ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Insilco screening, and Synthesis of 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one derivatives as novel potent leads as anti-cancer agents

Raghava Doonaboyina*, Abhilasha Mittal, Sridhar Babu Gummadi

Department of Pharmaceutical Chemistry, Institute of Pharmacy, NIMS University, Jaipur, Rajasthan,India

Article History:

Received on: 21.07.2018
Revised on: 05.12.2018
Accepted on: 09.12.2018

Keywords:

ADMET,
Anti cancer activity,
G361 cell line,
Sulforhodamine assay

ABSTRACT

Insilco technique in chemistry plays a major role in the development of new lead molecules. Computational tools docking, virtual screening, ADMET prediction are untilised in the identification of new lead molecules. A series of new benzofuranone derivatives IVA-F, containing a heterocyclic substituent linked to benzofuranone nucleus at C-2 were synthesized as potential antitumor agents. These products were synthesized starting with benzene 1.4 diol. The structures of all compounds were established on the basis of analytical and spectral data. The synthesized compounds were tested against human skin carcinoma cell line (G361) and all were found to be more potent of which compound IV B and IV F was the most active.

* Corresponding Author

Name: Raghava Doonaboyina
Phone: +91-9948567925
Email: Raghava.chinnu@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i1.1910

Production and Hosted by
IJRPS | https://ijrps.com

INTRODUCTION

Cancer has long been recognized as one of the most common causes of death (Varmus, H., 2006). Accordingly, many diverse strategies have been employed to develop new therapies or to improve existing treatments (Avendao, C., 2008). It is widely known that benzofuranone derivatives substituted at C-2 position show cytostatic and cytotoxic activity (Kossakowski, J., 2005). For example, luteolin derivative has been reported to have antiviral and antitumor activities. In addition, salvinal, isolated from *Salvia miltiorrhiza*, showed inhibitory activity against tumor growth and induced apoptosis in human cancer cells (Fuganti, 1998.,). Also, ebenfuran III (Chang, J.Y., 2004) and moracins O (Katsanou, E. S., 2007) are 2-phenyl benzofuran ones derived from natural compounds exhibiting potent cytotoxic activities against human breast cancer and hepatocellular carcinoma. Recently, in the process of new drug discovery, the anticancer and antiviral activities of a variety of benzofuran derivatives I (Dat, N., 2009), NSC725612 and NSC725616 have been reported. In addition, several reports have pointed out the value. In nature's collection of biologically active heterocyclic, benzofuran derivatives constitute a major group. The benzofuranone ring systems bearing various substituents at the C-2 position are widely distributed in nature. Luteolin is a flavonoid derivative, has been reported to have anti-cancer, anti-fungal, and other bacteriostatic activities (Galal, S. A., 2009). The broad spectrum of pharmacological activity in individual benzofurans indicates that this series of compounds is of an undoubted. In silico advances in the recent trends has grabbed a wide range of tools for the optimization of lead molecules. Which reduces a lot of researcher's expenses, the tools like docking studies, virtual screening and ADME predictors are used to optimize the lead molecules (Ganatra Sunil, H., 2013). Cyclin-Dependent Kinase (CDK) plays a vital role in control cell cycle progression from one phase to another. However, mutational changes in these molecules lead to the perturbed cell cycle leading to uncontrolled cellular proliferation. In human mutations CDK –II is responsible for cancers (Scott A., 2011).

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^{o} and validated for the Domain completeness.

Preparation of Protein: The selected protein 1GII has been was explored in Auto dock 4.0, the bonds and atoms in the protein are optimized, missing hydrogen are added, the non-polar centre between the hydrogens were 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: The basic benzofuranone was designed with modifications in the R^1, R^2, R^3, of the molecule given below in figure: 2.

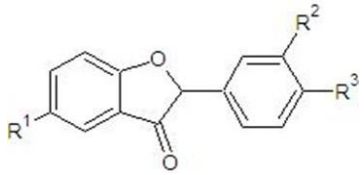


Figure 1: Substituted Benzofuranone

Table 1: Combinations of Ligands

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

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.

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 irritant 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 Alignment: 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 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.

Table 2: Virtual Screening results

S.No	Ligand	Target	Binding Energy	Date Created
1	208_uff_E=327.50	1GII	-7.9	2015.07.30 23:31:33
2	213_uff_E=365.73	1GII	-7.9	2015.07.30 23:37:26
3	214_uff_E=437.42	1GII	-7.9	2015.07.30 23:38:50
4	216_uff_E=341.47	1GII	-7.9	2015.07.30 23:41:01
5	226_uff_E=318.89	1GII	-7.9	2015.07.30 23:53:23
6	228_uff_E=365.59	1GII	-7.9	2015.07.30 23:55:33
7	23_uff_E=394.49	1GII	-7.9	2015.07.30 20:32:32
8	264_uff_E=387.93	1GII	-7.9	2015.07.31 00:26:25
9	273_uff_E=362.30	1GII	-7.9	2015.07.31 00:33:13
10	3_uff_E=287.99	1GII	-7.9	2015.07.30 20:14:21
11	324_uff_E=312.52	1GII	-7.9	2015.07.31 01:12:55
12	4_uff_E=287.02	1GII	-7.9	2015.07.30 20:15:02
13	50_uff_E=393.86	1GII	-7.9	2015.07.30 20:57:29
14	52_uff_E=447.95	1GII	-7.9	2015.07.30 20:59:39
15	66_uff_E=370.08	1GII	-7.9	2015.07.30 21:13:51
16	69_uff_E=350.28	1GII	-7.9	2015.07.30 21:16:26
17	7_uff_E=398.03	1GII	-7.9	2015.07.30 20:17:37
18	74_uff_E=356.52	1GII	-7.9	2015.07.30 21:20:36
19	78_uff_E=348.56	1GII	-7.9	2015.07.30 21:24:09
20	87_uff_E=331.38	1GII	-7.9	2015.07.30 21:31:30
21	89_uff_E=316.79	1GII	-7.9	2015.07.30 21:33:10
22	90_uff_E=316.22	1GII	-7.9	2015.07.30 21:33:53
23	91_uff_E=336.16	1GII	-7.9	2015.07.30 21:34:44
24	92_uff_E=400.39	1GII	-7.9	2015.07.30 21:35:42
25	94_uff_E=398.62	1GII	-7.9	2015.07.30 21:37:47
26	98_uff_E=378.54	1GII	-7.9	2015.07.30 21:41:57
27	99_uff_E=373.10	1GII	-7.9	2015.07.30 21:42:52
28	10_uff_E=367.68	1GII	-7.8	2015.07.30 20:19:54
29	12_uff_E=417.44	1GII	-7.8	2015.07.30 20:21:57
30	128_uff_E=354.66	1GII	-7.8	2015.07.30 22:09:30
31	129_uff_E=371.28	1GII	-7.8	2015.07.30 22:10:33
32	132_uff_E=360.19	1GII	-7.8	2015.07.30 22:13:43
33	134_uff_E=334.03	1GII	-7.8	2015.07.30 22:15:48
34	145_uff_E=313.76	1GII	-7.8	2015.07.30 22:27:24
35	164_uff_E=293.00	1GII	-7.8	2015.07.30 22:43:58
36	174_uff_E=365.79	1GII	-7.8	2015.07.30 22:53:56
37	175_uff_E=306.92	1GII	-7.8	2015.07.30 22:55:00
38	180_uff_E=326.74	1GII	-7.8	2015.07.30 23:01:00
39	19_uff_E=304.99	1GII	-7.8	2015.07.30 20:28:43
40	211_uff_E=360.22	1GII	-7.8	2015.07.30 23:34:58
41	215_uff_E=343.62	1GII	-7.8	2015.07.30 23:39:56
42	237_uff_E=366.55	1GII	-7.8	2015.07.31 00:04:42
43	253_uff_E=308.40	1GII	-7.8	2015.07.31 00:17:32
44	269_uff_E=381.15	1GII	-7.8	2015.07.31 00:30:29
45	308_uff_E=334.84	1GII	-7.8	2015.07.31 01:01:15
46	321_uff_E=327.60	1GII	-7.8	2015.07.31 01:10:46
47	47_uff_E=375.68	1GII	-7.8	2015.07.30 20:54:30
48	48_uff_E=339.74	1GII	-7.8	2015.07.30 20:55:33
49	49_uff_E=365.05	1GII	-7.8	2015.07.30 20:56:25
50	51_uff_E=378.47	1GII	-7.8	2015.07.30 20:58:30
51	65_uff_E=357.08	1GII	-7.8	2015.07.30 21:12:57
52	80_uff_E=357.08	1GII	-7.8	2015.07.30 21:26:02
53	9_uff_E=279.36	1GII	-7.8	2015.07.30 20:19:03
54	93_uff_E=392.86	1GII	-7.8	2015.07.30 21:36:44
55	96_uff_E=411.07	1GII	-7.8	2015.07.30 21:39:52

*Info: Vina

Table 2: Virtual Screening results (Contd……)

S.No	Ligand	Target	Binding Energy	Date Created
56	163_uff_E=296.28	1GII	-7.7	2015.07.30 22:43:01
57	183_uff_E=399.22	1GII	-7.7	2015.07.30 23:04:22
58	2_uff_E=364.06	1GII	-7.7	2015.07.30 20:13:37
59	210_uff_E=377.75	1GII	-7.7	2015.07.30 23:33:54
60	236_uff_E=303.11	1GII	-7.7	2015.07.31 00:03:38
61	252_uff_E=304.59	1GII	-7.7	2015.07.31 00:16:52
62	270_uff_E=375.62	1GII	-7.7	2015.07.31 00:31:14
63	296_uff_E=331.17	1GII	-7.7	2015.07.31 00:51:00
64	309_uff_E=348.25	1GII	-7.7	2015.07.31 01:02:00
65	8_uff_E=321.07	1GII	-7.7	2015.07.30 20:18:21
66	83_uff_E=354.81	1GII	-7.7	2015.07.30 21:28:27
67	165_uff_E=364.37	1GII	-7.6	2015.07.30 22:44:55
68	173_uff_E=300.57	1GII	-7.6	2015.07.30 22:52:39
69	249_uff_E=317.18	1GII	-7.6	2015.07.31 00:14:52
70	251_uff_E=303.41	1GII	-7.6	2015.07.31 00:16:15
71	46_uff_E=338.35	1GII	-7.6	2015.07.30 20:53:29
72	6_uff_E=337.21	1GII	-7.6	2015.07.30 20:16:42
73	64_uff_E=279.74	1GII	-7.6	2015.07.30 21:12:04
74	208_uff_E=327.50	1GII	-7.9	2015.07.30 23:31:33
75	213_uff_E=365.73	1GII	-7.9	2015.07.30 23:37:26
76	214_uff_E=437.42	1GII	-7.9	2015.07.30 23:38:50
77	216_uff_E=341.47	1GII	-7.9	2015.07.30 23:41:01
78	226_uff_E=318.89	1GII	-7.9	2015.07.30 23:53:23
79	228_uff_E=365.59	1GII	-7.9	2015.07.30 23:55:33
80	23_uff_E=394.49	1GII	-7.9	2015.07.30 20:32:32
81	264_uff_E=387.93	1GII	-7.9	2015.07.31 00:26:25
82	273_uff_E=362.30	1GII	-7.9	2015.07.31 00:33:13
83	3_uff_E=287.99	1GII	-7.9	2015.07.30 20:14:21

*Info: Vina

Table 3: ADMET results

Molecule Name	Absolute Weight	C LogP	C LogS	H-Acceptors	H-Donors	Total Surface Area	Polar Surface Area	Drug Likeness	LELP from Molecule Name
1	226.063	2.5051	-2.962	3	1	198	46.53	0.1775	4.7056
2	242.0579	2.1594	-2.666	4	2	196	66.76	0.1775	4.296
3	256.0736	2.4351	-2.98	4	1	229	55.76	0.2867	5.1149
4	240.0786	2.849	-3.306	3	1	213	46.53	0.14141	5.6708
5	271.0481	1.2605	-3.246	6	1	235	94.27	1.5659	2.7899
6	303.9735	3.2303	-3.796	3	1	218	46.53	-1.6125	6.4364
7	260.024	3.1111	-3.698	3	1	219	46.53	0.26995	6.2004
8	240.0786	2.7808	-3.276	3	0	224	35.53	0.25055	5.5436
9	256.0736	2.4351	-2.98	4	1	224	55.76	0.2867	5.1254
10	270.0892	2.7108	-3.294	4	0	232	44.76	0.25055	6.0075
11	254.0943	3.1247	-3.62	3	0	227	35.53	0.21835	6.5801
12	285.0637	1.5362	-3.56	6	0	225	83.27	1.6305	3.5782
13	317.9892	3.506	-4.11	3	0	241	35.53	-1.5395	7.3904
17	274.0397	3.3868	-4.012	3	0	235	35.53	0.34327	7.1409
18	224.0837	3.1947	-3.602	2	0	205	26.3	0.105	6.0283
19	254.0943	3.1247	-3.62	3	0	213	35.53	0.21835	6.5931
21	238.0994	3.5386	-3.946	2	0	208	26.3	0.105	7.0751
22	269.0688	1.9501	-3.886	5	0	230	74.04	-0.42042	4.3354
28	258.0448	3.8007	-4.338	2	0	217	26.3	0.19962	7.6083

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

Table 3: ADMET results (Contd……)

Molecule Name	Absolute Weight	C LogP	C LogS	H-Acceptors	H-Donors	Total Surface Area	Polar Surface Area	Drug Likeness	LELP from Molecule Name
30	238.0994	3.6103	-3.761	2	0	215	26.3	-0.10598	7.2289
38	254.0943	3.2646	-3.465	3	1	224	46.53	-0.05978	6.9015
39	268.1099	3.5403	-3.779	3	0	233	35.53	0.024994	7.8801
40	252.115	3.9542	-4.105	2	0	228	26.3	-0.10598	8.3632
43	283.0845	2.3657	-4.045	5	0	230	74.04	1.208	5.5341
44	225.079	2.1735	-3.334	3	1	196	52.32	0.31846	4.1188
46	255.0532	1.6062	-3.542	5	0	230	74.04	1.1005	3.4088
55	271.0481	1.2605	-3.246	6	1	230	94.27	1.7903	2.8166
56	285.0637	1.5362	-3.56	6	0	225	83.27	2.4307	3.6051
57	269.0688	1.9501	-3.886	5	0	225	74.04	0.31958	4.3594
58	300.0382	0.3616	-3.826	8	0	230	112.54	1.3609	0.88977
61	332.9637	2.3314	-4.376	5	0	236	64.8	-0.42907	5.2164
62	289.0142	2.2122	-4.278	5	0	236	64.8	1.448	4.9508
63	287.9786	3.576	-4.092	2	0	218	26.3	-1.6432	6.8039
64	303.9735	3.2303	-3.796	3	1	220	46.53	-1.6125	6.5091
65	317.9892	3.506	-4.11	3	0	240	35.53	-1.5395	7.4587
66	332.9637	2.3314	-4.376	5	0	240	64.8	-0.42907	5.2254
67	365.8891	4.3012	-4.926	2	0	228	26.3	-1.6432	8.6782
70	244.0291	3.4568	-3.994	2	0	210	26.3	0.2375	6.5898
71	260.024	3.1111	-3.698	3	1	217	46.53	0.26995	6.281
73	274.0397	3.3868	-4.012	3	0	231	35.53	0.34327	7.219
74	258.0448	3.8007	-4.338	2	0	218	26.3	0.19962	7.6764
75	289.0142	2.2122	-4.278	5	0	230	64.8	1.6334	4.9676
76	226.063	2.5051	-2.962	3	1	198	46.53	0.1775	4.7056
79	242.0579	2.1594	-2.666	4	2	196	66.76	0.1775	4.296
80	256.0736	2.4351	-2.98	4	1	229	55.76	0.2867	5.1149
81	240.0786	2.849	-3.306	3	1	213	46.53	0.14141	5.6708
82	271.0481	1.2605	-3.246	6	1	235	94.27	1.5659	2.7899
83	303.9735	3.2303	-3.796	3	1	218	46.53	-1.6125	6.4364

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

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

Preparation of 2-(2-bromo-2-phenylethanimidoyl) benzene-1, 4-diol

A fresh vacuum dried benzene-1,4-diol of (1.1g 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^{0}c than to this phenyl(bromo)acetonitrile of (1.9g 0.1mol), Lewis acid $Alcl_3$ (1g) and then Dry Hydrochloric acid gas

Table 4: Docking Results (Contd...)

S.no	Molecule No	Iupac name	Binding energy	Ic 50	Ic 50 units	No. of Confirmations
56	301	2-(4-methyl-3-nitrophenyl) -1-benzofuran-3(2*H*)-one	-7.51	3.11	MM	10
57	304	2-(3,4-dinitrophenyl) -1-benzofuran-3(2H)-one	-9.34	141.87	NM	6
58	305	2-(4-bromo-3-nitrophenyl) -1-benzofuran-3(2*H*)-one	-6.23	8.96	MM	9
61	306	2-(4-chloro-3-nitrophenyl) -1-benzofuran-3(2*H*)-one	-7.17	5.14	MM	9
62	307	2-(3-bromophenyl) -1-benzofuran-3(2*H*)-one	-7.39	3.84	MM	9
63	308	2-(3-bromo-4-hydroxyphenyl) -1-benzofuran-3(2*H*)-one	-7.28	2.63	MM	9
64	309	2-(3-bromo-4-methoxyphenyl) -1-benzofuran-3(2*H*)-one	-7.53	3.04	MM	10
65	313	2-(3-bromo-4-nitrophenyl) -1-benzofuran-3(2*H*)-one	-7.67	2.38	MM	9
66	316	2-(3-chlorophenyl) -1-benzofuran-3(2*H*)-one	-7.22	5.06	MM	8
67	317	2-(3-chloro-4-hydroxyphenyl) -1-benzofuran-3(2*H*)-one	-7.55	5.17	MM	10
70	318	2-(3-chloro-4-methoxyphenyl) -1-benzofuran-3(2*H*)-one	-7.3	4.42	MM	10
71	319	2-(3-chloro-4-methyl phenyl) -1-benzofuran-3(2*H*)-one	-7.6	2.7	MM	8
73	322	2-(3-chloro-4-nitrophenyl) -1-benzofuran-3(2*H*)-one	-7.54	2.5	MM	8
74	324	2-(3,4-dichlorophenyl) -1-benzofuran-3(2*H*)-one	-7.63	2.55	MM	8

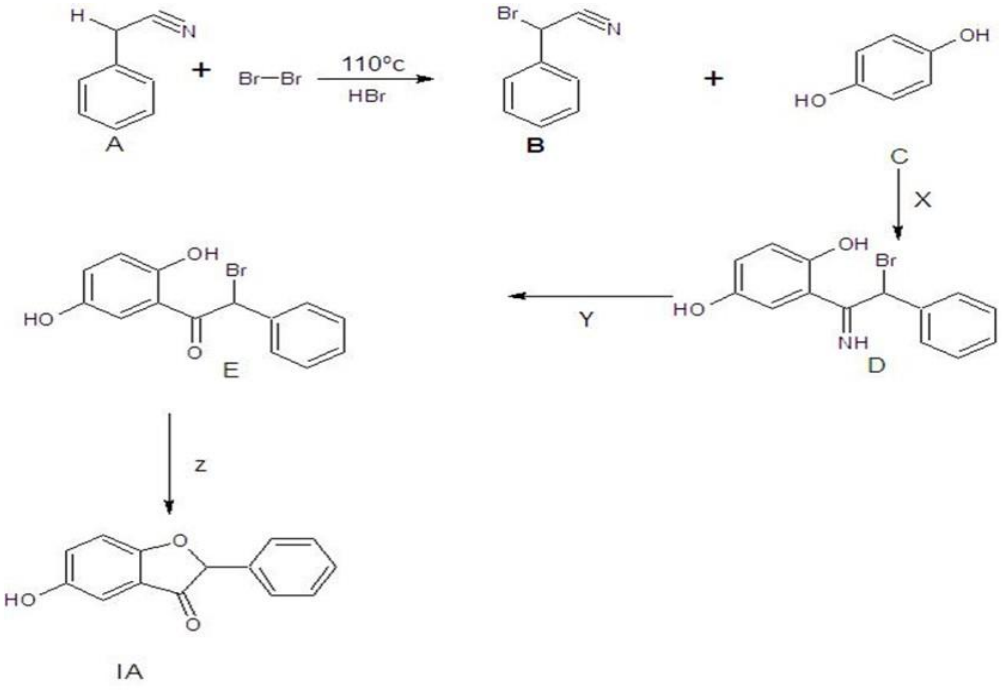
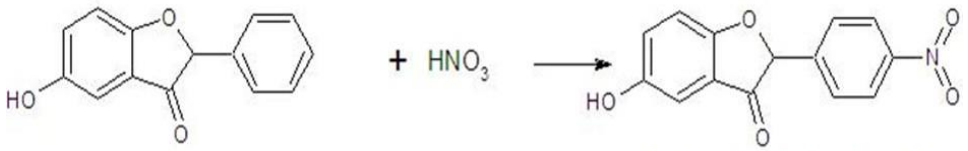


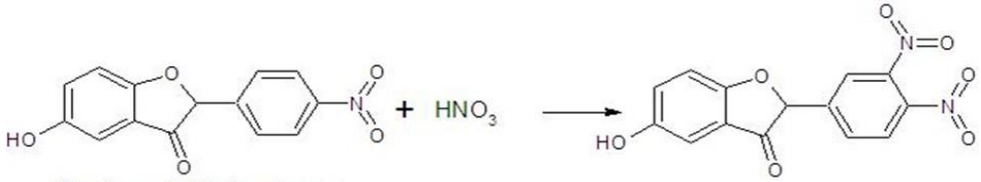
Figure 2: Scheme for Preparation of Benzofuranone derivatives: X ZnCl2, Dry HCL, Dry ether, at 0°C. Y 0.1N HCL Z Sodium acetate and ethanol. A-Phenyl acetonitrile, B-Phenyl(bromo) acetonitrile, C- benzene-1,4-diol, D 2-(2-bromo-2-phenylethanimidoyl) benzene-1, 4-diol, E 2-bromo-1-(2,5-dihydroxyphenyl)-2-phenylethanone,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



5-hydroxy-2-phenyl-1 -benzofuran-3(2*H*)-one

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

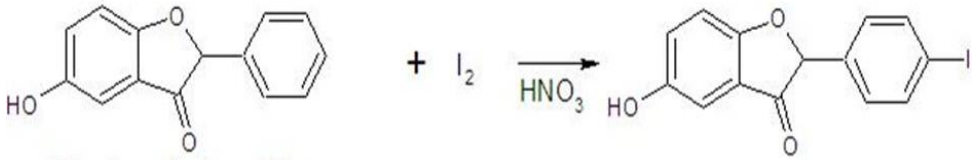
Figure 3: Preparation of 4,6-dihydroxy-2-(-4-nitrophenyl)-1-benzofuran-3(2H)-one



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

2-(3,4-dinitrophenyl)-5-hydroxy-1-benzofuran-3(2*H*)-one

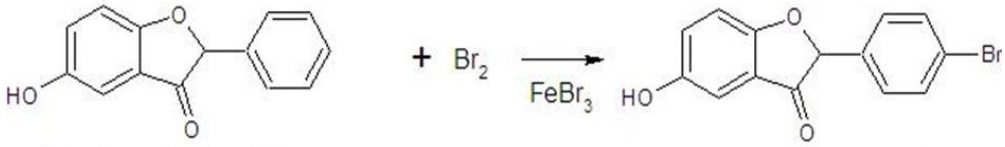
Figure 4: Preparation of 2-(-3,4-dinitrophenyl)-5-hydroxy-1-benzofuran-3(2H)-one



5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one

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

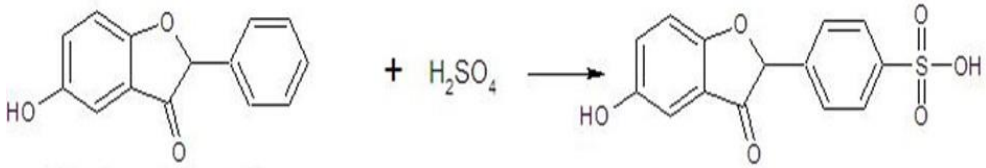
Figure 5: Preparation of 5-hydroxy-2-(4-iodophenyl)-1-benzofuran-3(2H)-one



5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one

2-(4-bromophenyl)-5-hydroxy-1-benzofuran-3(2*H*)-one

Figure 6: Preparation of 2-(4-bromophenyl)-5-hydroxy-1-benzofuran-3(2H)-one



5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one

4-(5-hydroxy-3-oxo-2,3-dihydro-1-benzofuran-2-yl)benzenesulfonic acid

Figure 7: Preparation of 4-(5-hydroxy-3-oxo-2,3-dihydro-1-benzofuran-2-yl)benzene sulfonic acid

as passed through the solution about three hours and then the reaction mixture was kept in an ice chest for one day, and then Dry Hydrochloric acid was again pumped into the reaction mixture for 3 hours and stored in an 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-(2,5-dihydroxyphenyl)-2-phenylethanone

2-(2-bromo-2-phenylethanimidoyl) benzene-1,4-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 white crystal was obtained which was dried.

Preparation of 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one:(IVA)

2-bromo-1-(2,5-dihydroxyphenyl)-2-phenylethanone (1gm) 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.

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 a cold bath and cooled, then 2.2g (0.2mol) of 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one compound IVA 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^{o}c (care to be taken such that temperature does not increase beyond 55^{o}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. then 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.

Dinitration

A 3.7ml of concentrated sulphuric acid was placed in 250ml round-bottomed flask fitted with a thermometer, few fragments of glazed porcelain are added then small portions 2.2gm(0.01mol) of 5-hydroxy-2-(-4-nitrophenyl)-1-benzofuran-3(2*H*)-one (compound-IVB) is added the reaction mixture was refluxed on a water bath for 30 minutes with occasional stirring and the reaction mixture was poured into 100 ml cold water and stirred and vacuum filtered dried as much as possible. Thus obtained product was recrystallised by hot ethanol solution, and then T.L.C Studies were performed.

Iodination

A 2.2g (0.1mol) of 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one (Compound IVA), 2.2gm 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.

Bromination

A 2.2g (0.1mol) of 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one (Compound IVA) 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.

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 2.2gm(0.01mol) of5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one (Compound IVA) 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 a product 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

5-hydroxy-2-phenyl-1-benzofuran-3(2H)-one: (IVA)

Yield: 65.62 %, Melting point 221°C, Rf Value: 0.70, Mol formula $C_{14}H_{10}O_3$ Mol Weight: 226.22; IR (Cm^{-1}) (KBr): 3422.18 (OH); 1559.05 (C=O); 1250.18 (C-O-C); ^{1}H NMR (400 MHz, MeOD-d_6): δ= 8.22 (d 1H Ar-H *J*=8) 7.57-7.55 (t 2H Ar-2`H and 6`H *J*=7.6) 7.35-7.34 (t =2H Ar-3`H and 5`H *J*= 7.2) 6.40-6.39 (d 2H Ar-6`H and 7H), 4.78(s 1H CH).

^{13}C NMR (100 MHz, MeOD): δ= 88.06 (C-2), 159.98 (C-5), 100.91 (C-7), 115.88 (C-9), 127.41 (C-1``), 128.41 (C-2``), 129.69 (C-2```), 136.59 (C-1`), 110.17 (C-4), 170.80 (C-8), 96.51 (C-6), 197.32 (C-3). Mass m/z: 228 (M+1), 225 (M-1);

5-hydroxy-2-(-4-nitrophenyl)-1-benzofuran-3(2H)-one: (IVB)

Yield: 54.5%, Melting point 242°C, Rf Value: 0.60, Mol formula $C_{14}H_9NO_5$Mol Weight: 271.22; IR (Cm^{-1}) (KBr): 3308.36 (OH); 1671.66 (C=O); 1113.06 (C-O-C); 1616.90 (NO2) Mass m/z: 271 (M+1), 270 (M-1);

2-(-3,4-dinitrophenyl)-5-hydroxy-1-benzofuran-3(2H)-one: (IVC)

Yield: 74.5%, Melting point 276°C, Rf Value: 0.47, Mol formula $C_{14}H_8N_2O_7$Mol Weight: 316.22; IR (Cm^{-1}) (KBr): 3393.63 (OH); 1654.39 (C=O); 1107.40 (C-O-C); 1602.36 (NO2); 1510.79 (NO2); Mass m/z: 317 (M+1), 315 (M-1);

5-hydroxy-2-(4-iodophenyl)-1-benzofuran-3(2H)-one: (IVD)

Yield: 66.5%, Melting point 243°C, Rf Value: 0.56, Mol formula $C_{14}H_9IO_3$Mol Weight: 352.12; IR (Cm^{-1}) (KBr): 3424.22 (OH); 1743.18 (C=O); 1115.37 (C-O-C); 571.05 (I) Mass m/z: 352 (M+1), 350 (M-1);

2-(4-bromophenyl)-5-hydroxy-1-benzofuran-3(2H)-one: (IVE)

Yield: 85.40%, Melting point 268°C, Rf Value: 0.58, Mol formula $C_{14}H_9O_3Br$ Mol Weight: 305.12; IR (Cm^{-1}) (KBr): 3447.98 (OH); 1735.39 (C=O); 1223.40 (C-O-C); 744.60 (Br); Mass m/z: 304 (M+1), 302 (M-1);

4-(5-hydroxy-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid: (IVF)

Yield: 82.33%, Melting point 207°C, Rf Value: 0.45, Mol formula $C_{14}H_{10}O_6S$ Mol Weight: 306.29; IR (Cm^{-1}) (KBr): 3322.92 (OH); 1753.06 (C=O); 1171.29 (C-O-C); 13390.39 (SO2); Mass m/z: 305 (M+1), 307 (M-1);

Anti-Cancer Activity Results

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

Table 4: Anti-Cancer Results Experiment – 1

	Experiment - 1			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IVA	100.0	100.0	100.0	30.1
IVB	100.0	100.0	100.0	-12.1
IVC	100.0	100.0	100.0	15.3
IVD	100.0	100.0	100.0	46.9
IVE	100.0	100.0	100.0	70.9
IVF	100.0	100.0	100.0	-12.1

Table 5: Anti-Cancer Results Experiment – 2

	Experiment - 2			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IVA	100.0	100.0	100.0	25.1
IVB	100.0	100.0	100.0	-37.6
IVC	100.0	100.0	100.0	12.2
IVD	100.0	100.0	99.5	43.8
IVE	100.0	100.0	100.0	61.8
IVF	100.0	100.0	100.0	-37.6

Table 6: Anti-Cancer Results Experiment – 3

	Experiment - 3			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IVA	100.0	100.0	100.0	35.1
IVB	100.0	100.0	100.0	-40.3
IVC	100.0	100.0	100.0	13.1
IVD	100.0	100.0	100.0	41.0
IVE	100.0	100.0	100.0	51.8
IVF	100.0	100.0	100.0	-40.3

Table 7: Anti-Cancer Results Average Values

	Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
IVA	100.0	100.0	100.0	30.1
IVB	100.0	100.0	100.0	-30.0
IVC	100.0	100.0	100.0	13.5
IVD	100.0	100.0	99.8	43.9
IVE	100.0	100.0	100.0	61.5
IVF	100.0	100.0	100.0	-30.0

Table 8: Results of anti-cancer activity for LC50, TGI, G150

G361	Molar Drug Concentration		
	LC 50	TGI	G150
IVA	$>10^{-4}$	$>10^{-4}$	$3.9*10^{-5}$
IVB	$>10^{-4}$	$>10^{-4}$	$2.4*10^{-6}$
IVC	$>10^{-4}$	$>10^{-4}$	$3.2*10^{-5}$
IVD	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IVE	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
IVF	$>10^{-4}$	$>10^{-4}$	$2.4*10^{-6}$
ADR	$1.7*10^{-7}$	$<10^{-7}$	$<10^{-7}$

DISCUSSION AND CONCLUSION

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 benzene-1,4-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 supernatant ether layer was removed and then washed with dry ether and then dried. Which yielded a crude product of 76.80 %. 2-(2-bromo-2-phenylethanimidoyl) benzene-1, 4-diol (1gm) was dissolved in 0.1N hydrochloric acid and then refluxed for two hours where the imine was oxidized which converts the imine to 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 76.80%. 2-bromo-1-(2,5-dihydroxyphenyl)-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 65.62%.

Nitration: Sulphuric acid and nitric acid was mixed in a flask and then cooled in an ice bath and then 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one and care have been taken such that temperature does not raise more than 55°C such that a 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 then 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one Then vigorously refluxed and dinitrate product 2-(-3,4-dinitrophenyl)-5-hydroxy-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 74.5%

Iodination: Iodine and nitric acid was mixed in a flask and then cooled in an ice bath and then 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one Then vigorously refluxed and dinitrate product **5**-hydroxy-2-(4-iodophenyl)-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 66.5%

Bromination: Bromine and pyridine was mixed in a flask and then cooled in an ice bath and then 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one Then pyridine (Freshly distilled) was added then the reaction temperature raises to 70°C and HBr gas evolution from the reaction mixture is ceased indicates the completion of reaction gives product 2-(4-bromophenyl)-5-hydroxy-1-benzofuran-3(2*H*)-one obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 85.40%

Sulphonation: Oleum (Fuming Sulphuric acid) was mixed in a flask and then boiled in a water bath and then 5-hydroxy-2-phenyl-1-benzofuran-3(2*H*)-one Then vigorously refluxed gives sulphonic product 4-(5-hydroxy-3-oxo-2,3-dihydro-1-benzofuran-2-yl) benzenesulfonic acid obtained is washed ice cold water finally extracted with ether and distilled gives a yield of 82.33%

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 IVB, IVF, 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 5-hydroxy-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 IVB, IVF **s**howed an excellent antiproliferative activity which serves as a lead to further optimization in a drug discovery process.

Acknowledgement

Mr Raghava Doonaboyina acknowledges Nims Institute of pharmacy to facilitating the resources for the research work, Authours acknowledge Laila Impex industry for providing spectral facilities for the research work and ACTREC (Tata Memorial Center) Mumbai to carry out anti-cancer activity.

REFERENCES

Avendao, C., Men e ndez, J.C., Medicinal Chemistry of Anticancer Drugs, Elsevier Amsterdam, (2008).

Chang, J. Y., Chang, C.Y., Kuo, C.C., Chen, L.T., Wein, Y.S., Kuo, Y.H., Salvinal, A novel microtubule inhibitor isolated from Salvia miltiorrhiza Bunge Danshen, with antimitotic activity in multidrug-sensitive and -resistant human tumor cells. Molecular Pharmacology. (2004); 65: 77–84.

Dat, N.T., Jin, X.J., Lee, K., Hong, Y.S., Kim, Y.H., Lee, J.J. Hypoxia-inducible factor-1 inhibitory benzofurans and chalcone-derived diels-alder adducts from Morus species. Journal of Natural Products, (2009); 2: 39 – 43.

Fuganti, C., Serra, S.A., New approach to 2-aryl-7-alkoxybenzofurans: synthesis of ailanthoidol, a natural neolignan, Tetrahedron Letters, (1998); 39: 5609 – 5610.

Galal, S.A., Abd El-All, A.S., Abdallah, M.M., El-Diwani, H.I. Synthesis of potent antitumor and antiviral benzofuran derivatives, Bioorganic & Medicinal Chemistry, (2009); 19: 2420 – 2428.

Ganatra Sunil H., Gurjar Shilpa A., Structure-Based Studies of 2-Bezylidine-Benzofuran-3-One Class of Compounds as the Cyclin-Dependent Kinases (CDKs) Inhibitor, (2013); 5(6): 712-717.

Katsanou, E.S., Halabalaki, M., Aligiannis, N., Mitakou, S., Skaltsounis, A. L., Alexi, X., Pratsinis, H., Alexis, M. N. Cytotoxic effects of 2-arylbenzofuran phytoestrogens on human cancer cells: modulation by adrenal and gonadal steroids, The Journal of Steroid Biochemistry and Molecular Biology, (2007); 104: 228 – 236.

Kossakowski, J., Ostrowska, K., Hejchman, E., Wolska, I., Synthesis and structural characterization of derivatives of 2-and 3-benzo[b]furan carboxylic acids with potential cytotoxic activity. IL Farmaco, (2005); 60: 519 – 527.

Scott A., Wildmanl., Xiange Zheng1., David Sept2., Jeffrey T., Auletta, Terrone L., Rosenberry and Garland R., Marshall Drug-like Leads for Steric Discrimination between Substrate and Inhibitors of Human Acetylcholinesterase, (2011); 01: 1747-1752.

Varmus, H., The new era in cancer research. Science (2006); 312: 1162 – 1165.