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Docking, Screening, Synthesis of 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one derivatives as new leads for anti-cancer activity

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ABSTRACT

Auroneis a bicyclic ring where a benzene ring fused with a furanone. Docking is an efficient tool in the development of new lead molecules. Docking, virtual screening, ADMET prediction are prominent devices in the identification of new lead molecules. Synthetic chemistry plays a major in developing a series of potent anti-cancer agents. Benzofuranone was synthesized by reacting benzene diols, and triols with bromo phenyl acetonitrile yielded an imine derivative are converted to a ketone with treatment with hydrochloric acid then cyclised with sodium acetate. The compounds identity and purity were confirmed by spectral and analytical methods. Benzofuranone derivatives are screened antineoplastic activity was performed against human skin cancer cell line G361 at micro molecular concentrations. The compound IIIA was found to be with potent activity.

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INTRODUCTION

Aurones constitute an important class of natural products in the family of flavonoid that is found in many plants (Haudecoeur, R 2012) Fruits and flowers are the common sources for flavonoids along with several flavones, isoflavones and chalcones. From the past several years' flavones and chalcones have been well studied for treatment against various diseases, but the biological activity of aurones has not been extensively studied. Aurones are mainly used as anti-cancer (Lawrence, N J 2003), antimalarial (Kayser, O 2001) and anti-microbial (Bandgar B P 2010) agents. In plants, aurones are synthesized from chalcones by oxidation, cyclisation and rearrangement involving the enzyme aureusidin synthase (Nakayama T 2000), (Nakayama T 2001), (Nakayama T 2002). Aurones exhibit a range of pharmacological activities including anti-cancer, antifeedant and antiparasitic activities via modulation of a variety of molecular targets such as G2/M phase cell-cycle arrest, arresting the cell cycle in G0/G1 phase and displayed apoptosis-inducing effect on Hep-2 cells (Huang W 2007), inhibition of Human Sphingosine Kinase (French KJ 2003), inhibition of P-gp related transport (Vaclavikova R 2006), high-affinity binding to cytosolic domain of p-glycoprotein (Boumendjel A 2002), modulation of ABCG2 activities (Sim HM 2011), etc. Literature reveals that several methods have been reported for the synthesis of aurones. The most commonly used method for the synthesis of aurones involves the aldol condensation of benzofuran-3(2H)- ones with an aromatic aldehyde. This reaction can be carried out by using alumina (Varma RS 1992), HCl, EDDA (Manjulatha K 2012), and deep eutectic solvent (Hawkins I 2013).

Computational Studies

Experimental Procedure: Selection of protein: CDK is the most abundantly found in tumor cell generation. So, CDK inhibitor has been selected for

Docking the lead molecules, the protein database was searched in the portal http://www.rcsb.org, the protein was searched from a group and 1GII protein was found with species of Homosapiens with X-ray method was used in determination of the protein, with lowest resolution of 2.00A° and validated for the Domain completeness.

Preparation of Protein

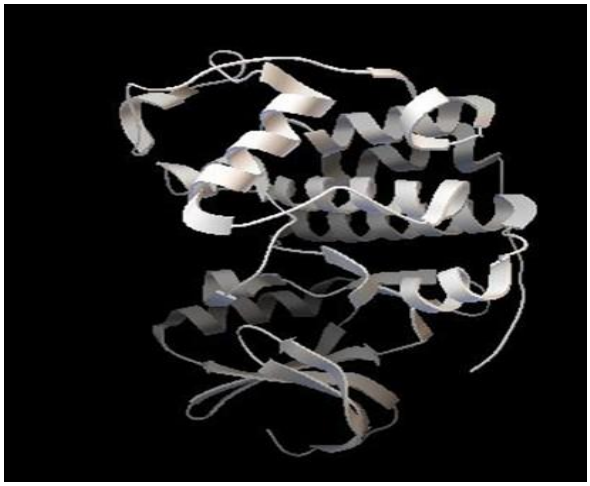


Figure 1: Protein Structure

The selected protein 1GII has been was explored in Auto dock 4.0, the bonds and atoms in the protein are optimized, missing hydrogens are added, the non-polar centre between the hydrogens was merged, and all the histidine hydrogens are protonated with +1charge. Kollaman and gastegier charges were added to the protein. All the missing atoms are repaired, and charges are applied to the protein.

Design of Ligands

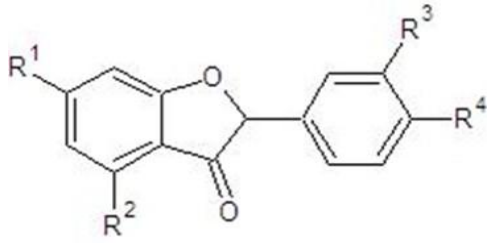


Figure 2: Benzofuranone

The basic benzofuranone was designed with modifications in the R^1, R^2, R^3, and R^4 of the molecule given below in figure: 2.

Preparation of Ligands: The above combinations of ligands were drawn with Chemsketch open source software obtained from http://www.acdlabs.com, as (. mol) file in a 2D structural format. Then the ligands are undergone for energy optimization and converted (.pdb) 3D structural format by using Discovery studio visualiser 4.0 from http://accelrys.com, and the angular forces between the bonds of ligands are minimized.

Table 1: Combinations of Ligands

R^1	CH3	1 combination
R^2	OH,	1 combination
R^3	H,OH,OCH_3,CH_3,C_2H_5, NH_2 , NO_2 ,BR, CL	9 combinations
R^4	H,OH,OCH_3,CH_3,C_2H_5, NH_2 , NO_2 ,BR, CL	9 combinations

Based on the above combination for pool contains 83 combinations were designed.

Virtual screening: The optimized protein is then explored in virtual screening software, and then ligand databank of the group is also linked, and quantitative optimization is performed. Then computational parameters like an auto grid, autovina, auto dock are applied, protein is fixed in the grid box, and Virtual screening is performed.

The screened results are given below.

Admet profile prediction of ligands

Ligands are preliminary are studied for Adsorption, Distribution, Metabolism, Elimination, and Toxicity for the search for best-fit ligands. Lipinski rule of 5 is the best fit parameter for prediction of ADMET of ligands, Lipinski rule of 5 parameters Log p (≤5), Molecular weight (≤500daltons), Hydrogen acceptors (≤5) and hydrogen acceptors (≤5). Molecules violate these parameters are found to be with poor bioavailability parameters. Data warrior of OSIRIS software is utilized for the prediction of the above parameters, and the best fit results are listed below. The molecules exhibiting mutagenicity, tumorigenicity, reproductive effect and irrant nature are found that molecules may be with toxicity and they are also removed from the database for the further insilico studies. The molecules with good ADMET properties are used for insilico screening such as virtual screening and docking studies further.

Docking studies: The best fit ligands from primary filtration by virtual screening and docking are then subjected to secondary Insilco studies (Docking).

Protein and Ligand Preparation: Auto dock 4.0 open source software is utilized for the docking studies. The optimized protein file (1GII) is explored in the auto dock 4.0, then optimized ligand is fit in it, and 3D structural energy is minimized, torrisions of the ligands are verified, adjusted, and the ligand is stored as (. pdbqt) parameter.

Grid Allignment: The protein (1GII) is explored in 3D space and grid box is fixed on the macromolecule protein and grid adjusted such that all binding pockets are aligned in the grid, and

Table 2: Virtual Screening results

S.No	Ligand	Target	Binding Energy	Date Created	Info
1	231_uff_E=349.76	1GII	-8.1	2015.07.30 23:58:38	Vina
2	232_uff_E=426.30	1GII	-8.1	2015.07.30 23:59:50	Vina
3	240_uff_E=349.40	1GII	-8.1	2015.07.31 00:08:01	Vina
4	241_uff_E=409.20	1GII	-8.1	2015.07.31 00:09:13	Vina
5	25_uff_E=415.80	1GII	-8.1	2015.07.30 20:34:43	Vina
6	260_uff_E=318.30	1GII	-8.1	2015.07.31 00:23:15	Vina
7	261_uff_E=317.86	1GII	-8.1	2015.07.31 00:24:02	Vina
8	262_uff_E=339.13	1GII	-8.1	2015.07.31 00:24:42	Vina
9	266_uff_E=430.24	1GII	-8.1	2015.07.31 00:28:01	Vina
10	267_uff_E=410.74	1GII	-8.1	2015.07.31 00:28:52	Vina
11	268_uff_E=509.59	1GII	-8.1	2015.07.31 00:29:45	Vina
12	271_uff_E=318.00	1GII	-8.1	2015.07.31 00:31:48	Vina
13	277_uff_E=432.52	1GII	-8.1	2015.07.31 00:36:05	Vina
14	29_uff_E=379.72	1GII	-8.1	2015.07.30 20:38:05	Vina
15	30_uff_E=386.03	1GII	-8.1	2015.07.30 20:38:57	Vina
16	305_uff_E=391.98	1GII	-8.1	2015.07.31 00:59:00	Vina
17	306_uff_E=388.81	1GII	-8.1	2015.07.31 00:59:53	Vina
18	307_uff_E=303.83	1GII	-8.1	2015.07.31 01:00:29	Vina
19	312_uff_E=328.68	1GII	-8.1	2015.07.31 01:04:15	Vina
20	314_uff_E=313.86	1GII	-8.1	2015.07.31 01:05:47	Vina
21	319_uff_E=330.49	1GII	-8.1	2015.07.31 01:09:15	Vina
22	45_uff_E=412.90	1GII	-8.1	2015.07.30 20:52:38	Vina
23	5_uff_E=369.85	1GII	-8.1	2015.07.30 20:15:51	Vina
24	81_uff_E=331.13	1GII	-8.1	2015.07.30 21:26:49	Vina
25	82_uff_E=330.26	1GII	-8.1	2015.07.30 21:27:38	Vina
26	84_uff_E=335.28	1GII	-8.1	2015.07.30 21:29:08	Vina
27	85_uff_E=335.77	1GII	-8.1	2015.07.30 21:29:49	Vina
28	97_uff_E=512.33	1GII	-8.1	2015.07.30 21:41:03	Vina
29	1_uff_E=348.49	1GII	-8	2015.07.30 20:12:40	Vina
30	100_uff_E=310.36	1GII	-8	2015.07.30 21:43:42	Vina
31	101_uff_E=329.59	1GII	-8	2015.07.30 21:44:39	Vina
32	114_uff_E=362.33	1GII	-8	2015.07.30 21:56:15	Vina
33	127_uff_E=320.39	1GII	-8	2015.07.30 22:08:28	Vina
34	13_uff_E=424.08	1GII	-8	2015.07.30 20:22:49	Vina
35	130_uff_E=349.83	1GII	-8	2015.07.30 22:11:37	Vina
36	150_uff_E=340.29	1GII	-8	2015.07.30 22:31:48	Vina
37	151_uff_E=402.21	1GII	-8	2015.07.30 22:32:47	Vina
38	168_uff_E=343.12	1GII	-8	2015.07.30 22:47:45	Vina
39	17_uff_E=405.70	1GII	-8	2015.07.30 20:26:56	Vina
40	170_uff_E=296.09	1GII	-8	2015.07.30 22:49:44	Vina
41	171_uff_E=327.24	1GII	-8	2015.07.30 22:50:35	Vina
42	177_uff_E=343.96	1GII	-8	2015.07.30 22:57:19	Vina
43	18_uff_E=400.94	1GII	-8	2015.07.30 20:27:53	Vina
44	182_uff_E=408.88	1GII	-8	2015.07.30 23:03:10	Vina
45	184_uff_E=406.19	1GII	-8	2015.07.30 23:05:27	Vina
46	186_uff_E=418.76	1GII	-8	2015.07.30 23:07:53	Vina
47	187_uff_E=518.87	1GII	-8	2015.07.30 23:09:11	Vina
48	189_uff_E=379.88	1GII	-8	2015.07.30 23:11:23	Vina
49	191_uff_E=326.39	1GII	-8	2015.07.30 23:13:16	Vina
50	209_uff_E=361.95	1GII	-8	2015.07.30 23:32:42	Vina
51	21_uff_E=387.93	1GII	-8	2015.07.30 20:30:40	Vina
52	219_uff_E=421.63	1GII	-8	2015.07.30 23:44:45	Vina
53	235_uff_E=319.26	1GII	-8	2015.07.31 00:02:33	Vina
54	244_uff_E=295.56	1GII	-8	2015.07.31 00:11:36	Vina
55	248_uff_E=340.68	1GII	-8	2015.07.31 00:14:11	Vina
56	265_uff_E=397.99	1GII	-8	2015.07.31 00:27:09	Vina

Table 2: Virtual Screening results (Contd...)

S.No	Ligand	Target	Binding Energy	Date Created	Info
57	300_uff_E=410.35	1GII	-8	2015.07.31 00:54:35	Vina
58	310_uff_E=330.26	1GII	-8	2015.07.31 01:02:38	Vina
59	313_uff_E=396.64	1GII	-8	2015.07.31 01:05:07	Vina
60	315_uff_E=313.49	1GII	-8	2015.07.31 01:06:28	Vina
61	318_uff_E=348.62	1GII	-8	2015.07.31 01:08:36	Vina
62	322_uff_E=393.82	1GII	-8	2015.07.31 01:11:36	Vina
63	323_uff_E=311.55	1GII	-8	2015.07.31 01:12:17	Vina
64	33_uff_E=380.41	1GII	-8	2015.07.30 20:41:26	Vina
65	39_uff_E=425.63	1GII	-8	2015.07.30 20:46:47	Vina
66	44_uff_E=383.79	1GII	-8	2015.07.30 20:51:45	Vina
67	53_uff_E=351.43	1GII	-8	2015.07.30 21:00:46	Vina
68	54_uff_E=350.15	1GII	-8	2015.07.30 21:01:41	Vina
69	71_uff_E=333.72	1GII	-8	2015.07.30 21:18:11	Vina
70	72_uff_E=332.70	1GII	-8	2015.07.30 21:19:00	Vina
71	73_uff_E=280.03	1GII	-8	2015.07.30 21:19:43	Vina
72	75_uff_E=370.25	1GII	-8	2015.07.30 21:21:30	Vina
73	11_uff_E=433.29	1GII	-7.9	2015.07.30 20:20:54	Vina
74	135_uff_E=332.83	1GII	-7.9	2015.07.30 22:16:43	Vina
75	147_uff_E=357.29	1GII	-7.9	2015.07.30 22:29:11	Vina
76	15_uff_E=439.45	1GII	-7.9	2015.07.30 20:24:53	Vina
77	154_uff_E=313.62	1GII	-7.9	2015.07.30 22:35:09	Vina
78	155_uff_E=336.73	1GII	-7.9	2015.07.30 22:36:02	Vina
79	156_uff_E=357.71	1GII	-7.9	2015.07.30 22:36:57	Vina
80	159_uff_E=339.78	1GII	-7.9	2015.07.30 22:39:34	Vina
81	178_uff_E=399.48	1GII	-7.9	2015.07.30 22:58:40	Vina
82	179_uff_E=328.91	1GII	-7.9	2015.07.30 22:59:51	Vina
83	181_uff_E=342.34	1GII	-7.9	2015.07.30 23:01:58	Vina

Table 3: ADMET results

Molecule Name	Absolute Weight	cLogP	cLogS	H-Acceptors	H-Donors	Total Surface Area	Polar Surface Area	Drugliken ess	LELP from Molecule Name
1	274.0397	3.455	-4.042	3	1	228	46.53	0.2372	7.0326
2	305.0091	1.8665	-3.982	6	1	215	85.03	-0.9675	4.2042
3	293.985	3.7171	-4.434	3	1	236	46.53	0.26995	7.5813
4	242.0579	2.1594	-2.666	4	2	206	66.76	0.1775	4.1741
5	258.0528	1.8137	-2.37	5	3	213	86.99	0.1775	3.7021
6	272.0685	2.0894	-2.684	5	2	228	75.99	0.2867	4.491
7	256.0736	2.5033	-3.01	4	2	221	66.76	0.14141	5.1136
8	270.0892	2.9189	-3.169	4	2	238	66.76	-0.05978	6.2788
9	257.0688	1.4821	-2.742	5	3	212	92.78	0.3527	3.0299
10	287.043	0.9148	-2.95	7	2	210	114.5	0.8705	2.0678
11	319.9684	2.8846	-3.5	4	2	234	66.76	-1.6125	5.9015
12	258.0528	1.8137	-2.37	5	3	210	86.99	0.1775	3.7134
13	274.0477	1.468	-2.074	6	4	220	107.22	0.1775	3.165
17	288.0634	1.7437	-2.388	6	3	229	96.22	0.2867	3.9488
18	272.0685	2.1576	-2.714	5	3	228	86.99	0.14141	4.6551
19	303.0379	0.5691	-2.654	8	3	210	134.73	0.61187	1.3521
21	335.9633	2.5389	-3.204	5	3	239	86.99	-1.6125	5.4858
22	292.0139	2.4197	-3.106	5	3	223	86.99	0.26995	5.2301
28	272.0685	2.0894	-2.684	5	2	230	75.99	0.2867	4.5178
30	288.0634	1.7437	-2.388	6	3	236	96.22	0.2867	3.9602
38	302.079	2.0194	-2.702	6	2	254	85.22	0.2867	4.8065

*Mutagenic: none; Tumorigenic: none; Reproductive Effective: none; Irritant: none

Table 3: ADMET results (Contd….)

Molecule Name	Absolute Weight	cLogP	cLogS	H-Accep-tors	H-Donors	Total Sur-face Area	Polar Sur-face Area	Druglike-ness	LELP from Mol-ecule Name
39	286.0841	2.4333	-3.028	5	2	240	75.99	0.259	5.5303
40	317.0536	0.8448	-2.968	8	2	210	123.73	0.7271	2.1051
43	349.979	2.8146	-3.518	5	2	239	75.99	-1.5033	6.4058
44	306.0295	2.6954	-3.42	5	2	239	75.99	0.38059	6.1366
46	256.0736	2.5033	-3.01	4	2	219	66.76	0.14141	5.1582
55	286.0841	2.4333	-3.028	5	2	245	75.99	0.259	5.5455
56	270.0892	2.8472	-3.354	4	2	228	66.76	0.14141	6.1819
57	301.0586	1.2587	-3.294	7	2	215	114.5	-1.3541	3.0092
58	290.0346	3.1093	-3.746	4	2	232	66.76	0.2372	6.7622
61	270.0892	2.9189	-3.169	4	2	245	66.76	-0.05978	6.3501
62	286.0841	2.5732	-2.873	5	3	242	86.99	-0.05978	5.8799
63	300.0998	2.8489	-3.187	5	2	254	75.99	0.073752	6.8221
64	284.1049	3.2628	-3.513	4	2	244	66.76	-0.05978	7.4605
65	315.0743	1.6743	-3.453	7	2	216	114.5	0.2574	4.1969
66	347.9997	3.6441	-4.003	4	2	249	66.76	-1.8498	8.3429
67	304.0502	3.5249	-3.905	4	2	250	66.76	0.043347	8.0726
70	257.0688	1.4821	-2.742	5	3	212	92.78	0.3527	3.0719
71	287.043	0.9148	-2.95	7	2	220	114.5	0.1349	2.1015
73	303.0379	0.5691	-2.654	8	3	215	134.73	0.86365	1.37
74	317.0536	0.8448	-2.968	8	2	220	123.73	1.6085	2.1268
75	301.0586	1.2587	-3.294	7	2	215	114.5	-0.61413	3.0319
76	332.0281	-0.329	-3.234	10	2	205	153	0.42923	-0.8673
79	364.9535	1.64	-3.784	7	2	210	105.26	-1.3608	3.955
80	321.004	1.5208	-3.686	7	2	210	105.26	0.52638	3.6686
81	319.9684	2.8846	-3.5	4	2	228	66.76	-1.6125	6.0113
82	335.9633	2.5389	-3.204	5	3	231	86.99	-1.6125	5.571
83	349.979	2.8146	-3.518	5	2	242	75.99	-1.5033	6.4866

*Mutagenic: none; Tumorigenic: none; Reproductive Effective: none; Irritant: none

other parameters are fixed, and grid parameter file (. gpf)is prepared.

Docking parameters

The macromolecule and ligand are exposed then genetic algorithm search; auto dock 4.2 parameters are fixed. A Lamarckian genetic algorithm for docking (. dpf) is prepared.

Docking: The prepared grid parameter file is then docked over with standard grid path database, and grid log file (. glg) is prepared. The docking parameter file is then docked with a comparison to the standard docking path file, and the docking score is obtained. The best fit molecules with docking score are analysed.

Analysing the docking results

After performing docking, the. dlg file is then opened by using analyse parameter in the software. No of dockings performed are recorded, and then the docking results are reviewed by using play ranked by energy options Best docking result with minimum binding energy and inhibition energy are recorded The recorded results are indexed below.

Synthesis experimental

Preparation of Phenyl(bromo)acetonitrile

11.7gm (0.1mole) was taken in 3 necked round bottomed flask in which one of the necks was connected to a flask containing bromine 17.6gm (0.1mol), to the second neck is fitted with a thermometer such that the tip was dipped in benzyl cyanide solution, the solution was heated up to 110^{o}C after preheating, third neck was fitted with a tube dipped in beaker of water to absorb excess hydrogen bromide evolved from the reaction, then slowly bromine was added drop by drop to hot benzyl cyanide until the Hydrogen bromide gas is completely evolved. Then the reaction mixture quenched into a separating funnel and washed with 5% sodium bicarbonate solution for twice, the product was extracted by ether and dried by magnesium sulphate. Finally

filtered and distilled gives a crude product of phenyl (bromo) acetonitrile.

Scheme

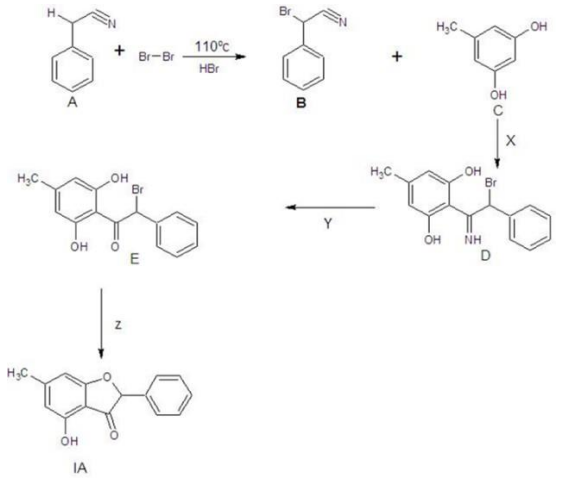


Figure 3: Scheme for Preparation of Benzofuranone derivatives: X ZnCl2, Dry HCL, Dry ether, at 0°C. Y 0.1N HCL Z Sodium acetate and ethanol. A-Phenyl acetonitrile, B-Phenyl(bromo) acetonitrile, C-Orcinol, D 2-(2-bromo-2-phenylethanimidoyl)-5-methylbenzene-1,3-diol, E2-bromo-1-(2,6-dihydroxy-4-methyl phenyl)-2-phenylethanone, IIIA 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one

Preparation of 2-(2-bromo-2-phenyl ethanimidoyl)- 5-methylbenzene-1, 3-diol

A freshly vacuum dried 5-methylbenzene-1,3-diol of (1.2g 0.1mol) was taken in a three-necked round-bottomed flask and 30ml of Dry ether was added then the solution was cooled to 0^0c than to this phenyl(bromo)acetonitrile of (1.9g 0.1mmol), Lewis acid $ZnCl_2$ (1.0g) and then Dry Hydrochloric acid gas was passed through the solution about 4 hours and the reaction mixture was kept in ice chest for one day and then Dry Hydrochloric acid was again pumped in to reaction mixture for 4 hours and stored in ice chest for 3 days. After three days' reaction mixture forms a strong cake, then excess ether removed and washed with freshly distilled dry ether for two times, and obtained solid crystals dried and filtered.

Preparation of 2-bromo-1-(2,6-dihydroxy-4-methyl phenyl)-2-phenylethanone

2-(2-bromo-2-phenylethanimidoyl)-5-methylbenzene-1,3-diol(1.0gm) was taken in a round-bottomed flask, and 0.1N of Hydrochloric acid (30 ml) was added to the reaction mixture and then refluxed for 2 hours, allowed to cool to room temperature and then the reaction mixture was distilled excess solvent was removed, the crude mixture is extracted by dry ether and the left overnight for evaporation at room temperature. A yellow crystal was obtained which was dried.

Preparation of 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one:(IIIA)

2-bromo-1-(2,6-dihydroxy-4-methylphenyl)-2-phenylethanone(0.8gm) was taken in a round-bottomed flask and sodium acetate (2 gm) [sodium acetate was freshly dried on Bunsen flame fine crystals were prepared] was added to the reaction mixture and dissolved in ethanol then refluxed for 10 min, ethanol was distilled and excess solvent was removed, crude was extracted by dry ether and evaporated then cream white precipitate was obtained which was filtered and dried. Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Preparation of 4-hydroxy-6-methyl-2-(-4-nitrophenyl)-1-benzofuran-3(2*H*)-one

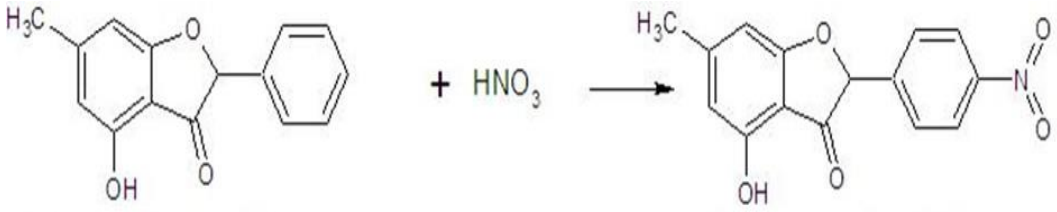
Nitration

A 5 gm (3.5ml 0.05mol) of concentrated nitric acid was placed in 250ml round-bottomed flask fitted with a thermometer, add small portions 7.4g (4 ml) of concentrated sulphuric acid the reaction mixture was kept in an ice cold bath and cooled, then 2.6g (0.03mol) of compound IA was added, stirred well and the temperature was controlled. A reflux condenser was fixed for the above reaction mixture and boiled up to 50-55°c (care to be taken such that temperature does not increase beyond 55°c) for about 1 hour and the reaction mixture was poured into 100 ml cold water and stirred and then supernatant layer of acid was discarded and the product was washed with ice-cold water for thrice until acid is completely washed out from the product, then transferred in to solution of 5% calcium chloride solution. The aqueous layer is separated in separating flask, and a trace amount of water is removed by heating on the flame. [74] Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Preparation of 2-(-3,4-dinitrophenyl)-4,6-dihydroxy-1-benzofuran-3(2*H*)-one

Dinitration

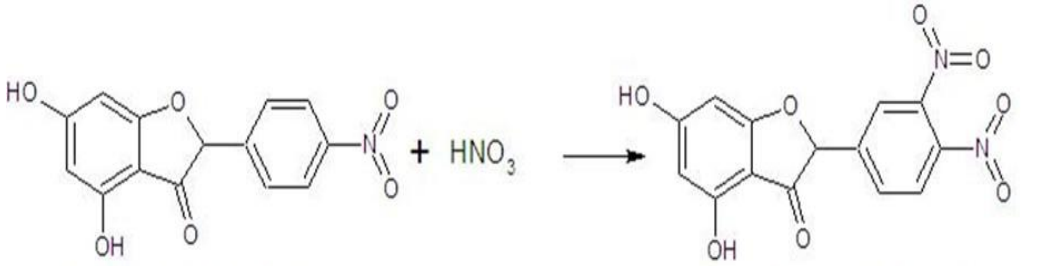
A 5 gm (3.5ml 0.05mol) of concentrated nitric acid was placed in 250ml round-bottomed flask fitted with a thermometer, add small portions 7.4g(4 ml) of concentrated sulphuric acid the reaction mixture was kept in an ice cold bath and cooled, then 2.4g (0.01mol) of 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one compound IIIA was added, stirred well and the temperature was controlled. A reflux condenser was fixed for the above reaction mixture and boiled up to 50-55°c(care to be taken such that temperature does not increase beyond 55°c) for about 1 hour and the reaction mixture



4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one

4-hydroxy-6-methyl-2-(4-nitrophenyl)-1-benzofuran-3(2*H*)-one

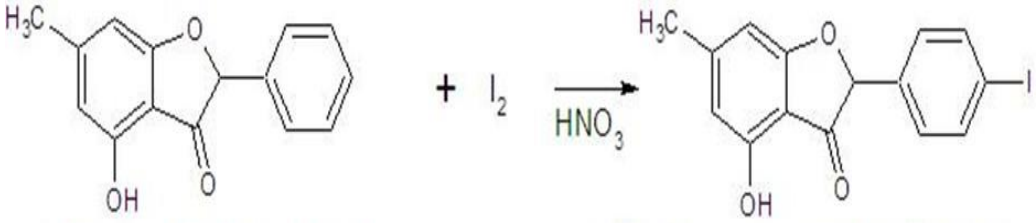
Figure 4: Preparation of 4-hydroxy-6-methyl-2-(-4-nitrophenyl)-1-benzofuran-3(2H)-one



4,6-dihydroxy-2-(4-nitrophenyl)-1-benzofuran-3(2*H*)-one

2-(3,4-dinitrophenyl)-4,6-dihydroxy-1-benzofuran-3(2*H*)-one

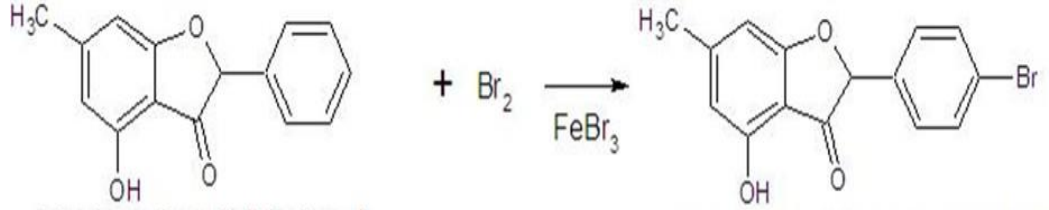
Figure 5: Preparation of 2-(-3,4-dinitrophenyl)-4,6-dihydroxy-1-benzofuran-3(2*H*)-one



4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one

4-hydroxy-2-(4-iodophenyl)-6-methyl-1-benzofuran-3(2*H*)-one

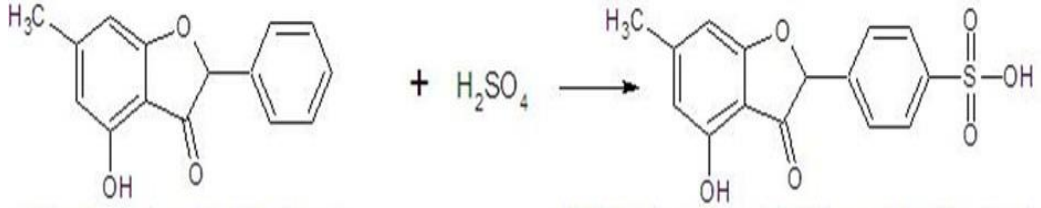
Figure 6: Preparation of 4-hydroxy-2-(4-iodophenyl)-6-methyl-1-benzofuran-3(2H)-one



4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one

2-(4-bromophenyl)-4-hydroxy-6-methyl-1-benzofuran-3(2*H*)-one

Figure 7: Preparation of 2-(4-bromophenyl)-4,6-dihydroxy-1-benzofuran-3(2H)-one



4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one

4-(4-hydroxy-6-methyl-3-oxo-2,3-dihydro-1-benzofuran-2-yl)benzenesulfonic acid

Figure 8: Preparation of 4-(4-hydroxy-6-methyl-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzene sulfonic acid

Table 4: Docking Results

S. no	Molecule No	Iupac name	Binding energy	Ic 50	Ic 50 units	No. of Confirmations
1	253	2-(3-hydroxyphenyl) -1-benzofuran-3(2H)-one	-7.39	5.83	MM	7
2	254	2-(3,4-dihydroxyphenyl) -1-benzofuran-3(2H)-one	-8	1.39	MM	9
3	255	2-(3-hydroxy-4-methoxyphenyl) -1-benzofuran-3(2H)-one	-7.62	6.08	MM	9
4	256	2-(3-hydroxy-4-methyl phenyl) -1-benzofuran-3(2H)-one	-7.39	3.84	MM	9
5	259	2-(3-hydroxy-4-nitrophenyl) -1-benzofuran-3(2H)-one	-7.86	1.39	MM	9
6	260	2-(4-bromo-3-hydroxyphenyl) -1-benzofuran-3(2H)-one	-7.51	3.11	MM	9
7	261	2-(4-chloro-3-hydroxyphenyl) -1-benzofuran-3(2H)-one	-6.54	16.12	MM	8
8	262	2-(3-methoxyphenyl) -1-benzofuran-3(2H)-one	-7.7	6.54	MM	10
9	263	2-(4-hydroxy-3-methoxyphenyl) -1-benzofuran-3(2H)-one	-6.09	35.36	MM	10
10	264	2-(3,4-dimethoxyphenyl) -1-benzofuran-3(2H)-one	-6.08	35.07	MM	10
11	265	2-(3-methoxy-4-methyl phenyl) -1-benzofuran-3(2H)-one	-7.63	2.55	MM	9
12	268	2-(3-methoxy-4-nitrophenyl) -1-benzofuran-3(2H)-one	-7.97	1.45	MM	7
13	269	2-(4-bromo-3-methoxyphenyl) -1-benzofuran-3(2H)-one	-8.22	946.25	MM	7
16	270	2-(4-chloro-3-methoxyphenyl) -1-benzofuran-3(2H)-one	-7.72	2.21	MM	10
17	271	2-(3-methyl phenyl) -1-benzofuran-3(2H)-one	-7.58	2.78	MM	7
18	273	2-(4-methoxy-3-methyl phenyl) -1-benzofuran-3(2H)-one	-7.3	4.49	MM	8
19	274	2-(3,4-dimethylphenyl) -1-benzofuran-3(2H)-one	-7.4	3.6	MM	7
21	277	2-(3-methyl-4-nitrophenyl) -1-benzofuran-3(2H)-one	-7.42	3.66	MM	9
22	279	2-(4-chloro-3-methyl phenyl) -1-benzofuran-3(2H)-one	-7.55	2.92	MM	9
28	281	2-(3-ethyl-4-hydroxyphenyl) -1-benzofuran-3(2H)-one	-7.07	6.51	MM	10
30	282	2-(3-ethyl-4-methoxyphenyl) -1-benzofuran-3(2H)-one	-6.01	39.41	MM	10
39	283	2-(3-ethyl-4-methyl phenyl) -1-benzofuran-3(2H)-one	-6.61	13.71	MM	7
40	286	2-(3-ethyl-4-nitrophenyl) -1-benzofuran-3(2H)-one	-7.44	3.53	MM	8
43	289	2-(3-aminophenyl) -1-benzofuran-3(2H)-one	-6.99	7.58	MM	9
44	298	2-(3-nitrophenyl) -1-benzofuran-3(2H)-one	-6.96	7.9	MM	6
46	299	2-(4-hydroxy-3-nitrophenyl) -1-benzofuran-3(2H)-one	-7.44	3.56	MM	10
55	300	2-(4-methoxy-3-nitrophenyl) -1-benzofuran-3(2H)-one	-7.86	1.72	MM	9

Table 4: Docking Results (Contd...)

S.no	Molecule No	Iupac name	Binding energy	Ic 50	Ic 50 units	No. of Confirmations
56	208	2-(3-aminophenyl)-4,6-dihydroxy -1-benzofuran-3(2H)-one	-7.52	3.02	MM	9
57	217	4,6-dihydroxy-2-(3-nitrophenyl) -1-benzofuran-3(2H)-one	-7.49	3.22	MM	10
58	218	4,6-dihydroxy-2-(4-hydroxy-3-nitro phenyl)-1-benzofuran-3(2H)-one	-7.47	3.36	MM	10
61	219	4,6-dihydroxy-2-(4-methoxy-3-ni-trophenyl)-1-benzofuran-3(2H)-one	-7.22	5.11	MM	9
62	220	4,6-dihydroxy-2-(4-methyl-3-nitro phenyl)-1-benzofuran-3(2H)-one	-7.23	4.25	MM	9
63	223	2-(3,4-dinitrophenyl)-4,6-di hydroxy-1-benzofuran-3(2H)-one	-9.21	176.58	NM	10
64	224	2-(4-bromo-3-nitrophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.56	2.86	MM	10
65	225	2-(4-chloro-3-nitrophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.55	2.92	MM	9
66	226	2-(3-bromophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-6.96	7.92	MM	10
67	227	2-(3-bromo-4-hydroxyphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.14	5.18	MM	9
70	228	2-(3-bromo-4-methoxyphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-6.36	21.94	MM	10
71	229	2-(3-bromo-4-methylphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.8	1.91	MM	10
73	232	2-(3-bromo-4-nitrophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-8.07	1.22	MM	10
74	233	2-(3,4-dibromophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-8.1	1.15	MM	10
75	235	2-(3-chlorophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-6.69	12.56	MM	8
76	236	2-(3-chloro-4-hydroxyphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-6.66	13.21	MM	10
79	237	2-(3-chloro-4-methoxyphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-5.7	56.55	MM	10
80	238	2-(3-chloro-4-methylphenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.33	5.55	MM	10
81	241	2-(3-chloro-4-nitrophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-7.15	5.15	MM	9
82	243	2-(3,4-dichlorophenyl)-4,6 -dihydroxy-1-benzofuran-3(2H)-one	-8.13	1.09	MM	9
83	244	2-phenyl-1-benzofuran-3(2H)-one	-7.12	6.09	MM	6

was poured into 100 ml cold water and stirred and then a supernatant layer of acid was discarded and the product was washed with ice-cold water for thrice until the acid is completely washed out from the product, then transferred into solution of 5% calcium chloride solution. The aqueous layer is separated in separating flask, and a trace amount of water is removed by heating on the flame. Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Preparation of 4-hydroxy-2-(4-iodophenyl)-6-methyl-1-benzofuran-3(2*H*)-one Iodination:

A 2.6g (0.3mol) of 4-hydroxy-6-methyl)-1-benzofuran-3(2*H*)-one compound IIIA, 2.4gm of iodine was taken in a three-necked round-bottomed flask fitted with reflux condenser and then to this add 3ml of nitric acid was added slowly through a separating funnel then the oxides of nitrogen is evolved, and the temperature is raised and refluxed for about 15 minutes, and then the solution is poured in to ice cold water, washed with sodium hydroxide solution, (care has been taken

such that reaction mixture is alkaline to litmus) finally washed with water and dried. Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Preparation of 2-(4-bromophenyl)-4,6-dihydroxy-1-benzofuran-3(2*H*)-one

Bromination

A 2.4g (0.3mol) of 4-hydroxy-6-methyl-1-benzofuran-3(2*H*)-one compound IIIA was taken in a three-necked round-bottomed flask fitted with reflux condenser and gas outlet tube connected to a beaker of water to collect the Hydrogen bromide gas, then to this add 3ml of pyridine (freshly dried over potassium hydroxide) then the apparatus is carefully arranged over a tripod stand with a ice cold water bath, then bromine was added with most care and then reaction started vigorously and slackens then the temperature is raised to 25^{o}C finally temperature raised to 70^{o}C and continued for 1 hour until evolution of bromine ceased (no red fumes from the reaction mixture) and then the solution is poured in to ice cold water, washed sodium hydroxide solution, (care has been taken such that reaction mixture is alkaline to litmus) finally washed with water and dried. Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Preparation of 4-(4-hydroxy-6-methyl-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid

Sulphonation

A 25ml of concentrated sulphuric acid was boiled in a flat bottomed flask, then added 10ml of oleum (fuming sulphuric acid) and boiled for few minutes and then a 1gm of 4-hydroxy-6-methyl-2-(-4-nitrophenyl)-1-benzofuran-3(2*H*)-one compound IIIA was added and then refluxed for 2 hours. Then the solution was cooled and then neutralized by sodium bicarbonate and to this mixture sodium chloride was until the total solution saturated by sodium chloride, then sodium salt of Compound (IIIF)was obtained and then extracted by absolute ethanol, the product is recovered by the evaporation of the solvent and then T.L.C Studies were performed.

Anti-cancer activity

The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein-dye sulforhodamine B to bind electrostatically. The activity is pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mildly acidic conditions it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement. The signal-to-noise ratio is favorable, and the resolution is 1000-2000 cells/well. Its performance is similar when compared to other cytotoxicity assays such as MTT or clonogenic assay. The SRB assay possesses a colorimetric endpoint and is nondestructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity even at large-scale application.

Parameters reported: GI_{50}, TGI, and LC_{50}

GI_{50}: Growth inhibition of 50 % (GI_{50}) calculated from drug concentration resulting in a 50 % reduction in the net protein increase; TGI: Drug concentration resulting in total growth inhibition (TGI); LC_{50}: Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment (concentration of drug causing lethality to 50 % of the cells as compared to that at the beginning) indicating a net loss of cells following treatment.

RESULTS

4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2H)-one: (IIIA)

Yield: 35.62%, Melting point 221^{o}C, Rf Value: 0.76, Mol formula $C_{15}H_{12}O_3$ Mol Weight: 240.22; IR (Cm^{-1}) (KBr): 3422.63 (OH); 2984.60 (CH3); 1740.40 (C=O); 1173.69 (C-O-C); ^{1}H NMR (400 MHz, MeOD-d_6):δ= 8.06-8.04 (d 2H Ar-2`H and 6`H *J*=8) 7.58-7.54 (t 2H Ar-3`H and 5`H *J*=8) 7.41-7.37 (t 1H Ar-4`H *J*= 4.8) 5-96 (d 1H Ar-H *J*= 12.4Hz), 5.85(d 1H Ar-H *J*= 1.6Hz) 5.40 (s 1H CH) 2.42 (d 3H CH3 *J*= 1.6Hz) ^{13} C NMR (100 MHz, MeOD): δ= 52.46 (C-10 CH3) 88.06 (C-2), 115.88 (C-5), 107.01 (C-7), 112.06 (C-9), 127.41 (C-1``), 128.41 (C-2``), 129.69 (C-2```), 136.59 (C-1`), 124.36 (C-4), 170.80 (C-8), 152.98 (C-6), 197.32 (C-3). Mass m/z: 241 (M+1), 239 (M-1);

4-hydroxy-6-methyl-2-(-4-nitrophenyl)-1-benzofuran-3(2H)-one: (IIIB)

Yield: 54.5%, Melting point 234^{o}C, Rf Value: 0.54, Mol formula $C_{15}H_{11}NO_5$ Mol Weight: 285.22; IR (Cm^{-1}) (KBr): 3442.78 (OH); 2926.50 (CH3); 1683.69 (C=O); 1114.30 (C-O-C); 1640.52 (NO2) Mass m/z: 286 (M+1), 284 (M-1);

2-(-3,4-dinitrophenyl)-4-hydroxy-6-methyl-1-benzofuran-3(2H)-one: (IIIC)

Yield: 64.5%, Melting point 246^{o}C, Rf Value: 0.42, Mol formula $C_{15}H_{10}N_2O_7$ Mol Weight: 330.22; IR (Cm^{-1}) (KBr): 3442.26 (OH); 2923.78 (CH3); 1583.65 (C=O); 1193.37 (C-O-C); 1498.69 (NO2); 1459.42 (NO2); Mass m/z: 331 (M+1), 329 (M-1);

4-hydroxy-6-methyl-2-(4-iodophenyl)-1-benzofuran-3(2H)-one: (IIID)

Yield: 64.5%, Melting point 237°C, Rf Value: 0.48, Mol formula $C_{15}H_{11}IO_3$ Mol Weight: 366.12; IR (Cm^{-1}) (KBr): 3422.24 (OH); 2924.85 (CH3); 1596.25 (C=O); 1112.27 (C-O-C); 686.42 (I); Mass m/z: 366 (M+1), 364 (M-1);

2-(4-bromophenyl)-4-hydroxy-6-methyl-1-benzofuran-3(2H)-one: (IIIE)

Yield: 60.80%, Melting point 249°C, Rf Value: 0.59, Mol formula $C_{15}H_{11}O_3Br$ Mol Weight: 319.12; IR (Cm^{-1}) (KBr): 3370.13 (OH); 2966.37 (CH3); 1624.18 (C=O); 1231.85 (C-O-C); 754.10 (Br); Mass m/z: 366 (M+1), 364 (M-1);

4-(4-hydroxy-6-methyl-3-oxo-2,3-dihydro-1-benzo furan-2-yl) benzenesulfonic acid: (IIIF)

Yield: 86.33%, Melting point 227°C, Rf Value: 0.58, Mol formula $C_{15}H_{12}O_6S$ Mol Weight: 320.29; IR (Cm^{-1}) (KBr): 3443.51 (OH); 2967.42 (CH3); 1612.15 (C=O); 1277.24 (C-O-C); 1353.71 (SO2H); Mass m/z: 321 (M+1), 318 (M-1);

Anti-Cancer Activity Results

Human Skin Cancer Cell Line G361, % Growth, Molar Drug Concentration

Table 5: Anti-Cancer Results Experiment – 1

	Experiment - 1			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IIIA	100	100	100	3.5
IIIB	100	100	100	61.2
IIIC	100	100	100	80.9
IIID	100	100	100	25.6
IIIE	100	100	100	70.9
IIIF	100	100	100	39.8

Table 6: Anti-Cancer Results Experiment – 2

	Experiment - 2			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IIIA	72.4	12.7	-11.2	100
IIIB	92.4	77.8	54.6	100
IIIC	100	99.2	63.7	100
IIID	100	86.5	15.8	100
IIIE	100	100	61.8	100
IIIF	100	100	14.5	100

Table 7: Anti-Cancer Results Experiment – 3

	Experiment - 3			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IIIA	100	100	17.5	-5.8
IIIB	100	100	99.1	39.3
IIIC	100	100	100	49.8
IIID	100	100	100	12
IIIE	100	100	100	51.8
IIIF	100	100	100	21.5

Table 8: Anti-Cancer Results Average Values

	Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IIIA	100	90.8	43.4	-4.5
IIIB	100	97.5	92.3	51.7
IIIC	100	100	99.7	64.8
IIID	100	100	95.5	17.8
IIIE	100	100	100	61.5
IIIF	100	100	100	25.3

Table 9: Results of anti-cancer activity for LC50, TGI, G150

G361	Molar Drug Concentration		
	LC 50	TGI	G150
IIIA	$>10^{-4}$	$3.7*10^{-5}$	$2.1*10^{-6}$
IIIB	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IIIC	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IIID	$>10^{-4}$	$>10^{-4}$	$3.2*10^{-5}$
IIIE	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IIIF	$>10^{-4}$	$>10^{-4}$	$3.6*10^{-5}$
ADR	$1.7*10^{-7}$	$<10^{-7}$	$<10^{-7}$

DISCUSSION

2-Phenyl Benzofuranone: The reactants phenylacetonitrile and bromine reacted where phenyl acetonitrile was preheated 110°C and bromine was added drop by drop where alpha hydrogen was replaced by bromine and HBr gas has been evolved which was trapped in water, and after ceasing of HBr gas the reaction has been completed, then after completion of the reaction it was washed with 5% sodium bicarbonate and then extracted by Dry ether and dry ether solution also washed with magnesium sulphate and the solvent is distilled off. Thus obtained product was confirmed by the reported boiling point of 242°C. And a good yield of 90.40% and IR spectrum has confirmed the existence of bromine in the molecule. Bromo(phenyl)acetonitrile was stirred in a three-necked round-bottomed flask immersed in ice-salt mixture which leads 0°c for the reaction then added 30ml of a dry ether solvent, to this mixture 5-methylbenzene-1,3-diol was added slowly then zinc chloride was (Lewis acid) added as a catalyst. Dry hydrochloric acid was prepared and pumped into the reaction mixture for about three hours and kept in ice-chest for three days. After three days' flask was removed from the ice chest and the supernatant ether layer was removed and then washed with dry ether and then dried. Which yielded a crude product of 86.80%. 2-(2-bromo-2-phenylethanimidoyl)-5-methylbenzene-1,3-diol was dissolved in 0.1N hydrochloric acid and then refluxed for two hours where the imine was oxidized which converts the mine to the ketone, then the reaction mixture was distilled, and the crude was extracted by dry ether and evaporated which yields a fine white powder with a yield of 76.10%. 2-bromo-1-(2,6-dihydroxy-

4-methylphenyl)-2-phenylethanone (1gm) was dissolved in freshly distilled ethanol, and sodium acetate which was freshly dried on a bunsen flame 2gm is added and then refluxed for 10 min then ethanol was distilled off, and the crude extract was dissolved in dry ether and evaporated yields a white cream crystals of 35.62%.

Nitration: Sulphuric acid and nitric acid was mixed in a flask and then cooled in an ice bath and then4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one and care has been taken such that temperature does not raise more than 55^{o}C such that an avoid multiple nitrations on the phenyl ring. Then slowly refluxed and the product obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 54.5%

Dinitration: Sulphuric acid and nitric acid was mixed in a flask and then cooled in an ice bath and then4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one then vigorously refluxed, and dinitrate product 2-(-3,4-dinitrophenyl)-4-hydroxy-6-methyl-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 64.5%

Iodination: Iodine and nitric acid was mixed in a flask and then cooled in an ice bath and then4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one Then vigorously refluxed and dinitrate product 4-hydroxy-2-(4-iodophenyl)-6-methyl-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 64.5%.

Bromination: Bromine and pyridine was mixed in a flask and then cooled in an ice bath and then4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one Then pyridine (Freshly distilled) was added then the reaction temperature raises to 70^{o}C and HBr gas evolution from the reaction mixture is ceased indicates the completion of reaction gives product 2-(4-bromophenyl)-4-hydroxy-6-methyl-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 60.80%

Sulphonation: Oleum (Fuming Sulphuric acid) was mixed in a flask and then boiled in a water bath and then4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3 (2*H*)-one Then vigorously refluxed gives sulphonic product 4-(4-hydroxy-6-methyl-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzene sulfonic acid obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 86.33%.

Anti-cancer activity

The newly synthesized compounds were screened for their anticancer activity against Human Skin Cancer Cell Line G361 by Sulforhodamine B assay. Doxorubicin was used as a standard reference drug and the results obtained were shown in (Table:2 &3). All compounds showed low antiproliferative activity. The % Growth inhibition of the compounds IIIA was found to be considered at a concentration of 10^{-4} M. TGI_{50} (Growth inhibition of 50 % cells, calculated from drug concentration resulting in a 50 % reduction in the net protein increase). As 2-phenylbenzofuranone derivatives are the most active compound, it serves as a lead to further optimization in a drug discovery process.

CONCLUSION

We had successfully developed a new series of 4-hydroxy-6-methyl-2-phenyl-1-benzofuran-3(2*H*)-one derivatives. Though benzofuranones were existed with prominent anti-cancer activity and in our developed a new series of phenyl substituted benzofuranones. These compounds are screened for antiproliferative activity against human skin cancer cell lines. The compounds IIIA showed an excellent antiproliferative activity which serves as a lead to further optimization in a drug discovery process.

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REFERENCES

Bandgar, B.P., Patil, S.A., Korbad, B.L., Biradar, S.C., Nile, S.N., Khobragade, C.N., Synthesis and biological evaluation of a novel series of 2, 2-bisaminomethylated aurone analogues as anti-inflammatory and antimicrobial agents. European Journal of Medicinal Chemistry, (2010); 45: 3223-27.

Boumendjel, A., Beney, C., Deka, N., Mariotte, A.M., Lawson, M.A., Trompier, D., Baubichon-Cortay, H., Di, Pietro, A. 4- Hydroxy-6-methoxyaurones with high-affinity binding to cytosolic domain of P-glycoprotein. Chemical and Pharmaceutical Bulletin, (2002); 50: 854-56.

French, K.J., Schrecengost, R.S., Lee, B.D., Zhuang, Y., Smith, S.N., Eberly, J.L., Yun, J.K., Smith, C.D., Discovery and evaluation of inhibitors of human sphingosine kinase. Cancer Research, (2003); 63: 5962-69.

Haudecoeur, R., Boumendjel, A., Recent advances in the medicinal chemistry of aurones. Current Medicinal Chemistry, (2012); 19: 2861- 75.

Hawkins, I., Handy, S.T., Synthesis of aurones under neutral conditions using a deep eutectic solvent. Tetrahedron, (2013); 69: 9200-04.

Huang, W., Liu, M.Z., Li, Y., Tan, Y., Yang, G.F., Design, syntheses, and antitumor activity of novel chromone and aurone derivatives. Bioorganic & Medicinal Chemistry, (2007); 15: 5191-97.

Kayser, O., Kiderlen, A.F., Brun, R., In vitro activity of aurones against Plasmodium falciparum strains K1 and NF54. Planta Medica. (2001); 67: 718-21.

Lawrence, N.J., Rennison, D., McGowan, A.T., Hadfield, J.A., The total synthesis of an aurone isolated from Uvaria hamiltonii: aurones and flavones as anticancer agents. ACS Medicinal Chemistry Letters, (2003); 13: 3759-63.

Manjulatha, K., Srinivas, S., Mulakayala, N., Rambabu, D., Prabhakar, M., Arunasree, K.M., Alvala, M., Basaveswara Rao, M.V., Pal, M., Ethylenediamine diacetate (EDDA) mediated synthesis of aurones under ultrasound: Their evaluation as inhibitors of SIRT1. Bioorganic & Medicinal Chemistry Letters, (2012); 22: 6160.

Nakayama, T., Enzymology of aurone biosynthesis, Journal of Bioscience and Bioengineering, (2002); 94: 487-91.

Nakayama, T., Sato, T., Fukui, Y., Yonekura-Sakakibara, K., Hayashi, H., Tanaka, Y., Kusumi, T., Nishino, T., Specificity analysis and mechanism of aurone synthesis catalysed by aureusidin synthase, a polyphenol oxidase homolog responsible for flower colouration. FEBS Letters, (2001); 499: 107-11.

Nakayama, T., Yonekura-Sakakibara, K., Sato, T., Kikuchi, S., Fukui, Y., Fukuchi-Mizutani, M., Ueda, T., Nakao, M., Tanaka, Y., Kusumi, T., Nishino, T., Aureusidin synthase: A polyphenol oxidase homolog responsible for flower coloration. Science (2000); 290: 1163-66.

Sim, H.M., Loh, K.Y., Yeo, W.K., Lee, C.Y., Go, M.L., Aurones as modulators of ABCG2 and ABCB1: Synthesis and structure-activity relationships, Chem Med Chem. (2011); 6: 713-24.

Vaclavikova, R., Boumendjel, A., Ehrlichova, M., Kovar, J., Gut, I., Modulation of paclitaxel transport by flavonoid derivatives in human breast cancer cells: Is there a correlation between binding affinity to NBD of P-gp and modulation of transport? Bioorganic & Medicinal Chemistry, (2006); 14: 4519-25.

Varma, R.S., Varma, M., Alumina-mediated condensation: A simple synthesis of aurones. Tetrahedron Letters, (1992); 33: 5937 -40.