ejpmr, 2024, 11(1), 544-553

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

THE DESIGN, SYNTHESIS AND ANTI-TUMOUR PROPERTIES OF AURONE ANALOGUES AS PRKACA INHIBITORS

Vibina K.[1], Dr. Shebina P. Rasheed[2]*, Krishnendu P. R.[3], Dr. Arun Rasheed[2] and Neethu Varghese[2]

[1]Department of Pharmaceutical Chemistry, Crescent College of Pharmaceutical Science College.
[2]Department of Pharmaceutical Chemistry, Al Shifa College of Pharmacy, Perinthalmanna. Affiliated to Kerala University of Health Sciences, Thrissur, Pin Code 679325, (Kerala) India.
[3]Department of Pharmaceutical Chemistry Amrita College of Pharmacy, Elamakkara Kochi, Kerala.

***Corresponding Author: Dr. Shebina P. Rasheed**

Department of Pharmaceutical Chemistry, Al Shifa College of Pharmacy, Perinthalmanna. Affiliated to Kerala University of Health Sciences, Thrissur, Pin Code 679325, (Kerala) India.

Article Received on 21/11/2023 **Article Revised on 11/12/2023** **Article Accepted on 01/01/2024**

ABSTRACT

The antiprostate cancer effect of Aurone analogues is revealed by inhibiting PRKACA in recent studies. PRKACA is the catalytic subunit of cAMP-dependent protein kinase A's alpha catalytic subunit. Overexpression of PRKACA induces severe cancer in prostate cells and plays a crucial role in cell signalling for numerous cellular activities. The compounds which inhibit PRKACA can be developed as anti-prostate cancer agents. Molecular docking studies were used to generate a panel of Aurone analogues, which were then synthesised by an oxidative cyclization of 2'-Hydroxy chalcones and their structures validated using several spectrometric methods. Commercially available 1'-Hydroxy-2'-acetonaphthones were used to make the chalocones. The compounds were then put through a series of pharmacological tests, including the MTT assay (PC-3 cells) and the DPPH assay. All the compounds show cytotoxic activity against PC-3 (prostate cancer cells). The results demonstrated that the compounds **AU-F(**2-(4-Fluorobenzylidene) naphtho[1,2-b] furan-3(2H)-one**)** and **AU-T(**2- (Thiophen-2-ylmethylene) naphtha [1,b]furan3 (2H)one**) with IC_{50} values of** 37.4461 µg/ml and 49.8939 µg/ml have shown better activity than the standard, Bicalutamide(**IC_{50}**.55.72µg/ml). And the compound AU-A((2-(4-Methoxybenzylidene) naphtho[1,2-b]furan3(2H)one) exhibits moderate activity with an **IC_{50} value of** 65.8055µg/ml. and In addition, the molecular docking study by using PDB ID- 2GU8 revealed that the compound **AU-T(**2-(Thiophen-2-ylmethylene) naphtha [1,b]furan3 (2H)one**) have a high binding affinity for the PRKACA subunit. Results of DPPH Assay shows that AU-F**, **AU-T** and **AU-A** have better activity than standard Ascorbic acid. The synthesized Aurone derivatives exhibit anti-cancer activity in different ratios. Among them the compounds **AU-F**, **AU-T** and **AU-A** are worthy of further development as anti-prostate cancer agents.

KEYWORDS: Aurones, Naphthofuranones, DPPH Assay, PRKACA inhibition, Prostate Cancer, PC-3, MTT Assay, 2'-Hydroxy chalcones.

INTRODUCTION

Aurones are heterocyclic chemicals found in nature that belong to the flavonoid family. They are isomeric to flavones and can be either natural or manufactured. The molecule has two isomers, with (E) and (Z) orientations. In position 2, the molecule comprises a benzofuran element connected to a benzylidene. A chalcone-like group in aurone is closed into a 5-membered ring rather than the 6-membered ring found in flavonoids.

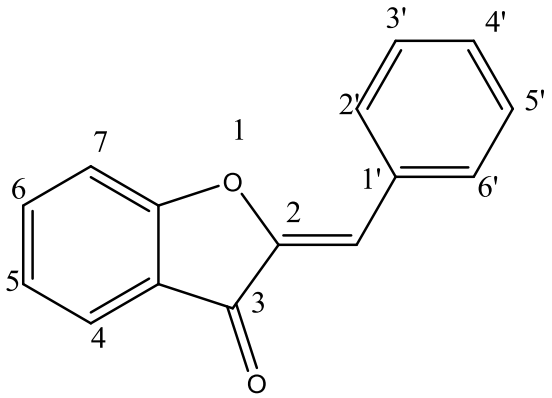


Figure 1: Basic structure of Aurone.

Flavonoids are a wide family of plant-derived natural compounds with a variety of biological activity. Aurones serve a crucial function in the pigmentation of several flowers and fruits, contributing to the vivid yellow colour of flowers in particular.[1] Aurones have been demonstrated to have anticancer, antioxidant, antimicrobial,[2,3] antidiabtic,[4] Xanthine oxidase inhibition, anti-inflammatory,[5,6] anti-hormonal, anti-fungal, anti-asthmatic, anti-obesity,[7] anti-parasitic,[8] AchE Inhibitory activities,[9] and other biological properties.

The goal of this research is to assess the anti-oxidant and anticancer properties of various Aurone analogues. Free radicals are created on a constant basis by normal or pathological cell metabolism, and they play a significant role in the immune system.[10] There are various forms of free radicals; reactive oxygen species [ROS] are those that are produced from oxygen. The unregulated production of reactive oxygen species (ROS) is linked to the start of a variety of diseases, including cancer, atherosclerosis, arthritis, Parkinsonism, cirrhosis, Alzheimer's disease, and ischemic heart disease.[11-17] The human body contains its own defense mechanism in the form of enzymes, such as superoxide dismutase, catalase and glutathione peroxidase etc.[18-19] Antioxidants are compounds that protect against auto-oxidation and minimize free radical damage. They reduce the risk factors of chronic diseases like cardiovascular disease and cancer.[20] Antioxidants are abundant in grains, fruits, and vegetables. Plant-based antioxidants like vitamin C, vitamin E, carotenes, and other carotenoids have been used as supplemental antioxidants. Flavonoids, for example, are secondary metabolites that aid in the protection of cells from free radicals. Aurones are flavonoids-like chemicals that have been shown in a number of studies to exhibit antioxidant properties.[21-24] A panel of aurone analogues with a Naphthofuranone ring were synthesised and their antioxidant potential was assessed using the DPPH Radical scavenging assay, followed by their anticancer potential. Cancer, a leading cause of death in humans, is receiving worldwide attention. Despite advances in our understanding of the regulatory systems involved in the beginning of cancer, current treatments are non-specific and hazardous to patients, resulting in a variety of adverse effects. According to the National Cancer Registry Program (NCRP), there were 6,92,704 males and 6, 95,693 women diagnosed with cancer in India in 2015. According to NCRP forecasts, there would be 8,71,756 men and 8,63,130 women diagnosed with cancer in India by 2020.[25] This highlights the need for novel anticancer drugs that are less hazardous and more effective. Aurones have been mentioned in several papers as having the potential to be used in cancer treatment. They block carcinogenesis by inhibiting P-gp expression and Cyclin Dependant Kinases Tyrosinase inhibition, HDAC inhibition, PRKACA inhibition.[26,30,33] We developed a new family of Aurone analogues as antiprostate cancer medicines in pursuit of new anticancer drugs. We explain their design, synthesis, characterisation, and anticancer potential against the PC-3 (prostate cancer) cell line in this paper The antioxidant activity of the produced compounds is further evaluated using the DPPH Radical Scavenging Assay. A panel of Aurone analoges were docked for their binding affinity towards PRKACA, which is the alpha catalytic subunit of cAMP-dependent protein kinase A and plays a key role in cell signaling to carry out a variety of physiological tasks. In prostate cells, overexpression of PRKACA produces severe tumorigenesis.[31] Docking is a method for predicting the binding orientation of small molecule drug candidates to their protein targets, which can then be used to estimate the small molecule's affinity and activity.[33-36] As a result, docking is critical in the rational design of medications. Molecular modelling experiments were carried out on a Window``s 7. Docking investigations were carried out with the use of the Schrodinger suite 2012, which included modules such as Ligprep, Glide, and Prime. The RCSB protein data bank provided the X-ray crystallographic 3D structure used in this investigation. We analysed the binding affinity of several Aurone analogues using the best suited X-ray structures of PRKACA Subunit (PDB ID: 2GU8).

Experimenital

MATERIAL AND METHODS

Docking Studies

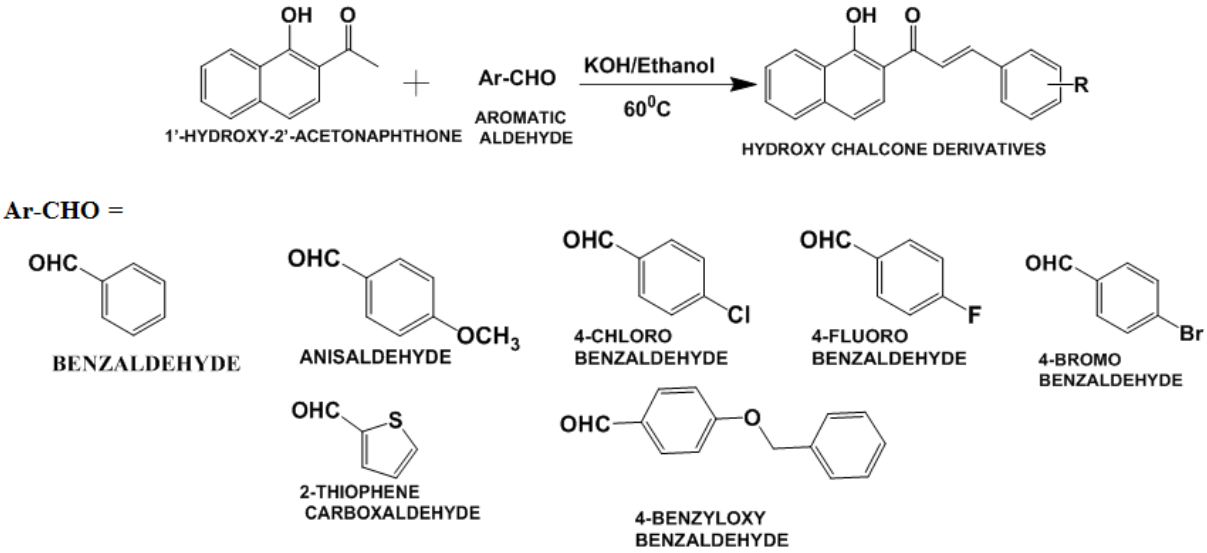
All compounds were docked into the binding site of the PDB ID: 2GU8 (PRKACA) using Schrodinger's Grid-Based Ligand Docking With Energetics (Glide) software to test the docking settings.[37] It searches for beneficial interactions between one or more normally tiny ligand molecules and a typically bigger receptor molecule, usually a protein, using grid-based ligand docking and energetics. Glide has three docking precision levels: The docking calculations were done first in HTVS mode, then in SP and XP mode.[37] All of the molecules were generated in Maestro using the Built module, and they were all subjected to an exhaustive conformational search using the OPLS-2005 force field, with a cut-off of total conformational energy permissible compared to the lowest-energy state.[37] Minimization cycles with default values of 0.05 for the initial step size and 1.00 for the maximum step size were used for conjugate gradient and steepest descent minimizations. With default values of 10-7 and 0.001kcal/mol, respectively, both the energy change and gradient criteria were used as convergence criteria for the minimization.[37]

The chemicals utilised in the synthesis were purchased from Sigma Aldrich USA, NICE Chemicals Pvt Ltd, Alfa aesar, and other suppliers. Melting point determination, Thin layer chromatography, and other spectrum analyses such as IR, 1H-NMR, C13-NMR Spectroscopy, and Mass spectrometry were used to confirm the characterisation of the anticipated structures of the synthesised derivatives. The open-capillary tube method was used to determine the melting points of all the synthesised derivatives, and the results were

uncorrected. TLC was checked on a regular basis for the formation of compounds, and spots were visualised using an Iodine chamber. The IR spectra of all synthesised compounds were documented using Bruker ATR instruments, Al Shifa College of Pharmacy, 1H NMR, and C13 NMR. The Brucker Avance II 400 NMR Spectrometer at the Sophisticated Analytical Instrument Facility at the University of Punjab and the ESI-MS Q-Tof Microwaters Mass Spectrometer at IIT Ropar were used to record the spectra of all synthesised derivatives

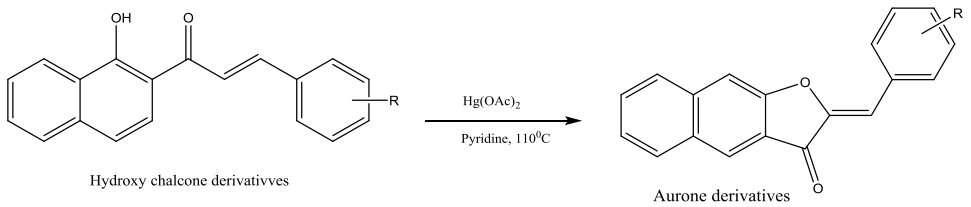
General Method Of Synthesis For 2'-Hydroxychalcone Derivatives[38]

A equimolar quantity of 1'-Hydroxy-2'-acetonaphthone was mixed with a KOH solution in ethanol, then an equimolar quantity of substituted benzaldehydes was added and thoroughly stirred. The reaction mixture was neutralised with 0.1 N HCl after vigorous stirring to facilitate precipitation, and it was kept in the refrigerator for the entire night. The crude chalcones produced were dried in the air and recrystallized with ethanol.

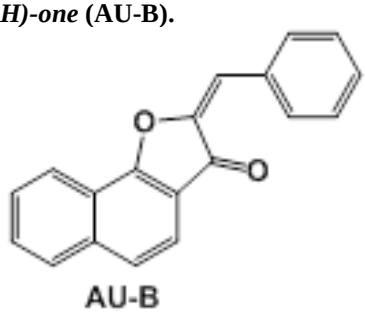


General Method of Synthesis For Aurone Analogues[39]

At room temperature, hydroxy chalcones (1equiv) were added to a solution of mercuric acetate (1equiv) in pyridine, and the mixture was stirred at 110°C. The reaction mixture was cooled before being put into ice cold water and acidified with HCl (10% aqueous solution)[39] Using ethanol, the precipitated solid was dried and recrystallized.[39]



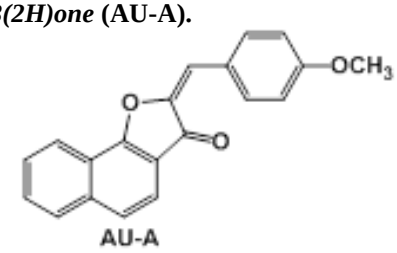
2-*Benzylidenenaphtho [1, 2-b] furan -3(2H)-one* (AU-B).



Yield: 87.5%, mp-84-90°C, IR (ν_{max}); 1698 cm-1(C=O), 3109 cm-1(aromatic C=H), 1648 cm-1(aromatic C=C), 1019,1152,1195,1238 cm^{-1} (C-O-C), ^{1}H-NMR (500MHz, $CDCl_3$) δppm; 6.994(S,1H,CH=C),7.446(dd,J= 7.5 Hz, 1H,ArH),7.502-7.532 (d, J= 7.5 Hz, 2H, ArH), 7.583(J= 8.5 Hz, ArH), 7.667.69(d, J= 7 Hz, 2H,ArH), 7.739(dd, J= 5.5 Hz, 1H,ArH),8.008 8.023(d, J= 7.5 Hz, 2H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm; 184.15, 166.16, 147.55, 138.35, 132.38, 131.74, 130.42, 130.08, 129.05,

128.74, 127.16, 123.81, 121.95, 120.85, 119.16, 116.82, 113.67, ESI MS; m/z: 273.09 [M+1]+

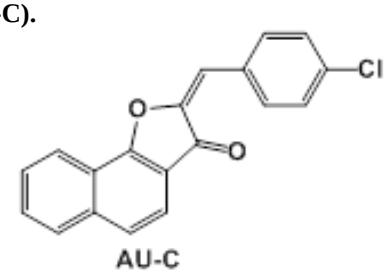
***2-(4-Methoxybenzylidene)naphtho[1,2-b]furan3(2H)one* (AU-A).**



AU-A

Yield:77.4%, mp-110-118°C, IR (ν_{max}):1692cm^{-1} (C=O), 3048 cm^{-1} aromatic(C-H) stretching,1590 cm^{-1} (C=C), 1013, 1108, 1174 cm^{-1} (C-O-C),^{1}H-NMR($CDCl_3$), δppm: 3. 90-3.926(S,3H,CH_3) 6.99(S,1H,CH=C), 7.0417.059 (d, J= 9 Hz 2H,ArH),7.606(d, J= 8.5 Hz 1H,ArH),7.68(d, J= 7 Hz 1H,ArH),7.7127.744(dd, J= 8 Hz H,ArH),7.954(d, J= 8 Hz 1H,ArH), 7.990-8.008(d, J= 9Hz, 2H,ArH), 8.384(d, J= 8 Hz, 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.15, 165.52, 161.20, 146.54, 138.15, 133.60, 130.16, 128.70, 127.02, 125.11, 123.56,121.87, 120.89, 119.19, 117.09, 114.64, 113.89, 55.43, ESI MS; *m/z*: 303.10 [M+1]$^{+}$

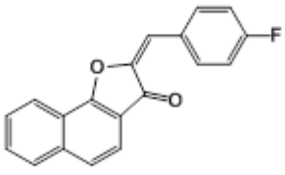
***2-(4-Chlorobenzylidene)naphtho[1,2-B]furan-3(2H)-one* (AU-C).**



AU-C

Yield: 65.7%, mp-148-156°C, IR (ν_{max}): 1703 cm^{-1} (C=O), 3061 cm^{-1} aromatic (C-H) stretching, 1625 cm^{-1} (C=C), 1092,1123,1176,1212 cm^{-1} (C-O-C), ^{1}H-NMR ($CDCl_3$), δ ppm: 6.95 (S,1H,CH=C), 7.484(d, J= 7.92 Hz 1H, ArH),7.501(d, J= 8.52 Hz 1H,ArH),7.61(d, J= 1.28 Hz 1H,ArH), 7.63(d, J= 1.2 Hz 1H,ArH), 7.768(dd, J= 5.96 Hz 1H,ArH), 7.75(dd, J= 1.08 Hz 1H,ArH), 7.76(d, J= 8.44 Hz 1H, ArH), 7.96(d, J= 5.04 Hz 2H,ArH), 8.38(d, J= 1.72 Hz 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.02, 166.09, 147.55, 138.35, 132.38, 131.74, 130.42, 130.08, 129.05, 128.74, 127.16, 123.81, 121.95, 120.85, 119.16, 116.82, 113.67, ESI MS; *m/z*: 307.02 [M+1]$^{+}$

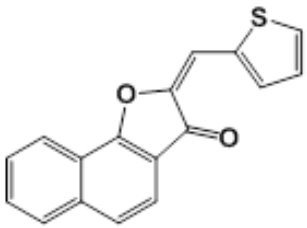
***2-(4-Fluorobenzylidene)naphtho[1,2-b]furan-3(2H)-one* (AU-F).**



AU-F

Yield: 69.9%, mp-128-134°C, IR (ν_{max}): 1698cm^{-1} (C=O), 3056 cm^{-1} aromatic (C-H) stretching, 1622 cm^{-1} (C=C), 1123,1178, 1206,1245 cm^{-1} (C-O-C), ^{1}H-NMR ($CDCl_3$), δ ppm: 6.95 (S,1H,CH=C), 7.484(d, J= 7 Hz 1H, ArH),7.501(d, J= 5.5 Hz 1H,ArH),7.61(d, J= 5.5 Hz 1H,ArH), 7.63(d, J= 9 Hz 1H,ArH), 7.768(dd, J= 7 Hz 1H,ArH), 7.75(dd, J= 7.5 Hz 1H,ArH), 7.76(d, J= 8.5 Hz 1H, ArH), 7.96(d, J= 8 Hz 2H,ArH), 8.38(d, J= 8 Hz 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.29, 162.51, 137.40, 133.70, 133.63, 130.42, 130.05, 128.74,128.68, 127.41, 127.17, 125.93, 124.92, 124.45, 12.86, 121.82, 119.09, 118.32, 116.35, 112.28, ESI MS; *m/z*: 291.08 [M+1]$^{+}$

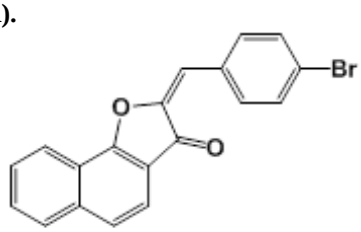
***2-(Thiophen-2-ylmethylene)naphtho[1,b]furan3(2H)one* (AU-T).**



AU-T

Yield: 75.5%, mp-64-70°C, IR (ν_{max}):1692 cm^{-1} (C=O), 3063 cm^{-1} aromatic (C-H) stretching, 1616 cm^{-1} (C=C), 1077,1115,1169,1210cm^{-1} (C-O-C), ^{1}H-NMR ($CDCl_3$), δ ppm: 7.18 (S,1H,CH=C), 7.29(d, J= 8 Hz 1H), 7.59-7.60(dd J= 7.5 Hz,2H,ArH), 7.708(d, J= 3.5 Hz 1H,ArH), 7.711(d, J= 7 Hz 1H,ArH), 7.73-7.74(d, J= 1 Hz 2H,ArH), 7.94(d, J= 8 Hz 1H,S=CH), 8.405(d, J= 8 Hz 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.19, 165.46, 146.00, 138.19, 135.72, 133.41, 132.13, 130.30, 128.63, 128.04, 127.15, 123.74, 122.05, 120.83, 119.09, 117.40, 107.62, ESI MS; *m/z*- 279.02 [M+1]$^{+}$

***2-(4- Bromobenzylidene)naphtho[1,2-b]furan-3(2H)-one* (AU-R).**

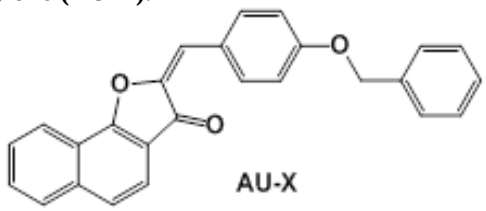


AU-R

Yield: 66.7%, mp-136-146°C, IR (ν_{max}): 1680 cm^{-1} (C=O), 3057 cm^{-1} aromatic (C-H) stretching, 1622 cm^{-1} (C=C), 1070,1116,1205,1250cm^{-1} (C-O-C), ^{1}H-NMR ($CDCl_3$), δ ppm: 6.877 (S,1H,CH=C),7.570(dd, J= 8 Hz 1H,ArH),7.612-7.629(d, J= 8.5 Hz 2H,ArH),7.696-7.707(d, J= 4.5 Hz 2H,ArH), 7.717(dd, J= 6.5 Hz 1H,ArH), 7.824-7.841(d, J= 8.5 Hz 2H,ArH), 7.927(d, J= 8 Hz 1H, ArH), 8.319(d, J= 8 Hz 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.06, 166.01, 147.69, 138.35, 132.89, 132.29, 131.29, 130.49, 128.75, 127.23,

124.48, 123.96, 121.79, 120.70, 119.07, 116.08, 112.06, ESI MS; *m/z*: 351 $[M]^+$

2-(4-(benzyloxy)benzylidene)naphtho[1,2-b]furan-3(2H)-one **(AU-X).**



Yield: 67.8%, mp-154-162°C, IR (ν_{max}): 1688 cm^{-1} (C=O), 3053 cm^{-1} aromatic (C-H) stretching, 1588 cm^{-1} (C=C), 1108,1173,1245 cm^{-1} (C-O-C), ^{1}H-NMR ($CDCl_3$), δ ppm: 5.17(S,2H, $-CH_2$), 6.99 (S,1H,CH=C)7.10-7.12(d, J= 8.5 Hz 2H,ArH), 7.361(dd, J= 7 Hz 1H,ArH), 7.42-7.43(d, J= 7.5 Hz 2H,ArH), 7.46-7.47(d, J= 7 Hz 2H,ArH), 7.6(dd, J= 8.5 Hz 1H,ArH), 7.68-7.69(dd, J= 6.5 Hz 2H,ArH), 7.72(dd, J= 8 Hz 1H, ArH), 7.95(d, J= 8 Hz 1H,ArH), 7.98-8.003(d, J= 9 Hz 2H,ArH), 8.38(d, J= 8 Hz 1H,ArH), ^{13}C NMR (500MHz) $CDCl_3$ δ ppm: 184.05, 165.65, 160.35, 146.58, 138.15, 136.42, 133.61, 130.18, 128.72, 128.22, 12748, 127.03, 125.33, 121.88, 120.88, 119.18, 117.07, 115.52, 113.83, 70.14, ESI MS, *m/z*: 379.10 $[M+1]^+$

Dpph Radical Scavenging Assay

A 0.2M DPPH solution in methanol was prepared, and 1.0 ml of this solution was added to different concentrations of compound in ethanol (40, 120, 240, 360 g/ml)[40] The absorbance was measured at 517 nm 30 minutes later.[41] A blank was prepared without adding the compound. Ascorbic acid was employed as a standard at various doses (40–360 g/ml). Higher free radical scavenging activity is shown by a reaction mixture with a lower absorbance value. The following equation[24] was used to calculate the ability to scavenge the DPPH radical: Compounds' antioxidant activity was measured in terms of IC50 and compared to that of standard Ascorbic acid.[41]

$$\% \text{ Antiradical activity} = \frac{\text{Control absorbance - Sample absorbance} \times 100}{\text{Control absorbance}}$$ [41]

MTT Assay

DMEM medium containing 10% FBS was used to adjust the cell count to 1.0x105 cells/ml after centrifugation[42]. A diluted cell solution of 100µl (about 10,000 cells per well) was added to each well of a 96 well flat bottom micro titre plate. The cells were centrifuged and the pellets were suspended in 100µl of different test sample quantities made in maintenance medium after 24 hours, when the cell population was determined to be adequate.[42] After that, the plates were incubated at 37°C for 48 hours in a 5% CO_2 environment, with microscopic examination and observations taken every 24 hours. The plates were lightly shaken before being incubated at 37°C for 2 hours in a 5% CO_2 environment. The plates were gently shaken to solubilize the formed formazan after adding 100µl of DMSO/isopropanol. A microplate reader was used to measure the absorbance at a wavelength of 540nm.[42] The percentage cell viability was computed using the formula below, and the dose response curves were used to generate the concentrations of drug or test samples required to inhibit cell growth by 50%.[42]

$$\% \text{ Cell Viability} = \frac{\text{Mean OD of individual sample}}{\text{Mean OD of control}} \times 100$$ [42]

RESULTS AND DISCUSSION

The seven compounds with the highest binding affinity for PRKACA were chosen for synthesis based on docking experiments. Table 1 displays the docking scores of the seven compounds chosen. The compound AU-T has a higher glide score (-8.028) than the others in this group of seven compounds (AU-B to AU-X). AU-F and AU-A were likewise found to have good docking values.

Please see the examples of all types of References

Journal's Reference

H. Matsumoto, S. Hara, N. Nagata and K. Ikeda, *Heterocyclic*, **41**, 47 (1995)

Book Reference

M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, Springer-Verlag, New York, p.78,91(1984).

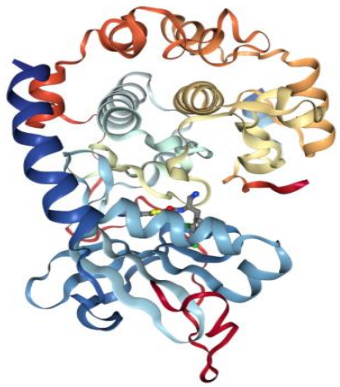


Figure 6; 3D Structure of PRKACA Subunit.

Table 1: Docking Scores of different Aurone analogues.

SL.NO	COMPOUND	GLIDE SCORE
1	**AU-B**	-6.193
2	**AU-A**	-7.129
3	**AU-C**	-6.908
4	**AU-F**	-7.434
5	**AU-T**	**-8.028**
6	**AU-R**	-6.904
7	**AU-X**	-5.682

The ligand interaction diagram of AU-T with PRKACA is shown in **Figure 2**, which showed high binding affinity with a docking score of -8.028.

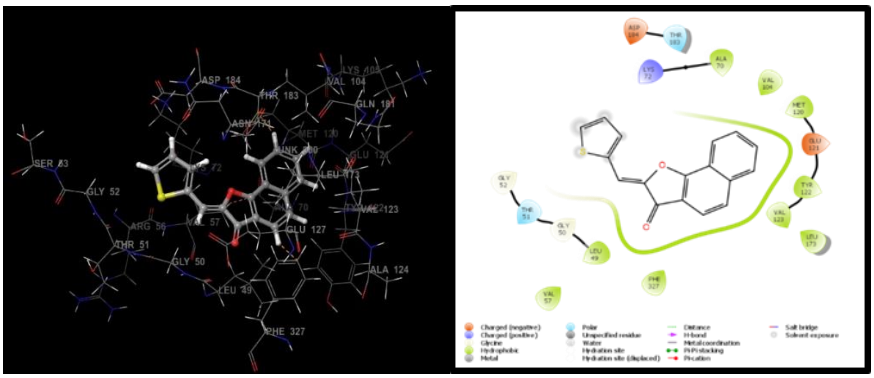


Figure 2: Ligand interaction diagram of AU-T with 2GU8.

The compounds were synthesised via an oxidative cyclization of 2'-Hydroxy chalcones in the presence of Mercuric acetate in pyridine, which yielded seven Aurone derivatives with strong binding affinity (SCHEME 2). In the presence of KOH, 2'-Hydroxy chalcones were prepared from 1'-Hydroxy-2'-acetonaphthone and diverse aromatic aldehydes (SCHEME 1).

All the synthesized compounds were evaluated for their antioxidant and anticancer activities. The antioxidant capabilities of the newly synthesized Aurone analogues were assessed using a 2, 2-diphenyl-1-picrylhydrazyle (DPPH) radical scavenging assay with Ascorbic acid as the reference antioxidant. The IC 50 values were used to conduct a comparative research. The DPPH radical is scavenged from the antioxidants in this approach, resulting in a drop in absorbance at 517 nm. The absorbance obtained is used for calculating %inhibition and the results are given in the **Table 2.** A comparison on percentage DPPH Radical scavenging activity of various Aurone analogues and standard Ascorbic acid was depicted in the **Figure 3**

Table 2: % Inhibitory activity of different compounds.

Compound	% INHIBITION			
	40 µg/ml	120 µg/ml	240 µg/ml	360 µg/ml
Ascorbic acid	26.5	50.2	75	99.2
AU-B	33.5	43.2	65	83.4
AU-A	39.8	65.3	78.4	89.2
AU-C	22.3	45.6	71.4	87.5
AU-F	33.4	59.8	74.2	89.1
AU-T	35.2	67.6	76	90.2
AU-R	18.56	42	65.3	93.1
AU-X	21.1	51.86	81	92.6

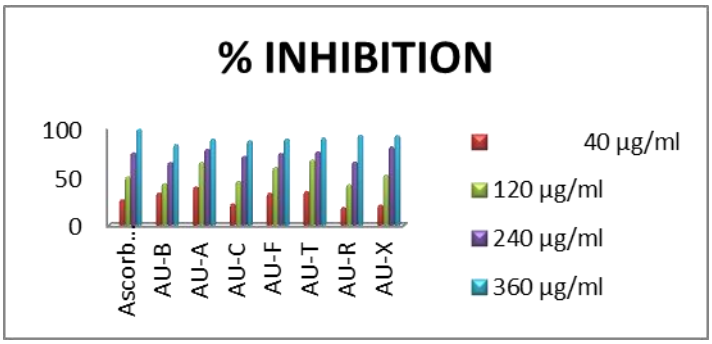


Figure 3: Comparison on percentage DPPH Radical scavenging activity of various Aurone analogues with standard Ascorbic acid.

The IC_{50}values of each compound were calculated from the graph and the results are given in the **Table 3.** A comparison on IC_{50}values of different Aurone analogues and standard Ascorbic acid was depicted in the **Figure 4.** Among the synthesized Aurone analogues compounds **AU-A, AU-T,** and **AU-F** (**64.9 µg/ml, 78.82 µg/ml,**

103.74µg/ml respectively) displayed a better radical scavenging activity than the standard, Ascorbic acid**(133.001 µg/ml)** and compound **AU-X(137.50 µg/ml**) has got a 50% inhibitory concentration comparable to that of Ascorbic acid.

Table 3: IC_{50} Values of different compounds.

Compound	IC_{50} (µg/ml)
Ascorbic acid	**133.001**
AU-B	150.66
AU-A	**64.9**
AU-C	156.92
AU-F	**103.74**
AU-T	**78.82**
AU-R	169.18
AU-X	**137.50**

Compounds AU-A, AU-T, and AU-F (64.9 g/ml, 78.82 g/ml, and 103.74 g/ml, respectively) displayed better radical scavenging action than the reference, Ascorbic acid(133.001 g/ml), and compound AU-X(137.50 g/ml) had a 50% inhibitory concentration comparable to Ascorbic acid.

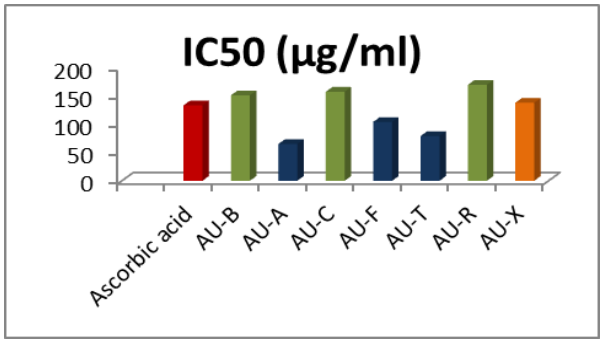


Figure 4: Comparison on IC_{50}values of different Aurone analogues with standard Ascorbic acid.

The cytotoxicity studies were carried out on the PC-3 human prostate cancer cell line. The conventional MTT test was used to evaluate cellular viability in the presence and absence of experimental compounds.[43] **Table 4** shows the results of the cytotoxic potential of synthesized Aurone analogues.

Table 4: % Viability of different compounds.

COMPOUND	% Viability			
	25µg/ml	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**
AU-B	68.12143	62.91428	58.61429	40.39286
AU-A	68.64286	44.06429	48.85712	34.37857
AU-C	80.52857	72.75	57.25714	50.02143
AU-F	70.10714	30.69286	20.07143	18.57138
AU-T	61.85714	49.64286	36.9	29.7
AU-R	83.49286	73.23571	72.29286	49.43571
AU-X	79.47857	78.16429	68.09286	46.30586

The IC_{50} values of the compounds were calculated from the graph and the results are given in **Table 5** comparison of the IC_{50} values were depicted in the **Figure 5.**

All the synthesized Aurone analogues show activity towards the prostate cancer cells. The IC_{50} values of the compounds compared with the IC_{50} value of standard drug Bicalutamide. The results demonstrate that among the synthesized Aurone analogues, **AU-F (37.44µg/ml)** and **AU-T (49.89µg/ml)** have better activity than the standard and **AU-A (65.80µg/ml)** shows a moderate activity comparable to the standard, Bicalutamide **(55.72µg/ml).** The results also indicates that among the halogenated derivatives, compound with fluorine at 4'-position have better activity than the 4'-chloro and 4'-bromo derivatives. Bicalutamide is an Non-steroidal antiandrogen medication which is primarily used to treat Prostate cancer.

Table 5: IC_{50} values of different compounds.

Sl. No.	Sample Description	IC_{50} µg/ml
1	**AU-B**	128.278
2	**AU-A**	**65.80556**
3	**AU-C**	188.4796
4	**AU-F**	**37.4461**
5	**AU-T**	**49.89389**
6	**AU-R**	263.9978
7	**AU-X**	117.7254
8	**Bicalutamide**	**55.72**

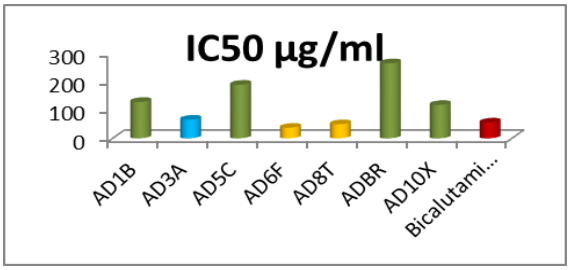


Figure 5: comparison of the IC_{50} values in MTT Assay.

The molecular modeling helps to identify the pharmacologically active species of Aurones and the glide scores were in good agreement with the observed IC50 values. In computational studies seven compounds shows promising binding affinity with the PRKACA subunit, among them three compounds (AU-F, AU-T and AU-A) displayed comparatively better activity than the others with a docking score of -8.028, -7.434 and -7.129 respectively. The seven compounds were selected for the synthesis. All the compounds were synthesized from 1'-Hydroxy-2'-acetonaphthones and various aldehydes such as Benzaldehyde (AU-B), 4- Methoxybenzaldehyde (AU-A), 4-Clorobenzaldehyde (AU-C), 4-Fluorobenzaldehyde (AU-F), and 2-Thiophene carboxaldehyde (AU-T), 4- Bromobezaldehyde (AU-R), 4-Benzyloxy benzaldehyde (AU-X) by a twostep reaction and all the compounds obtained as yellow colored solid in good yield(65.7-87.5%). Characterization of Aurone analogues confirms the anticipated structures of the synthesized Aurone analogues. All reactions were routinely monitored by TLC and spots were visualized by Iodine and Melting point of the compounds was recorded by using open capillary tube method. The IR spectra showed absorption bands ranges from 1688-1703 cm-1C=O Stretching), 3053-3063 cm-1 (Aromatic CH), 1588-1625 cm-1 (C=C), 1070- 1250 cm-1 (C-O-C) thereby confirming the presence of C=O, C-O-C and C=C linkages in synthesized compounds. 1H NMR spectra of all analogues showed chemical shift in the range of 6.887- 7.18ppm (s, 1H) CH=C, 7.10-8.405ppm (multiplicity of relative number of different proton of benzene ring), confirm the structure of synthesized Aurone analogues. The 13C NMR spectra of all the compounds ranges from 184.02-184.39ppm (C=O), 107.62-113.89ppm (=CH) and 137.40-147.69ppm (C=) establishing the formation of desired compounds. Mass spectrometric results were confirmed the molecular weight of the synthesized Aurone analogues. All the analogues of Aurones displayed promising antioxidant activity with the IC50 values in the range of 64.9-169.18 µg/ml. The results of DPPH Assay demonstrates that compounds AU-F, AU-T and AU-A with IC50 values 64.9 µg/ml, 78.82 µg/ml, 103.74µg/ml respectively have better antioxidant potential than the standard and AU-X (133.001 µg/ml) shows a moderate activity as compared to the standard, Ascorbic acid (137.50 µg/ml). The synthesized Aurone analogues displayed promising anticancer activity in MTT Assay with 50% inhibitory concentration ranges from 37.44-263.9 µg/ml. The compounds AU-F(37.44µg/ml) and AU-T(49.89µg/ml) exhibited more potent activity against PC-3 Cell line and AU-A(65.80µg/ml) shows a moderate activity as compared to the standard, Bicalutamide(55.72µg/ml) which is a non-steroidal antiandrogen drug primarily used for treatment of Prostate cancer. The two most active Aurones (AU-F and AU-T) are structures synthesized from 4-Fluoro benzaldehyde and 2-Thiophene carboxaldehyde. Among the halogenated derivatives of Aurones the activity was observed as F> Cl >Br.

CONCLUSION

In search of new anticancer agents we synthesized different Aurone analogues containing naphthofuranone ring as PRKACA inhibitors was synthesized from commercially available 1'-Hydroxy-2'-acetonaphthones and evaluated the cytotoxic activity against PC-3 (prostate cancer cells) and from the results we can conclude that the synthesized Aurone analogues have better antioxidant and antiprostate cancer activities.

ACKNOWLEDGEMENTS

We wish to thank Dr. Ashish Wadhwani, M.Pharm, Ph.D, Dept. of Pharmaceutical Biotechnology, JSS Academy of Higher Education &Research, College of Pharmacy for performing the cytotoxic studies of this work and SAIF, Panjab University for performing NMR spectral analysis and Indian Institute of Technology, Ropar for performing the Mass spectrometry and for helping me to complete my thesis, are also gratefully acknowledged.

REFERENCES

1. Ono E, Fukuchi M, Nakamura N, Fukui K. Yonekura-Sakakibara, M. Yamaguchi, *Proc. Natl. Acad., Sci.U.S.A.*, 2006; 103: 29,1075-80. doi: 10.1073/pnas.0604246103.
2. Chu. W.L.A, Jensen. F.R, Jensen. T.B, Mc Alpine. J.B, Sokilde. B, Santana-Sorensen. A.M, Ratnayake. S, Jiang. J.B, Noble. C, Stafford. A.M, Patent. U.S. US 6307070.
3. Zhang. X, Li. H, Feng. H, Xiong. H, Zhang. L, Song. Y, Yu. L, Deng. X. Int. Immunopharmacol, 2009; 9: 7-8, 810-816 doi: 10.1016/j.intimp.2009.02.018.
4. Gurevich-Panigrahi.T, Panigrahi. S, Wiechec.E, Los.M. Curr. Med. Chem, 2009; 16: 506-521 doi: 10.2174/092986709787315568.

5. Shin. J.-S, Park Y.M, Choi. J.-H, Park. H.-J, Shin. M.C, Lee Y.S, Lee. K.T, Int. Immunopharmacol, 2010; 10: 8,943-950. doi: 10.1016/j.intimp.2010.05.007.
6. Shin. S.Y, Shin. M.C, Shin. J.-S, Lee. K.-T, Lee. Y. Bioorg. Med. Chem. Lett., 2011; 21: 15. 4520-4523. doi: 10.1016/j.bmcl.2011.05.117.
7. Boumendjel. A, Bois.F, Beney. C, Mariotte. A.M, Conseil. G, Di Pietro. A, Bioorg. Med. Chem. Lett., 2001; 11: 75-77. doi: 10.1016/s0960-894x (00)00595-3.
8. Chu. W.L.A, Jensen. F.R, Jensen. T.B, Sokilde B, Santana Sorenson. A.M, Mc Alpine. J.B. PCT Int. Appl. WO 9904789 A1 19990204, 1998.
9. Brownlee, M, Nature., 2001; 414: 6865, 813-820. doi: 10.1038/414813a.
10. Elmastas M, Isildak O, Turkekul I, Temur N, *J. Food Compos. Anal*, 2007; 20: 337–345. doi:10.1016/j.jfca.2006.07.003.
11. Spallolz J.E, Free Radic. Biol.Med, 1994; 17(1): 45-64. doi: 10.1016/0891-5849(94)90007-8.
12. Kumar A, Preeti K S. *RJPBCS*., 2013; 4(2): 247-269.
13. Halliwell B, Gutteridge J.M.C, Oxford University Press, Oxford, 1999; 3: 1-25. doi:10.1093/acprof:oso /9780198717478.001.0001.
14. Yagi K, Chem. Physics Lipids, 1987; 45(2): 337-351. doi: 10.1016/0009-3084(87)90071-5.
15. Harman D, Mol. Cell. Biol, 1984; 84: 155–161. doi: 10.1007/BF00421050.
16. Kong S, Davidson A.J, J. Biol. Chem, 1980; 204: 18–29. doi: 10.1016/0003-9861(80)90003-x.
17. Davies K, Biochem. Soc. Symp, 1995; 61: 1–31. doi: 10.1042/bss0610001.
18. Dalery-Usmar V, Halliwell B, Pharm. Res, 1996; 13: 649-662. doi: 10.1023/a:1016079012214
19. Gutteridge JMC, Quinlan GJ, *Biochem. Biophys. Acta.*, 1992; 1159: 249–254. doi.org:10.1016/0167-4838(92)90052-F.
20. Foroumadi A, Samzade KA, Emami S, Dehghan Gh, Sorkhi M, Arabsorkhi F, Heidari M, Abdollahi M, Shafiee A, Bioorg. Med. Chem. Lett., 2007; 17: 6764-6769. doi: 10.1016/j.bmcl.2007.10.034.
21. Adibi. H, Foroumadi. A, Khodarahmi. A, Najafi. K, Tavirani. R M, JPS Spring, 2013; 4(2): 87-92. DOI:10.22037/JPS.V4I2.4416.
22. Nenadis. N, Sigalas. P M, Food Res., 2011; 44: 114-120. doi:10.1016/j.foodres.2010.10.054.
23. Pan. G, Li. X, Zhao. L, Wu. M, Su. C, Li. X, Zhang. Y, Yu. P, Teng. Y, Lu. K, Eur. J. Med. Chem, 2017; 138: 577-589. doi: 10.1039/D0RA00822B.
24. Narsinghani. T, Sharma. C M, Bhargav. S, Med Chem Res, 2013; 22(9): 4059–4068. DOI10.1007/s00044-012-0413-3.
25. ICMR National Cancer Registry Programme, Consolidated report of the population-based cancer registries, 2014.
26. Boumendjel. A, Di Pietro. A, C. Dumontet, D. Barron. Med. Res. Rev, 2002; 22(5): 512-29. doi: 10.1002/med.10015.
27. Boumendjel. A, Curr. Med. Chem, 2003; 10: 2621-2630. doi: 10.2174/0929867033456468.
28. Joseph Schoepfer, Heinz Fretz, Bhabatosh Chaudhuri, Lionel Muller, Egge Seeber, Laurent Meijer, J.Med.Chem., 2002; 45(9): 1741-47. doi: 10.1021/jm0108348.
29. Okombi S, Rival D, Bonnet S, Marriotte AM, Perrier E, Boumendjel A, J.Med.Chem, 2006; 49(1): 329-33. doi:10.1021/jm050715i.
30. Vincent Zwick, Alkiviadis-Orfefs, Chatzivasileiou, Nathalie Deschamps, Marina Roussaki, Claudia A.Simões-Pires, Alessandra Nurisso, 2014; 24(23): 5497-5501. doi: 10.1016/j.bmcl.2014.10.019.
31. S. Sandeep, V. Priyadarshini, D. Pradhan, M. Munikumar, A.Umamaheswari, J Clin Sci Res., 2012; 1: 15-23. doi:10.15380/2277-5706.JCSR.12.005.
32. Ballinari Dario, Bonomini Luisella, Ermoli Antonella, Gude Markus, Menichincheri Maria, Moll Jurgen, United States Patent Application, 2002; 20040132807.
33. Lawrence J, Rennison D, McGown T A, Hadfield A J, Bioorg.Med.Chem.Lett, 2003; 13: 3759-3763. doi: 10.1016/j.bmcl.2003.07.003.
34. Hendlich. M, 1998; 54: 1178–1182. doi: 10.1107/s0907444998007124.
35. Hu. L, Benson. M.L, Smith. R.D, Lerner. M.G, Carlson. H.A, *Proteins*, 2005; 60(3): 333–340.
36. Irwin. J.J, Shoichet. B.K. ZINC—a free database e of commercially available compounds for virtual screening. J. Chem Inf Model, 2005; 45(1): 177–182. doi: 10.1021/ci049714.
37. Sanal Dev, Sunil. R. Dhaneshwar, Bijo Mathew, Comb Chem High Throughput Screen, 2018; 21(2): 138-148. doi: 10.2174/1386207321666 180228113925.
38. Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., and Bourne, P.E, The Protein Data Bank. Nucleic Acids Res, 2005; 28(1): 235–242. doi: 10.1093/nar /28.1.235.
39. S Demirayak, L Yurttas, N Gundogdu- Karaburun, A Cagri Karaburun, and I Kayagil J, Enzyme Inhib Med Chem, 2015; 30,5,816-825. doi: 10.3109/14756366.2014.976568.
40. M Elmastaş, I Dermirtas, O Isildak & Hassan Y, Liq. Chromatogr. Relat, 2009; 29,10: 1465-1475. doi:10.1080/10826070600674893.
41. N tamanna & S mukesh & B sakshi, medicinal chemistry research, 2012; 22(10): 4059–4068. doi:10.1007/s00044-012-0413-3.
42. S. Jubie, U Durai, S Latha, S Ayyamperumal, A Wadhwani, T Prabha, Curr Drug Res Rev., 2021; 13(1): 73-83. doi: 10.2174/2589977512 999200821170221.
43. R Elancheran & K Saravanan & C Bhaswati & S Divakar & Kabilan, S. & R Muthiah & D Babulal & D Ranjan & K Jibon. Medicinal Chemistry Research, 2016; 25: 539–552. doi:10.1007/s00044-016-1504-3.

44.