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Design, Synthesis, and Evaluation of Anticancer Potential of Some New Benzopyran Schiff Base Derivatives

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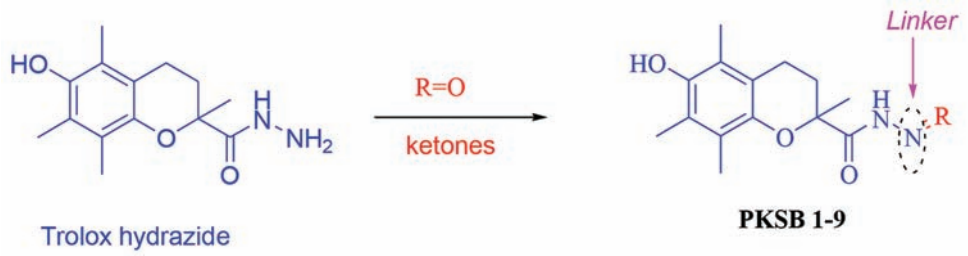
ABSTRACT The present study was carried out to design, synthesize, and evaluate the anticancer activity of newly synthesized benzopyran Schiff base derivatives (**PKSB 1-9**) on MCF-7 and MDA-MB-231 cell lines. The compounds were designed by docking, *in silico* absorption, distribution, metabolism, elimination (ADME), and predicted toxicity studies. Designed compounds were synthesized by condensing substituted Trolox hydrazides with different substituted ketones. These compounds were characterized by proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, Fourier transform infrared, and high-resolution mass spectrometry. Then, all synthesized compounds were tested on MCF-7 and MDA-MB-231 cell lines for anticancer activity. All compounds (except **PKSB-7**) showed better docking scores than standard. *In silico*, ADME, and toxicity studies were also found as significant for most of the compounds. The majority of the compounds displayed good to potent anticancer activity on MCF-7 and MDA-MB-231 cell lines.



KEYWORDS Anticancer, Absorption, Distribution, Metabolism, and Elimination, Benzopyran, Docking, Schiff Base, Toxicity.

INTRODUCTION

Among all the chronic diseases which are known till date, cancer is considered as one of the most common diseases in human community, affecting almost 9.6 million deaths world while in 2018. Breast cancer is known as the most common cancer in women, which accounts for 25.1% out of all cancers.[1] It has been seen that heterocyclic compounds play a significant role in designing new drugs having medicinal assistance.[2] Benzopyrans are members of the pyran heterocycles that include benzene and pyran. These heterocyclic compounds possess various pharmacological activities such as neuroprotective,[3] analgesics,[4] antibacterial,[5] insulin release process inhibitor,[6] human rhinovirus capsid-binding inhibitor,[7] and antiepileptic.[8] In recent years, compounds substituted with hydrazides have shown significant pharmacological activities, such as antiepileptic[8] and anticancer.[9,11] This gives a huge motivation to the exploration for potential pharmacologically active candidates carrying benzopyran

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ring. The search for novel anticancer agents devoid of resistance and adverse effects remains a major area of research in medicinal chemistry. Although many new drugs have been synthesized and found to be clinically active, their cost is very high. Therefore, there have been tremendous needs for searching for new chemical entities, which will be more potent, economical, and safe anticancer drugs.

Molecular docking is an essential part of rational drug design. The main function of molecular docking is to determine the preferable position of one molecule to another molecule, whenever they would bind to one another to form a stable complex. Here, benzopyran Schiff base derivatives have been docked to the estrogen receptor. Absorption, distribution, metabolism, elimination (ADME) and toxicity studies were also performed to predict the possible pharmacokinetic and toxic variables in the compounds. **Figure 1** represents the pharmacophoric pattern of some anticancer drugs having benzopyran ring and model compound.

Therefore, we hereby report the design, synthesis, and anticancer property of some new benzopyran Schiff base derivatives **(PKSB 1-9)**. These compounds were evaluated for their anticancer properties on MCF-7 and MDA-MB-231 cell lines.

RESULTS AND DISCUSSION

Docking studies

Docking of all compounds **(PKSB 1-9)** was performed by AutoDock 1.4.6 software on estrogen receptor for anti-breast cancer screening (PDB: 2POG; **Table 1**). The docking score of compounds was ranging between −11.82 and −5.91. Compound **PKSB-6** having an adamantane ring showed the highest docking score (−11.82). All compounds possessed better docking scores than the standard drug, adriamycin (ADR) (−6.90) except compound **PKSB-7** (−5.91). Compound **PKSB-2** exhibited good score −9.36. The results showed that the presence of electronegative atoms (**PKSB-2, PKSB-3, PKSB-4,** and **PKSB-8)** is responsible for significant docking score. The best docked compound and their binding pocket are shown in **Figures 2** and **3**. The H of -OH in highest docking scored compound **PKSB-6** involved in hydrogen bonding interaction with GLU353 through O of CO.

Predicted ADME studies

According to the pharmacokinetic properties, all compounds followed druglikeness prediction such as Lipinski, Ghose, and Veber rules **[Table 2]**. Their bioavailability scores were also significant. All compounds showed high gastrointestinal absorption. Most of the compounds had no BBB permeability except compounds **PKSB-1, PKSB-2,** and **PKSB-6** that had yes BBB permeability. None of the compounds inhibited to cytochrome P450 isomers (CYP1A2 and CYP2D6).

Toxicity studies

All compounds were tested for predicted toxicity study by Osiris Property Explorer **[Table 3]**. Compound **PKSB-8** was predicted to have moderate mutagenic (MUT) toxicity, whereas **PKSB-7** was predicted to be highly carcinogenic. Most of the compounds were considered as better drug scores as compared to compounds **PKSB-4, PKSB-5,** and **PKSB-7**. Compounds **PKSB-1, PKSB-2, PKSB-3, PKSB-4, PKSB-5, PKSB-8,** and **PKSB-9** showed positive

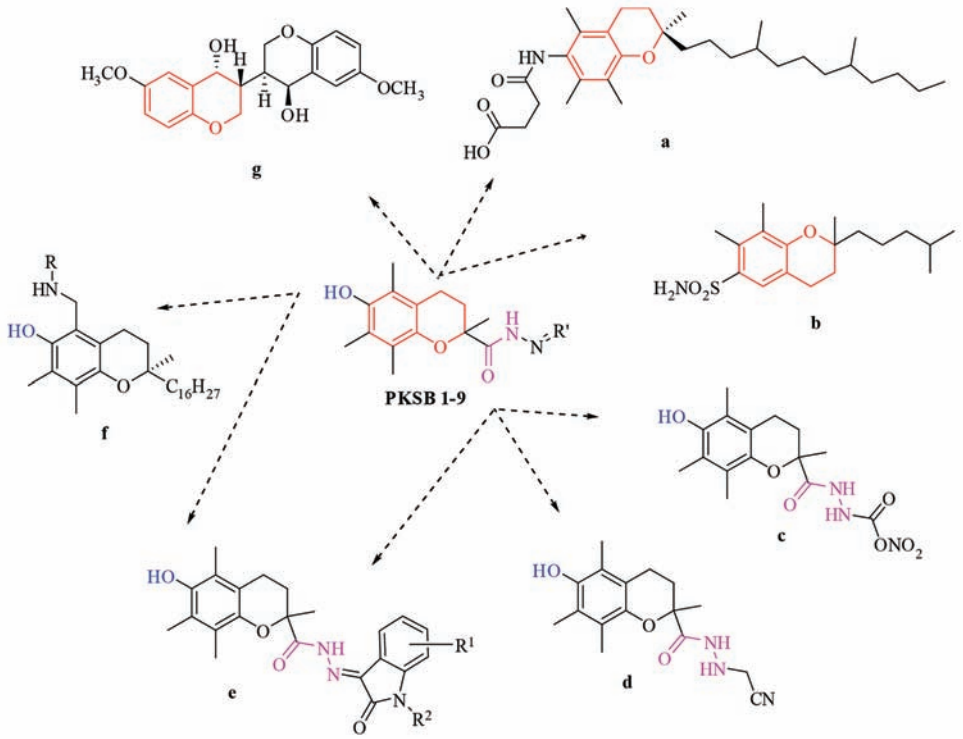
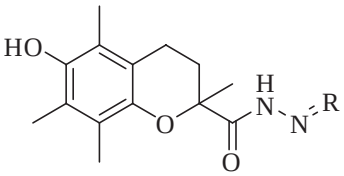
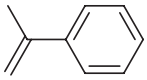
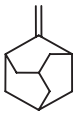
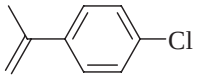
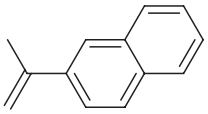
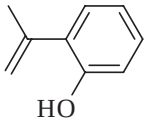
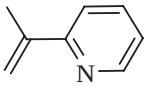
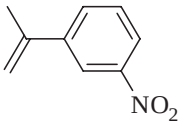
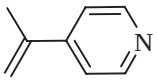
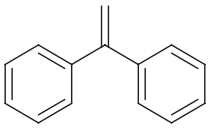


Figure 1: Pharmacophoric pattern of anticancer drugs (a-g) and model compound (PKSB 1-9)

Table 1: Docking scores of designed compounds PKSB 1-9 and standard drug (adriamycin)



PKSB 1-9

Compounds	R	Docking score	Compounds	R	Docking score
PKSB-1		−8.65	**PKSB-6**		−11.82
PKSB-2		−9.36	**PKSB-7**		−5.91
PKSB-3		−8.86	**PKSB-8**		−8.10
PKSB-4		−8.86	**PKSB-9**		−7.15
PKSB-5		−8.20	**Adriamycin**	-	−6.90

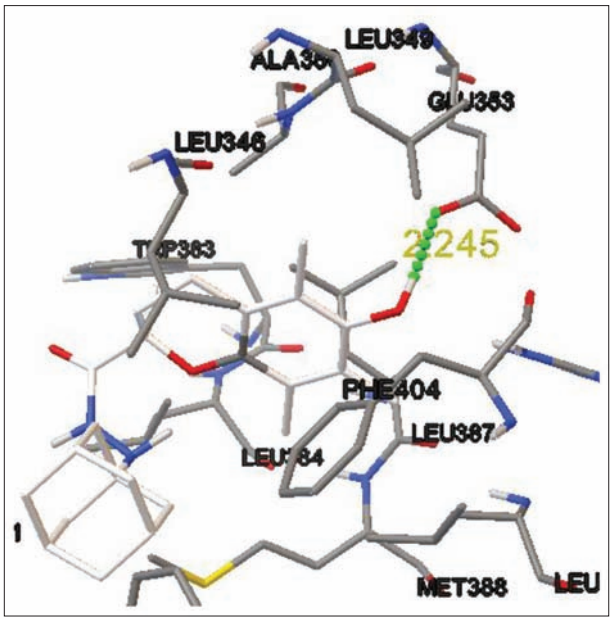


Figure 2: AutoDock-predicted pose of compound PKSB-6 with estrogen receptor (PDB: 2POG). The H-bond interaction of the compound with active site amino acid residue GLU353 was represented as green dotted lines

DL values, which indicate that the compounds include mostly fragments which are part of many commercial drugs. Compounds **PKSB-6, PKSB-8,** and **PKSB-9** displayed better solubility than other compounds. High logP (logarithm of partition coefficient between n-octanol and water $\log(c_{octanol}/c_{water})$ [CLP]) values are an estimation of low hydrophilicity, therefore, accounting poor absorption as well as permeation. Compounds **PKSB-4, PKSB-8,** and **PKSB-9** displayed significant CLP values.

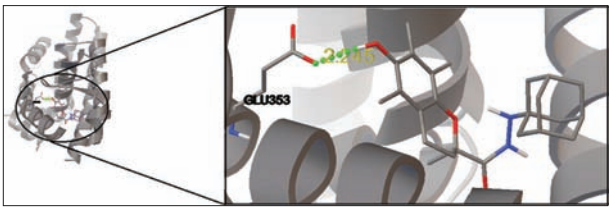


Figure 3: AutoDock-predicted visualization pose of the compound PKSB-6 with ER*α* binding pocket of the estrogen receptor

Chemistry

The first step involved the synthesis of Trolox **(III)** prepared through hetero Diels–Alder reaction. The trimethylhydroquinone **(I)**, methyl methacrylate **(II)**, paraformaldehyde, and dibutylamine were refluxed in the presence of acetic acid.[12] Then, the ester group present in **III** underwent a nucleophilic attack by hydrazine molecule

Table 2: Pharmacokinetic properties (ADME) of compounds (PKSB 1-9)

Compounds	Pharmacokinetics						Drug-likeness			
	GI absorption	BBB permeant	P-gp	CYP1A2	CYP2D6	Log K_p (skin permeation), cm/s	Lipinski	Ghose	Veber	Bioavailability score
PKSB-1	High	Yes	No	No	No	−5.38	Yes	Yes	Yes	0.55
PKSB-2	High	Yes	No	No	No	−5.15	Yes	Yes	Yes	0.55
PKSB-3	High	No	Yes	No	No	−5.73	Yes	Yes	Yes	0.55
PKSB-4	High	No	No	No	No	−5.78	Yes	Yes	Yes	0.55
PKSB-5	High	No	Yes	No	No	−4.58	Yes	Yes	Yes	0.55
PKSB-6	High	Yes	Yes	No	No	−5.44	Yes	Yes	Yes	0.55
PKSB-7	High	No	Yes	No	No	−4.79	Yes	Yes	Yes	0.55
PKSB-8	High	No	Yes	No	No	−5.91	Yes	Yes	Yes	0.55
PKSB-9	High	No	Yes	No	No	−6.14	Yes	Yes	Yes	0.55

P-gp: P-glycoprotein; GI: Gastrointestinal; 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)

Table 3: Toxicity analysis results of compounds PKSB 1-9

Compounds	Toxicity risks		Osiris calculations				
	MUT	TUMO	MW	CLP	S	DL	D-S
PKSB-1	Green	Green	352	4.60	−5.42	1.0	0.27
PKSB-2	Green	Green	386	5.21	−6.16	1.02	0.23
PKSB-3	Green	Green	368	4.26	−5.12	0.77	0.28
PKSB-4	Green	Green	413	3.66	−6.04	2.42	0.17
PKSB-5	Green	Green	428	5.51	−6.82	1.39	0.18
PKSB-6	Green	Green	344	4.07	−4.9	−1.8	0.21
PKSB-7	Green	Red	416	5.45	−7.01	−2.41	0.12
PKSB-8	Yellow	Green	367	3.31	−4.63	2.71	0.31
PKSB-9	Green	Green	367	3.25	−4.61	2.49	0.39

resulting in the formation of compound **IV**.[13] The latter compound was subjected to imine formation by the condensation reaction with different substituted ketones **(V)** to afford final compounds **(PKSB 1-9, Scheme 1, Table 4)**.[9]

The structures of all synthesized compounds were verified based on melting point analysis, Fourier transform infrared (FT-IR), proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), and mass spectroscopy. The obtained data were inconsistent with the proposed structures.

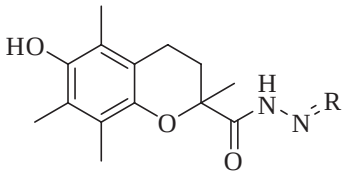
The final products **PKSB 1-9** were identified by its IR spectra. The amide (-$CONH_2$) showed its characteristic absorption band at 1673–1691 cm^{-1} (stretch). All compounds displayed a broad absorption band at 3375–3424 cm^{-1} of the hydroxyl group (-OH, stretch). In ^{1}H-NMR, a singlet at δ 9.13–9.66 ppm was observed by the amide group (-$CONH_2$). The sharp singlet at around δ 4.31–4.57 ppm confirmed the hydroxy group (-OH) in compounds **(PKSB-3, PKSB-4, PKSB-5, PKSB-6, and PKSB-9)** except in compounds **PKSB-1, PKSB-2, PKSB-7,** and **PKSB-8** (singlet at δ 7.55–7.57 ppm) due to $CDCl_3$. All of the other aromatic and aliphatic protons were present at their distinct place. In ^{13}C-NMR, the signal due to the carbonyl of the amide group (C=O) appeared at δ 169.7–170.6 ppm. Other signals were found corresponding to their established structures. The mass spectra of almost all compounds showed $(M+H)^+$ (m/z) peaks according to their molecular weight (MW). Complete details were provided in the experimental section.

In vitro anticancer screening

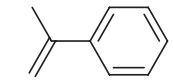
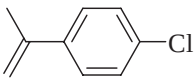
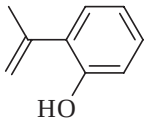
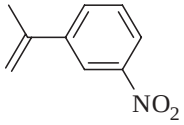
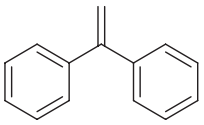
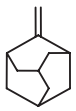
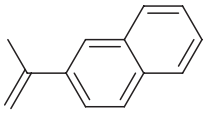
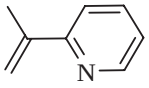
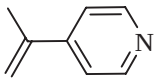
The *in vitro* anticancer activity of synthesized compounds **(PKSB 1-9)** was checked on human mammary adenocarcinoma (MCF-7) and MDA-MB-231 using sulforhodamine B assay.[14] For each compound, GI_{50} concentration was calculated from sigmoidal dose-response curves and edited in **Table 5**. For reference purposes, ADR data were included in the table.

The resultant data showed that some compounds exhibited remarkable cytotoxic effects toward the MCF-7 cell line. The result presented showed that compounds **PKSB-2, PKSB-4, PKSB-5,** and **PKSB-7** exhibited almost similar activity (GI_{50} ≤10 µg/ml) as ADR. Compound **PKSB-1** showed significant activity with GI_{50} of 23.8 µg/ml, while compound **PKSB-9** demonstrated promising activity (GI_{50} = 59.5 µg/ml), the remaining three compounds **PKSB-3, PKSB-6,** and **PKSB-8** showed GI_{50} above 80 µg/ml on the MCF-7 breast cancer cells **(Figure 4)**. It had been observed that activity was enhanced by the presence

Table 4: Compounds (PKSB1-9) differing in the substitution at R



PKSB 1-9

Compounds.[a,b]	R	Reaction time (h)	Mol. formula	Mol. weight	Yield (%)[c]	MP (°C)	R_f[d]
PKSB-1		10	$C_{22}H_{22}N_2O_5$	366.45	85	147	0.7
PKSB-2		10	$C_{22}H_{18}Cl_4N_2O_5$	400.89	82	163	0.7
PKSB-3		12	$C_{22}H_{18}Br_4N_2O_5$	382.45	89	140	0.7
PKSB-4		12	$C_{22}H_{21}FN_2O_5$	411.45	90	133	0.6
PKSB-5		10	$C_{21}H_{21}N_3O_5$	428.52	86	136	0.6
PKSB-6		11	$C_{26}H_{23}N_3O_7$	396.52	82	82	0.7
PKSB-7		11	$C_{18}H_{22}N_2O_5$	416.51	89	166	0.6
PKSB-8		12	$C_{19}H_{22}N_2O_5$	367.44	83	86	0.5
PKSB-9		12	$C_{18}H_{18}Cl_2N_2O_5$	367.44	87	132	0.7

[a]Reagents and conditions: IV (1mmol), V (1 mmol), ethanol (10 ml). [b]Product was analyzed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS. [c]Yield calculated of pure products after chromatography. [d]R_f=EtOAc/Hexane (40:60)

of electronegative atoms such as chlorine or nitro group at 4 and 3 positions, respectively, on phenyl of the ketone molecule, whereas in the presence of hydroxyl group on phenyl of ketone molecule reducing activity. However, the increase in the ring system (naphthyl) and ring number (two phenyl ring) accounted for more activity. Pyridine ring system appeared in less cytotoxicity than the phenyl ring system, whereas compounds showed very less cytotoxic effects (GI_{50} ≥80 μg/ml) toward the MDA-MB-231 cell line. MCF-7 is ER +ve and MDA-MB-231 is ER -ve breast cancer cell lines. The results revealed that compounds (**PKSB 1-9**) were active on the MCF-7 cell line; therefore, all compounds were active against ER-positive breast tumors. About 80% of breast cancers were reported as ER positive. It involves the high concentration of hormone estrogen and progesterone leads to cancer cell growth.

EXPERIMENTAL

Molecular docking

The AutoDockTool (AutoDock 4.2) was used for creating PDBQT files from traditional PDB files. The sequence of ((3AS,4R,9BR)-4-(4-Hydroxyphenyl)-1,2,3,3a,4,9b-hexahydrocyclopenta[c]chromen-9-ol) (WST) was retrieved

Table 5: Anti-breast cancer activity of compounds (PKSB 1-9)

Compound	GI_{50} (μg/ml)[a]	
	MCF-7	MDA-MB-231
PKSB-1	23.8	>80
PKSB-2	<10	>80
PKSB-3	>80	>80
PKSB-4	<10	>80
PKSB-5	<10	>80
PKSB-6	>80	>80
PKSB-7	<10	>80
PKSB-8	>80	>80
PKSB-9	59.5	>80
Adriamycin	<10	<10

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

from UniProt database.[14] The three-dimensional structure of WST was downloaded from the PDB database. The drug compound structures were drawn using ACD-ChemSketch and converted into PDB format using an Open Babel tool. The three-dimensional structures of above targeted proteins were docked with designed compounds (**PKSB 1-9**) 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.

Predicted ADME studies

ADME properties of compounds (**PKSB 1-9**) such as predicted P-glycoprotein, gastrointestinal absorption, druglikeness prediction, and blood–brain barriers such

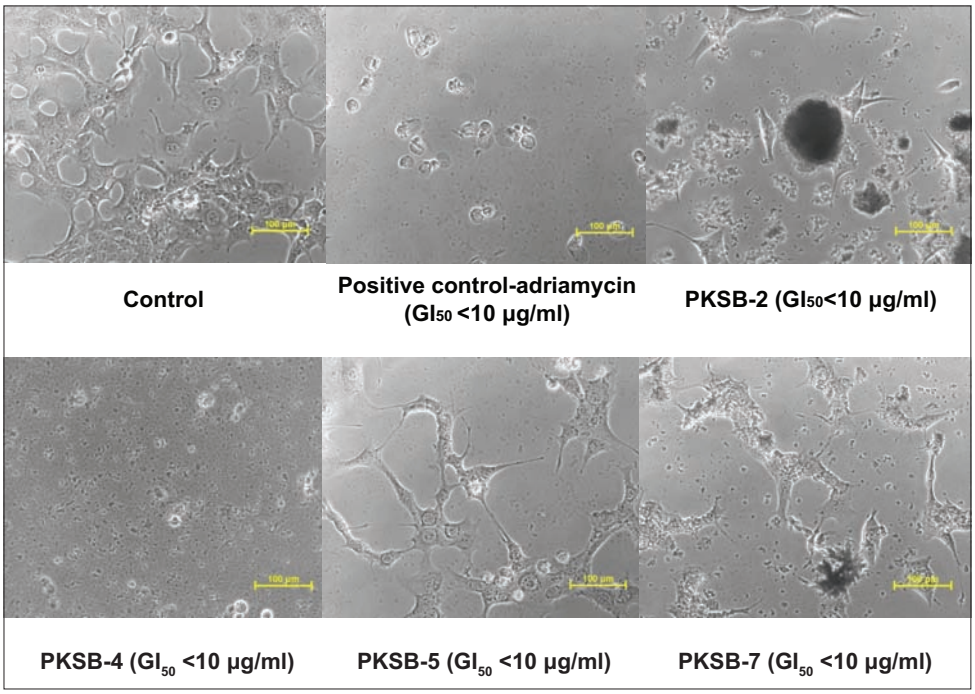
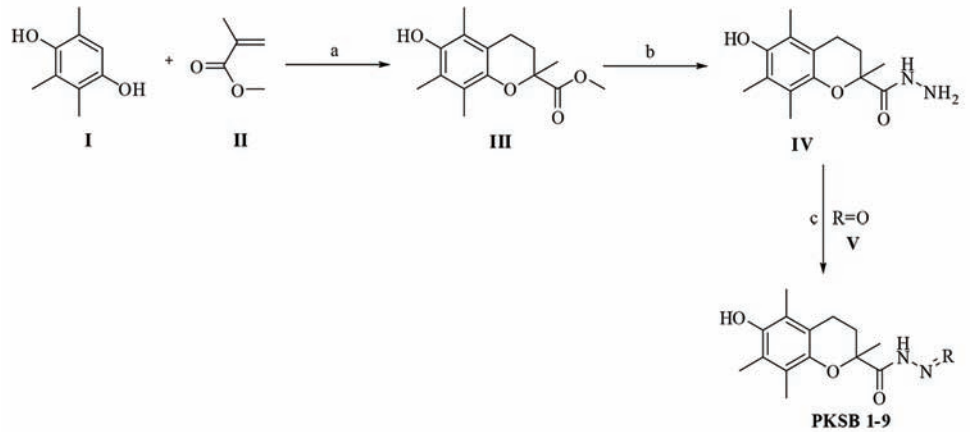


Figure 4: The inhibition of growth of cancer cells on MCF-7 cell line by compounds PKSB-2, PKSB-4, PKSB-5, PKSB-7, and positive control (adriamycin)



Scheme 1: Synthetic pathway for compounds PKSB1-9. (a) $(HCHO)_n$, $[CH_3(CH_2)_3]_2NH$, CH_3COOH, reflux, 20 h; (b) NH_2NH_2. H_2O, EtOH, 80°C, 10 h; (c) EtOH, CH_3COOH, reflux, 10–12 h

as Lipinski, Ghose, and Veber rules and bioavailability score were predicted by online software SwissADME of Swiss Institute of Bioinformatics (http://www.sib.swiss). ChemBioDraw Ultra version 15.0 (Cambridge Software) was used for drawing of 2D structural models.

Toxicity studies

Toxicity prediction studies were performed by Osiris Property Explorer. The Osiris Property Explorer includes the Molinspiration software through which the data may obtain. MUT and tumorigenic properties were predicted using Osiris molecular property explorer. Green color assigns for low toxicity; yellow color for moderate toxicity, while the red color shows a high tendency for toxicity. Drug score (D-S) of a compound predicts its potential as a drug. It provides results based on MW, cLogP, log S, druglikeness, and toxicity risks. Druglikeness (DL) values are based on topological descriptors, the fingerprint of molecular structure, or other properties such as MW, solubility, and cLogP.

MW and aqueous solubility (S) were also predicted. The compound having low aqueous solubility affects its absorption and distribution patterns. High logP (CLP) values are estimation of low hydrophilicity and therefore cause poor absorption or permeation.

Materials

All chemicals used were procured commercially from Sigma-Aldrich and Spectrochem Pvt. Ltd. The progress of the reaction was observed by thin-layer chromatography on E Merck silica gel GF-254 pre-coated plates and the visualization was done by spraying charring solution. Melting points were measured in open capillaries. NMR spectra were recorded on the JEOL ECX-500 spectrometer in dimethyl sulfoxide (DMSO)-*d6* and $CDCl_3$ at 400 MHz and 500 MHz for 1H and 125 MHz for 13C. Chemical shifts (δ) were determined in δ parts per million (ppm) relative to tertramethyl silane. The mass spectra were analyzed with a Waters-Q-T of Premier-HAB213 spectrometer and Microscopic II Triple Quadrupole mass spectrometer (MS) using EI and the *m/z* values were indicated in Dalton. The IR spectra (KBr) were analyzed on a Bruker FT/IR Vector 22 spectrophotometer.

Synthesis

Synthesis of compounds III and IV

Intermediates **III** and **IV** were synthesized through known procedures.[9]

General procedure for the synthesis of final compounds **(PKSB 1-9)**

A mixture of Trolox hydrazide (**IV**; 1 mmol) and different ketones (**V**; 1 mmol) in ethanol and containing 3-4 drops of acetic acid was heated to reflux for 10–12 h. After completion of the reaction, the reaction mass was cooled at room temperature. The obtained solid was then filtered, washed with cold ethanol, and dried. The compounds were further recrystallized by ethanol to obtain final compounds (**PKSB 1-9**).

(E)-6-hydroxy-2,5,7,8-tetramethyl-N'-(1-phenylethylidene)chroman-2-carbohydrazide (PKSB-1)

White solid IR (KBr, ν, cm^{-1}): 3375 (-OH), 1685 (CO); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 9.58 (s, 1H,-CONH-), 7.72-7.70 (m, 2H, Ar-H), 7.55 (s, 1H, OH), 7.37-7.35 (m, 3H, Ar-H), 2.58-2.39 (m, 2H, >CH_2), 2.33-2.27 (m, 1H, >CH_2), 2.15 (s, 3H, -CH_3), 2.05 (s, 3H, -CH_3), 2.04 (s, 3H, -CH_3), 1.95 (s, 3H, -CH_3), 1.81-1.74 (m, 1H, >CH_2), 1.50 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 169.7 (>C=O), 154.3, 146.7, 144.0, 138.1, 130.0, 128.8, 126.9, 123.5, 121.4, 121.2, 117.6, 78.1, 29.6, 25.0, 20.6, 13.5, 13.3, 12.5, 12.3; high-resolution MS (HR-MS): 367.2021 (M+H)+, calcd. 367.2022.

(E)-N'-(1-(4-chlorophenyl)ethylidene)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carbohydrazide (PKSB-2)

White solid IR (KBr, ν, cm^{-1}): 3377 (-OH), 1676 (CO); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 9.60 (s, 1H,-CONH-), 7.73 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.56 (s, 1H, OH), 7.42 (d, *J* = 8.7 Hz, 2H, Ar-H), 2.57-2.39 (m, 2H, >CH_2), 2.32-2.28 (m, 1H, >CH_2), 2.14 (s, 3H, -CH_3), 2.04 (s, 3H, -CH_3), 2.03 (s, 3H, -CH_3), 1.95 (s, 3H, -CH_3), 1.81-1.74 (m, 1H, >CH_2), 1.50 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):169.8 (>C=O), 153.1, 146.7, 144.0, 136.9, 134.7, 128.9, 126.6, 123.5, 121.4, 121.2, 117.6, 78.1, 29.6, 24.9, 20.5, 13.3, 13.2, 12.4, 12.3; HR-MS: 401.1628 (M+H)+, calcd. 401.1631.

(E)-6-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)-2,5,7,8-tetramethylchroman-2-carbohydrazide (PKSB-3)

White solid IR (KBr, ν, cm^{-1}): 3384 (-OH), 1686 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.37 (s, 1H,-CONH-), 7.73 (dd, *J* = 8.2, 9.6 Hz, 2H, Ar-H), 7.28-7.23 (m, 1H, Ar-H), 6.98 (dd, *J* = 8.2, 9.6 Hz, 2H, Ar-H), 6.85-6.81 (m, 1H, Ar-H), 4.38 (s, 1H, OH), 2.71-2.58 (m, 2H, >CH_2), 2.49-2.42 (m, 1H, >CH_2), 2.25 (s, 3H, -CH_3), 2.19 (s, 3H, -CH_3), 2.11 (s, 3H, -CH_3), 2.08 (s, 3H, -CH_3), 2.03-1.95 (m, 1H, >CH_2), 1.63 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):169.7 (>C=O), 159.4, 155.5, 146.0, 143.9, 131.7, 127.6, 121.7, 121.5, 119.4, 118.8, 118.6, 118.3, 118.0, 78.6, 29.6, 24.7, 20.4, 12.3, 12.1, 11.8, 11.4; HR-MS: 383.1977 (M+H)+, calcd. 383.1970.

(E)-6-hydroxy-2,5,7,8-tetramethyl-N'-(1-(3-nitrophenyl) ethylidene)chroman-2-carbohydrazide (PKSB-4)

Light yellow solid IR (KBr, ν, cm^{-1}): 3374 (-OH), 1691 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.47 (s, 1H,-CONH-), 8.49 (s, 1H, Ar-H), 8.25-8.19 (m, 2H, Ar-H), 7.52 (t, *J* = 8.0 Hz, 1H, Ar-H), 4.40 (s, 1H, OH), 2.68-2.64 (m, 2H, >CH_2), 2.52-2.47 (m, 1H, >CH_2), 2.25 (s, 3H, -CH_3), 2.19 (s, 3H, -CH_3), 2.12 (s, 3H, -CH_3), 2.07 (s, 3H, -CH_3), 2.03-1.96 (m, 1H, >CH_2), 1.64 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):170.6 (>C=O), 149.7, 148.4, 146.0, 143.9, 139.3, 132.6, 129.5, 124.3, 121.7, 121.5, 119.4, 118.1, 78.8, 29.6, 24.8, 20.5, 12.7, 12.3, 12.1, 11.4; HR-MS: 412.1872 (M+H)+, calcd. 412.1865.

N'-(diphenylmethylene)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carbohydrazide (PKSB-5)

Off white solid IR (KBr, ν, cm^{-1}): 3424 (-OH), 1673 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.32 (s, 1H,-CONH-), 7.56-7.54 (m, 2H, Ar-H), 7.50-7.46 (m, 1H, Ar-H), 7.42-7.38 (m, 2H, Ar-H), 7.34-7.25 (m, 3H, Ar-H), 7.06-7.04 (m, 2H, Ar-H), 4.31 (s, 1H, OH), 2.60-2.57 (m, 2H, >CH_2),

2.43-2.38 (m, 1H, >CH_2), 2.10 (s, 3H, -CH_3), 2.06 (s, 3H, -CH_3), 1.90-1.84 (m, 1H, >CH_2), 1.52 (s, 3H, -CH_3), 1.47 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): 170.6 (>C=O), 155.4, 145.6, 143.7, 136.8, 131.9, 130.0, 129.6, 128.2, 128.1, 122.1, 121.1, 117.6, 78.4, 29.6, 24.5, 20.4, 12.3, 11.4, 11.3; HR-MS: 429.2171 (M+H)+, calcd. 429.2178.

N'-(2-admantone)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carbohydrazide (PKSB-6)

White solid IR (KBr, ν, cm^{-1}): 3386 (-OH), 1686 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.13 (s, 1H,-CONH-), 4.34 (s, 1H, OH), 2.74 (s, 1H), 2.65-2.61 (m, 3H), 2.46-2.41 (m, 1H, >CH_2), 2.16 (s, 3H, -CH_3), 2.15 (s, 3H, -CH_3), 2.07 (s, 3H, -CH_3), 1.99-1.89 (m, 8H), 1.88-1.77 (m, 5H), 1.59 (s, 3H, -CH_3), 1.47-1.44 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$):170.2 (>C=O), 168.7, 145.8, 144.1, 121.4, 119.3, 118.3, 78.6, 39.5, 39.3, 38.9, 38.8, 37.6, 37.4, 36.2, 31.3, 29.6, 27.7, 27.6, 27.4, 20.5, 12.2, 11.9, 11.3; HR-MS: 397.2505 (M+H)+, calcd. 397.2491.

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (1-naphthalen-1-yl-ethylidene)-hydrazide (PKSB-7)

Off white solid IR (KBr, ν, cm^{-1}): 3380 (-OH), 3290, 1685 (CO); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 9.66 (s, 1H,-CONH-), 8.21 (s, 1H), 7.99-7.93 (m, 2H, Ar-H), 7.87-7.85 (m, 2H, Ar-H), 7.57 (s, 1H, OH), 7.51-7.49 (m, 2H, Ar-H), 2.57-2.41 (m, 2H, >CH_2), 2.36-2.29 (m, 1H, >CH_2), 2.18 (s, 3H, -CH_3), 2.17 (s, 3H, -CH_3), 2.06 (s, 3H, -CH_3), 1.96 (s, 3H, -CH_3), 1.83-1.76 (m, 1H, >CH_2), 1.53 (s, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6):169.8 (>C=O), 153.9, 146.7, 144.1, 135.4, 133.8, 133.2, 129.1, 128.2, 128.0, 127.5, 127.1, 127.0, 123.9, 123.5, 121.4, 121.2, 117.6, 78.2, 29.7, 25.06, 20.6, 13.3, 13.2, 12.5, 12.3; HR-MS: 415.2018 (M+H)+, calcd. 415.2021.

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (1-pyridin-2-yl-ethylidene)-hydrazide (PKSB-8)

Off white solid IR (KBr, ν, cm^{-1}): 3374 (-OH), 1688 (CO); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 9.66 (s, 1H,-CONH-), 8.54 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.96 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.82-7.77 (m, 1H, Ar-H), 7.57 (s, 1H, OH), 7.38-7.35 (m, 1H, Ar-H), 2.61-2.42 (m, 2H, >CH_2), 2.33-2.26 (m, 1H, >CH_2), 2.16 (s, 3H, -CH_3), 2.15 (s, 3H, -CH_3), 2.05 (s, 3H, -CH_3), 1.95 (s, 3H, -CH_3), 1.84-1.77 (m, 1H, >CH_2), 1.51 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):170.0 (>C=O), 154.9, 154.1, 149.2, 146.7, 144.0, 137.2, 124.8, 123.6, 121.4, 121.2, 120.8, 117.6, 78.1, 29.6, 24.8, 20.5, 13.2, 12.4, 12.3, 11.3; HR-MS: 368.1973 (M+H)+, calcd. 368.1974.

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (1-pyridin-4-yl-ethylidene)-hydrazide(PKSB-9)

White solid IR (KBr, ν, cm^{-1}): 3377 (-OH), 1684 (CO); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): 9.49 (s, 1H,-CONH-), 8.60 (d, *J* = 6 Hz, 2H, Ar-H), 7.63 (dd, *J* = 4, 6 Hz, 1H, Ar-H), 4.54 (s, 1H, OH), 2.67-2.62 (m, 2H, >CH_2), 2.51-2.45 (m, 1H, >CH_2), 2.24 (s, 3H, -CH_3), 2.19 (s, 3H, -CH_3), 2.07 (s, 3H, -CH_3), 2.05 (s, 3H, -CH_3), 2.01-1.94 (m, 1H, >CH_2), 1.64 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$):170.6 (>C=O), 150.1, 149.5, 146.0, 144.7, 140.0, 121.8, 121.4, 120.6, 119.5, 118.0, 78.8, 29.6, 24.8, 20.5, 12.3, 12.2, 12.1, 114.4; HR-MS: 368.1975 (M+H)+, calcd. 368.1974.

In vitro anticancer activity by sulforhodamine b assay

The *in vitro* cytotoxic activity was checked using sulforhodamine B stain (SRB) assay.[15] Cells were plated in 96 multi-well plates (104 cell/ well) for 24 h before treatment with the compounds. Different concentrations of the test compounds (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) were put into the cell monolayer. For each individual dose, triplicate wells were made. Test compounds were incubated into monolayer cells for almost 48 h at 37˚ C and in an atmosphere of 5% CO_2. Then after completion of 48 h, cells were fixed, washed, and stained with SRB. If there made the excess stain, it would be washed with acetic acid. The Tris-EDTA buffer was used for recovering the attached stain. ELISA reader was used for measuring color intensity. The relation between surviving fraction and drug concentration was plotted and GI_{50} was calculated for each compound.

CONCLUSION

Hence, from results obtained in the present study, it can be concluded that all compounds (**PKSB 1-9**) exhibited remarkable anticancer activity on MCF-7 and MDA-MB-231 cell lines. As compounds were more active on MCF-7 cell line, so based on the results, it might be proposed that the synthesized compounds were worked by acting against ER-positive breast tumor. Docking, *in silico* ADME, and toxicity studies were also favorable for the synthesis of compounds. Further evaluation of the detail mechanism pathway involved in an activity needs to be investigated.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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