Journal Pre-proofs

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Graphical Abstract

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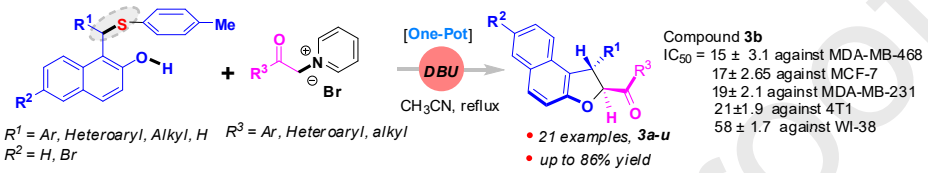
Kobirul Islam,[a,b] Kunal Pal,[a] Utsab Debnath,[a] R. Sidick Basha,[b] Abu Taleb Khan,[b] Kuladip Jana[a]* and Anup Kumar Misra[a] *



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Kobirul Islam,[a,b,c] Kunal Pal,[a,c] Utsab Debnath,[a] R. Sidick Basha,[b] Abu Taleb Khan,[b] Kuladip Jana[a]* and Anup Kumar Misra[a]*

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ABSTRACT

A series of 1,2-dihydronaphtho[2,1-b]furan derivatives were synthesized by cyclizing 1-(aryl/alkyl(arylthio)methyl)-naphthalen-2-ol and pyridinium bromides in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in very good yield. The synthesized compounds were evaluated for their anti-proliferative potential against human triple negative MDA-MB-468 and MCF-7 breast cancer cells and non-cancerous WI-38 cells (lung fibroblast cell) using MTT experiments. Among 21 synthesized compounds, three compounds (**3a**, **3b** and **3s**) showed promising anti-cancer potential and compound **3b** was found to have best anti-proliferative activities based on the results of several biochemical and microscopic experiments.

Cancer is most lethal disease which is characterized by abnormal proliferation of cells, which invades other parts of the body through metastasis and affects the normal functioning of vital organs of the body.[1] Various therapeutic strategies such as chemotherapy, immunotherapy, radiation therapy, targeted therapy, gene therapy have been explored for the treating of the disease.[2] Despite of several advanced treatment strategies, metastasis and cancer relapsed with resistance remain a significant challenge in cancer treatment. Recently, the reports show that more or less 8.2 million people around the globe are suffering from the irreversible metastatic condition of malignant tumors with drug resistance.[3] Among several organ specific cancer complications, breast cancer is frequently found in women throughout the world.[4] Because of its metastatic nature, breast cancer usually transfers to distant organs such as the bone, liver, lung and brain. Early diagnosis of the disease can lead to a good prognosis and a high survival rate. Although several anticancer therapeutics are available in clinics to arrest breast cancer, they are suffering from undesirable side-effects and toxicities.[5-7] Several new molecular signaling and pathways in oncogenic transformation, tumor progression and metastasis have been identified utilizing several bio-chemical techniques like protein-protein interaction, protein-DNA/RNA interactions.[8,9] Therefore, targeting the pathways in oncogenic transformation, tumor progression and metastasis through chemically synthesized small molecules may be effective to develop chemoprevention of cancer.

A wide range of biologically active natural products contain 2,3-dihydrobenzofuran as the active framework for their biological activities.[10] Several compounds having 2,3-dihydrobenzofuran scaffold within were reported as antibacterial,[11] antitumor,[12] antifungal,[13] and insecticidal agents[14] (Figure 1).

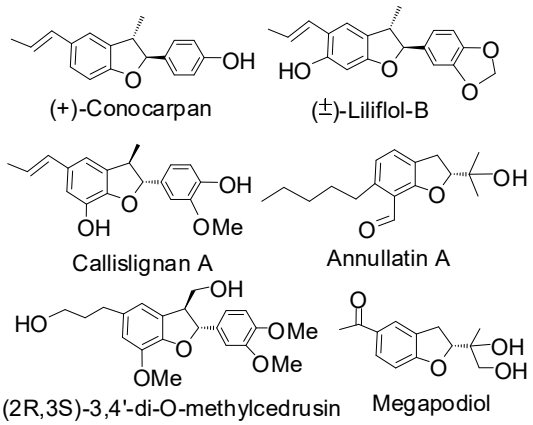


Figure 1: Biologically active compounds having 1,2-benzofuran scaffold.

However, reports on the synthesis of 1,2-dihydronaphthofuran derivatives[15] and their biological activity are rather scarce. In spite of their versatile pharmaceutical potential, only a few reports on their enzyme inhibitory activities[16-18] are available (Figure 2).

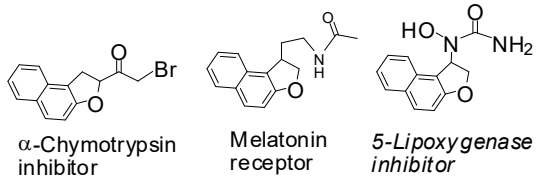
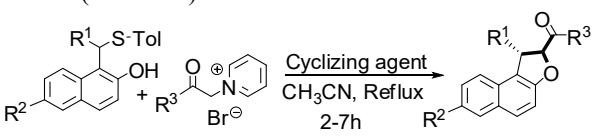


Figure 2: 1,2-Dihydronaphthofuran derivatives as enzyme inhibitors.

It was decided to synthesize a library of 2,3-dihydronaphthofuran derivatives to evaluate their anti-proliferative potential against different breast cancer cells towards the development of novel anti-cancer agents. As an extension of the recent report[19] on the synthesis of unsymmetrical ethers by the cleavage of C-S bond in napthalen-2-ol sulphide, it was envisaged that combination of C-S bond cleavage in 1-(aryl/alkyl(arylthio)methyl)-naphthalen-2-ol along with tandem C-C and C-O bond cyclization might lead to the synthesis of 2-acyl-1,2-dihydronaphtho [2,1-*b*]furan derivatives (Scheme 1).

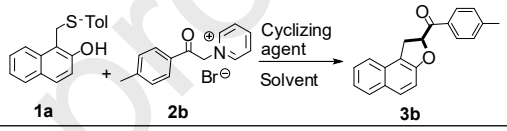


R^1, R^2, R^3 = H, alkyl and aryl

Scheme 1: Cleavage of C-S bond along with tandem C-C and C-O bond cyclization for the synthesis of 1,2-dihydronaphtho[2,1-b]furan derivatives.

In a set of initial experiments, cyclization of 1-(*p*-tolylthiomethyl)naphthalen-2-ol (**1a**) with 1-(2-oxo-2-*p*-tolylethyl)pyridinium bromide (**2b**) was attempted using a variety of organic bases such as DBU, DABCO, Et_3N, $(iPr)_2NH$, K_2CO_3 in different organic solvents such as EtOH, CH_3CN, DMF, THF, toluene at variable reaction temperature. The reactions were continued till the formation of the desired product in maximum yield. The temperature and time of the reactions were changed to obtain maximum yield of the product. After a set of 1,2-dihydronaphtho[2,1-b]furan-2-yl)(*p*-tolyl)methanone (**3b**) can be obtained by refluxing the reaction mixture in CH_3CN using DBU (1.0 equiv.) as the cyclizing agent (Table 1; entry 4). Use of other bases as cyclizing agents did not give satisfactory yield of the product. The substrate scope of the reaction condition was excellent since a series of (1,2-dihydronaphtho[2,1-b]furan-2-yl)methanone derivatives (**3a-u**) were synthesized using a variety of 1-(alkyl/arylthio)methyl)-naphthalen-2-ol (**1a-o**) and pyridinium bromides (**2a-f**) following similar reaction condition as mentioned in Table 2. It is noteworthy that the reaction condition is highly stereoselective and use of substituted 1-(alkyl/arylthio)methyl)-naphthalen-2-ol furnished exclusively single diasteriomeric products (**3g-u**) without formation of the other isomer. The starting materials napthalen-2-ol sulfides (**1a-o**) were prepared following reported reaction conditions.[20] All synthesized compounds were purified using flash chromatography and obtained with high purity. Most of the compounds tested for biological potential were in the range of 96-98% purity and characterized by their NMR and mass spectral analysis.

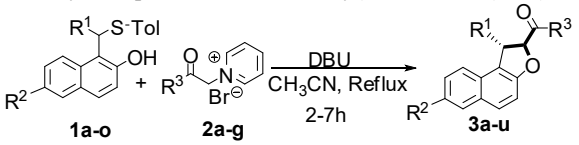
Table 1: Optimization of reaction condition for the synthesis of (1,2-dihydronaphtho[2,1-b]furan-2-yl)(*p*-tolyl)methanone (**3b**).



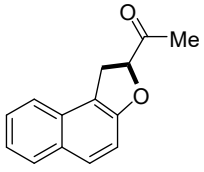
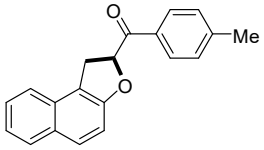
1a **2b** **3b**

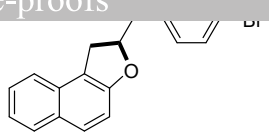
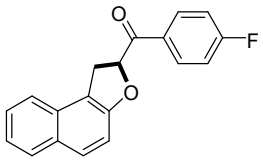
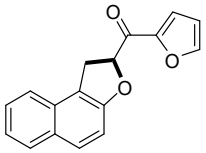
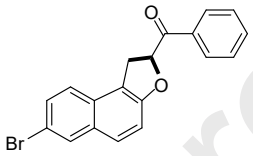
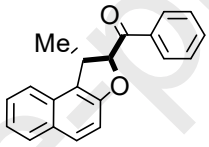
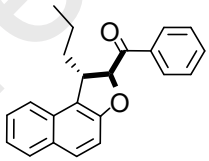
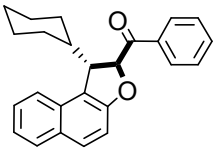
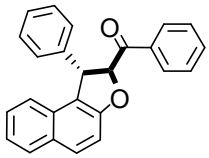
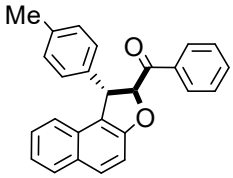
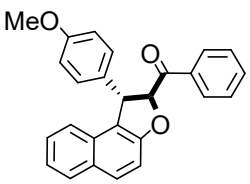
Sl. No.	Base	Solvent	Temp (°C)	Time (h)	Yield (%)
1	--	CH_3CN	r t	5	ND
2	--	CH_3CN	reflux	5	ND
3	DBU (0.5 equiv)	CH_3CN	Reflux	8	60
4	DBU (1.0 equiv)	CH_3CN	Reflux	5	80
5	DBU (1.0 equiv.)	THF	Reflux	10	50
6	DBU (1.0 equiv)	DMF	80	8	72
7	DBU (1.0 equiv)	EtOH	80	10	35
8	DBU (1.0 equiv)	Toluene	Reflux	10	20
9	DABCO (1.0 equiv)	CH_3CN	Reflux	10	50
10	Et_3N (1.0 equiv)	CH_3CN	Reflux	10	45
11	iPr_2NH (1.0 equiv)	CH_3CN	Reflux	10	55
12	K_2CO_3 (1.0 equiv)	CH_3CN	Reflux	8	40

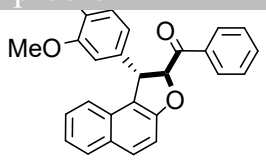
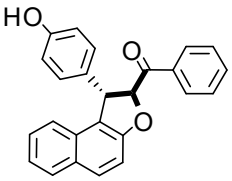
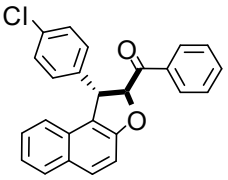
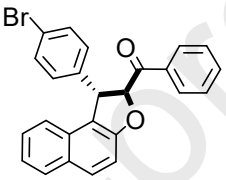
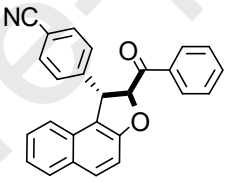
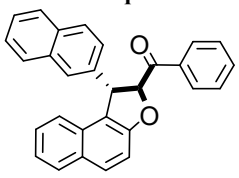
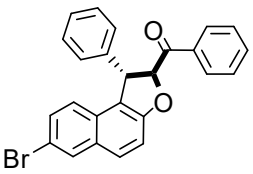
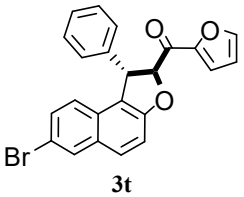
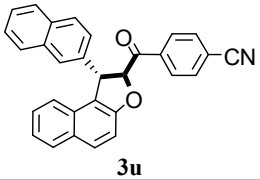
Table 2: Synthesis of (aryl)(1-(aryl/alkyl-1,2-dihydronaphtho-[2,1-*b*]furan-2-yl) methanone (**3a-u**).



1a-o **2a-g** **3a-u**

Sl. No.	R^1	R^2	R^3	Product	Time (h)	Yield (%)
1	H	H	Me	 **3a**	0.75	68
2	H	H	4-MeC_6H_4	 **3b**	3	80

				 **3c**		
4	H	H	4-FC_6H_4	 **3d**	5	86
5	H	H	2-furyl	 **3e**	6	75
6	H	Br	C_6H_5	 **3f**	5	71
7	Me	H	C_6H_5	 **3g**	5	85
8	Pr	H	C_6H_5	 **3h**	3	70
9	Cyclohexyl	H	C_6H_5	 **3i**	5	69
10	C_6H_5	H	C_6H_5	 **3j**	5	70
11	4-MeC_6H_4	H	C_6H_5	 **3k**	2	75
12	4-$MeOC_6H_4$	H	C_6H_5	 **3l**	4	79

				 **3m**		
14	4-HOC_6H_4	H	C_6H_5	 **3n**	4	71
15	4-ClC_6H_4	H	C_6H_5	 **3o**	4	81
16	4-BrC_6H_4	H	C_6H_5	 **3p**	3	79
17	4-CN-C_6H_4	H	C_6H_5	 **3q**	6	85
18	2-naphthyl	H	C_6H_5	 **3r**	7	63
19	C_6H_5	Br	C_6H_5	 **3s**	3	78
20	C_6H_5	Br	2-furyl	 **3t**	4	75
21	2-naphthyl	H	4-CN-C_6H_4	 **3u**	12	41

Biology

Compound 3b showed selective cytotoxicity towards the cancer cells

Primarily, **21** synthesized compounds along with Etoposide, a well known anticancer agent, were assessed for their ability to block proliferation of human triple negative MDA-MB-468 [(ER-, PR- and HER2-) breast cancer cells], MCF-7 [human (ER+, PR+ and HER2-) breast cancer cells] and non-cancerous WI-38 cells (lung fibroblast cell) using MTT experiments.[21] For this purpose, cells were treated with varying concentrations of the compounds (0-50 μM) and cell proliferation was measured. The anti-proliferative activities of the tested compounds in terms of LD_{50} were presented in Table 3. From the MTT assay it was found that three ntly higher efficacy in blocking proliferation of MDA-MB-468 cells with LD_{50} values of (15 ± 3.1, 18 ± 1.2, 18 ± 2.4 μM respectively). Similar anti-proliferative efficacy of Compounds **3b, 3c** and **3s** was also found in the case of MCF-7 cells with LD_{50} values of (17 ±2.65, 21 ± 3.9 and 19 ±2.2 μM respectively). Subsequently, when the selectivity index (SI) compounds were calculated by comparing the cytotoxic LD_{50} value of the compound in normal non-cancerous lung fibroblast cells (WI-38) versus cancer cells (MDA-MB-468 and MCF-7) it was observed that compound **3b** possessed higher SI (SI = 3.87±0.058 and 3.11 ±0.062) as compared to compound **3c** (SI = 3.11±0.062 and 2.66 ±0.075) and compound **3s** (SI = 2.89±0.076 and 2.74±0.027) (Figure 3). Therefore, amongst the 21 screened compounds, compound **3b** showed significant anti-cancer activity based on its highest selectivity index.

Table 3: The anti-proliferative activities of the synthesized compounds and standard anticancer agent expressed in terms of LD_{50} (μM).

Sl. No.	Compound	MDA-MB-468	MCF-7	WI-38	S.I. (WI-38/ MDA-MB 468)	S.I. (WI-38/ MCF-7)
1	**3a**	22 ± 1.7	26± 2.5	47± 2.8	2.13± 0.056	1.8± 0.056
2	**3b**	**15 ± 3.1**	**17± 2.65**	**58 ± 1.7**	**3.87± 0.058**	**3.41± 0.054**
3	**3c**	**18± 1.2**	**21± 3.9**	**56± 0.59**	**3.11± 0.062**	**2.66± 0.075**
4	**3d**	27 ± 2.5	29± 2.9	54± 2.37	2.00± 0.056	1.86± 0.045
5	**3e**	34 ±1.9	37± 1.16	51 ± 1.7	1.5± 0.026	1.38± 0.066
6	**3f**	32±1.87	54 ± 2.17	73± 1.23	2.28± 0.036	1.35± 0.067
7	**3g**	34 ± 1.9	38 ± 2.36	64 ± 1.7	1.88± 0.051	1.68± 0.084
8	**3h**	69 ± 1.87	74 ± 1.27	79 ± 2.13	1.14± 0.048	1.07± 0.044
9	**3i**	46± 3.4	42 ± 1.14	74 ± 2.9	1.6± 0.036	1.76± 0.064
10	**3j**	72 ± 1.7	68 ± 1.8	89 ± 2.8	1.24± 0.026	1.30± 0.071
11	**3k**	63± 2.3	71 ± 1.6	72 ± 0.8	1.14± 0.032	1.01± 0.036
12	**3l**	118 ± 1.8	84 ± 2.9	83± 1.4	0.70± 0.023	0.98± 0.028
13	**3m**	88 ± 3.1	86 ± 1.9	128± 0.93	1.45± 0.036	1.49± 0.068
14	**3n**	81 ± 2.4	77 ± 2.1	115 ± 1.9	1.42± 0.054	1.49± 0.059
15	**3o**	87± 2.5	9 1± 2.4	96 ± 3.27	1.1± 0.087	1.05± 0.076
16	**3p**	63 ± 3.2	56 ± 3.2	77 ± 0.59	1.22± 0.032	1.37± 0.068
17	**3q**	53 ± 2.3	51 ± 2.2	72 ± 0.7	1.35± 0.045	1.41± 0.043
18	**3r**	23± 2.3	28 ± 2.6	59 ± 0.8	2.56± 0.086	2.10± 0.032
19	**3s**	**18 ± 2.4**	**19± 2.2**	**52 ± 0.68**	**2.89± 0.076**	**2.74± 0.027**

21	**3u**	54 ± 1.8	57± 3.9	88 ± 1.4	1.63± 0.076	1.54± 0.027
22	**Etoposide**	27.88± 3.62	45.31± 2.41	29.63 ± 2.19	1.06± 0.046	0.65± 0.066

In order to evaluate the cytotoxic effects of the active compounds (**3b, 3c** and **3s**) against other triple negative breast cancer cell lines, a comparative MTT assay was also performed using the active compounds against MDA-MB-231 (Human) and 4T1 (Murine) cells. Compounds **3b, 3c** and **3s** showed similar trends in cytotoxicity against other cell lines such as MDA-MB 231, 4T1 and as presented in Table 4.

Table 4: The anti-proliferative activities of compounds **3b, 3c** and **3s** against MDA-MB-231 (Human), 4T1 (Murine) and WI-38 cells expressed in terms of LD_{50} (µM).

Compd.	MDA-MB 231	4T1	WI-38	SI(WI-38/ MDA-MB 231)	SI(WI-38/ 4T1)
3b	19±2.1	21±1.9	58 ± 1.7	3.05±0.037	2.76±0.051
3c	23±1.6	25±2.4	56± 0.59	2.43±0.058	2.24±0.068
3s	22±1.8	24±1.7	52 ± 0.68	2.36±0.054	2.16±0.048

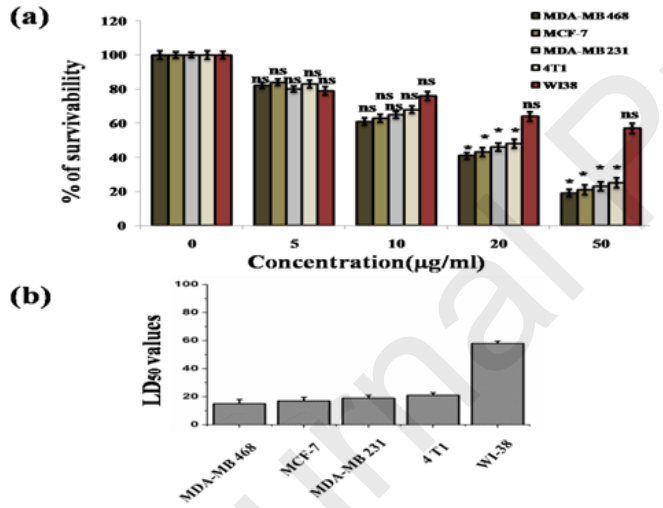


Figure 3: (a) Assessment of cytotoxicity of compound **3b** on triple negative breast cancer cell lines, MDA-MB 468, MDA-MB-231, 4T1, MCF-7 and non-cancerous cell line WI-38 by MTT assay which shows dose dependent decrease in cell survivability in cells treated with compound **3b**, (b) LD_{50} values of compound **3b** in MDA-MB 468, MDA-MB-231, 4T1, MCF-7 and WI38 cells.

In order to study the mechanism of action of the cytotoxicity against the cancer cells of the most promising compound **3b** based on the selectivity index (SI= 3.87±0.058 and 3.41 ±0.054), the induction of apoptosis was measured using Annexin V FITC/PI staining.[22] After treatment of MDA-MB-468 and MCF-7 cells with different concentrations of compound **3b** (0, 10, 20 and 30 µM) for 24 h, the flow cytometry analysis showed significant induction of apoptosis in a concentration dependent manner in the cancer cells in comparison with the non-cancer cells (WI-38). Quite evidently, compound **3b** was able to induce nearly 69% apoptosis in MDA-MB-468 and 61% in MCF-7 cells at 30 µM dose as shown in Figure 4. From the AnnexinV-FITC/PI staining on the cancer cells (MDA MB-468 and MCF-7) and non-cancer cell lines (WI-38), it was observed that there was no substantial apoptosis took place in case of the non-cancer cells treated with compound **3b** while in case of the cancer cells the percentage of apoptotic cell is significantly high. Therefore, it was decided to identify the mechanism of the induction of apoptosis in cancer cells by compound **3b** employing a series of biochemical and microscopic analysis.

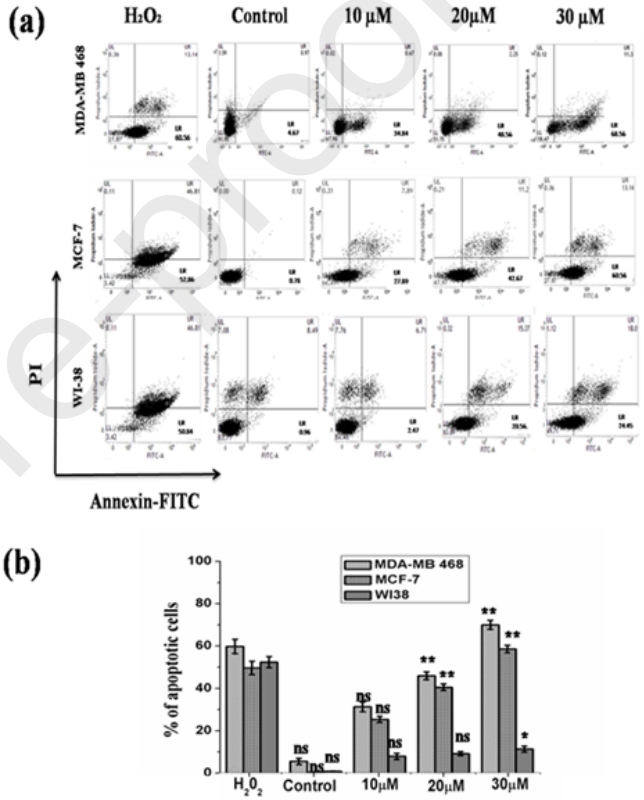


Figure 4: (a) Dot plot of Annexin V-FITC/PI for evaluation of apoptosis in MDA-MB 468, MCF-7 and WI-38 cells treated with the compound **3b**; (b) Quantification of apoptotic cells for evaluation of apoptosis treated with compound **3b** at doses 10 µM, 20 µM and 30 µM.

Treatment of compound 3b promotes ROS generation, mitochondrial permeability transition (MPT), DNA damage and apoptosis in cancer cells.

Generation of reactive oxygen species (ROS) is a critical phenomenon linked with several anti-proliferative processes.[23] The ROS production in cancer cells in response to the treatment with compound **3b** has been assessed using the DCFDA method.[24] Briefly, cells were treated with varying doses (0, 10, 20 and 30 µM) of compound **3b** for 12 h followed by 30 min of DCFDA (100 µM) staining and microscopy. The photomicrographs demonstrated concentration dependent increase in the intensity of the green colour in response to the treatment with compound **3b** suggesting significant induction of ROS generation in MDA-MB-468 and MCF-7 cells as shown in Figure 5. Pre-treatment with 10 mM of cellular ROS scavenger, *N*-acetyl cysteine (NAC)

sign by staining the cells with MitoSOX™ Red reagent.[27] The high red fluorescence due to the presence of oxidized products indicates the induction of mitochondrial ROS generation.[28] In the present study an enhanced high red fluorescence was observed in case of cancer cells treated with different concentrations of compound **3b** (10 µM, 20 µM and 30 µM) (Figure 7). Hence it can be concluded that compound **3b** mediated cancer cell apoptosis took place due to increased mitochondrial ROS production.

MCF-7 cells treated with the 30 µM of Compound **3b** as seen in Figure 5a. The WI-38 cells also exhibits no such significant enhancement in ROS production when they were treated with the doses of 10, 20 and 30µM as shown in Figure 1 in supporting information. Therefore, it can be confirmed that intracellular ROS was generated in presence of the compound **3b** selectively in the cancer cells.

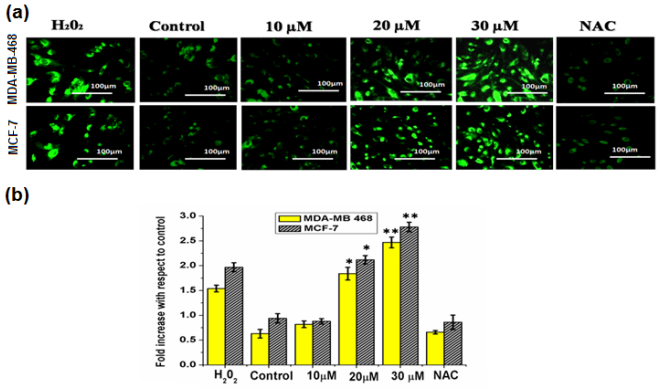


Figure 5: Determination of cellular ROS with DCFDA method. (a) Fluorescence microscopic image of treated cells and (b) Spectrophotometric fluorescence intensity measurement which indicates the enhancement of ROS in cells treated with the compound **3b** at doses 10 µM, 20 µM and 30 µM. NAC is used as a ROS inhibitor. The data is the average of three experiments ±SD. * represents P value < 0.05, ** represents P value < 0.01.

Since ROS plays critical role in changing the mitochondrial permeability transition (MPT),[25] the effect of compound **3b** on mitochondrial damage in MDA-MB-468 and MCF-7 cells was investigated by JC-1 staining.[26] A drastic alteration of the redox status of cellular mitochondria in response to the treatment of compound **3b** was observed in a concentration and time dependent manner (Figure 6). However, the WI-38 cells treated with compound **3b** at the same doses did not exhibit any significant transition in mitochondrial membrane potential as seen in Figure 2 in supporting information. The shift of fluorescence from red to green or a decrease in the red/green ratio indicated the increase in the mitochondrial permeability in response to compound **3b** only in cancer cells. These results altogether implies that compound **3b** mediated generation of ROS may have decisive role in the mitochondrial permeability transition in cancer cells.

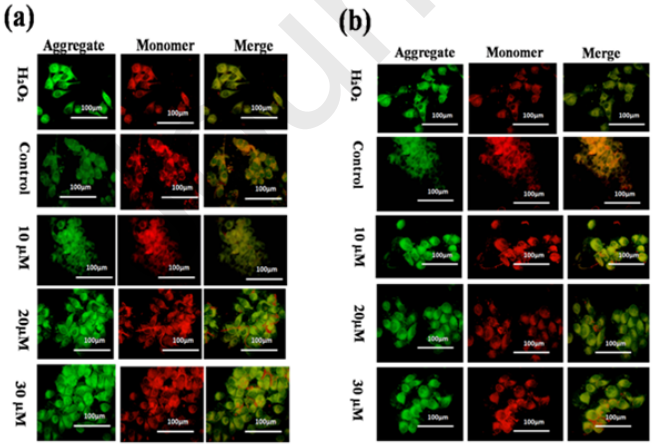


Figure 6: Mitochondrial membrane potential measurement by JC1 on (a) MDA-MB-468 and (b) MCF-7, which clearly indicates the changes in the mitochondrial membrane potential transition in cancer cells treated with the compound **3b** at doses 10 µM, 20 µM and 30 µM.

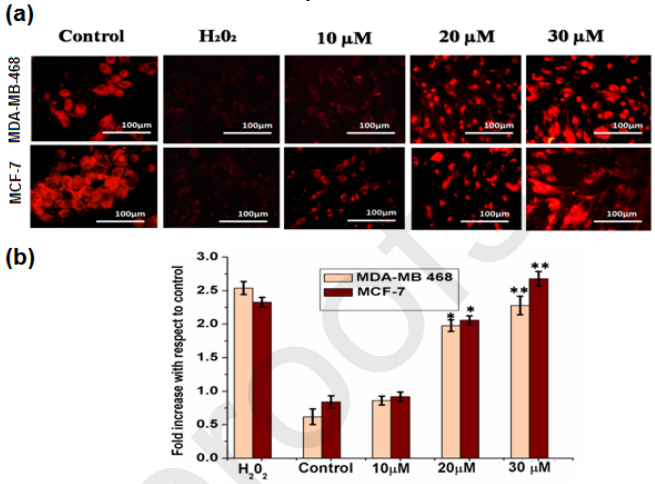


Figure 7: (a) Mitochondrial ROS determination by Mitosox™ Red on MDA-MB-468 and MCF-7 cells which clearly indicates the generation of mitochondrial ROS in cells treated with compound **3b** at doses 10 µM, 20 µM and 30µM; (b) Spectroscopic fluorescence intensity of the induced mitochondrial ROS in MDA-MB- 468 and MCF-7 cells. The data is the average of three experiments ± SD. * represents P value < 0.05, ** represents P value < 0.01.

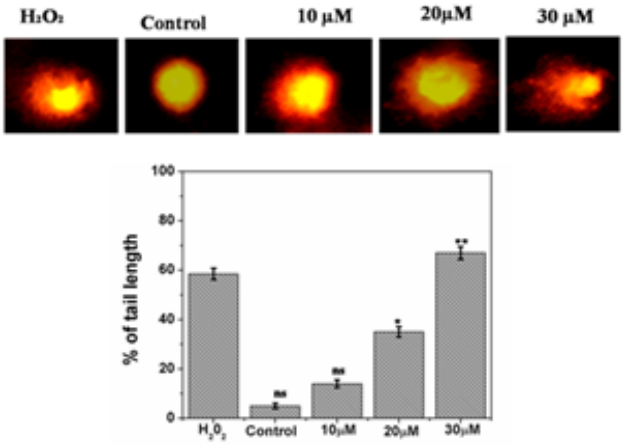


Figure 8: Comet assay of MDA-MB-468 cells following treatment with compound **3b** at the doses of 10 µM, 20 µM and 30 µM.

In order to accumulate more evidences to support cancer cell apoptosis mediated by the treatment of compound **3b**, damage of DNA in MDA-MB-468 cancer cells in response to the treatment with compound **3b** was assessed using Comet Assay.[29] Comet assay (also known as Single Cell Gel Electrophoresis Assay) is a facile method for measuring DNA strand breaks in eukaryotic cells.[30] The term "comet" refers to the pattern of DNA migration through the electrophoresis gel, which often resembles a comet. It was found that the fluorescence was confined mostly to the nucleus in control cells because of the undamaged DNA, whereas negatively charged DNA fragments were released from the nucleus of MDA-MB-468 cells treated with compound **3b** due to DNA damage and migrate toward the anode leading to a prolonged tail formation. It was observed that tail length upon treatment with

com ...osis, 32 ± 3 nm and 63 ± 2 nm respectively. This increase of tail length is an indication of DNA damage in cancer cells in comparison to the untreated cells which had no or negligible tail length (Figure 8).

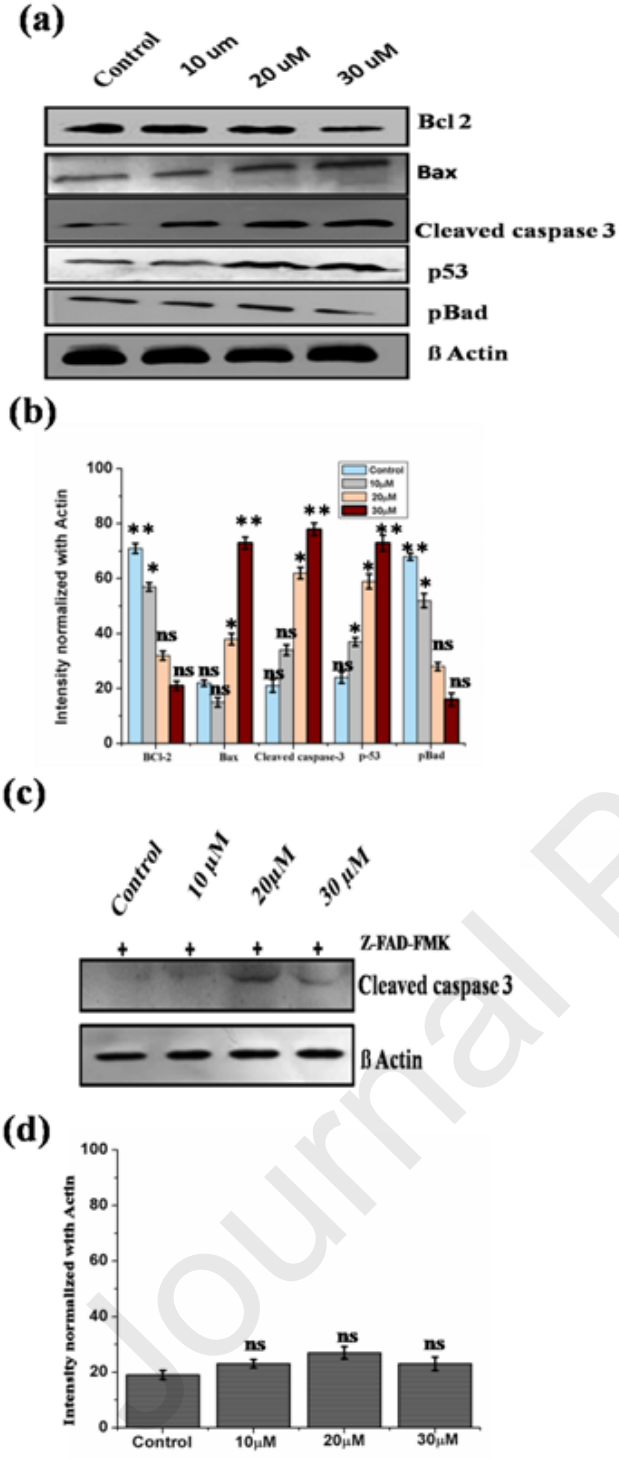


Figure 9: (a) Western blot analysis of apoptotic proteins like Bcl2, Bax, cleaved caspase-3, p53, pBad suggesting up-regulation of apoptic pathway upon treatment of the MDA-MB 468 cells with compound **3b** at doses 10 µM, 20 µM and 30 µM; (b) Quantification of the apoptotic protein expression; (c) Western blot of MDA-MB 468 cells exposed to Caspase inhibitor Z-FAD-FMK and then estimating its cleaved caspase-3 expression with Compound **3b** at doses 10µM, 20 µM and 30 µM; (d) Quantification data of cleaved caspase-3.

It is well established that generation of ROS and change in MPT are linked with apoptosis which is a process that controls cellular expression of different pro-apoptotic and anti-apoptotic proteins were carried out using western blot analysis.[32] The data showed increase in the expression of pro-apoptotic protein Bax, whereas the expression of anti-apoptotic protein Bcl-2 and pBad was decreased significantly in MDA-MB-468 cells following treatment with compound **3b**. The expression of p53 in MDA-MB-468 cells was also enhanced upon treatment with the compound **3b**. Moreover, upon treatment with compound **3b** showed cleavage mediated activation of caspase-3 and thereby suggesting caspase mediated induction of apoptosis.[33] Altogether these results suggested that there was a significant change occurred in Bax/Bcl-2 ratio and the expression of p53, pBad, caspase-3 in MDA-MB-468 cancer cells due to the treatment with compound **3b**, which play critical roles in apoptosis (Figure 9). It was observed that a general caspase-3 inhibitor Z-VAD-FMK was able to diminish the expression of the cleaved caspase-3 which confirmed the cell death was caspase dependent. However the WI38 cells that were treated with a dose of 10, 20 and 30 µM of compound **3b** did not show any significant enhanced expression of cleaved caspase-3 protein (as shown in Figure-3 in supporting information), which suggested that compound **3b** was selectively effective against the cancer cells only.

Molecular docking study of three selective compounds

In order to elucidate the structure activity relationship (SAR) between the biological activity of compound **3b** and its binding affinity towards the active domain of caspase-3 enzyme, molecular docking experiment was carried out.[34,35] It is clear that hydrogen bonding interactions with the binding pocket of caspase-3 enzyme play crucial roles for its caspase-3 inhibitory activity (Figure 10a). Nevertheless, Gibbs free energy (ΔG) indicates that π-π stacking and CH-π hydrophobic interactions with surrounding amino acids (i.e. Tyr 204, Arg 207, Trp 206, Gly 122 and Thr 166) of the binding site are also crucial to improve the biological activity (Table 5, Figure 10). The 3D docked conformation of compound **3b** showed that naphthafuran scaffold is important to increase their common π-π as well as CH-π stacking hydrophobic interactions. Also, the tetrahydrofuran group of naphthafuran scaffold provide a strong H-bond interaction with Cys 163 (compound **3b**, ΔG = −8.15 kcal/mol, IC_{50}: 15±3.10 for MDA-MB-468 and 17±2.65 for MCF-7). In addition, a carbonyl (C=O) linker in between napthafuran and 4-methyl phenyl group showed another H-bond interaction with Tyr 204 which was used to bind the molecule more tightly with the caspase-3 enzyme.[36] From 2D view, it can be concluded that naphthalene is a very important part of naphthafuran as it binds with Arg 207 closely which may induces its better potential activity against the enzyme (Figure 10b). Furthermore, Figure 10C revealed that compound **3b** is nicely superimposed with the indole-2,3-dione group of the co-crystal ligand. These superimposed structures give significant information to compare their anti-cancer activity. Therefore, it can be concluded that (a) targeting of Cys 163, Thr 166 and Arg 207 amino acids of caspase-3 enzyme's binding pocket are vital for anti cancer activity of compound **3b** and (b) naphthofuran moiety acts a central role to develop the potency of the molecule.[34,37,38]

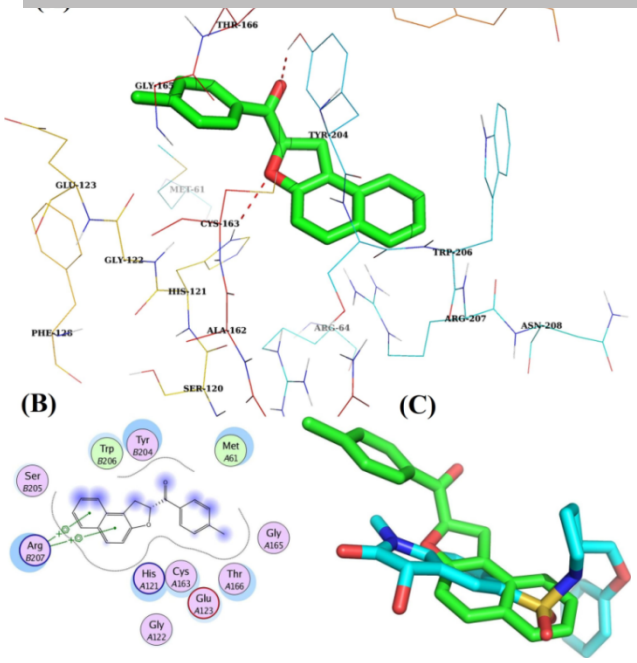


Figure 10: Molecular docking study of compound **3b** with caspase-3 enzyme co-crystal (PDB ID: 1GFW). (a) Docked conformation of compound **3b** (Green), into the binding pocket of caspase-3. Red dotted lines (---) indicate the H-bond interactions; (b) 2D diagram of protein binding interaction of compound **3b**; (c) The superimposed structure of co-crystal ligand (cyan) with compound **3b.**

Table 5: Binding interactions of compound **3b** (most active) with caspase-3 enzyme.

Compound	ΔG (-Kcal/mole)	H-bond interacting sites	Hydrophobic binding sites
3b	8.15	Cys 163 and Thr 166	Arg 207, Trp 207, Met 61, Ser 205, Gly 165, Thr 166, His 121, Cys 163, Gly 122
Reference (Ligand)	8.26	Gly 122, Cys 163 and Arg 207	Phe 256, Tyr 204, Trp 206, Arg 207, His 121, Ser 209

In summary, a series of (1,2-dihydronaphtho[2,1-b]furan-2-yl)methanone (**3a-u**) derivatives were synthesized and evaluated for their anti-proliferative activities against cancer cells (MDA-MB-468 and MCF-7) and normal cells (WI-38; human lung fibroblast). Three compounds (**3b**, **3c** and **3s**) showed significant cytotoxic effects on MDA-MB-468 and MCF-7 cancer cell lines as well as other triple negative cancer cells such as MDA-MB-231 and 4T1 cells. Based on the selectivity index, compound **3b** was considered as most promising anticancer agent. Further biochemical and microscopic studies established that compound **3b** mediated cytotoxicity appeared due to apoptotic pathways. QSAR docking studies have also been carried out to establish the structure-activity relationships of the active compounds with apoptotic proteins.

Acknowledgements

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Conflicts of interest

There are no conflicts to declare.

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Supplementary Material

Detailed experimental conditions for the synthesis of compounds and their analytical data. Copies of the NMR spectra of s

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An [illegible] derivatives

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Research highlights:

- 1,2-dihydronaptho[2,1-b]furan derivatives were synthesized.
- One-pot condition has been used to prepare compounds in high yield.
- Synthetic compounds were evaluated for their potential as anticancer agents.
- Three compounds showed promising cytotoxicity against human breast cancer.
- Biochemical and microscopic experiments were carried out using the best compound.