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Antiproliferative Activity of Diarylnaphthofurans through Microtubule Destabilization

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Abstract The present communication deals with pharmacophore designing based on "Fragment-Based Drug Discovery" approach. Two series of compounds were synthesized and evaluated against human cancer cell lines by sulforhodamine assay, target studies through tubulin kinetics, *in silico* predictions of binding pocket, and druggability and safety studies by acute oral toxicity in mice model. Out of 24 varied analogs, seven compounds exhibited significant antiproliferative activity against both hormone-dependent and hormone-independent breast cancer cell lines. Among these, two of the most active representative compounds **7d** and **13** showed potential antiproliferative activity against MDA-MB-231 with IC_{50} at 10.04 μM and 10.70 μM and significant anti-tubulin effect at their half IC_{50} and IC_{50}. In molecular docking studies, both the compounds occupied colchicine binding pocket at β-tubulin with high binding energies of –8.3 Kcal/mol and –7.9 Kcal/mol, respectively. Both the identified investigational leads were found to be safe and non-toxic in rodent model up to 1000 mg/kg oral dose. Optimization of these lead compounds may yield some better candidates in future.

KEYWORDS Antiproliferative, Fragment-based drug discovery, Molecular docking studies, Acute oral toxicity.

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INTRODUCTION

Breast cancer is the most common cancer diagnosed in women. It is a multifactorial disease with diverse morphology and complications. Early, accurate, and affordable diagnosis is one of the major problems associated with the disease.[1] In general, breast cancer is defined with three biomarkers, estrogen receptor (ER), progesterone receptor (PR), and human oncogene human epidermal growth factor receptor 2. Hormone-dependent breast cancer is the most abundant type breast cancer which covers about 65% of total breast cancer cases. Estrogen antagonists such as tamoxifen and raloxifen and aromatase inhibitors such as anastrozole, exemestane, and fulvestrant as ER downregulator are used to tackle such type of breast cancer.[2] On an average, the hormone responsive breast cancer can be managed more than 15–20 years. On the other

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hand, triple-negative breast cancer (TNBC) (ER –ve, PR –ve, and HER-2 –ve) is very aggressive type cancer which occurs in about 15–20% of all breast cancer cases. TNBC is fatal and considered difficult to treat. Various types of cytotoxic drugs are used to tackle this type of breast cancer with meager success only. Some of the notable drugs are paclitaxel and docetaxel (antimitotic), doxorubicin (topoisomerase-II inhibitor), cisplatin (DNA intercalation), 5-fluorouracil (antimetabolite), abemaciclib (cyclin-dependent kinases inhibitor), olaparib (Poly-ADP ribose polymerase inhibitor), and gefitinib (EGFR inhibitor), etc. [**Figure 1**]. However, TNBC is very difficult to treat due to lack of prognostic biomarkers.[3] There is no systematic and accurate treatment available for this type of breast cancer cases. However, clinicians are using mixed targeted therapies to tackle it.[4]

Fragment-based drug discovery (FBDD) is a systematic and relatively a new approach to design pharmacophores in drug discovery. This approach identifies a small fragment (or motif) in a pharmacophore to have quality interactions with the biological target. It is well adopted by pharmaceutical industries and conferred some early successful drug candidates.[5]

We applied FBDD approach in designing the pharmacophores. A small fragment, that is, 3,4,5-trimethoxyphenyl has been established as an anti-tubulin motif, which is also present in some of the natural anti-tubulins such as colchicine, podophyllotoxin, and combretastatin A4.[6] Benzofurans and naphthofurans are important biodynamic agents exhibiting diverse pharmacological activities.[7a-e-9] To have a combretastatin A4 type stilbene arrangement, we planned to select both of these as basic cores and placed 3,4,5-trimethoxyphenyl fragment at C3 position in pharmacophores I (1,2-diarylnaphtho-[2,1b] furan) and II (2,3-diaryl-naphtho [2,3b]furan-4,9-dione) on naphthofuran core **[Figure 2]**.[10] Twenty-two diverse new naphthofuran derivatives were prepared and evaluated for antiproliferative activity against several human cancer cell lines. Two of the analogs exhibiting potent cytotoxicity were further undertaken for target studies. Finally, both these potential leads were evaluated for safety studies in rodent model.

RESULTS

Chemistry

Synthetic strategy for pharmacophore-I-based compounds is depicted in Scheme 1. While Scheme 2

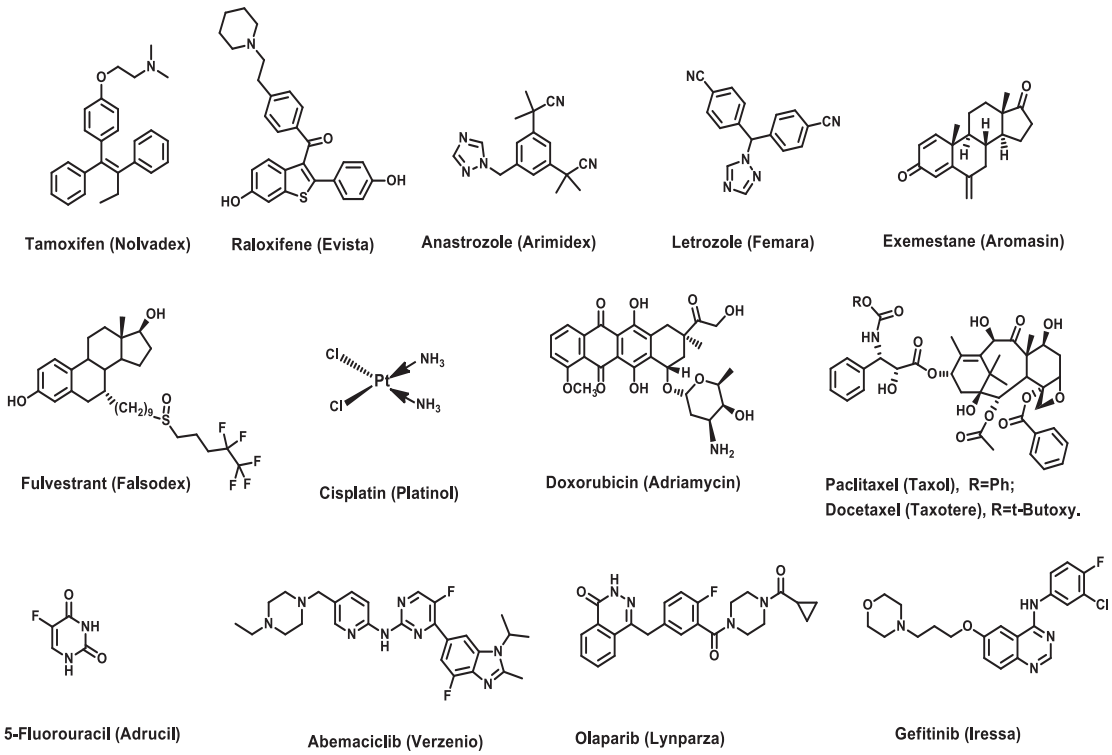


Figure 1: Some of the anti-breast cancer drugs (generic names in parentheses) in clinics

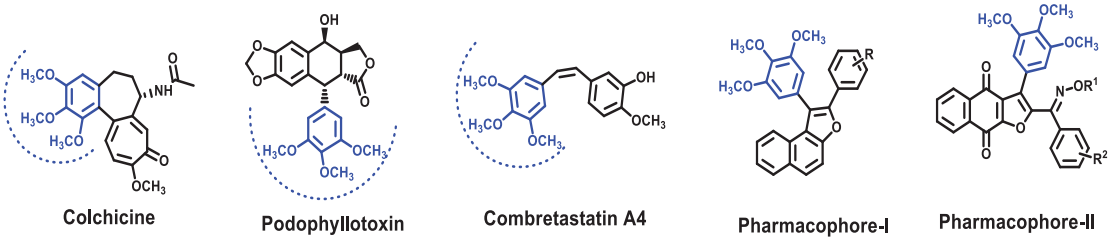


Figure 2: Natural tubulin polymerization inhibitors, fragment, and pharmacophores I and II

shows synthesis of compounds as per pharmacophore-II. In Scheme 1, 3,4,5-trimethoxyacetophenone (**1**) was taken as starting substrate which on treatment with bromine in diethyl ether underwent α-bromination to afford corresponding bromoacetophenone **2**. Bromoacetophenone **2** was hooked up with 2-naphthol in the presence of potassium carbonate in DMF to get 2-naphthyloxyacetophenone **3** in quantitative yield which on treatment with trifluoroacetic acid was cyclized to 2-arylnaphthofuran derivative **4**.

1,2-Diaryl naphthofurans (Scheme 1, Series **5, 6a** *and* **6b***)*

Naphthofuran derivative **4** was modified to three different series of compounds, that is, **6a**, **6b**, **7a-g**, and **8a-e**. Naphthofuran **4** underwent Heck coupling reaction to get 2,3-diarylnaphthofuran 5. Ester 5 was hydrolyzed to corresponding phenolic derivative **6a** on saponification. Phenolic derivative **6a** was methylated with dimethyl sulfate to get corresponding 4-methoxy derivative **6b**. **6a** was further benzylated with benzyl bromide in anhydrous potassium carbonate-acetone system to get corresponding benzyl derivative **6c**.

1,2-Diaryl naphthofurans (Scheme 1, Series **7a-7g***)*

Naphthofuran derivative **4** underwent palladium catalyzed coupling reaction with aryl halides to get 1,2-diaryl naphthofurans **7a-7g** in excellent yields.

1,2-Diaryl naphthofurans (Scheme 1, Series **8a-8e***)*

Similarly, naphthofuran derivative **4** underwent palladium catalyzed coupling reaction with aroyl halides to afford 1-aroyl-2-aryl naphthofurans **8a-8e** in excellent yields.

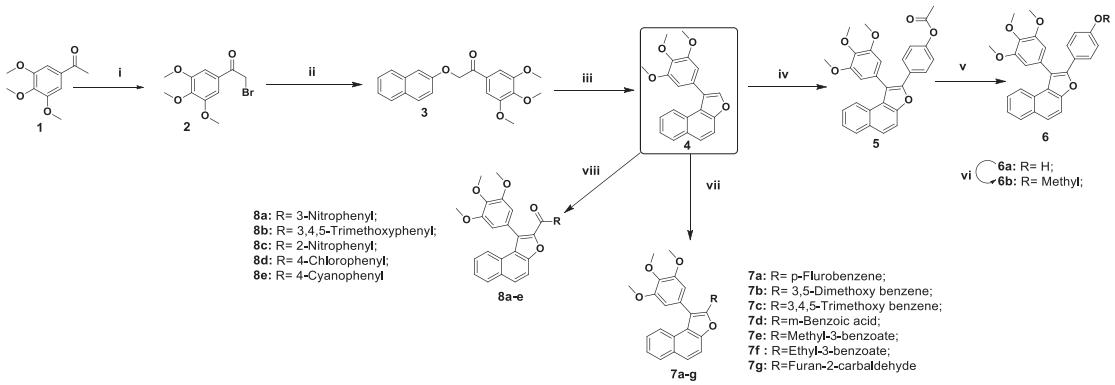
Reagents and conditions: (i) Br_2, diethyl ether, RT, 2h, 88%; (ii) 2-Naphthol, anh. K_2CO_3, DMF, RT, 4h, 95%; (iii) trifluoroacetic acid, RT, 5h, 90%; (iv) p-Acetoxybromobenzene, dimethylacetamide (DMA), $Pd(OAc)_2$, 80°C, 4 h, 92%; (v) 5% KOH, methanol/H_2O (9:1), 80°C, 30 min, 98%; (vi) Me_2SO_4, anh. K_2CO_3, dry acetone, reflux, 2h, 92%; (vii) aryl halide, DMA, $Pd(OAc)_2$, KOAc, 80°C, 4h, 69–91%; (viii) benzoyl halide, DMA, $Pd(OAc)_2$, KOAc, 80°C, 4 h, 84–89%.

2,3-Diaryl-naphtho[2,3b]furan-4,9-dione (Scheme 2, Series **14a-14g***)*

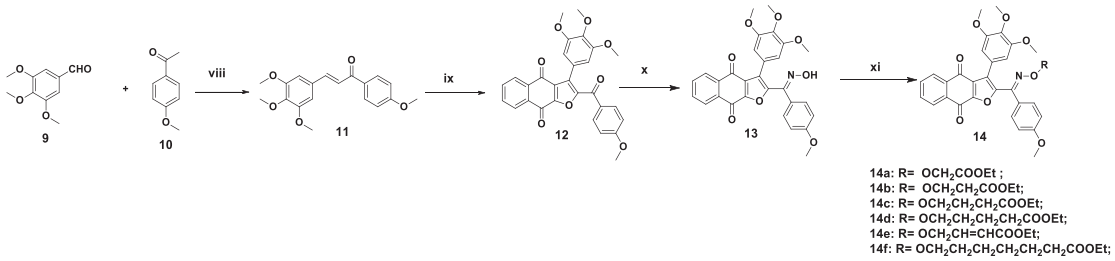
Scheme 2 represents the synthetic strategy adopted for the preparation of compounds **14a-14g**. 3,4,5-Trimethoxybenzaldehyde (**9**) was condensed with p-methoxyacetophenone (**10**) in 3% alcoholic alkali to get chalcone derivative **11** in excellent yield. On treatment with N-iodosuccinimide (NIS) in acetonitrile and DABCO base, chalcone **11** underwent Michael addition followed by *in situ* oxidative cyclization to afford 2,3-diarylnaphtho [2,3b] furan-4,9-dione moiety as compound **12**. The keto group of **12** was treated with hydroxylamine to get corresponding ketoxime **13**. Several fatty acid ester chains were hooked-up at the hydroxyl group of ketoxime **13** to yield final naphthofuran oxime esters **14a-14g**. All the intermediates and final compounds were confirmed by spectroscopy.

Reagents and conditions: (viii) 3% NaOH, methanol, 70°C, RT, 10h, 94%; (ix) Lawsone, NIS, DABCO, acetonitrile, sealed tube, 100°C, 18h, 87%; (x) hydroxylammonium HCl, pyridine, RT, 3h, 90%; (xi) NaH, dry THF, ethyl bromoester, reflux, 2–4 h, 62–73%.

Both the series of compounds were evaluated against two human breast cancer cell lines MCF-7 and MDA-MB-231 by sulforhodamine assay. Out of these, seven compounds exhibited significant antiproliferative activity, that is, IC_{50}<20 μM [**Table 1**]. Rest of compounds exhibited higher



Scheme 1



Scheme 2

IC_{50}, that is, low antiproliferative activity (**Tables S1-S4**, Supplementary information). Further, all these compounds were also evaluated against normal cell line HEK-293T to assess the toxicity of the compounds. Compounds did not show any toxicity up to 50 μM concentration.

Structure–activity relationship

Among both the series of compounds of pharmacophore-I and pharmacophore-II, Series-1 compounds possessed better cytotoxicity as compared to Series-II compounds. However, both series of compounds exhibited better activity against MDA-MB-231 (10–17 μM) as compared to MCF-7 (15.13–20 μM) cell line. Series I compounds exhibited inhibition of both the breast cancer cell lines but Series II compounds were only effective against MDA-MB-231 cell line. In Series-I, only four compounds showed significant activity, that is, 2,3-diarylnapthofuran derivatives (**7c** and **7d**) and 2-aroyl-3arylnaphthofurans (**8a** and **8e**). Compound **7c** possessing 3,4,5-trimethoxyphenyl unit (IC_{50}, 15.5 μM and 16.2 μM) and compound **7d** possessing 3-carboxyphenyl unit (IC_{50}, 15.1 μM and 10 μM) at C2 position showed significant activities. While, other two compounds **8a** with 3-nitrobenzoyl unit and **8c** as 4-cyanobenzoyl unit possessed cytotoxicity as IC_{50} at 20uM & 12uM and 18uM & 9.9uM respectively. No further trend could be established with varied substitutions in this series. In both the series, benzoyl group at 2-position exhibited poor cytotoxicity against MCF-7 cell line (compounds **8a**, **8e**, **12**).

In Series-II, benzoyl group at 2-position exhibited poor cytotoxicity (compound **12**, IC_{50}>20 μM) against both the breast cancer cell lines, but its corresponding oxime derivative **13** exhibited better activity against both the cell lines (IC_{50} at 17.2 μM and 10.7 μM). Further modification by a pendant chain of fatty acid ester lowered the cytotoxicity (compounds **14a-14f**, IC_{50}>20 μM) of the series except **14e** (IC_{50}=17 μM) which might be due to conjugated crotonate ester chain in **14e**.

Tubulin polymerization inhibition

Tubulin kinetics provides conversion of tubulins to polymer unit microtubules. The rate of polymerization is either stabilized or destabilized by antimitotic agents. Both the lead compounds **7d** and **13** destabilized the polymerization process which is evident from the tubulin kinetic curves. In **Figure 3a**, the curves of paclitaxel (PAC) are above the control curves (GTB and DMSO) showing stabilization effect. While podophyllotoxin (PDT) curves are below the control curves showing destabilization effect on microtubules. Compound **7d** curves also exist below the control curves, clearly indicating destabilization effect but less than PDT. Similarly, in **Figure 3b**, compound **13** induced curves are below the control curves, showing destabilization effects. Thus, both the naphthofuran derivatives exhibited tubulin polymerization inhibition. Modulation of tubulin-microtubules dynamics is one of the most effective targets for cancer chemotherapeutics.

Molecular docking studies

Molecular docking is a computational approach to know the mechanistic insights of protein and ligand interaction. The interaction of the both the naphthofuran derivatives **7d** and **13** was assessed with target protein, that is, β-tubulin to know the binding pocket. Further, absorption, distribution, metabolism, and excretion (ADME) properties were also predicted to assess their drug-like properties.

Interaction with β-tubulin

Analysis of docking results against target 4O2B revealed that both naphthofuran derivatives, namely, **7d** and **13** showed good binding affinity [**Table 2**]. Derivatives **7d** showed better binding affinity of −8.3 kcal/mol than derivative **13** with binding energy of −7.9 kcal/mol. However, the redocking study of colchicine, a known β-tubulin inhibitor, displayed highest binding affinity of −8.5 kcal/mol [**Table 2**]. Further, the ligand-receptor interaction analysis [**Figure 4**] indicated that both naphthofuran derivatives bind well at inhibitor binding site of β-tubulin. Several residues, namely, ALA316, LYS254, LEU255, ALA250, LEU248, ASP251, LYS352, and ASN258 were conserved within the binding pocket (4Å). Furthermore, the interaction results showed in **Table 2**, indicated that the derivatives **7d** form hydrogen bond with amino acid residue ASP251. While, **13**

Table 1: Antiproliferative activity of diaryl naphthofurans and diaryl naphthodifurandiones against human cancer cell lines

Sample code	Cytotoxicity IC_{50} (μM)			
	MCF-7	MDA-MB-231[a]	HEK-293T[b]	Selectivity index (SI)[#]
7c	15.54±0.1111	16.19±0.083	>50	>3.09
7d	15.13±0.1515	10.04±0.067	>50	>4.98
8a	>20	12.00±0.028	>50	>4.17
8e	18.05±0.1368	9.921±0.063	>50	>5.04
13	17.28±0.1011	10.70±0.027	>50	>4.67
14e	>20	17.06±0.056	>50	>2.93
Podophyllotoxin	3.5±0.6	35.73±11.80	50.0±0.22	1.40
Doxorubicin	2.585±0.1213	4.127±0.0739	>20	>4.84
Tamoxifen	10.74±0.2730	9.501±0.1163	>20	>2.11

#SI (calculated against MDA-MB-231)=a/b; *n*=2 independent experiments in triplicates

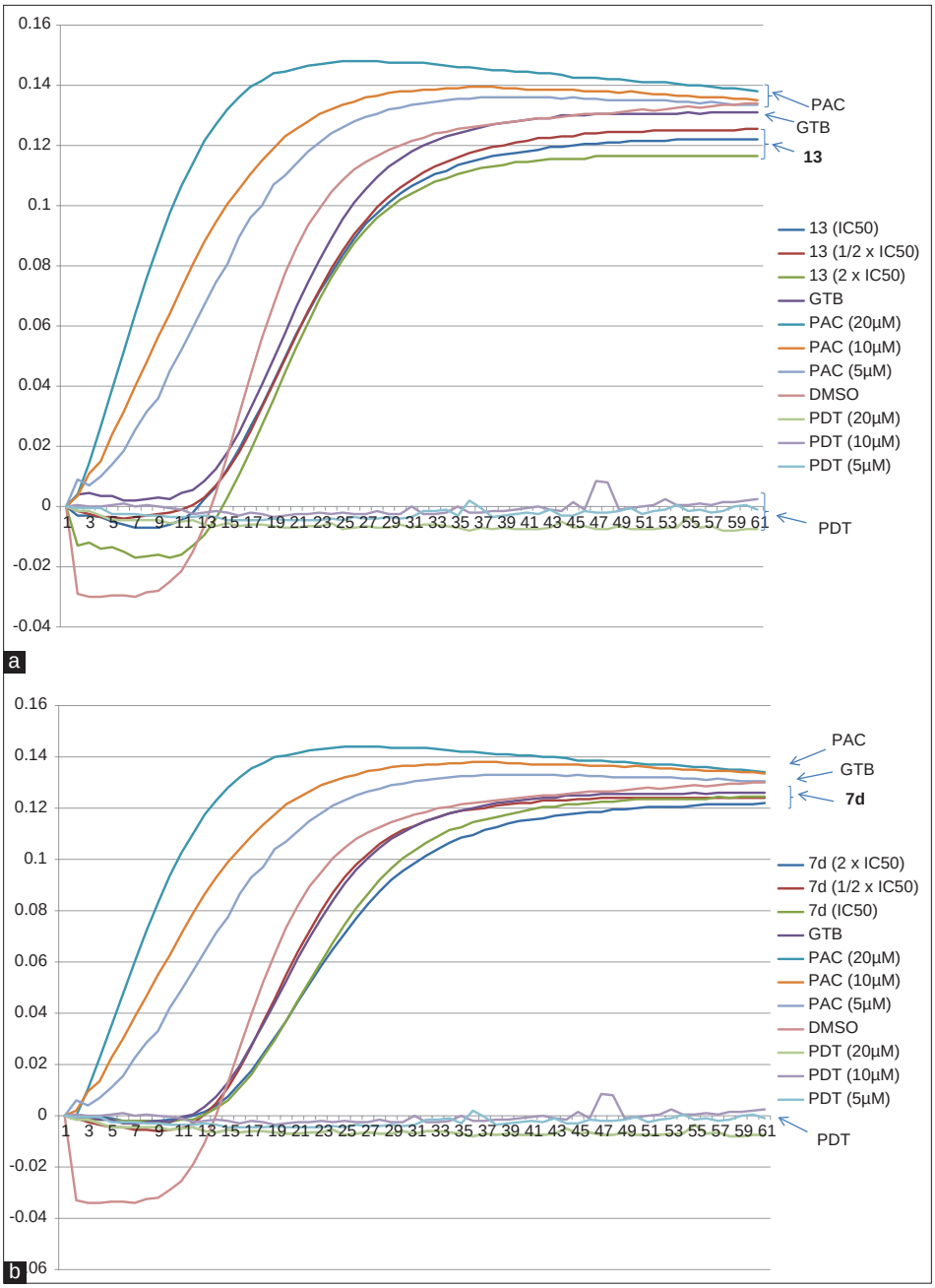


Figure 3: (a) Tubulin polymerization kinetic curve GTB and DMSO are control curves, PAC is paclitaxel (stabilizer), PDT (destabilizer), and diarylnaphthofuran derivative 7d at half IC_{50}, IC_{50}, and double IC_{50} (*n* = 1, duplicate). (b) Tubulin polymerization kinetic curve GTB and DMSO are control curves, PAC is paclitaxel (stabilizer), PDT (destabilizer), and diarylnaphthodione derivative 13 at half IC_{50}, IC_{50}, and double IC_{50} (*n* = 1, duplicate)

Table 2: Docking energy and residual amino acids within 4Å of colchicine (positive control) and naphthofuran derivatives 7d and 13 with the protein target β-tubulin (PDB: 4O2B)

Compound	Binding energy (kcal mol^{-1})	Binding pocket interacting amino acid (within 4 Å)	Key amino acid	H-bond length (Å)
Colchicine (positive control)	–8.5	LYS254, LEU255, ALA250, LEU248, ASP251, ILE378, LEU242, VAL238, CYS241, ILE318, ALA354, ALA316, LYS352, ALA317, VAL315, THR314, ASN350, MET259, ASN258	-	-
7d	–8.3	LYS254, LEU248, LEU255, ASP251, ALA316, ALA250, LYS352, MET259, THR314, ASN350, ASN258	ASP251	5.42
13	–7.9	ASN258, LYS254, ASN249, GLN247, THR353, LYS352, ALA316, ALA354, LEU248, CYS241, LEU255, ASP251, ALA250	ASN258	4.98
			LYS254	4.60
			GLN247	5.36

forms hydrogen bond with amino acid residues ASN258, LYS254, and GLN247. These interactions provide a strong affinity/binding toward the targeted anticancer β-tubulin.

In silico prediction of ADME properties

Physicochemical properties of a drug candidate give an idea about the druggability of the molecule. It is necessary for a drug candidate to reach to the site of action in sufficient concentration and interacts with the biological target to induce pharmacological effect.

Pharmacokinetic properties of both the naphthofuran derivatives **7d** and **13** were determined using online Swiss ADME software program. Important physicochemical was calculated. Both the compounds showed satisfactory bioavailability. Most of the required parameters were within the defined limits **[Table 3]**. Compound **7d** did not possess PAINS (Pan-assay interference compounds) structure while **13** showed one warning due to quinone type structure. However, both the lead compounds violated Lipinski's rule of five but within acceptable limit. Overall, with these physiochemical parameters, both the compounds possess low oral bioavailability and hence moderate druggability.

Safety studies

Both the identified lead compounds **7d** and **13** were further evaluated for safety studies through acute oral toxicity at four different oral doses, that is, 5 mg/kg, 50 mg/kg, 300 mg/kg, and 1000 mg/kg in Swiss albino mice. No morbidity and mortality and observational changes could be recorded during the experimental period. All the hematological (total hemoglobin level and differential leukocyte count), lipid (serum total cholesterol and triglycerides), kidney function (creatinine level), liver function (serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, and ALKP) activity, biochemical and pathological parameters studied showed non-significant changes **[Tables 4 and 5]**. Both the compounds **7d** and **13** were well tolerated by the experimental animals up to 1000mg/kg as single acute oral dose. Animals on gross pathological study showed no changes in any of the organs studied including their absolute and relative weights **[Figures 5-8]**. There were some elevated levels of alkaline phosphatase and cholesterol as minor issues. However, the difference in levels was statistically non-significant. However, for future development of both the compounds, these should further be assessed for subacute and chronic experiments to look for any adverse effect on all these parameters on repeated exposure.

DISCUSSION

Naphthofuran derivatives **7d** and **13** exhibited significant antiproliferative activity against human breast cancer cell lines. Further, both the naphthofuran derivatives exhibited moderate tubulin polymerization inhibition. In our previous study, we synthesized 3-arylnaphthofurans as cytotoxic agents.[8]

Both naphthofurans **7d** and **13** possessed moderate anti-tubulin effect as compared to standard destabilizer podophyllotoxin. Previously, Simoni *et al*. reported some benzofuran analogs of combretastatin A4 exhibiting potential cytotoxicity against several human cancer cell lines through microtubule destabilization.[9] The modulation of tubulin-microtubule dynamics is regarded as prime target for the development of cancer chemotherapeutics. Induction of interference in this dynamic equilibrium suppresses spindle dynamics which subsequently leads to cell death.[11]

In docking studies, both naphthofuran derivatives showed good affinity with β-tubulin that was comparable

Table 3: Various druggability parameters of naphthofuran derivatives 7d and 13

Parameter	7d	13	Acceptable range	Parameter	7d	13	Acceptable range
Physicochemical properties				Lipophilicity			
Molecular formulae	$C_{28}H_{22}O_6$	$C_{29}H_{23}NO_8$	-	Log $P_{o/w}$	5.18	4.27	≤5
M. Wt.	454.47	513.29	≤500	Pharmacokinetics			
Rotatable bonds	06	07	≤10	GI absorption	Low	Low	-
H-bond acceptors	6	9	≤10	BBB permeability	No	No	-
H-bond donors	1	1	≤5	P-gp substrate	Yes	No	-
Molar refractivity	131.03	137.29	40–130	CYP1A2 inhibition	No	No	-
C_{Sp3} hybridization fraction	0.11	0.14	Not less than 0.25	CYP2C9/19 inhibition	Yes	Yes	-
TPSA	78.13 Å^2	116.79 Å^2	20–130 Å^2	Druglikeness			
Water solubility				Lipinski rule	No violation	1 violation	Up to 1 violation
Water solubility	0.08 µg/mL	0.13 µg/mL	**7d**-low, **13**-moderate	Bioavailability score	0.56 good	0.55	moderate
Solubility class	Low	Moderate	acceptable	Medicinal chemistry			
Log S	–7.73	-6.61	>-6	PAINS (false bioactivity)	No	1, alert quinone	-
			Synthetic accessibility	3.96	4.47	1–10 easiest-most difficult	

Table 4: Effect of naphthofuran derivative 7d as single acute oral dose at 5, 50, 300, and 1000 mg/kg on body weight hematological and serum biochemical parameters in Swiss albino mice (Mean±SE; *n*=6)

Parameters	Dose of compound 7d at mg/kg body weight as a single oral dose				
	Control	5 mg/kg	50 mg/kg	300 mg/kg	1000 mg/kg
Body weight (g)	27.68±0.88	26.85±1.48	30.33±1.37	27.58±1.13	29.82±0.88
Hematological profile					
Hemoglobin (g/dL)	12.66±0.623	12.03±0.42	13.26±0.568	11.90±0.52	11.52±0.66
RBC (million/mm^3)	7.37±0.23	6.99±0.19	7.03±0.35	7.75±0.32	7.56±0.26
WBC (1000*/mm^3)	4.69±0.64	4.50±0.24	5.60±0.48	4.11±0.42	4.93±0.28
Liver function test					
Alkaline phosphatase (U/L)	170.63±15.68	152.43±5.45	151.32±15.92	172.15±15.62	176.17±8.92
Serum glutamic-oxaloacetic TRANSAMINASE (U/L)	25.54±1.45	31.24±3.14	27.08±2.06	26.59±2.85	24.02±1.72
Serum glutamic pyruvic transaminase (U/L)	11.19±0.58	16.22±2.67	16.12±1.07	13.77±2.03	13.57±1.05
Albumin (g/dL)	2.82±0.156	2.77±0.16	3.01±0.136	3.06±0.11	2.97±0.13
Bilirubin (mg/dL)	0.19±0.008	0.18±0.011	0.182±0.012	0.167±0.011	0.162±0.007
Serum protein mg/ml)	6.44±0.09	6.09±0.08	6.48±0.30	6.15±0.09	6.005±0.25
Lipid profile					
Triglycerides (mg/dL)	162.15±4.72	184.24±6.00	192.11±5.79	174.72±14.55	187.40±8.51
Cholesterol (mg/dL)	149.83±9.82	166.42±5.52	166.72±10.03	162.25±3.64	181.63±7.41
Kidney function test					
Creatinine (mg/dL)	0.33±0.03	0.36±0.09	0.31±0.05	0.33±0.02	0.35±.02

Table 5: Effect of naphthofuran derivative 13 as single acute oral dose at 5, 50, 300, and 1000 mg/kg on body weight hematological and serum biochemical parameters in Swiss albino mice (Mean±SE; *n*=6)

Parameters	Dose of compound 13 at mg/kg body weight as a single oral dose				
	Control	5 mg/kg	50 mg/kg	300 mg/kg	1000 mg/kg
Body weight (g)	29.67±1.42	28.92±1.19	31.02±1.54	29.40±0.86	28.85±1.21
Hematological parameters					
Hemoglobin (gm/dL)	12.90±0.34	14.15±0.45	14.20±0.51	13.20±1.02	13.39±0.66
RBC (million/mm^3)	7.13±0.51	6.90±0.77	7.86±0.53	8.43±0.40	8.31±0.58
WBC (1000*/mm^3)	4.77±0.36	4.96±0.38	4.63±0.49	4.14±0.33	5.37±0.47
Liver function test					
Alkaline phosphatase (U/L)	153.91±14.22	151.63±9.01	156.88±8.54	163.42±2.39	183.22±5.10
Serum glutamic-oxaloacetic transaminase (U/L)	35.11±3.71	31.87±2.51	32.73±2.12	34.86±1.97	35.58±1.58
Serum glutamic pyruvic transaminase (U/L)	25.94±1.60	24.21±2.24	24.91±3.15	25.77±2.87	26.50±1.81
Albumin (g/dL)	3.49±0.09	3.49±0.06	3.77±0.11	3.68±0.16	3.88±0.05
Serum protein (mg/ml)	3.31±0.21	3.37±0.08	3.6±0.11	3.17±0.09	3.20±0.06
Bilirubin (mg/dL)	0.21±0.03	0.221±0.02	0.206±0.07	0.301±0.04	0.250±0.04
Lipid profile					
Triglycerides (mg/dL)	131.16±7.37	149.39±9.41	134.85±6.63	126.91±8.50	133.79±7.10
Cholesterol (mg/dL)	99.70±5.48	106.99±1.81	113.22±6.92	111.31±3.05	116.48±2.73
Kidney function test					
Creatinine (mg/dL)	0.44±0.04	0.37±0.02	0.33±0.01	0.50±0.02	0.41±0.03

to colchicine. Both the compounds possessed hydrogen bonds, that is, ASP-251 in **7d** and LYS254, ASN249, and GLN247 with **13**. There exists π-π stalking between the aromatic rings of compounds **7d** with another residue LYS β:352. The common interacting amino acid residues revealed that both the ligands lie at exact binding position as colchicine within the β-tubulin. It was observed that ALA316, LYS254, LEU255, ALA250, LEU248, ASP251, LYS352, and ASN258 were conserved amino acid found within the binding pocket (4Å). Among these, interactions with β:316 are very crucial for the induction of anti-tubulin effects. In a study, it was apprised that both β:316 and β:318 interactions by a trimethoxyphenyl fragment are very crucial for microtubule destabilizers acting at colchicine binding pocket and β:316 is directly involved in binding with 3,4,5-trimethoxyphenyl fragment.[12]

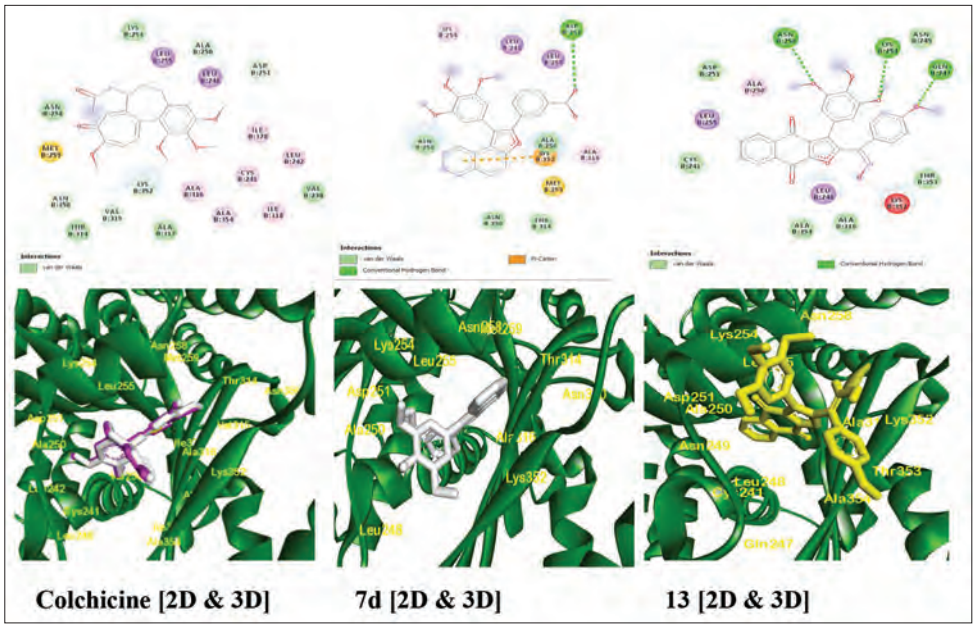


Figure 4: Docked view (2D and 3D) of naphthofuran derivatives 7d and 13 at active site showing crucial interactions with amino acids

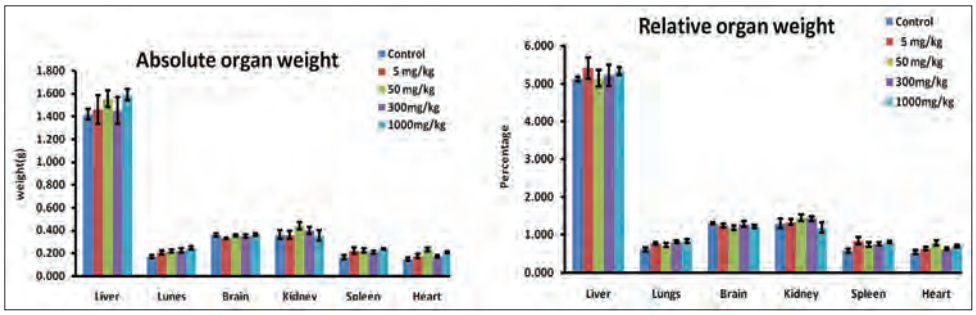


Figure 5: Effect of naphthofuran derivative 7d as single acute oral dose at 5, 50, 300, and 1000 mg/kg body weight on absolute and relative organ weight in Swiss albino mice (mean ± SE; *n* = 6)

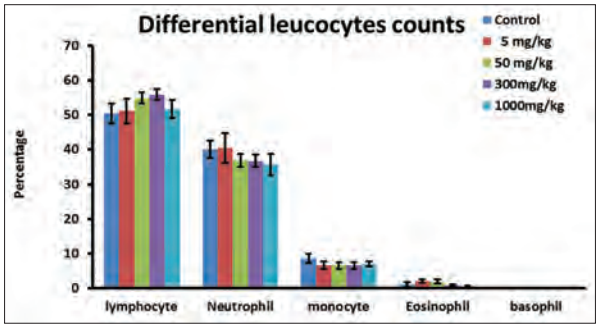


Figure 6: Effect of naphthofuran derivative 7d as single acute oral dose at 5, 50, 300, and 1000 mg/kg body weight on differential leucocytes counts in Swiss albino mice (mean ± SE; *n* = 6)

The ADME properties predicted for both naphthofurans **7d** and **13** through SwissADME software were quite satisfactory. Compound **7d** had no deviation in Lipinski rule parameters while **13** showed one deviation. Inadequate ADME properties can otherwise be devastating to any good drug candidate.[13] Many a time, the progress of ADME profiling has decreased the proportion of drug candidates failing in clinical trials.

Acute toxicity attributes to the adverse effects that occur on first exposure to a single dose of a substance. In acute oral toxicity studies, both the investigational lead compounds were found to be safe. There were no significant changes in various parameters of experiment mice. However, toxicity of a test compound can be affected by several different factors such as route of administration, time of exposure, physical form of compound, and genetic makeup of experimental animal. Systematic subacute and chronic experiments should also be done for assessing repeated exposure of the compounds to the experimental animals. However, both the compounds were found safe also in *in vitro* evaluation (safety index>4.6) against HEK-293T normal cells. Due to its importance in drug development, the WHO has defined drug safety as "Pharmacovigilance."[14]

EXPERIMENTAL

Chemical synthesis

*Synthesis of 1-(3,4,5,-trimethoxy)phenyl,2-(4-hydroxy) phenyl naphtho[2,1b]furan (**6a**)*

To a stirred solution of diarylnaphthofuran **5** (200 mg, 0.43 mmol) in methanol (20 mL), potassium hydroxide (1 g,

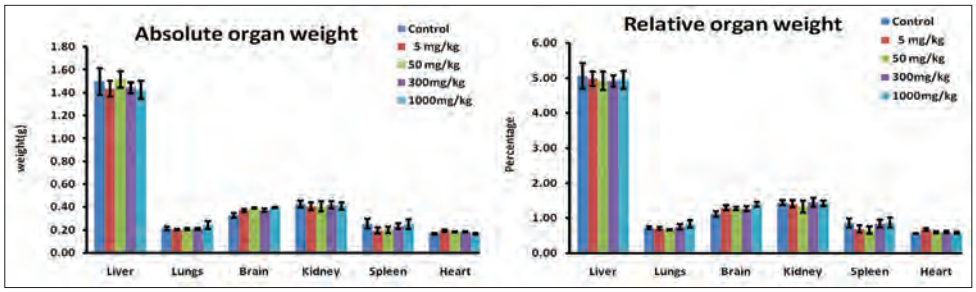


Figure 7: Effect of naphthofuran derivative 13 as single acute oral dose at 5, 50, 300, and 1000 mg/kg on absolute and relative organ weight in Swiss albino mice (mean ± SE; *n* = 6)

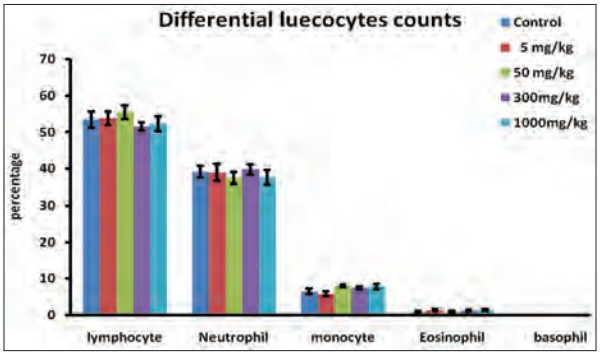


Figure 8: Effect of naphthofuran derivative 13 as single acute oral dose at 5, 50, 300, and 1000 mg/kg on differential leukocyte counts in Swiss albino mice (mean ± SE; *n* = 6)

17.8 mmol) was added and further stirred at RT for 30 min. Solvent was concentrated under vacuum and crude mass which was purified through a filter column of silica gel to get compounds **6a**.

Yield = 82%; MP: gummy mass; in ^{1}H-NMR (500MHz, $CDCl_3$): δ3.76 (s, 6H, 2xOCH_3), 3.92 (s, 3H, OCH_3), 4.87 (bs, 1H, exchangeable, OH), 6.71 (m, 2H, Ar-H), 6.79 (s, 2H, Ar-H), 7.25 (t, 1H, Ar-H), 7.35 (t, 1H, Ar-H), 7.43 (d, 2H, Ar-H), 7.59 (d, 1H, Ar-H, J = 8.40 Hz,), 7.70 (dd, 2H, Ar-H, J = 8.90 Hz), 7.89 (d, 1H, Ar-H, J = 8.10 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$): δ55.40, 60.08, 109.02, 112.15, 113.02, 116.47, 118.37, 118.64, 123.58, 124.15, 124.83, 126.92, 128.89, 130.15, 132.11, 132.58, 139.31, 152.09, 152.34, 155.63, 158.98, electrospray mass for $C_{27}H_{22}O_5$ (MeOH): 465 $[M+K]^+$; HRMS (ESI-TOF): calcd. for $C_{27}H_{22}O_5$ [M+H], 427.1467, found 427.1971.

*Synthesis of 1-(3,4,5,-trimethoxy)phenyl,2-(4-methoxy) phenyl naphtho[2,1b]furan (***6b***)*

To a stirred solution of diarylnaphthofuran **6a** (100 mg, 0.2 mmol) in dry acetone (12 mL), anhydrous potassium carbonate (1 g, 7.2 mmol) and dimethyl sulfate (0.1 ml, 1mmol) were added and refluxed for 2 h. Solvent was concentrated under vacuum and crude mass which was purified through a filter column of silica gel to get compound **6b**.

Yield: 89%; MP: gummy mass; in ^{1}H-NMR (500MHz, $CDCl_3$) ppm: δ 3.82 (s, 6H, 2xOCH_3), 3.84 (s, 6H, OCH_3), 6.49 (s, 2H, Ar-H), 6.82 (s, 2H, Ar-H), 7.30 (d, 1H, Ar-H, J = 9.15 Hz), 7.44 (d, 2H, Ar-H, J = 7.50 Hz), 7.63 (d, 2H, Ar-H), 7.67 (d, 1H, Ar-H), 7.80 (d, 2H, Ar-H, J = 9.05 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$): δ55.45, 55.50, 56.71, 106.53, 109.58, 113.41, 118.82, 124.65, 124.95, 125.49, 128.18, 128.25, 128.49, 128.58, 130.09, 135.12, 135.16, 159.60, 160.61, 160.63. HRMS (ESI-TOF): Calcd. for $C_{28}H_{24}O_5$ [M+H], 441.1624, found 441.6603.

*General procedure for the synthesis of 1,2-diarylnaphtho[2,1b] furans (***7a-7g***)*

Trimethoxyphenylnaphtho furan (**4**) (1g, 3.0 mmol) was stirred in DMA (30 mL). To this, different aryl benzyl halide (3.1 mmol), palladium acetate (135 mg, 0.6 mmol), and potassium acetate (588 mg, 6 mmol) were added and reaction mixture was heated at 80°C for 4–6 h to get compound **7** (**a-g**) in 83–90% yield.

*1-(3,4,5,-Trimethoxy)phenyl, 2-(4-fluoro)phenyl naphtho[2,1b]furan (***7a***)*

Yield = 87%; M.P. = gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.74 (s, 6H, 2xOCH_3), 3.98 (s, 3H, OCH_3), 6.59 (s, 2H, Ar-H), 6.80 (d, 2H, Ar-H, J = 5.10 Hz), 7.37 (t, 1H, Ar-H), 7.46 (t, 1H, Ar-H), 7.64 (d,1H, Ar-H, J = 8.90 Hz), 7.79 (d, 1H, Ar-H), 7.88 (d, 2H, Ar-H), 7.95 (d, 2H, Ar-H, J = 8.00 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$): δ56.11, 61.05, 107.21, 112.27, 114.08, 116.49, 119.05, 121.97, 123.67, 126.29, 127.00, 128.12, 128.19, 129.09, 130.97, 137.65, 139.29, 142.61, 152.61, 153.05, 153.54, 154.25; HRMS (ESI-TOF) m/z calcd. for $C_{27}H_{21}FO_4$ [M+H] 429.1424, found 429.7975.

*1-(3,4,5,-Trimethoxy)phenyl, 2-(3,5-dimethoxy)phenyl naphtho[2,1b]furan (***7b***)*

Yield = 82%; M.P. = gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$) ppm: δ 3.71 (s, 9H, 3xOCH_3), 3.97 (s, 6H, 2xOCH_3), 6.57 (s, 4H, Ar-H), 7.35 (t, 1H, Ar-H), 7.44 (t, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.63 (d, 1H, Ar-H, J = 8.90 Hz), 7.80 (d, 1H, Ar-H, J = 8.90 Hz), 7.86 (d, 1H, Ar-H, J = 8.35 Hz), 7.94 (d, 1H, Ar-H, J = 8.05 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$): δ55.32, 55.88, 56.02, 60.84, 98.99, 106.76, 107.27, 112.06, 112.45, 113.89, 120.42, 121.72, 126.83, 127.92, 127.96, 127.99, 128.31, 128.78, 130.63, 132.11, 135.02, 137.51, 141.54, 152.39, 152.82, 152.88, 153.12, 160.93. ESI-MS (MeOH) negative mode: For $C_{29}H_{26}O_6$ **(**MeOH**):** 469 $[M-H]^-$.

*1-(3,4,5,-Trimethoxy)phenyl, 2-(3,4,5-trimethoxy)phenyl naphtho[2,1b]furan (***7c***)*

Yield = 89%; M.P.=183–185°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ3.68 (s, 9H, 3xOCH_3), 3.82 (s, 6H, 2xOCH_3),

6.81 (s, 2H, Ar-H), 7.89 (s, 2H, Ar-H), 7.30 (t, 1H, Ar-H), 7.37 (t, 1H, Ar-H), 7.58 (d, 1H, Ar-H), 7.73 (d, 1H, Ar-H), 7.85 (m, 1H, Ar-H), 7.86 (m, 1H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.38, 55.95, 59.97, 60.63, 102.76, 107.00, 111.73, 111.88, 118.73, 122.80, 124.09, 126.71, 127.76, 128.63, 128.74, 130.58, 137.53, 137.58, 139.38, 150.74, 152.71, 152.78, 153.07, 153.98. ESI-MS (MeOH): For $C_{30}H_{28}O_7$, 523 $[M+Na]^+$, HRMS (ESI-TOF) m/z [M+H] calcd. for $C_{30}H_{28}O_7$, 501.1835, found 501.7712.

*3-(1-(3,4,5,-Trimethoxy)phenyl naphtho[2,1b]furan-2-yl) benzoic acid (***7d***)*

Yield = 77%; M.P.=211°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.73 (s, 6H, 2xOCH_3), 3.97 (s, 3H, OCH_3), 6.58 (s, 2H, Ar-H), 7.37 (t, 1H, Ar-H), 7.41 (t, 1H, Ar-H), 7.44 (t, 1H, Ar-H), 7.57 (d, 1H, Ar-H, J = 9.05 Hz), 7.61 (d, 2H, Ar-H, J = 8.90 Hz), 7.85 (dd, 2H, Ar-H, J = 8.30 Hz), 7.93 (d, 1H, Ar-H, J = 8.10 Hz), 8.00 (d, 1H, Ar-H, J = 2.95 Hz), 8.09 (bs, 1H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ56.10, 61.08, 107.49, 112.28, 121.96, 123.34, 123.63, 124.68, 124.83, 126.30, 127.01, 128.15, 128.17, 128.28, 129.08, 130.24, 130.95, 131.19, 133.72, 134.67, 137.58, 142.60, 152.59, 153.03, 170.03; HRMS (ESI-TOF) m/z [M+H] calcd. for $C_{28}H_{22}O_6$ 455.1416, found 455.1792.

*Methyl-3-(1-(3,4,5,-trimethoxy)phenyl naphtho[2,1b] furan-2-yl)benzoate (***7e***)*

Yield = 69%; M.P.=gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ3.71 (s, 9H, 3xOCH_3), 3.97 (s, 3H, OCH_3), 6.57 (s, 2H, Ar-H),7.35 (d,1H, Ar-H), 7.36 (t, 1H, Ar-H, J = 5.06 Hz), 7.42 (d, 1H, Ar-H), 7.45 (t, 1H, Ar-H, J = 3.93 Hz), 7.62 (d, 1H, Ar-H, J = 8.90 Hz), 7.79 (d, 2H, Ar-H, J = 8.95 Hz), 7.86 (d, 1H, Ar-H, J = 8.35 Hz), 7.94 (d, 1H, Ar-H, J = 8.05 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$): δ56.10, 56.22, 61.05, 107.50, 112.27, 123.34, 124.67, 126.28, 129.07, 130.95, 133.22, 137.61, 142.59, 152.59, 153.03, 172.40; ESI-MS (MeOH): For $C_{29}H_{24}O_6$, 507 $[M+K]^+$.

*Ethyl-3-(1-(3,4,5,-trimethoxy)phenyl naphtho[2,1b]furan-2-yl)benzoate (***7f***)*

Yield = 87%; M.P.=gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ1.42 (t, 3H, CH_3), 3.72 (s, 6H, 2xOCH_3), 3.97 (s, 3H, OCH_3), 4.37 (m, 2H, OCH_2), 6.57 (s, 2H, Ar-H) 7.36 (t, 1H, Ar-H, J = 7.87 Hz), 7.40 (t, 1H, Ar-H, J = 7.05 Hz), 7.45 (t, 1H, Ar-H, J = 7.87 Hz), 7.53 (d, 1H, Ar-H, J = 10.05 Hz), 7.63 (d, 1H, Ar-H, J = 8.95 Hz), 7.84 (d, 1H, Ar-H, J = 8.30 Hz), 7.79 (d, 1H, Ar-H, J = 8.95 Hz), 7.92 (d, 1H, Ar-H, J = 8.10 Hz), 7.94 (d, 1H, Ar-H, J = 8.00 Hz), 8.02 (bs, 1H, Ar-H), ^{13}C-NMR (125 MHz, $CDCl_3$): δ14.40, 56.09, 61.42, 66.22, 107.47, 112.28, 115.91, 121.96, 123.34, 123.63, 124.67, 126.30, 127.00, 128.13, 128.17, 129.08, 129.64, 129.66, 130.95, 132.23, 132.86, 137.56, 142.59, 152.59, 153.02, 174.39. ESI-MS (MeOH): For $C_{30}H_{26}O_6$, 453 $[M-Et]^-$.

*5-(1-(3,4,5,-Trimethoxy)phenyl naphtho[2,1b]furan-2-yl) furan-2-carbaldehyde (***7g***)*

Yield = 83%; M.P.= gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ3.85 (s, 6H, 2xOCH_3), 4.03 (s, 3H, OCH_3), 6.36 (d, 1H, Ar-H), 6.79 (s, 2H, Ar-H), 7.22 (d, 1H, Ar-H, J = 3.80 Hz), 7.38 (t, 1H, Ar-H), 7.48 (t, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.85 (d, 1H, Ar-H, J = 9.00 Hz), 7.96 (d, 1H, Ar-H, J = 8.05 Hz), 9.64 (s, 1H, CHO); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.32, 61.25, 106.90, 110.23, 112.36, 114.07, 122.61, 123.21, 123.45, 125.03, 126.73, 127.74, 127.94, 128.05, 129.13, 131.06, 138.38, 139.30, 141.62, 150.36, 151.95, 152.60, 154.03, 177.51; ESI-MS (MeOH): For $C_{26}H_{20}O_6$, 429 $[M+H]^+$.

*Synthesis of phenyl (1-(3,4,5-trimethoxyphenyl) naphtho[2,1-b]furan-2-yl)methanone derivatives (***8a-8e***)*

Arylnaphtho [2, 1-b] furan **4** (500 mg, 1.5 mmol) was taken in N,N-dimethyl acetamide (20 mL). To this, different aryl benzoyl halides (1.6 mmol), palladium acetate (0.3 mmol), and potassium acetate (3 mmol) were added and reaction temperature was raised up to at 80°C for 4-6 h to get the final compounds **8a-8e**.

*3-Nitrophenyl(1-(3,4,5-trimethoxyphenyl)naphtho[2,1-b] furan-2-yl)methanone (***8a***)*

Yield = 85%; M.P.=174–176°C; ^{1}H-NMR (500 MHz, $CDCl_3$) ppm: δ 3.72 (s, 6H, 2xOCH_3), 3.96 (s, 3H, OCH_3), 6.55 (s, 2H, Ar-H), 7.44 (d, 1H, Ar-H), 7.45 (d, 1H, Ar-H), 7.46 (t, 1H, Ar-H), 7.46 (t, 1H, Ar-H), 7.65 (t, 2H, Ar-H, J = 0.97 Hz,), 8.39 (m, 1H, Ar-H), 8.40 (d, 1H, Ar-H), 8.41 (d,1H, Ar-H), 8.90 (s,1H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.09, 61.07, 112.26, 121.11, 123.30, 124.67, 125.00, 125.72, 127.42, 132.39, 135.65, 148.28, 152.98, 167.38, 171.89; ESI-MS (MeOH): For $C_{28}H_{21}NO_7$, negative mode: 482 $[M-H]^-$.

*3,4,5-Trimethoxyphenyl(1-(3,4,5-trimethoxyphenyl) naphtho[2,1-b]furan-2-yl)methanone (***8b***)*

Yield = 87%; gummy solid; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.72 (s, 6H, 2xOCH_3), 3.96 (s, 3H, OCH_3), 6.57 (s, 2H, Ar-H), 7.28 (d,1H, Ar-H), 7.34 (t, 1H, Ar-H), 7.43 (t, 1H, Ar-H), 7.46 (d, 1H, Ar-H), 7.62 (d, 1H, Ar-H, J = 8.90 Hz), 7.79 (d, 1H, Ar-H, J = 9.0 Hz), 7.86 (d, 2H, Ar-H, J = 8.35 Hz), 7.94(d, 1H, Ar-H, J = 8.0 Hz), in ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.12, 61.01, 107.54, 112.26, 114.06, 121.97, 123.34, 123.65, 124.66, 126.28,126.99, 128.18, 130.96, 137.66, 142.60, 152.59, 153.04,160.05, 167.00; ESI-MS (MeOH): For $C_{31}H_{28}O_8$, 527 $[M-H]^-$, HRMS (ESI-TOF) m/z [M+H] calcd. for $C_{31}H_{28}O_8$, for [M+H]+ 529.1784, found 529.7737.

*2-Nitrophenyl(1-(3,4,5-trimethoxyphenyl)naphtho[2,1-b] furan-2-yl)methanone (***8c***)*

Yield = 85%; gummy solid; ^{1}H-NMR (500 MHz, $CDCl_3$) ppm: δ 3.73 (s, 6H, 2xOCH_3), 3.97 (s, 3H, OCH_3), 6.57 (s, 2H, Ar-H), 7.37 (t, 1H, Ar-H), 7.45 (t, 1H, Ar-H), 7.61 (m, 1H, Ar-H), 7.78 (d,1H, Ar-H), 7.80 (m, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.10, 61.06, 107.47, 112.28, 114.08, 121.96, 124.67, 126.30, 127.00, 128.13, 128.17, 129.08, 130.95, 137.58, 142.59, 152.59, 153.03,161.00, 168.76; ESI-MS (MeOH): Negative mode for $C_{28}H_{21}NO_7$, 482 $[M-H]^-$,

*4-Chlorophenyl(1-(3,4,5-trimethoxyphenyl)naphtho[2,1-b] furan-2-yl)methanone (***8d***)*

Yield = 87%; gummy solid; ^{1}H-NMR (500 MHz, $CDCl_3$) ppm: δ 3.73 (s, 6H, OCH_3), 3.97 (s, 3H, OCH_3), 6.57 (s, 2H,

Ar-H), 7.33 (d, 1H, Ar-H), 7.36 (t, 1H, Ar-H), 7.42 (t, 1H, Ar-H), 7.45 (d, 2H, Ar-H), 7.45 (d, 1H, Ar-H), 7.62 (d, 1H, J = 8.95 Hz, Ar-H), 7.78 (d, 1H, J = 8.95 Hz, Ar-H), 7.84 (d, 2H, J = 8.35 Hz, Ar-H), in ^{13}C-NMR (125 MHz, $CDCl_3$) ppm: δ 56.10, 61.05, 103.96, 111.25, 119.52, 124.82, 127.08, 130.69, 131.62, 134.03, 135.37, 140.72, 142.24, 150.98, 151.94, 162.83, 166.17; ESI-MS (MeOH): For $C_{28}H_{21}ClO_5$, 494 $[M+Na-H]^+$.

*4-Cyanophenyl(1-(3,4,5-trimethoxyphenyl)naphtho[2,1-b] furan-2-yl)methanone (**8e**)*

Yield = 89%; M.P.=200–202°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.73 (s, 6H, 2xOCH_3), 3.97 (s, 3H, OCH_3), 6.57 (s, 2H, Ar-H), 7.35 (t, 1H, Ar-H), 7.44 (t, 1H, Ar-H), 7.63 (d, 1H, Ar-H, J = 8.90 Hz), 7.71 (d, 1H, Ar-H, J = 8.45 Hz), 7.78-7.76 (m, 4H, Ar-H), 8.19 (d, 2H, Ar-H, J = 8.50 Hz), in ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.10, 61.08, 107.50, 112.28, 117.02, 117.87, 121.96, 123.33, 123.60, 124.68, 126.30, 127.01, 128.16, 129.08, 130.61, 130.96, 133.30, 137.56, 142.61, 152.60, 153.01, 168.76; HRMS (ESI-TOF) m/z [M+H] calcd. for $C_{29}H_{21}NO_5$, 464.1420, found 464.3072.

Synthesis of 2-(4-methoxy)benzoyl,3-(3,4,5,-trimethoxy) phenylnaphtho[2,3b]furan-4,9-dione (12)

To a stirred solution of Lawsone (174 mg, 1.0 mmol) in acetonitrile (30 mL), chalcone **11 (**244 mg, 0.75 mmol), N-iodo succinimide (NIS, 818 mg, 3.65 moles), and DABCO (1120 mg, 10.00 moles) were added and stirred at 100°C for 20 h. The reaction mixture was concentrated in rotavapor. The residue was taken in EtOAc (40 mL), washed with water (20mLx2), organic layers were combined, dried over anhydrous sodium sulfate, and concentrated under vacuum to get compound **12** in (80%) yields.

Yield = 94%; M.P.=166-167°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.78 (s, 6H, 2xOCH_3), 3.84 (s, 6H, 2xOCH_3), 6.72 (s, 2H, Ar-H), 6.85 (d, 2H, Ar-H), 7.78 (m, 2H, Ar-H), 7.84 (m, 2H, Ar-H), 8.23 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.58, 56.22, 108.10, 113.89, 127.54, 128.78, 130.60, 132.15, 134.48, 138.75, 150.98, 152.65, 164.22, 174.09, 178.08, 180.09, 188.69; ESI-MS (MeOH): For $C_{29}H_{22}O_8$, 521 $[M+Na]^+$,

*Synthesis of 2-(hydroxyimino)(4-methoxyphenyl)methyl,3-(3,4,5,-trimethoxy)phenylnaphtho [2,3b] furan-4,9-dione (**13**)*

Compound **12 (**100 mg, 0.20 mmol) and hydroxylamine hydrochloride (94 mg, 1.36 mmol) were taken in pyridine (3 mL) at RT for 3h. After completion, the reaction was extracted with EtOAc (10 mL × 3), washed with water. All organic layers were combined, dried over Na_2SO_4, and concentrated by a rotavapor. To get compound **13** in (90%) yields.

Yield = 90%; M.P.= 226–227°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.80 (s, 6H, 2xOCH_3), 3.83 (s, 6H, 2xOCH_3), 6.74 (s, 2H, Ar-H), 6.87 (d, 2H, Ar-H), 7.78 (m, 2H, Ar-H), 7.85 (m, 2H, Ar-H), 8.23 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.58, 56.06, 106.75, 114.18, 125.63, 128.20, 131.25, 132.06, 132.15, 132.34, 133.38, 138.29, 139.29, 147.78, 152.01, 153.37, 161.17, 164.25, 173.79, 180.36; ESI-MS (MeOH): For $C_{29}H_{23}NO_8$, 536 $[M+Na]^+$ and 512 $[M-H]^+$.

*Synthesis of 2-benzoyloxime esters (**14a-14g**)*

To a stirred solution of oxime **13 (**50 mg, 0.10 mmol) in dry tetrahydrofuran (10 mL), ethyl bromoacetate (80 mg, 1.0 mmol) and NaH (pre-washed, 50 mg, 2 mmol) were added and refluxed for 2–4 h. Excess of sodium hydride was quenched by adding cold water dropwise. Reaction mixture was extracted with ethyl acetate (10 mL × 3), organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The crude mass thus obtained was purified through filter column of silica gel to get desired product **14a**. Similarly, other fatty acid ester chains were hooked up at oxime **13** to get **14b-14g** in 62–73% yields, respectively.

*Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-acetate (**14a**)*

Yield = 68%; M.P.=66–68°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.30 (m, 3H, CH_3), 3.88 (s, 6H, 2xOCH_3), 3.90 (s, 6H, 2xOCH_3), 4.172 (m, 2H, OCH_2), 4.64 (s, 2H, OCH_2), 6.75 (s, 2H, Ar-H), 6.88 (d, 2H, Ar-H), 7.70 (m, 2H, Ar-H), 7.93 (m, 2H, Ar-H), 8.46 (m, 2H, Ar-H); In ^{13}C-NMR (125 MHz, $CDCl_3$): δ14.15, 55.98, 56.05, 61.95, 65.24, 105.81, 114.46, 126.19, 130.48, 130.80, 131.14, 131.28, 132.05, 138.16, 140.36, 144.45, 152.75, 153.48, 161.44, 161.74, 166.69, 168.27, 176.32, 188.74; ESI-MS (MeOH): Negative mode; for $C_{33}H_{29}NO_{10}$, 598 $[M-H]^-$.

Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-propionate (14b)

Yield = 62%; M.P.= 52–54°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.66 (t, 3H, CH_3), 2.74 (t, 2H, CH_2), 3.79 (t, 2H, CH_2), 3.80 (s, 6H, 2xOCH_3), 3.83 (s, 6H, 2xOCH_3), 4.18 (m, 2H, OCH_2), 6.85 (s, 2H, Ar-H), 6.96 (d, 2H, Ar-H), 7.40 (m, 2H, Ar-H), 7.92 (m, 2H, Ar-H), 8.00 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.29, 29.69, 56.00, 56.06, 61.83, 72.57, 105.85, 114.74, 126.27, 130.53, 130.94, 131.27, 131.85, 138.03, 140.43, 144.35, 152.77, 153.51, 161.33, 169.09, 171.53, 176.14, 188.73; ESI-MS (MeOH): For $C_{34}H_{31}NO_{10}$, 631 $[M+H_2O]^+$.

*Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-butyrate (**14c**)*

Yield = 69%; M.P. = gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.37 (t, 3H, CH_3), 1.56 (m, 2H, CH_2), 2.28 (t, 2H, CH_2), 3.57 (t, 2H, CH_2), 3.79 (s, 6H, 2xOCH_3), 3.90 (s, 6H, 2xOCH_3), 4.16 (m, 2H, OCH_2), 6.67 (s, 2H, Ar-H), 6.87 (d, 2H, Ar-H), 7.78 (m, 2H, Ar-H), 7.85 (m, 2H, Ar-H), 8.23 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.22, 24.45, 29.69, 56.00, 56.26, 61.01, 67.01, 105.65, 114.28, 121.29, 126.51, 130.75, 130.81, 131.31, 137.69, 144.16, 152.77, 153.50, 161.17, 164.25, 173.79, 180.36; ESI-MS (MeOH): Negative mode: For $C_{35}H_{33}NO_{10}$, 626 $[M-H]^-$.

*Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-pentanoate (**14d**)*

Yield = 73%; M.P. = gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.31 (bs, 3H, CH_3), 1.33 (m, 2H, CH_2), 1.66 (bs,

2H, CH_2), 2.31 (m, 2H, CH_2), 3.88 (s, 6H, OCH_3), 3.90 (s, 6H, 2xOCH_3), 4.00 (t, 2H, OCH_2), 4.06 (m, 2H, OCH_2), 6.67 (s, 2H, Ar-H), 6.84 (d, 2H, Ar-H), 7.42 (m, 2H, Ar-H), 7.94 (m, 2H, Ar-H), 8.03 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.11, 22.69, 28.50, 31.93, 55.58, 56.06, 67.65, 73.90, 106.98, 114.27, 125.63, 128.20, 131.32, 132.06, 132.15, 132.34, 133.38, 138.29, 139.29, 147.78, 152.01, 153.37, 161.17, 164.25, 173.79, 180.36; ESI-MS (MeOH): For $C_{36}H_{35}NO_{10}$, 642 $[M+H]^+$. HRMS (ESI-TOF) m/z [M+H] calcd. for $C_{36}H_{35}NO_{10}$, 642.2295, found 642.2445.

*Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-crotonate (**14e**)*

Yield = 66%; M.P. = 55–57°C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.32 (bs, 3H, CH_3), 3.84 (s, 6H, 2xOCH_3), 3.89 (s, 6H, 2xOCH_3), 4.18 (bs, 2H, OCH_2), 4.29 (d, 2H, OCH_2), 6.78 (m, 1H, CH), 6.88 (s, 2H, Ar-H), 6.87 (d, 2H, Ar-H), 6.96 (m, 1H, CH), 7.89 (m, 2H, Ar-H), 7.92 (m, 2H, Ar-H), 8.38 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.22, 56.00, 56.26, 60.53, 67.01, 105.41, 114.28, 121.10, 126.51, 130.75, 130.81, 131.31, 137.69, 143.92, 152.77, 153.50, 161.17, 164.25, 173.79, 180.36; ESI-MS (MeOH): Negative mode: For $C_{35}H_{31}NO_{10}$, 626 $[M-H]^-$.

*Ethyl-2-((((4,9-dioxo-3-(3,4,5-trimethoxy)phenyl-4,9-dihydronaphtho[2,3b]furan-2-yl)(4-methoxy)phenyl) methylene)amino)oxy-heptanoate (**14f**)*

Yield = 69%; M.P.= gummy mass; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.26 (bs, 3H, CH_3), 1.28 (m, 4H, CH_2), 1.41 (m, 2H, CH_2), 1.66 (m, 2H, CH_2), 2.02 (m, 2H, CH_2), 3.63 (t, 2H, CH_2), 3.89 (s, 6H, 2xOCH_3), 3.93 (s, 6H, 2xOCH_3), 4.12 (m, 2H, OCH_2), 6.84 (s, 2H, Ar-H), 6.96 (d, 2H, Ar-H), 7.42 (m, 2H, Ar-H), 7.72 (m, 2H, Ar-H), 8.02 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 14.25, 25.67, 25.70, 27.79, 28.93, 34.21, 55.58, 56.06, 61.00, 73.90, 107.00, 114.31, 126.59, 128.20, 131.31, 137.58, 138.29, 139.69, 140.11, 144.86, 147.78, 152.75, 153.51, 163.02, 163.32, 173.71, 188.72; ESI-MS (MeOH): Negative mode; for $C_{38}H_{39}NO_{10}$, 668 $[M-H]^-$.

Biological assays

The detailed protocols have been given in Supplementary information.

Antiproliferative activity

The antiproliferative activity was determined by sulforhodamine assay (S3). Tamoxifen and doxorubicin were used as standard drugs (positive control) for cytotoxicity.

Tubulin polymerization assay

Tubulin polymerization assay was performed on porcine brain tubulin (>99% pure) using "assay kit-BK006P" from Cytoskeleton, USA.[15] Podophyllotoxin (PDT) was used as standard inhibitor and paclitaxel as standard stabilizer of tubulin polymerization process.

Molecular docking studies

ChemSpider software was used for sketching the ligands and simultaneously saved as SMILES. Further, the SMILES was converted into 3D structures using online SMILES translator and structure file generator software and saved as.pdb. Afterward, the site directed docking of both the compounds (**7d** and **13**) was performed at colchicine binding pocket of β-tubulin using AutoDock Vina (http://www.vina.scripps.edu/), (Molecular Graphics Lab at the Scripps Research Institute, USA).[16] The binding site was determined based on the binding pocket of colchicine cocrystallized with tubulin. The 3D crystal structure of β-tubulin (PDB: 4O2B) was downloaded from RCSB (Protein Data Bank) to serve as docking template for the docking experiment. The grid center and grid dimensions (Å) were as follows X: 17.0213, Y: 65.9976, Z: 43.3909 and X: 25.0841, Y: 22.7296, Z: 15.7054, respectively. The redocked pose of the ligand and its corresponding crystallized structure was superimposed to calculate the root mean square deviation (RMSD) and the RMSD was 0.2 Å. ADME properties of both the naphthofuran derivatives were predicted on SwissADME online software.[17]

Safety studies

The safety assessment of both the naphthofuran derivatives **7d** and **13** was done in accordance with the Organization for Economic Cooperation and Development test guideline No. 423 (1987) and following the IAEC (Institutional Animal Ethical Committee) approved protocols vide ref. No. CIMAP/IAEC/2016-19/01, dated 09/2/2017 as per reported method.[18]

CONCLUSION

The present study provided two naphthofuran derivatives possessing potential anticancer activity against TNBC. The antiproliferative activity was through microtubule destabilization. Docking studies showed that both the compounds occupied colchicine binding pocket of β-tubulin with comparable affinity to standard ligands. Both the compounds possessed moderate bioavailability in *in silico* predictions. In safety studies, both the naphthofuran derivatives were well tolerate by rodents and were non-toxic to them. These naphthofuran derivatives may further be optimized for better efficacy properties in future.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

SUPPLEMENTARY INFORMATION

Chemistry of some compounds, selected spectra, and detailed biological assays are available in Supplementary information.

REFERENCES

[1] Giampietro, R.R., Cabral, M.V.G., Lima, S.A.M., dos Weber, S.A.T., Nunes-Nogueira, S.V. Accuracy and effectiveness of mammography versus mammography and tomosynthesis for population-based breast cancer screening: A systematic review and meta-analysis, *Sci. Rep*., **2020**, *10*, 7991.

[2] Chan, H.J., Patrossian, K., Chen, S. Structural and functional characterization of aromatase, estrogen receptor, and their genes in endocrine-responsive and resistant breast cancer cells, *J. Steroid Biochem. Mol. Biol*., **2016**, *161*, 73–83.

[3] da Silva, J.L., Nunes, N.C.C., Izetti, P., de Mesquita, G.G., de Melo, A.C. Triple negative breast cancer: A thorough review of biomarkers, *Crit. Rev. Oncol. Hematol*., **2020**, *5*, 102855.

[4] Pado, F., Buydens, L.M.C., Degani, H., Hilhorst, R., Klipp, E., Gribbestad, I.S., Huffel, S.V., van Laarhoven, H.W.M., Luts, J., Monleon, D., Postma, G.J., Schneiderhan-Narra, N., Santoro, F., Wouters, H., Russnes, H.G., Sorlie, T., Tagliabue, E., Borresen-Dale, A.L. Triple negative breast cancer: Present challenges and new perspectives, *Mol. Oncol.*, **2010**, *4*, 209–229.

[5] Taylor, R.D., MacCoss, M., Lawson, A.D.G. Combining molecular scaffolds from FDA approved drugs: application to drug discovery, *J. Med. Chem.*, **2017**, *60*, 1638–1647.

[6] Li, L., Jiang, S., Li, X., Liu, Y., Su, J., Chen, J. Recent advances in trimethoxyphenyl (TMP) based tubulin inhibitors targeting the colchicine binding site, *Eur. J. Med. Chem.*, **2018**, *151*, 482–494.

[7] (a) Khanam, H., Shamsuzzaman. Bioactive benzofuran derivatives: A review, *Eur. J. Med. Chem.*, **2015**, *97*, 483–504. (b) Lumb, J.P., Trauner, D. Biomimetic synthesis and structure elucidation of rubicordifolin, a cytotoxic natural product from *Rubia cordifolia*, *J. Am. Chem. Soc*., **2005**, *127*, 2870–2871. (c) Wong, K.S., Houry, W.A. Chemical modulation of human mitochondrial ClpP: Potential application in cancer therapeutics, *ACS Chem. Biol*., **2019**, *14*, 2349–2360. (d) Gatti, R., Chiarini, A., Casaroni, M.R., Garuti, L., Brigidi, P. Synthesis and antimicrobial activity of some 2-(2-nitroethenyl)naphthofuran and -benzofuran derivatives, *Arch. Pharm.* (*Weinheim*), **1983**, *316*, 849–851. (e) Jimenez-Alonso, S., Guasch, J.J., Estevez-Braun, A., Ratera, I.I., Veciana, J.J., Ravelo, A.G. Electronic and cytotoxic properties of 2-amino-naphtho[2,3-b]furan-4,9-diones, *J. Org. Chem*., **2011**, *76*, 1634–1643.

[8] Srivastava, V., Negi, A.S., Kumar, J.K., Faridi, U., Sisodia, B.S., Darokar, M.P., Luqman, S., Khanuja, S.P.S. Synthesis of 1-(3',4',5'-trimethoxyphenyl) phenyl naphtho[2,1b]furan as a novel anticancer agents, *Bioorg. Med. Chem. Lett*., **2006**, *16*, 911–914.

[9] Simoni, D., Romagnoli, R., Baruchello, R., Rondanin, R., Rizzi, M., Pavani, M.G., Alloatti, D., Giannini, G., Marcellini, M., Riccioni, T., Castorina, M., Guglielmi, M.B., Bucci, F., Carminati, P., Pisano, C. Novel combretastatin analogues endowed with antitumor activity, *J. Med. Chem*., **2006**, *49*, 3143–3152.

[10] Negi, A.S., Gautam, Y., Alam, S., Chanda, D., Luqman, S., Sarkar, J., Khan F., Konwar, R. Natural antitubulin agents: Importance of 3,4,5-trimethoxyphenyl fragment, *Bioorg. Med. Chem.*, **2015**, *23*, 373–389.

[11] Borisy, G., Heald, R., Howard, J., Janke, C., Musacchio, A., Nogales, E. Microtubules: Fifty years on from the discovery of tubulin, *Nature*, **2016**, *17*, 322–328.

[12] Burns, R.G. Analysis of the colchicine-binding site of beta-tubulin, *FEBS J*., **1992**, *297*, 205–208.

[13] Kenakin, T.P. Chapter; Pharmacokinetics-I. In: *Pharmacology in Drug Discovery and Development. Understanding Drug Response*. 2nd ed. Academic Press, Elsevier Inc., Cambridge, Massachusetts, **2017**.

[14] World Health Organization. *WHO Pharmacovigilance Indicators; a Practical Manual for the Assessment of Pharmacovigilance Systems*. WHO Publications, France, **2015**.

[15] Lee, J.C., Timassheff, S.N. *In vitro* reconstitution of calf brain microtubules: Effects of solution variables. *Biochemistry*, **1997**, *16*, 1754–1764.

[16] Prota, A.E., Danel, F., Bachmann, F., Bargsten, K., Buey, R.M., Pohlmann, J., Reinelt, S., Lane, H., Steinmetz, M.O. The novel microtubule-destabilizing drug BAL27862 binds to the colchicine site of tubulin with distinct effects on microtubule organization. *J. Med. Chem.*, **2014**, *426*, 1848–1860.

[17] Diana, A., Michielin, O., Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, **2017**, *7*, 42717.

[18] Chanda, D., Shanker, K., Pal, A., Luqman, S., Bawankule, D.U., Mani, D.N., Darokar, M.P. Safety evaluation of Trikatu: A generic Ayurvedic medicine in Charles Foster rats. *J. Toxicol. Sci.*, **2009**, *34*, 99–108.

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