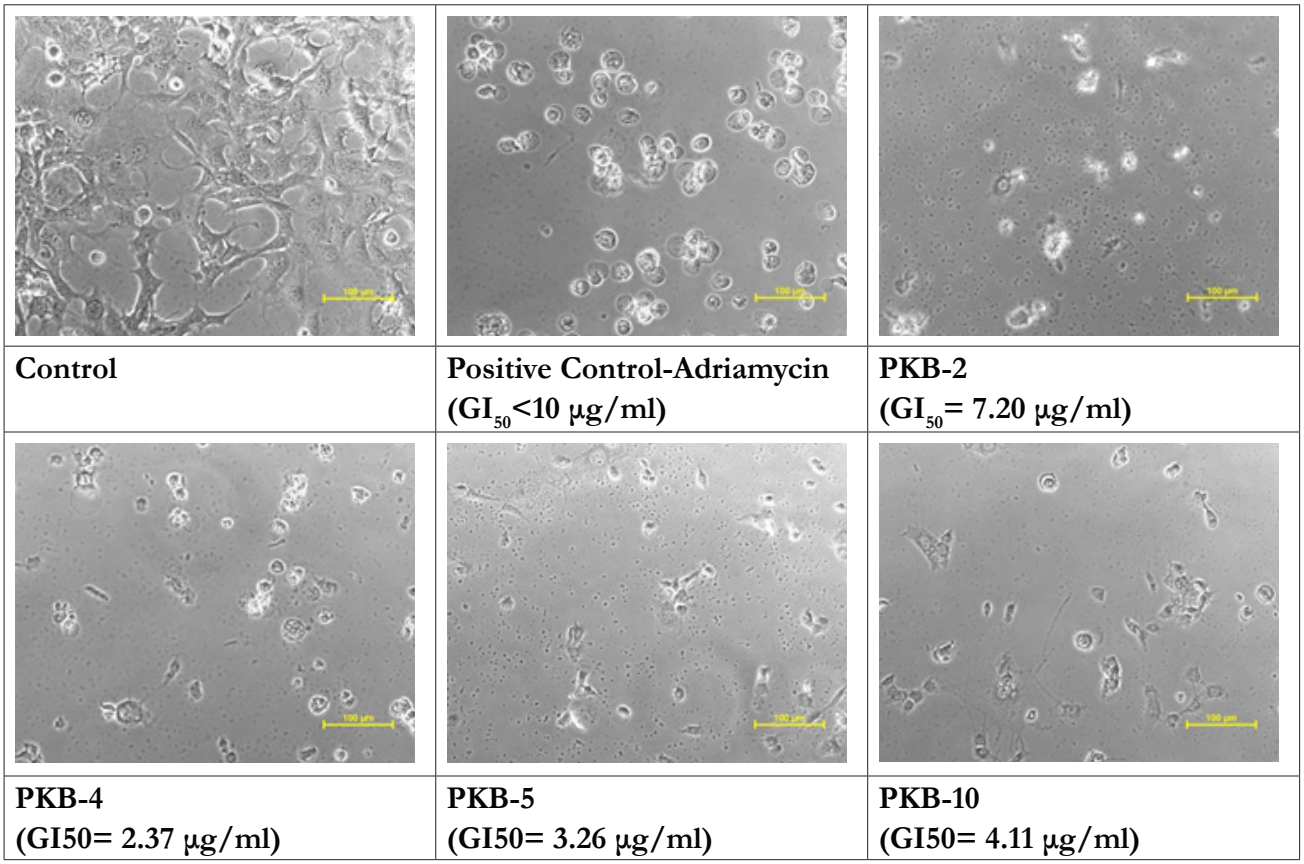


Figure 4: The inhibition growth (GI_{50}) of MCF-7 cancer cells by PKB-2, PKB-4, PKB-5, PKB-10 and positive control (adriamycin).

ADME Studies

ADME properties of compounds (PKB 1-10) such as predicted gastrointestinal absorption, P-glycoprotein, blood-brain barrier and drug-likeness prediction such as Lipinski, Ghose and Veber rules and bioavailability score were predicted by online tool Swiss ADME of Swiss Institute of Bioinformatics (http://www.sib.swiss). ChemBioDraw Ultra version 15.0 (Cambridge Software) was used for drawing of 2D structural models and SMILES of each compound was translated into molfile by online Smiles translator and structure file generator found in Online tool Swiss ADME.

Toxicity Studies

Toxicity prediction studies were performed by Osiris Property Explorer. The Osiris Property Explorer includes the mol inspiration software through which the data may obtain. Mutagenic (MUT) and tumorigenic (TUMO) properties were predicted using Osiris molecular property explorer. Green colour predicts low toxicity; yellow shows moderate toxicity, while the red color predicts a high tendency for toxicity. Drug Score (D-S) of a compound predicts the compound's overall potential to qualify for a drug. It provides results based on molecular weight, cLogP, log S, drug-likeness and toxicity risks. Druglikeness (DL) values are based on topological descriptors, the fingerprint of molecular structure or other properties like molecular weight, solubility and cLogP. Molecular weight (MW) and aqueous solubility (S) were also predicted. The low aqueous solubility of a compound affects its absorption and distribution characteristics.

Synthesis

Physical and analytical data of synthesized derivatives were presented in Table 5.

Synthesis of substituted ethyl benzylidiene oxobutanoates (3)

Intermediate compounds **3** were synthesized via known procedures.[25]

Synthesis of substituted 4H-Chromene derivatives (PKB 1-10)

The targeted final compounds (PKB 1-10) were synthesized via known procedures.[25]

Ethyl 7-hydroxy-4-(2-hydroxyphenyl)-2-methyl-4H-chromene-3-carboxylate (PKB-1): Yield: White solid (71%); IR (KBr, ν, cm^{-1}): 3385 (-OH), 2986, 2941, 1742, 1719, 1627, 1598, 1464; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.14 (d, *J=8,* 1H), 7.09-7.05 (m, 1H, Ar-H), 6.98

(d, *J=8,* 1H), 6.85 (m, 2H, Ar-H), 6.35 (s,1H), 6.32 (s, 1H), 5.30 (bs, 1H, -OH), 4.20 (s, 1H, >CH), 4.12-4.06 (m, 2H, $>CH_2$), 3.14 (bs, 1H, -OH), 1.97 (s, 3H, $-CH_3$), 1.14 (t, *J=8*, 3H, $-CH_3$).

Ethyl 4-(5-chloro-2-hydroxyphenyl)-7-hydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-2): Yield: White solid **(**67%); IR (KBr, ν, cm^{-1}): 3373 (-OH), 3001, 2939, 1718, 1627, 1506, 1462, 1258; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.12 (s, 1H), 7.04-6.97 (m, 2H), 6.77 (d, *J=8*, 1H), 6.37-6.34 (m, 2H), 4.16 (s, 1H, >CH-), 4.08 (q, *J=8*, 2H, $>CH_2$), 3.10 (bs, 1H, -OH), 1.96 (s, 3H, $-CH_3$), 1.14 (t, *J=8*, 3H, $-CH_3$).

Ethyl 4-(5-bromo-2-hydroxyphenyl)-7-hydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-3): Yield: Red solid **(**66%); IR (KBr, ν, cm^{-1}): 3371 (-OH), 2982, 2938, 1718, 1627, 1506, 1464, 1380, 1147; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.26 (d, 1H, *J=4*), 7.16 (dd, *J=4*; *12*, 1H), 6.97 (d, *J=8,* 1H), 6.72 (d, *J=8,* 1H), 6.37-6.35 (m, 2H), 4.16 (s,1H, >CH-), 4.11-4.06 (m, 2H, $>CH_2$), 3.09 (bs, 1H, -OH), 1.96 (s, 3H, $-CH_3$), 1.14 (t, *J=8*, 3H, $-CH_3$).

Ethyl 4-(5-bromothiophen-2-yl)-7-hydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-4): Yield: Brown solid **(**65%); IR (KBr, ν, cm^{-1}): 3426 (-OH), 2982, 2899, 1690, 1629, 1507, 1459, 1261, 1146; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.01 (d, *J=8*, 1H), 6.76 (d, *J=4*; 1H, thiophenyl), 6.57 (dd, *J=2.5; 8*, 1H), 6.52 (s, 1H), 6.45 (d, *J=4*, 1H, thiophenyl), 5.18 (s,1H, >CH-), 4.23-4.16 (m, 2H, $>CH_2$), 2.43 (s, 3H, $-CH_3$), 1.28 (t, *J=8*, 3H, $-CH_3$); ^{13}C NMR (200 MHz, $CDCl_3$): 166.9, 161.1, 155.6, 152.4, 150.4, 129.9, 129.3, 124.1, 115.8, 112.5, 110.6, 105.8, 103.2, 60.7, 36.1, 19.7, 14.3.

Ethyl 5,7-dihydroxy-2-methyl-4-phenyl-4H-chromene-3-carboxylate (PKB-5): Yield: White solid **(**76%); IR (KBr, ν, cm^{-1}): 3446 (-OH), 3350 (-OH), 2987, 2909, 1662, 1624, 1519, 1336,1127; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.29-7.22 (m, 3H), 7.22-7.13 (m, 2H), 6.17 (d, *J=2.3*, 1H), 6.01 (d, *J=2.3*, 1H), 5.01 (s, 1H, >CH-), 4.13-4.07 (m, 2H, $>CH_2$), 2.43 (s, 3H, $-CH_3$), 2.10 (bs, 1H, -OH), 1.22 (t, *J=8*, 3H, $-CH_3$).

Ethyl 5,7-dihydroxy-4-(2-hydroxyphenyl)-2-methyl-4H-chromene-3-carboxylate (PKB-6): Yield: White solid **(**77%); IR (KBr, ν, cm^{-1}): 3339 (-OH), 2985, 2949, 1717, 1632, 1513, 1485, 1381, 1145; ^{1}HNMR (400MHz, DMSO-d^6, ppm): 9.50 (bs, 1H, -OH), 9.09 (bs, 1H, -OH), 7.27 (d, *J=8*, 1H), 7.06 (t, *J=8*, 1H), 6.84 (t, *J=8*, 1H), 6.78 (d, *J=8*, 1H), 5.85 (s, 1H, >CH-), 5.67 (s, 1H), 4.50 (s, 1H), 4.09-3.97 (m, 2H), 3.28 (bs, 1H, -OH), 1.87 (s, 3H), 1.08 (t, *J=8*, 3H, $-CH_3$); ^{13}C NMR (200 MHz, DMSO-d^6): 169.2, 157.1, 155.4, 152.9, 151.4, 128.2, 127.8, 127.5, 121.4, 115.9, 103.1, 98.1, 96.3, 94.4, 61.1, 30.1, 25.9, 14.3.

Ethyl 5,7-dihydroxy-4-(2-hydroxy-5-methoxyphenyl)-2-methyl-4H-chromene-3-carboxylate (PKB-7): Yield: White solid **(**73%); IR (KBr, ν, cm^{-1}): 3443 (-OH), 2982, 2943, 1743, 1726, 1623, 1513; ^{1}HNMR (400MHz, DMSO-d^6, ppm): 9.47 (bs, 1H, -OH), 9.08 (bs, 1H, -OH), 6.88-6.86 (m, 1H), 6.80-6.77 (m, 2H), 5.84 (s, 1H, >CH-), 5.67 (s, 1H), 4.50 (s, 1H), 4.49-3.96 (m, 2H), 3.71 (s, 3H, $-OCH_3$), 3.26 (bs, 1H, -OH), 1.87 (s, 3H), 1.07 (t, *J=8*, 3H, $-CH_3$); ^{13}C NMR (200 MHz, DMSO-d^6): 169.2, 157.1, 155.4, 153.0, 147.5, 140.5, 128.4, 121.3, 119.3, 110.9, 103.0, 97.9, 96.3, 94.4, 61.1, 55.9, 43.6, 30.09, 25.9, 14.3.

Ethyl 4-(5-chloro-2-hydroxyphenyl)-5,7-dihydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-8): Yield: Red solid **(**74%); IR (KBr, ν, cm^{-1}): 3398 (-OH), 2994, 1714, 1626, 1613, 1512, 1479, 1374, 1149; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.31 (d, *J=4*, 1H), 6.99 (dd, *J=4*, 8, 1H), 6.74 (d, *J=8*, 1H), 5.95 (s, 1H), 5.85 (s, 1H), 4.57 (s, 1H, >CH-), 4.14-4.08 (m, 2H), 3.05 (bs, 1H, -OH), 2.59 (bs, 1H, -OH), 1.93 (s, 3H, $-CH_3$), 1.17 (t, *J=8*, 3H, $-CH_3$).

Ethyl 4-(5-bromo-2-hydroxyphenyl**)-5,7-dihydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-9):** Yield: Red solid **(**74%); IR (KBr, ν, cm^{-1}): 3404 (-OH), 2925, 1714, 1624, 1512, 1477, 1374, 1249; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 7.46 (d, *J=2*, 1H), 7.15 (dd, *J= 4, 8,* 1H), 6.69 (d, *J=8*, 1H), 5.96 (d, *J=4*, 1H), 5.83 (d, *J=4*, 1H), 4.57 (s, 1H, >CH-), 4.14-4.09 (m, 2H), 3.05 (bs, 1H, -OH), 2.57 (bs, 1H, -OH), 1.93 (s, 3H,-CH_3), 1.17 (t, *J=8*, 3H,-CH_3).

Ethyl 4-(5-bromothiophen-2-yl)-5,7-dihydroxy-2-methyl-4H-chromene-3-carboxylate (PKB-10): Yield: Off white solid **(**75%); IR (KBr, ν, cm^{-1}): 3512 (-OH), 3333 (-OH), 2980, 1695, 1528, 1494, 1375, 1211; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 6.76 (d, *J=4*, 1H), 6.54 (d, *J=4*; 1H, thiophenyl), 6.15 (d, *J=4*, 1H), 6.09 (d, *J=4*, 1H, thiophenyl), 5.32 (s, 1H, >CH-), 4.24-4.19 (m, 2H, $>CH_2$), 2.41 (s, 3H, $-CH_3$), 1.30 (t, *J=8*, 3H, $-CH_3$); ^{13}C NMR (200 MHz, $CDCl_3$): 166.9, 161.2, 156.0, 153.9, 151.5, 150.8, 129.2, 124.7, 110.7, 106.1, 104.4, 99.5, 96.1, 60.7, 31.3, 19.7, 14.3.

In vitro Anticancer Activity by Sulforhodamine B Assay

The *in vitro* cytotoxic activity was checked by using Sulforhodamine B staining (SRB) assay.[26] The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. Cells were plated in 96 multiwell plates (104 cell/ well) and plates were incubated at 37°C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental compounds. Experimental compounds were initially

solubilized in dimethyl sulfoxide and prepared different molar concentrations. Different molar concentrations of the test compounds (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) were put into the cell line monolayer. For each individual dose, triplicate wells were made. Test compounds were incubated into monolayer cells for almost 48 hr at 37°C and in an atmosphere of 5% CO_2. Then after completion of 48 hr, cells were fixed, washed and stained with Sulforhodamine B stain. If there made the excess stain, it would be washed with acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The relation between surviving fraction and different drug concentration was plotted and GI_{50} was calculated for each compound.

CONCLUSION

It could be concluded that most of the compounds in series PKB 1-10 exhibited potent anticancer activity on MCF-7 cell line. Compound PKB-4 (GI_{50}= 2.37 µg/ml) possessed most potent activity as compared to standard, Adriamycin (GI_{50} <10 µg/ml). Docking, *in silico* ADME and toxicity studies were also favorable for the synthesis of compounds. The presence of 5-bromothiophen moiety (PKB-4 and PKB-10) was favorable for binding as well as anti-breast cancer activity. All compounds (PKB 1-10) were found to be active on the MCF-7 cell line, therefore, compounds might be active against ER-positive breast cancer. Further evaluation of the detail mechanism pathway involved in an activity needs to be investigated

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ADME: Absorption, Distribution, Metabolism, Excretion; **GI:** Gastro Intestinal; **P-gp:** P-glycoprotein; **BBB:** Blood Brain Barrier; **CYP1A2:** Cytochrome P450 family 1 subfamily A member 2; **CYP2D6:** Cytochrome P450 family 2 subfamily D member 6; **FT-IR:** Fourier-transform infrared spectroscopy; **^{1}H-NMR:** Proton nuclear magnetic resonance; **^{13}C-NMR:** Carbon-13 nuclear magnetic resonance; **GABA:** Gamma aminobutyric acid; **TMS:** Tertramethyl Silane; **TLC:** Thin layer chromatography; **WST**: ((3AS,4R,9BR)-4-(4-Hydroxyphenyl)-1,2,3,3a,4,9b-hexahydrocyclopenta[c] chromen-9-ol).

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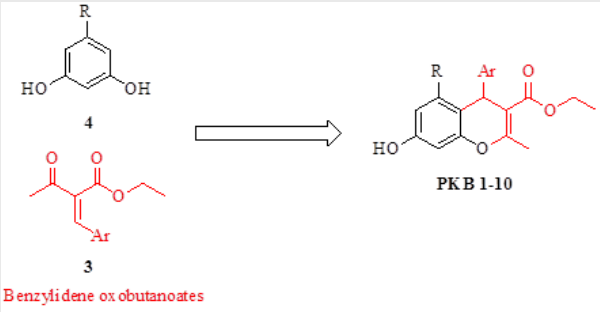
PICTORIAL ABSTRACT



SUMMARY

The 4H-chromenes are important chemical synthon, associated with a broad range of biological activities, including anticancer activity. The computational studies like docking, ADME and toxicity were performed and based on their results, compounds were designed and synthesized. The anticancer study of 4H-chromene derivatives (PKB 1-10) was carried out by Sulforhodamine B stain (SRB) assay on MCF-7 cell line. The compounds PKB-2, PKB-4, PKB-5 and PKB-10 displayed similar activity (GI_{50} = < 10 μg/ml) as standard drug, Adriamycin. Most of the compounds displayed significant activity.

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