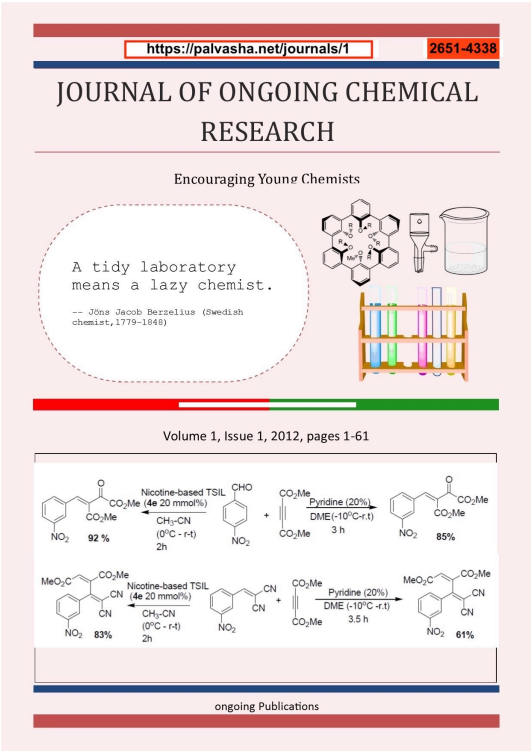
SYNTHETIC BISCOUMARIN ANALOGS: THEIR PC3 CELL LINE AND ANTIOXIDANT INHIBITORY POTENTIALS



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Research Article

Synthetic Biscoumarin Analogs: Their PC3 Cell Line and Antioxidant Inhibitory Potentials

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Abstract

Prostate cancer is perhaps the most dominant cancer in men. The increased resistance to therapeutic agents and deficiency of targeted therapy for prostate cancer cells offer motivation to find new scaffolds for the treatment. The current study investigates substituted biscoumarin analogs (**1-19**) anticancer activity in the prostate cancer (PC3) cell line and its antioxidant potential. Out of these nineteen analogs, the compounds **1**, **8**, **9**, **10**, **12**, **14**, **16**, **18** and **19** showed good PC3 cell line inhibitory potential when compared with the standard doxorubicin. Compounds **1-19** were also checked for DPPH radical scavenging potential. Compounds **2**, **5**, and **17** showed good potential. All analogs were also evaluated for superoxide anion scavenging activity and found to be inactive. The docking analysis shows that the biscoumarin is an anti-cancer ligand and has the ability to inhibit the activity of the human histone acetyltransferase. Overall this study has contributed for anticancer and antioxidant agents.

Keywords: Molecular Docking, Biscoumarin, PC3 Cell Line, DPPH Radical, Superoxide Anion Scavenging

INTRODUCTION

Prostate cancer (CaP) is the most common cancer-causing death in men. According to the American Cancer Society in the USA in 2012 almost 28,000 peoples died from CaP [Siegel et al, 2012]. Prostate cancer frequently exists in several sites within the prostate and has mutable characteristics [Boyd et al, 2012]. The great complexities of prostate cancer bring phenotypic variation, which results in major hurdles in prostate cancer treatment [Drake et al, 2013]. To date no such complete and reliable therapy exists for the remedy of this disease. Therefore there is an urgent need to explore alternate chemo-therapeutic approaches. The medicinal chemists are continuously in struggles to ascertain inventive molecular objectives at several stages of clinical development for CaP [George et al, 2012; Higano et al, 2011].

Reactive oxygen species have been allied with cancer, neurodegenerative and heart diseases. Moreover, it also causes DNA damage to cells in the body. Antioxidants have the ability to scavenge or prevent the production of ROS, thus can defend against the free radicals formation and hinder the development of many long-lasting diseases, including cancer [Joseph et al, 2013]. Free radicals are vital to numerous biochemical processes and be a dynamic portion of metabolism and aerobic life [Ashok et al, 2001]. Major reactive oxygen species like superoxide anion, hydrogen peroxide radicals and peroxyl radicals have been also reported to be involved in many diseases like arthritis, physical injury, aging, carcinogenesis, HIV, and infection [Joyce, 1987]. Antioxidant therapy gained an enormous significance in the cure of these ailments by inhibiting free radicals either through chelating or by acting as oxygen scavengers [Buyukokurouglu et al, 2001; Shahidi et al, 1992]. A monotherapy of an anticancer drug with antioxidant properties will probably become more beneficial from the pharmacoeconomic point of view.

Biscoumarin has received considerable attention in the past few years for its versatile biological properties, such as antioxidant, anti-inflammatory, antibacterial, and anticancer activities [Au et al, 2008]. It was reported that biscoumarin derivatives can strongly inhibit tubulin aggregation and played an efficient role against cancer. Other mechanism of anti-cancer

activity was due to the promotion of apoptosis and anti-angiogenesis [Kim et al, 2012]. In this study herein we are going to report substituted biscoumarin analogs, their anticancer and antioxidant potentials, and molecular docking studies.

RESULTS AND DISCUSSION

Chemistry

Various coumarin derivatives were mixed and refluxed for few hours with a different aromatic aldehyde in distilled water in the presence of tetraethylammonium bromide to give biscoumarin analogs **1-19 as shown in** Fig-1. The reaction completion was monitored by Thin Layer Chromatography. To obtain a pure product, the reaction mixture was filtered and washed with distilled water. In some cases, the column chromatography technique was used for the purification process [Khan et al, 2014].

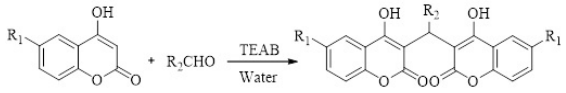

Figure 1. Synthesis of biscoumarin derivatives **(1-19)**

Table 1. Different Substituents of Biscoumarin Derivatives (1-19)

S. No	R_1	R_2	S. No	R_1	R_2	S. No	R_1	R_2
1	Cl	3-nitrophenyl	8	Cl	2,4-dichlorophenyl	15	CH_3	3-hydroxyphenyl
2	Cl	(4-hydroxy,3-methoxy) phenyl	9	Cl	2-nitrophenyl	16	CH_3	2-nitrophenyl
3	Cl	3-hydroxyphenyl	10	Cl	4-methoxyphenyl	17	CH_3	(4-hydroxy,3-ethoxy) phenyl
4	Cl	(3-hydroxy,4-methoxy) phenyl	11	CH3	3,4-dimethoxyphenyl	18	CH_3	2-nitrophenyl
5	Cl	(4-hydroxy,3-ethoxy) phenyl	12	CH3	4-nitrophenyl	19	CH_3	4-methoxyphenyl
6	Cl	3,4,5-trimethoxyphenyl	13	CH3	3,4,5-trimethoxyphenyl			
7	Cl	3,4-dimethoxyphenyl	14	CH3	4-thioanisoyl			

Biological Activity

Prostate Cancer Inhibition of Biscoumarin Compounds

Biscoumarin compounds (**1-19**) were evaluated for their activity PC3 cell line. Out of nineteen, compounds **1**, **8**, **9**, **10**,**12**, **14**, **16**, **18** and **19** showed good PC3 cell line inhibitory potential with IC_{50} value of 21.27 ± 0.43, 19.47± 0.64, 28.443 ± 0.96, 27.03± 0.51, 14.80± 0.81, 23.15± 0.04, 21.45 ± 0.46, 11.98 ± 0.34 and 28.65 ± 0.29 µM, respectively when compared with the standard doxorubicin (IC_{50} = 0.91 ± 0.12 µM). A closer view of the structure-activity relationship suggests that the cytotoxic potency was not surprisingly depending on the substitution patterns on the phenyl ring of aldehyde like in case of antioxidant activity. The different substituents on the phenyl ring of coumarin can also slightly change the cytotoxicity against the cancer cell lines checked. Compound **18** showed good inhibition having methyl group on each coumarin part while one nitro group on the aldehydic phenyl part at position-2. Compound **12** is the second analog among the series which showed good inhibition having methyl group on each coumarin part while one nitro group on the aldehydic phenyl part at position-4. Compound **8** is the third analog among the series which showed good inhibition having chloro group on each coumarin part and two chloro groups on the aldehydic phenyl part at 2,4-positions. The slight difference in the potential of analogs **18** and **12** might be due to the difference in the position of the nitro group. Similarly, compounds **1**, **16**, **14**, **10**, **9** and **19** also have either electron-withdrawing group or electron-donating groups on aldehydic phenyl part as well as on coumarin showed good to moderate inhibition. We observed here that the electron-withdrawing group greatly affects the potential of compounds. This is possibly due to greater lipophilicity, physicochemical properties, pharmacokinetic properties, durability for metabolic destruction and electronegativity.

Table 2. Results of PC3 Cell line Inhibition of Biscoumarin Compounds (1-19)

S. No.	IC_{50} ± SEM[a](µM)	S. No.	IC_{50} ± SEM[a] (µM)	S. No.	IC_{50} ± SEM[a](µM)
1	21.27± 0.434	**8**	19.47 ± 0.64	**15**	NA[b]
2	NA[b]	**9**	28.45 ± 0.96	**16**	21.45 ± 0.46
3	NA[b]	**10**	27.03 ± 0.51	**17**	NA[b]
4	NA[b]	**11**	NA[b]	**18**	11.98 ± 0.34
5	NA[b]	**12**	14.80 ± 0.81	**19**	28.65± 0.29
6	NA[b]	**13**	NA[b]	**Doxorubicin**[c]	0.91± 0.12
7	NA[b]	**14**	23.15 ± 0.04	-	-

Molecular Docking Study

The docking results described that the biscoumarin buried well in the binding pocket of the enzyme (**Fig 2**). The biscoumarin formed five interactions with the active site residues of the enzyme. Among the five interactions, it was observed that three were polar hydrogen bond interactions, one arene-arene, and an

arene cation interaction. Arg124 is a basic amino acid and acts as a hydrogen bond donor observed in the docking analysis. Arg124 interacted with the oxygen atom of the carbonyl group of the 4-hydroxy-6-methyl-2H-chromen-2-one (**Compound 18**) of the biscoumarin compound. Tyr128 acts as a hydrogen bond donor and hydrogen bond formed between the hydrogen atom of the Tyr128 and the carbonyl oxygen atom of the 4-hydroxy-6-methyl-2H-chromen-2-one moiety of the same inhibitor. Gln169 showed in interaction with the oxygen atom of the hydroxyl group of the 4-hydroxy-6-methyl-2H-chromen-2-one moiety. Trp180 is a hydrophobic amino acid and formed an arene-arene interaction with the benzene ring of the nitro-benzene. Arene cation interaction was detected between the basic amino acid His165 and benzene ring of the 4-hydroxy-6-methyl-2H-chromen-2-one of the ligand. This docking analysis has shown that the biscoumarin is an anti-cancer ligand and has the ability to inhibit the activity of the human histone acetyltransferase. Further investigations might be very useful for developing more novel and potent inhibitors of the enzyme.

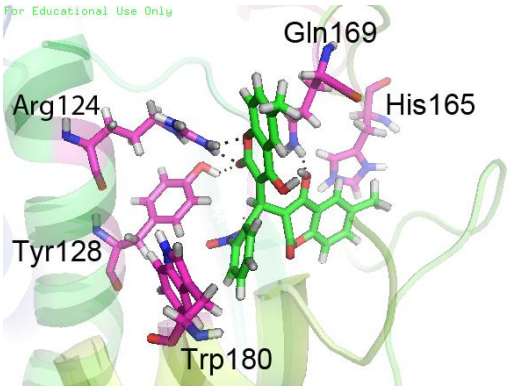

Figure 2. Binding mode of compound **(18)** in the active site of the enzyme.

The activity difference between compounds **4** and **2** having the same functional group on aldehydic phenyl ring might be due to the position difference of methoxy and hydroxyl functionality. Compound **17** also showed inhibition having an ethoxy group at 3-position and hydroxy group at 4-position. The slight activity difference between **5** and **17** might be due to the difference of methyl and chloro groups on coumarin. Here we observed that the presence of hydroxyl group along with methoxy and ethoxy groups greatly affects the activity. The free radical scavenging potential of the compounds seems as a capability to stabilize the free radicals of the compounds. All active compounds among the series possess hydroxyl groups on the phenyl ring of aldehyde, which on the abstraction of hydrogen radical by DPPH results in the formation of oxygen radical on the aromatic ring. The resulted oxygen radical go through to resonance thus accomplishing stability. Extended conjugation might be involved here to such radicals for their stabilization as in this case. This ability of a compound to stabilize the free radicals in fact determines the free radical scavenging strength. The remaining all compound showed no inhibition and this is because of having no hydroxyl along with methoxy or ethoxy functionality. Structure-activity relationship (SAR) reveals that the presences of hydroxyl group along with electron-donating groups on aldehydic phenyl ring are generally more beneficial.

DPPH Radical Scavenging Activity of Biscoumarin Compounds

Biscoumarin compounds (**1-19)** were screened for DPPH radical scavenging potential. A perusal of Table-2 reveals that out of nineteen compounds, the analogs **2**, **5** and **17** with IC_{50} value of 36.75 ± 1.2, 38.6 ± 1.64 and 38.93 ± 2.01 µM, respectively showed good DPPH radical scavenging activity when compared with the standard *n*-propyl gallate (IC_{50} value of 30. 27 ± 1.6 μM). Compound **4** (IC_{50} value 53.65 ± 2.9 µM) showed moderate DPPH radical scavenging activity. It is clear from the results that the activity of the compounds is connected with the nature and position of substituents on aldehydic phenyl ring. Compound **2** is the most active analog among the series having methoxy group at position-3 and hydroxyl group at position-4 on aldehydic phenyl part. Similarly compound **5** also showed good inhibition having ethoxys group at position-3 and hydroxyl at position-4 on aldehydic phenyl part. The very minute activity difference among these two analogs might be due to the difference of methoxy and ethoxy functionality. The compound **4** showed moderate inhibition having a hydroxy group at position-3 and methoxy group at position-4 on aldehydic phenyl part.

Table 3. Results of DPPH Radical Scavenging of Biscoumarin Derivatives (1-19)

S. No.	IC_{50} ± SEM[a](μM)	S. No.	IC_{50} ± SEM[a](μM)	S. No.	IC_{50} ± SEM[a](μM)
1	NA[b]	**8**	NA[b]	**15**	NA[b]
2	36.75 ± 1.2	**9**	NA[b]	**16**	NA[b]
3	NA[b]	NA[b]	**17**	38.93±2.01	
4	53.65 ± 2.9	**11**	NA[b]	**18**	NA[b]
5	38.6 ± 1.64	**12**	NA[b]	**19**	NA[b]
6	NA[b]	**13**	NA[b]	***n*-Propyl gallate**[c]	**30.27 ± 1.6**
7	NA[b]	**14**	NA[b]	-	-

Superoxide Inhibition of Biscoumarin Derivatives

Biscoumarin derivatives **1-19** were screened for superoxide inhibition. All compounds exhibited less than 50% inhibition, therefore not further screened for IC_{50}. Quercetin was used as standard with IC_{50} value of 94.1 ± 1.1*m*M.

EXPERIMENTAL

NMR spectra were recorded on Avance Bruker AMX-400 and AM-300 MHz. EI-MS were recorded on a Finnegan MAT-311, Germany. TLC was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm. Column chromatography was carried out on silica gel (E. Merck, type 60, 70-230 mesh).

General Procedure for the Synthesis of Biscoumarin Derivatives (1-19)

Different aromatic aldehyde (2.0 mmol) were mixed and refluxed for 1-2 hours with various coumarin derivatives (4.0 mmol) in distilled water in the presence of tetraethyl ammonium bromide to give biscoumarin analogs **1-19**. The reaction completion was checked by TLC. The reaction mixture was filtered and washed with distilled water. In some cases column chromatography technique was used for the purification process [Khan et al, 2014].

Characterization of representative compound 3,3′-{(2-nitrophenyl)methylene}bis(6-chloro-4-hydroxy-2H-Chromen-2-one) (1)

Yield: 0.21 g (80%); ^{1}H-NMR (DMSO, 300 MHz); δ 7.69 (d, 2H, *J* = 2.7Hz, H-5/5′), 7.52 (m, 4H, H-7/7′/3″/6″),7.33 (m, 4H, H-8/8′/4″/5″), 6.42 (s, 1H, Ar_3CH). EI-MS m/z (rel. int. **%):**526 (M^+, 42), 298 (67), 283 (58), 196(27), 154 (100).

Prostate Cancer Cell Line Activity Assay

PC-3 and C4-2B cells and PBMCs were plated at a density of 3 x105 cells/well in six-well plates in a medium having 10% FBS, cultured for 24 h, and cured by adding of different concentrations of pso to the medium. The control cells were treated with the same volumes of DMSO alone which never exceeded 0.002% of the total volume of the medium. After each treatment, cells were incubated in an atmosphere of 5% CO_2 at 37 °C for 24 hrs. Viable cells were counted by Trypan blue exclusion using a hemocytometer. Results were expressed as a percentage of the number of cells in DMSO-treated control cultures, and the IC_{50} values were calculated 133 using non-linear regression analysis [Lim et al, 2005].

DPPH Radical Scavenging Assay

The DPPH radical scavenging potential of biscoumarin analogs was measured in terms of hydrogen donating ability by the stable radical DPPH [Blois, 1958]. DPPH solution 0.1 mM in ethanol was set from which 1.0 mL was added to 3 mL solution of biscoumarin analogs and standard in the water at different concentrations (10-100 μg/mL). Absorbance was measured at 517 nm after 30 mins. The higher free radical scavenging potential showed lower absorbance of the reaction mixture. DPPH radical scavanging calculaiton were done as shown in Fig-3.

$$\% \text{ inhibition} = \frac{A_{conc} - A_{test}}{A_{conc}} \times 100$$

Figure 3. DPPH radical scavenging activity calculation equation.

Superoxide Anion Scavenging Assay

The superoxide anion radicals scavenging potential of biscoumarin analogs were measured by Ni-Shimiki *et al.* method and with slight changes. About 0.1mL of different concentrations of biscoumarin analogs and 1.0 mL of nitro blue tetrazolium solution and standard in water were assorted. The reaction was started by adding 100 µL of phenazinemethosulphate solution in 100mMphosphate buffer (pH7.4) to the mixture. The reaction mixture was incubated for 5 min at room temperature and the absorbance was measured at 560nm against reagent blank in the spectrophotometer. Quercetin was used as a standard.

Molecular Docking Simulation

PASS (Prediction of Activity Spectra) is an online tool [Lagunin et al, 2000] that predicts almost 900 types of activities based on the structure of a compound. PASS analysis (**Table-4**) of the biscoumarin predicts anti-cancer activity (human histone acetyltransferase) with Pa (probability to be active) value of 0.173. To evaluate the inhibitory nature of the title compound against human histone acetyltransferase, molecular docking simulations were carried out. Molecular docking is an efficient method to get an insight into

ligand-receptor interactions. Molecular docking studies were performed using Molecular Operating Environment (MOE) software (www.chemcomp.com). The 3D crystal structure of human histone acetyltransferase was downloaded from Protein Data Bank (PDB ID: 4PZS) [Oikonomakos et al, 2000]. Before the docking experiment, the 3D structure of biscoumarin was modeled using MOE. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was done prior to docking using Protonate 3D tools implemented in MOE. Protonation was followed by energy minimization up to 0.05 Gradient using the Amber99 force field. The docking protocol predicted the same conformation as was present in the crystal structure with RMSD value close to the allowed range [Paul et al, 2004] and surrounded by the same active site residues of the enzyme. Amongst the generated docking conformations the top-ranked conformation was visualized for ligand-enzyme interaction using PyMol.

Table 4. PASS prediction of the compound, Pa represents probability to be active and Pi represents probability to be inactive.

Pa	Pi	Predicated activity
0.206	0.126	2-Hydroxymuconate-semialdehyde hydrolase inhibitor
0.111	0.032	Phosphomevalonate kinase inhibitor
0.185	0.106	CYP2A2 substrate
0.127	0.048	L-ascorbate peroxidase inhibitor
0.160	0.082	4-Chlorobenzoyl-CoA dehalogenase inhibitor
0.238	0.160	Manganese peroxidase inhibitor
0.136	0.059	UGT2B1 substrate
0.173	0.096	Histone acetyltransferase inhibitor
0.174	0.097	Rhizopuspepsin inhibitor
0.137	0.060	Glucosamine-6-phosphate deaminase inhibitor
0.132	0.057	Antineoplastic (uterine cancer)
0.177	0.102	Cyclopropane-fatty-acyl-phospholipid synthase inhibitor
0.110	0.036	Muconatecycloisomerase inhibitor
0.122	0.049	6-Methylsalicylate decarboxylase inhibitor
0.147	0.075	Glutamin-(asparagin-)ase inhibitor

CONCLUSION

In conclusion, all synthetic analogs of biscoumarin were screened for PC3 cell line inhibitory potential, DPPH radical scavenging, Superoxide anion. Among the series compounds **2**, **5** and **17** showed good DPPH radical scavenging activity, when compared with the standard *n*-propyl gallate (IC_{50} value = 30. 27 ± 1.6μM). All biscoumarin derivatives were found inactive in superoxide anion inhibition. Furthermore, all compounds were also evaluated for their PC3 cell line activity. Compounds **1**, **8**, **9**, **10**, **12**, **14**, **16**, **18**, and **19** showed good PC3 cell line inhibitory potential when compared with standard doxorubicin. The binding interactions of active compound **18** were confirmed through molecular docking, which showed interaction with amino acids Arg124, Tyr128, Gln169, His165, and Trp180. Moreover, the pragmatic antioxidant and anticancer activities may be viewed as vital steps for the designing of effective new scaffolds with greater pharmacological potential compared to that of the standard drugs.

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