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Research paper

Antiproliferative activity of diarylnaphthylpyrrolidine derivative *via* dual target inhibition

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ABSTRACT

Breast cancer is the second leading cause of deaths in women globally. Present communication deals with design and synthesis of a few diarylnaphthyls as possible anti-breast cancer agents. Among the thirty three representatives with significant antiproliferative activity compounds 23 and 50 were quite efficacious against human breast cancer cells. Compound 50 induced apoptosis in both MCF-7 and MDA-MB-231 cells and exerted S phase and G2/M phase arrest respectively via distinct mechanistic pathways. It showed moderate microtubule destabilization. Further, it exhibited DNA topoisomerase-II inhibition effect in MCF-7 cells. It was well tolerable and found safe up to 300 mg/kg dose in Swiss albino mice. The dual action antiproliferative effect of compound 50 is quite interesting and warrants for future development.

1. Introduction

Breast cancer is the most prevalent among all cancer types in women around the world [1]. It is the second leading cause of cancer deaths in women. Early stage diagnosis is very important for the patient to control and eradicate the disease. Mammography is quite successful technique though unaffordable to common person. However, mammography is significantly associated in drastic reduction of breast cancer mortality [2,3.] Nevertheless, due to heterogeneity of the disease, the effective treatment is still a challenge. There are several successful clinical anticancer drugs with varied cancer targets which are used alone or in combinations to treat breast cancer [4]. Some of the important drugs are tamoxifen (Antiestrogen), anostrozole and letrozole (Aromatase inhibitors), doxorubicin (Topo-II inhibitor), 5-fluorouracil (Anti-metabolite), cisplatin (Alkylating agent), fulvestrant. (Estrogen receptor down-regulator, SERD), paclitaxel and docetaxel (Anti-tubulins), abemaciclib (Cyclin dependent kinase inhibitor, CDKI), and olaparib (PARP inhibitor) etc. (Fig. 1) [5, 6]. In advanced stage the breast cancer cells may metastasise to spread to distant organs such as lungs, bones, and brain [7,8]. The treatment of advanced stage breast cancer is very difficult and restricted due to chemo-therapy resistance [9]. Although several effective drugs have been developed, yet development of safe, effective and affordable anti-breast cancer drug is still a challenge to scientific fraternity around the globe.

Breast cancer is multifactorial in nature. Hence, it exhibits numerous targets due to genetic, epigenetic and transcriptional factors [4]. Among various targets, tubulin is considered an important target for anticancer drug development. α/β-Tubulin dimer polymerises to microtubules and then microtubules depolymerise to tubulin [10,11]. This conversion is always in a dynamic equilibrium. Any disturbance exerted on this equilibrium leads to cell cycle arrest and induces apoptosis [12]. Microtubule targeting agents are one of the most reliable anticancer drugs with clinical efficacy. Paclitaxel, docetaxel (microtubule stabilizers) and vincristine, vinblatine (microtubule destabilizers) are some of the

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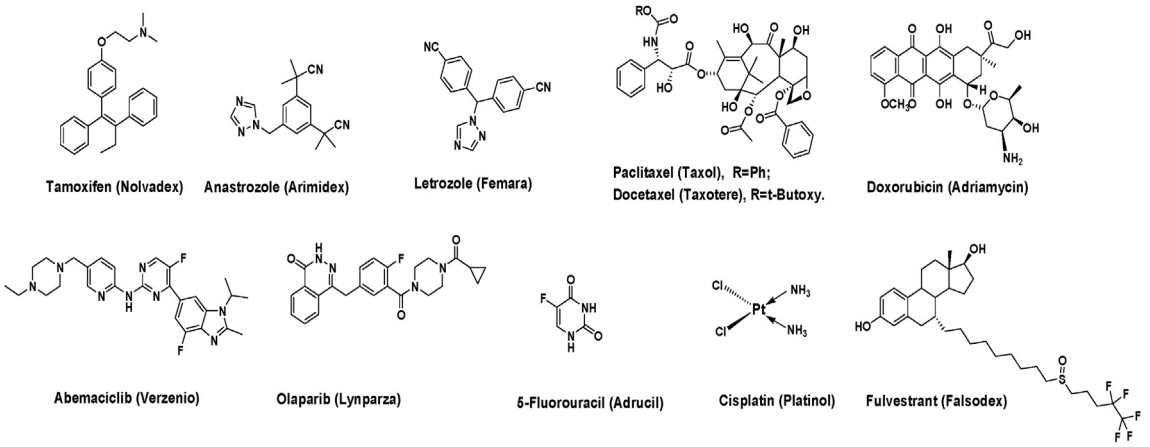


Fig. 1. Some of the clinical drugs for breast cancer.

clinical drugs of this class [13,14].

In the present study, we synthesized some naphthylstibenes and diarylnaphthylmethanes as possible antibreast cancer agents. We designed these pharmacophores (I & II) based on structure activity relationship of combretastatin A4 (CA4), a potent antitubulin agent [15–17]. According to SAR of CA4, two aryl rings must be separated by a linker group preferably in cis orientation, and one of the aryl rings must possess a 3,4,5-trimethoxy system to induce antitubulin effect [14,18.] This 3,4,5-trimethoxyphenyl fragment is also present is some of the naturally occurring antitubulin agents *i.e.* podophyllotoxin and colchicine. Further, we planned to have some aminoalkyl chains at one of the aryl rings to induce antiestrogenic effect. In hormone dependent breast cancer, there is a high level of estrogenicity in tumour cells. Based on this hypothesis, we synthesized thirty four diverse compounds as per prototypes-I & II compounds exhibited significant anticancer activity. Compound **50**, was the best representative of the series very close to our hypothesis. It was extensively evaluated for cancer biology and safety aspects.

2. Results

2.1. Chemistry

The synthetic strategy was as depicted in Scheme 1. Pyrogallol (**1**) was fully methylated with dimethyl sulphate in presence of anhydrous potassium carbonate to give 1,2,3-trimethylpyrogallol (**2**). Trimethoxybenzene **2**, underwent Friedel-Craft acylation on heating at 80 °C with 1-naphthoic acid in presence of polyphosphoric acid to yield an inseparable mixture of products i.e. trimethoxynaphthophenone (**3a**, 67%) and 3,4,-dimethoxy-2-hydroxynapnthophenone (**3b**, 14%). This mixture was as such again methylated with methyl iodide in presence of sodium hydride to get exclusively **3a**. Naphthophenone **3a** was diversified

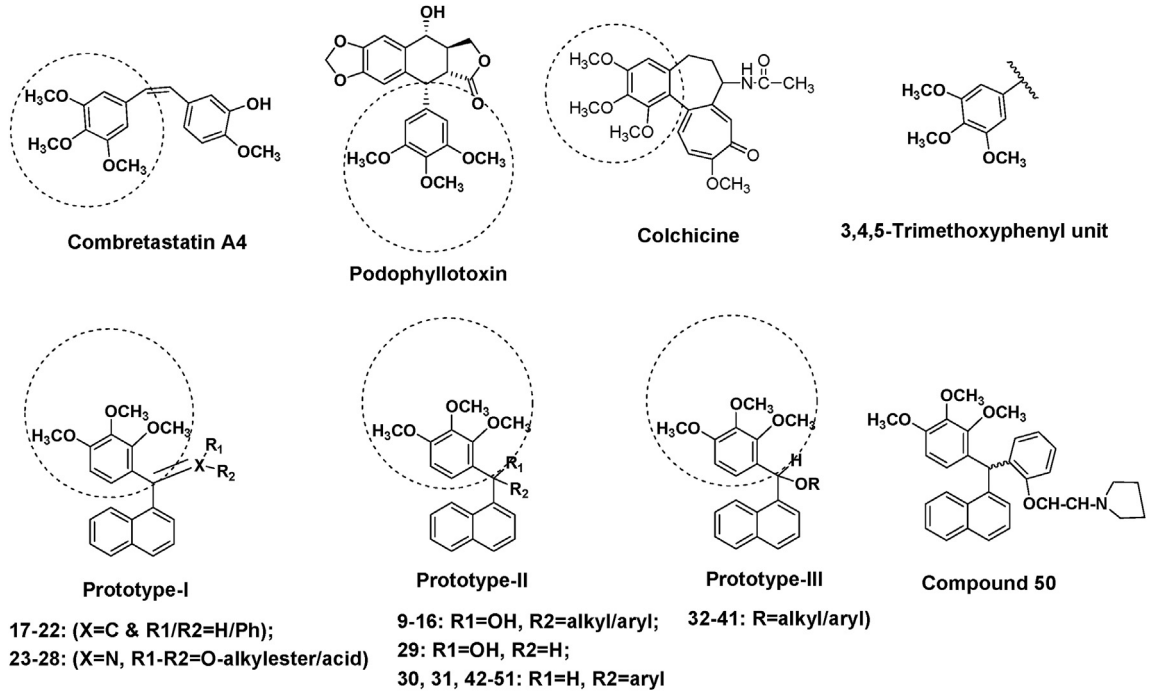
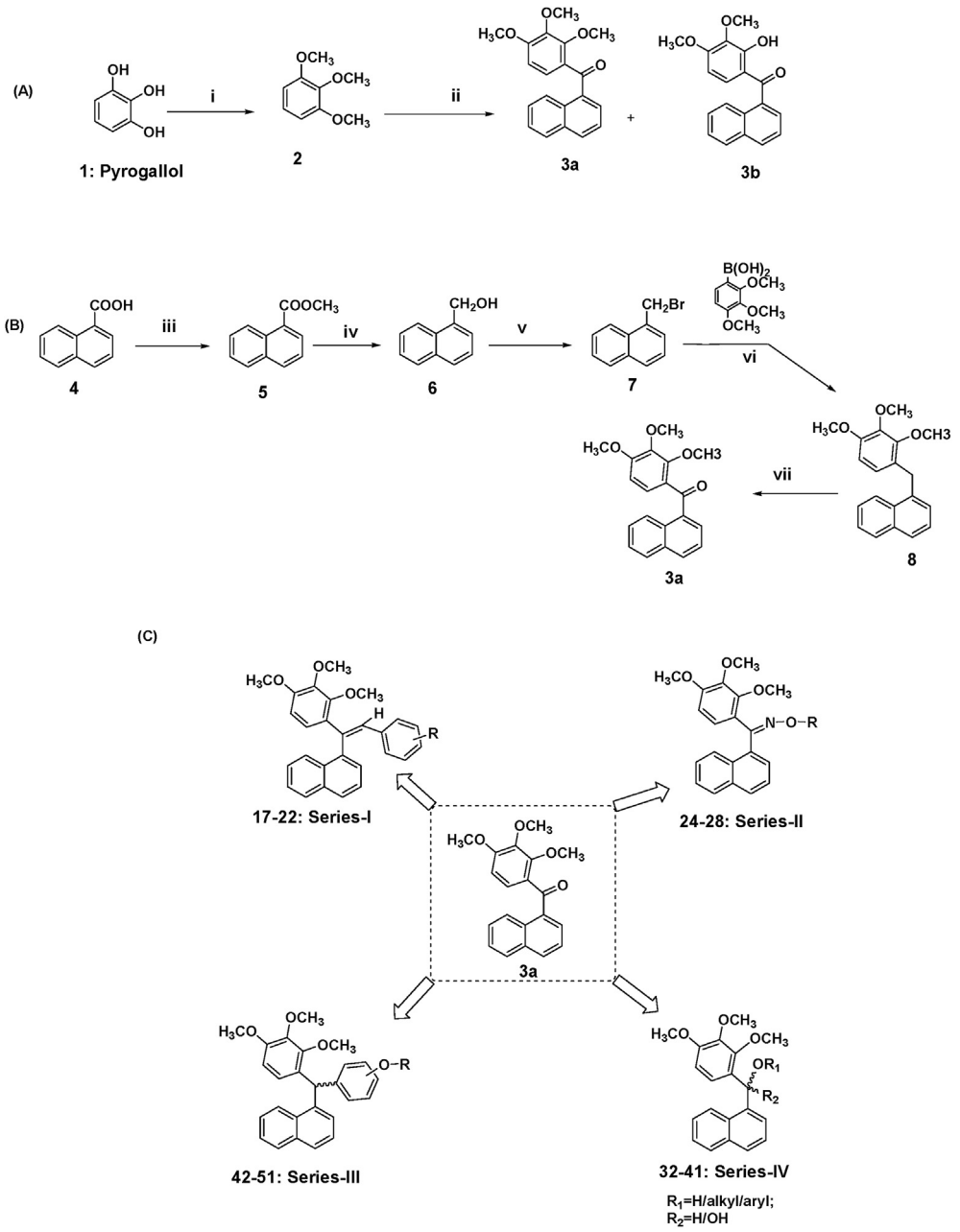


Fig. 2. Some of natural microtubule destabilizers, trimethoxyphenyl fragment and pharmacophores-I, II, & III.

towards the synthesis of four different pharmacophores I, II, IIIand IV. The yield of the product **3a** was very poor in three steps. Alternatively, acid catalysed methylation of 1-naphthoic acid was done to get the corresponding methyl ester **5**, which was reduced with lithium borohydride in dry THF to afford 1-naphthyl alcohol (**6**). Alcohol **6** was treated with phosphorus tribromide to get corresponding 1-bromomethylnaphthalene (**7**). Bromo derivative **7** underwent palladium chloride catalysed Suzuki coupling with 2,3,4-trimethoxyphenylboronic acid in presence of anhydrous potassium carbonate to afford 2,3,4-trimethoxybenzylnaphthalene (**8**). Compound **8** was oxidised with Collins reagent to get naphthophenone **3a** in very good yield.

Prototype-I (Scheme 1D): Naphthophenone **3a** underwent Grignard reaction with various Grignard reagents in dry



Scheme 1. i) Me_2SO_4, K_2CO_3, dry acetone, reflux, 3h, 85%; ii) PPA, 1-Naphthoic acid, 80 °C, 3h, **3a** (67%), **3b** (14%); iii) MeOH, Conc. H_2SO_4, 65 °C, 2h, 91%; iv) $LiBH_4$, dry THF, 4h, 60 °C, 86%; v) PBr_3, dry THF, 0–10 °C, 2h, 74%; vi) $PdCl_2.(PPh_3)_2$, K_2CO_3, DMF:H_2O(3:2), 80 °C, 4h, 51%; vii) Collins reagent (CrO_3 in DCM), pyridine, 0–10 °C (1h) then RT (28–30 °C), 9h, 56%.

viii) RMgX, diethylether, THF, RT (26–30 °C), 30–60min, 68–90%; ix) MeOH, Conc. HCl, reflux, 2h, 72–90%;

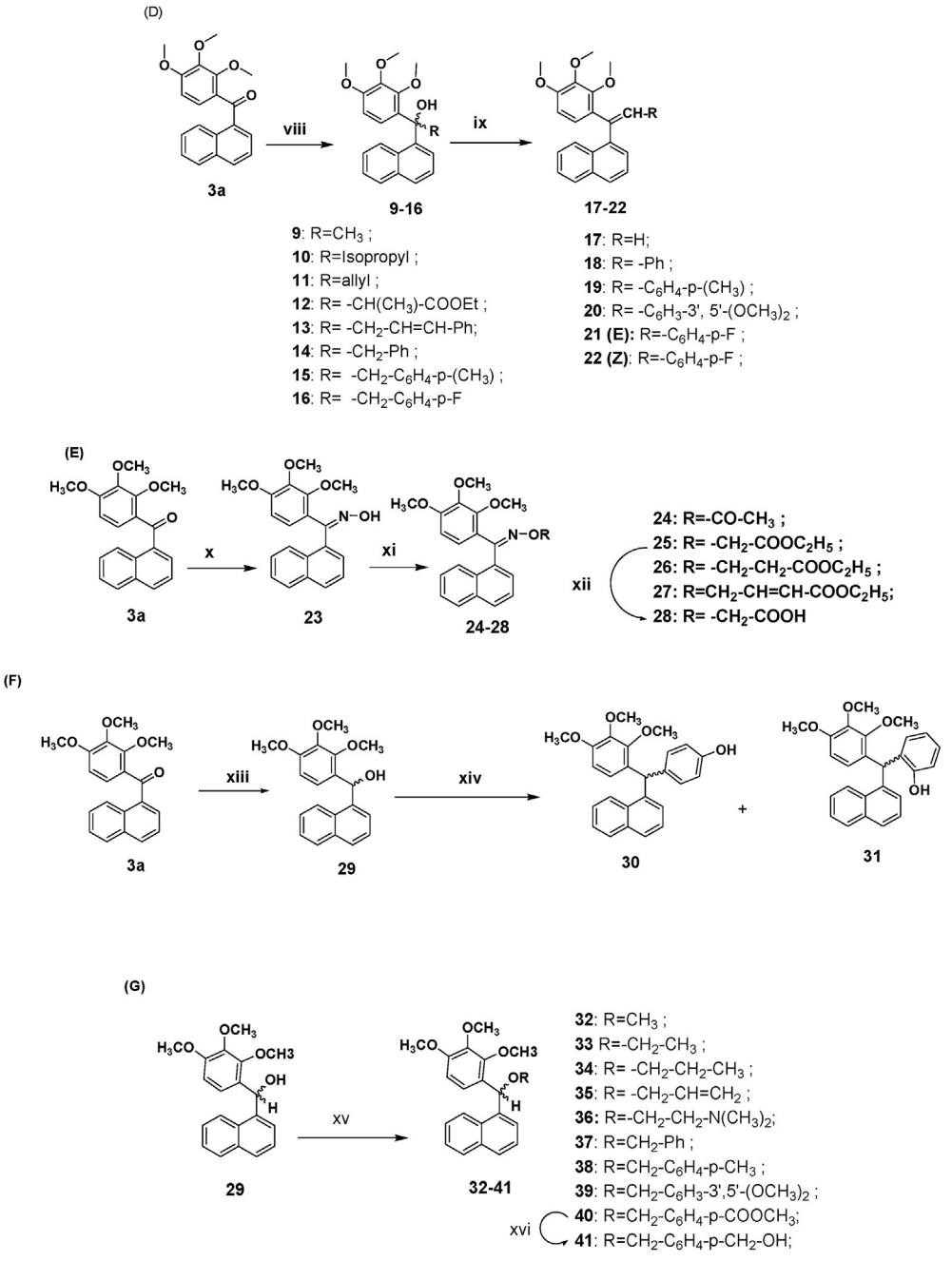
x) $NH_2OH{\cdot}HCl$, EtOH, DMAP, reflux, 18h, 87%; xi) For **24**: Ac_2O, DMAP, dry $CHCl_3$, RT, 2h, 86%, for **25**–**27**: ethylbromoester, dry THF, NaH, 65 °C, 2h, 86–92%; xii) 6% KOH in MeOH, reflux, 2h, 66%.

xiii) EtMgBr, diethyl ether, THF, RT (1h), reflux (3h), 86%; xiv) Phenol, dry benzene, Conc. HCl, 5–6h, **30**: 48%, **31**: 26%.

xv) NaH, dry THF, alkyl halide/aryl halide/benzylbromide, reflux, 1–2h, 63–86%; xvi) $LiBH_4$, THF, reflux, 83%.

xvii) NaH, dry THF, aminoalkylchain (HCl), 65 °C, 74–82%; xviii) Ethylbromoacetate, dry THF, NaH, 65 °C, 85%; xix) $LiBH_4$, dry THF, 50 °C, 3h, 85%; xx) 6% KOH in MeOH-H_2O (9:1), 50 °C, 72%;

xxi) NaH, aminoalkyl chain (HCl), dry THF, reflux, **50**: 87%, **51**: 81%.



Scheme 1. (*continued*).

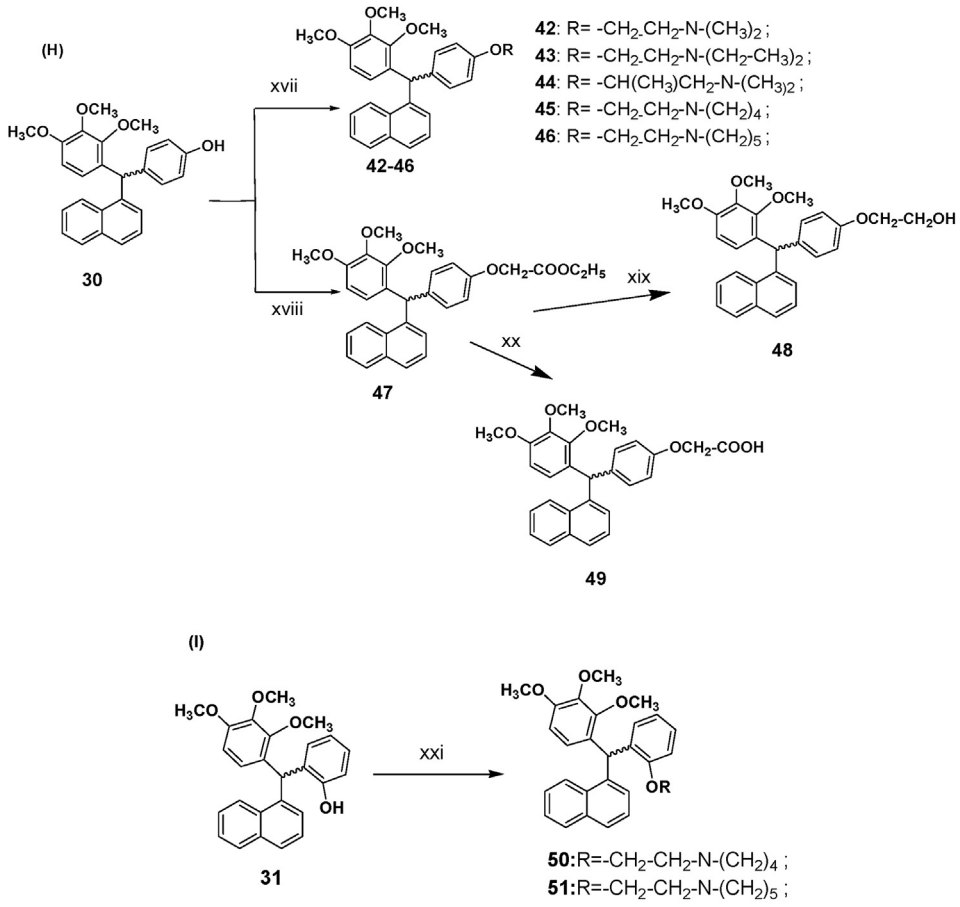
diethylether to get various naphthyl-methyl alcohols (**9**—**16**), which on dehydration with MeOH-HCl afforded the desired olefinic products (**17**—**22, Series-I**) in excellent yields.

Prototype-II (Scheme 1E) Further, naphthophenone **3a** was treated with hydroxylamine in presence of DMAP to get corresponding oxime **23**. Various fatty acid esters were hooked up at hydroxyl group of the oxime **23** to afford oxime esters (**24**—**27, Series-II**) and product **28** was obtained on hydrolysis of ester **25**.

Prototype-III (Scheme 1F): Naphthophenone **3a** was treated with ethyl magnesium bromide (EtMgBr, diethyl ether) in dry THF to get a Grignard product at carbonyl carbon. But, a reduced product i.e. 2,3,4-trimethoxyphenyl, 1′-naphthyl methanol (**29**) was achieved in excellent yield. Alcohol **29** on treatment with conc. hydrochloric acid in dry benzene underwent Friedel-Craft arylation with phenol to get a mixture of *para* and *ortho* substituted products **30** and **31** as diarylnaphthalenes.

Prototype-IV (Scheme 1G): Aryl-naphthylmethanol **29** was treated with diverse alkyl halides (MeI/EtBr/n-PrBr/Allyl bromide), aryl halides (BnBr/*p*-Me-BnBr/3,5-diMeO-BnBr/*p*-COOMe-BnBr/*p*-CH_2OHBnBr) to get corresponding ethers (**32**—**41, Series-III**) at alcoholic hydroxyl.

Further, diarylnaphthalenes **30** and **31** were modified by utilizing the *o*/*p*-phenolic hydroxyl group (Scheme 1H & 1I). Compound **30** was condensed with various aminoalkyl chains to get aliphatic amino chains at *p*-phenolic group (**42**—**46, Series-IV**). A fatty acid ester chain was attached to phenolic group to get ethyl



Scheme 1. (*continued*).

acetate chain at phenolic group (**47**). Ester **49** was reduced with lithium borohydride in dry THF to get corresponding alcohol **48** in good yield. Ester **49** was hydrolysed with 6% alkali to get free carboxylic acid derivative **49** in excellent yield. Similarly, *o*-phenolic group of compound **31** was hooked up with two amino alkyl chains to get corresponding *o*-aminoalkyl ethers **50** and **51** in excellent yields (Series-IV). All the intermediates and final products were confirmed by spectroscopy.

2.2. Purity profile of compound ***50***

Chromatographic conditions were optimised to get optimum separation. The peak integration was performed at λ_{max} (210 nm). The column eluent was monitored in PDA range (190–400 nm) for co-elution of possible impurities. The peak area and the retention time of compound **50** and other unknown peaks were accounted to define the purity. Under optimised chromatographic conditions, compound **50** was eluted at 4.295 min, while other impurities appeared at 3.331 min without any interference. As a reference methodology, the functions for peak purity analysis in the chromatographic data processing software (Empower®, 3 version 7.40, Waters, USA) were applied. The purity of compound **50** was 97.9%.

2.3. Biological evaluation

2.3.1. Cytotoxicity evaluation by sulphorhodamine assay

All the synthesized compounds of prototypes-I, II, III, and IV (**17**–**48**) were evaluated for antiproliferative activity against a panel of seven human cancer cell lines *i.e.* MCF-7 (hormone dependent breast cancer), MDA-MB-231 (breast cancer, triple negative), HCT-116 (Colon cancer), A549 (lung cancer), and THP-1 (acute leukemia) by employing sulphorhodamine assay (Table 1).

Among the four diverse series of compounds i.e. series I-IV, compounds of series I (**17**–**22**) and IV (**42**–**51**) exhibited significant antiproliferative activity against both MCF-7 and MDA-MB-231 breast cancer cell lines. While, series II compounds (**24**–**28**) showed efficacy only against MDA-MB-231 cells. Further, series III compounds were not much effective and only three compounds (**36**–**38**) exhibited significant activity against MCF-7 cell lines. It will be worth to mention that there was a distinct selective activity in series II compounds against MDA-MB-231 and series-III compounds against MCF-7 cell lines. Compound **18** of series I exhibited best cytotoxicity against MCF-7 while compound **23** from series II was the best against MDA-MB-231 cell line. Compound **50** possessing potent cytotoxicity against both type of breast cancer cell lines, was selected for detailed pharmacology.

In series IV (**42**–**51**), only the compounds with aminoalkyl

group were active (**42**–**46** & **50**, **51**). Compounds **47**–**49** without this group were found inactive. Among the active compounds, compounds bearing amino alkyl chain at *ortho* position (**50** & **51**) were more effective as compared to *para* substitution (**42**–**46**). The five member amino ring compound **50** was more effective as compared to six member amino ring compound **51**.

2.3.2. Soft agar colony formation assay

The cytotoxicity of compound **50** was further authenticated by Soft agar colony formation in MCF-7 cells, a well-established surrogate assay for tumorigenesis. This experiment estimates the anchorage-independent growth ability of cells which is considered as gold standard assay for cellular transformation *in-vitro* [19]. It gives an accurate quantitative measurement of antiproliferative activity [20].

Compound **50** inhibited growth formation of MCF-7 cells in concentration dependent manner (Table 2). At 6.25 μg/mL concentration 29.2% cells were killed, while at 50 μg/mL concentration, 87% MCF-7 cells were killed. It exhibited moderate cytotoxicity (IC_{50} = 11.23 μg/mL). However, this effect was less than the standard drug tamoxifen (IC_{50} = 1.31 μg/mL). The experiment confirmed the antiproliferative activity of compound **50** against MCF-7 cells.

2.3.3. Cell cycle analysis

Cell division cycle is an ordered process having series of events which ultimately yields to DNA duplication. Compound **50**, exhibited S phase arrest at its half IC_{50} (2.17 μM) and IC_{50} (4.33 μM) and induced apoptosis in MCF-7 cells (Table 3, Fig. 3). However, at higher concentration (double IC_{50}) S phase arrest was less, but apoptosis was enhanced significantly.

Interestingly, in cell cycle analysis in MDA-MB-231 cells, compound **50**, exerted mainly G2/M phase arrest at all the three concentrations (Half IC_{50}, IC_{50} and double IC_{50}). However, the induction of apoptosis was less up to IC_{50} concentration and it was significantly high at double IC_{50}. There was slight effect at G0/G1 phase.

Compound **50** behaved distinctly with both the cell lines in cell division cycle, which indicates independent mechanism in these cell lines for inducing cytotoxicity.

2.3.4. Apoptosis vs necrosis induction by **50** by Annexin V-FITC assay

Compound **50** substantially induced late apoptosis in MCF-7 cells at its half IC_{50} (2.17 μM) and IC_{50} (4.33 μM). There was no early apoptosis in the treated cells. However, it also induced necrosis at its IC_{50}. However, induction of necrosis was much less than the apoptosis in MCF-7 cells (Fig. 4a).

Surprisingly, compound **50** behaved differently with MDA-MB-231 (Fig. 4b). It induced both early and late apoptosis significantly. However, effect on late apoptosis was much higher than the early apoptosis. There was some increase in necrosis also. But the induction of apoptosis was much higher than necrosis. Nevertheless, induction of necrosis by compound **50** was much less than the control drug doxorubicin.

Table 1
In-vitro cytotoxicity and tubulin polymerisation inhibition of synthesized compounds.

S. no.	Compd. No.	Cytotoxicity IC_{50} (μM)[a]					Tubulin polymerisation inhibition IC_{50} (μM)
		MCF-7	MDA-MB-231	HCT-116	A549	THP-1	
1	**11**	–	–	–	10.66	–	ND
2	**12**	–	–	–	**4.00**	–	ND
3	**13**	10.19	12.76	–	17.60	19.42	ND
4	**14**	10.63	**4.78**	–	–	12.38	6.02
5	**15**	–	–	–	18.71	–	ND
6	**16**	**7.56**	**5.44**	–	5.94	5.86	ND
7	**18**	11.69	**4.66**	–	19.81	12.54	9.03
8	**19**	–	**8.31**	–	19.63	–	ND
9	**21**	17.72	16.16	–	**–**	**9.08**	ND
10	**22**	10.53	–	–	18.64	18.85	ND
11	**23**	–	**2.50**	**5.88**	13.13	–	**5.58**
12	**24**	–	17.00	**4.98**	17.23	–	ND
13	**25**	–	16.36	**3.36**	6.72	–	ND
14	**26**	–	15.15	10.03	19.63	–	ND
15	**27**	–	–	–	14.01	–	ND
16	**28**	–	–	**4.98**	17.23	–	ND
17	**30**	17.54	17.35	15.97	16.39	9.45	ND
18	**31**	16.63	17.74	–	13.58	–	ND
19	**35**	–	–	–	**9.71**	–	ND
20	**36**	11.14	–	–	17.90	–	ND
21	**37**	14.07	–	–	13.51	–	ND
22	**38**	10.71	–	–	15.73	–	ND
23	**39**	–	–	–	17.50	–	ND
24	**40**	–	–	–	11.99	–	ND
25	**42**	15.16	14.29	**3.30**	10.21	7.95	ND
26	**43**	15.49	15.60	**8.34**	11 .47	13.88	ND
27	**44**	13.08	**4.34**	**8.41**	11.36	15.01	ND
28	**45**	15.27	14.39	10.83	15.78	**5.40**	ND
29	**46**	15.44	15.16	11.94	15.00	16.58	ND
30	**48**	–	–	–	–	16.64	ND
31	**49**	–	–	**5.02**	16.44	–	ND
32	**50**	**4.33**	**5.21**	**2.03**	**5.85**	**12.57**	**5.65**
33	**51**	12.42	**7.18**	**4.63**	9.86	19.13	ND
34	Tamoxifen	**11.55**	–	45.0	10.08	11.91	ND
35	Podophyllotoxin	10.86	5.73	–	7.34	19.55	**0.72**
36	Doxorubicin	**3.89**	**5.12**	ND	**0.63**	ND	ND

[a] IC_{50} > 20 μM was considered as inactive, — means inactive.

Table 2
Effect of compound **50** on Colony Formation of MCF-7 cells.

Condition	Concentration (μg/mL)	Avg. % live cells	MCF-7 (% dead cells)	MCF-7 IC_{50} (μg/mL)
Control	—————	100	———	————
Compound **50**	6.25	70.77	29.23 ± 3.39	11.23
	12.5	47.20	52.80 ± 1.22	
	25	20.64	79.36 ± 2.28	
	50	12.98	87.02 ± 1.50	
Tamoxifen	1.07	70.50	29.50 ± 2.18	1.31
	5.39	42.20	57.80 ± 1.03	
	26.95	5.69	94.31 ± 0.43	

No. of cells = 12369 ± 836; **p < 0.01 (Dunnett test).

The induction of apoptosis effectively in both MCF-7 and MDA-MB-231 cells, indicate that the cytotoxic effect of compound **50** is *via* a systematic mechanism.

2.3.5. Molecular docking studies

Molecular docking studies of both the enantiomers of compound **50** i.e. **50R** and **50S** for the interaction with β-tubulin showed good affinity and comparable binding energy of compound **50R** (−8.0 kcal/mol), **50S** (−8.8 kcal/mol) with standard destabilizers colchicine (−8.5 kcal/mol) and podophyllotoxin (−7.2 kcal/mol) (Table 4). Docked view showed that there were seven residual amino acids (LEU B:248, LEU B:255, ASN B:258, MET B:259, ALA B:316, LYS B:352, and ALA B:354) common to all three within 4 Å. It clearly indicates that all the four ligands occupied the same binding pocket of β-tubulin (Fig. 5).

2.3.6. Tubulin polymerisation inhibition activity

The effect of potent compounds **14**, **18**, **23**, and **50** was determined on polymerisation of tubulin protein. All these compounds exhibited moderate to weak antitubulin effect (IC_{50} = 5.58–9.44 μM) by inhibiting polymerisation of tubulin to microtubules (Table 1). However, the standard inhibitor, podophyllotoxin had potential antitubulin effect (IC_{50} = 0.78 μM) (See Table 1).

2.3.7. DNA-topoisomerase-II inhibition activity

In DNA topoisomerase inhibition activity in MCF-7 cells, compound **50** showed concentration dependent effect on enzyme inhibition (Table 5). The maximum inhibition of 25.89% was observed at 17.32 μM concentration (4*IC_{50}). The standard inhibitor etoposide caused 47.66% inhibition at 10.76 μM (4*IC_{50}) while podophyllotoxin, a known poor inhibitor of Topo-II exhibited 26.86% inhibition at 50 μM concentration.

2.3.8. Safety studies

Compound **50** was further evaluated for safety studies by acute oral toxicity at three different oral doses *i.e.* 5 mg/kg, 50 mg/kg, and 300 mg/kg in Swiss-albino mice. There were no observational changes, morbidity and mortality throughout the experimental period up to the dose level of 300 mg/kg body weight. Blood and serum samples upon analysis showed non-significant changes in all the parameters studied like haematological (total haemoglobin level, differential leucocyte count), lipid (serum total cholesterol, triglycerides), Kidney function (creatinine level), Liver function (SGOT, SGPT, and ALKP) activity (Table 6 and Fig. 7). Animals on gross pathological study showed no changes in any of the organs studied including their absolute and relative weights (Fig. 6). Therefore, the experiment showed that compound **50** is well tolerated by the Swiss albino mice up to the dose level of 300 mg/kg body weight as a single acute oral dose. However, the compound should be evaluated for sub-acute and chronic experiments to look for any adverse effect on repeated exposure to the compound **50** and changes in biochemical parameters for its future development [21].

3. Discussion

Breast cancer is a complex and heterogeneous disease. On an average 65–70% of breast cancer cases are hormone dependent, 15–20% cases are hormone independent and 10–15% are metastatic breast cancer. Among the various treatment options chemotherapy is one of the most effective strategy to tackle the disease. However, at advanced stages metastatic breast cancer is difficult to treat which is one of the greatest challenges encountered by the clinicians. Drug resistance is another problem which is frequently faced due to prolonged treatments.

During Grignard reaction on naphthophenone derivative **3a**, no adduct was formed rather ketone was reduced to alcohol **29**. It was the case only when there was a β-hydrogen available in Grignard reagent (RMgX). In case of MeMgX and BnMgBr only Grignard product was obtained. However, EtMgBr and PrMgBr yielded exclusively reduced products. In **3a**, carbonyl group is flanked with phenyl and naphthyl ring and presence of β-hydrogen in Grignard reagent resulted in reduction of ketone to alcohol and no Grignard adduct was formed. We also tried some more naphthophenones and benzophenones to give similar results (see Fig. 1).

Compound **50** was designed as microtubule destabilizer applying Fragment Based Drug Discovery (FBDD) approach [22]. In FBDD, a fragment is identified that has quality interactions with the target protein. When this fragment is incorporated at an appropriate position in a pharmacophore, it may induce desired biological response [23]. We incorporated a trimethoxyphenyl fragment

Table 3
Cell cycle analysis of compound **50** in MCF-7 and MDA-MB-231 cells.

Compd.	Conc. (μM)	Average %Population of MCF-7 Cells				Conc. (μM)	Average %Population of MDA-MB-231 Cells			
		Apoptosis	G0/G1	S	G2/M		Apoptosis	G0/G1	S	G2/M
Control	–	2.1	74.5	6.9	15.2	–	2.6 ± 0.28	77.7 ± 0.28	11.35 ± 0.07	8.35 ± 0.07
Compd. **50**	2.17 (half IC_{50})	2.2	74.6	10.9	12.1	2.60 (half IC_{50})	2.7 ± 0.21	80.2 ± 0.14	6.75 ± 0.21	10.35 ± 0.21
	4.33 (IC_{50})	4.7	67.4	13.9	13.4	5.21 (IC_{50})	2.8 ± 0.14	79.1 ± 0.14	7.9 ± 0.14	10.2 ± 0.14
	8.66 (2xIC_{50})	11.4	66.5	9.2	12.8	10.42 (2xIC_{50})	16.7 ± 0.28	56.9 ± 0.00	11.35 ± 0.35	15.05 ± 0.07

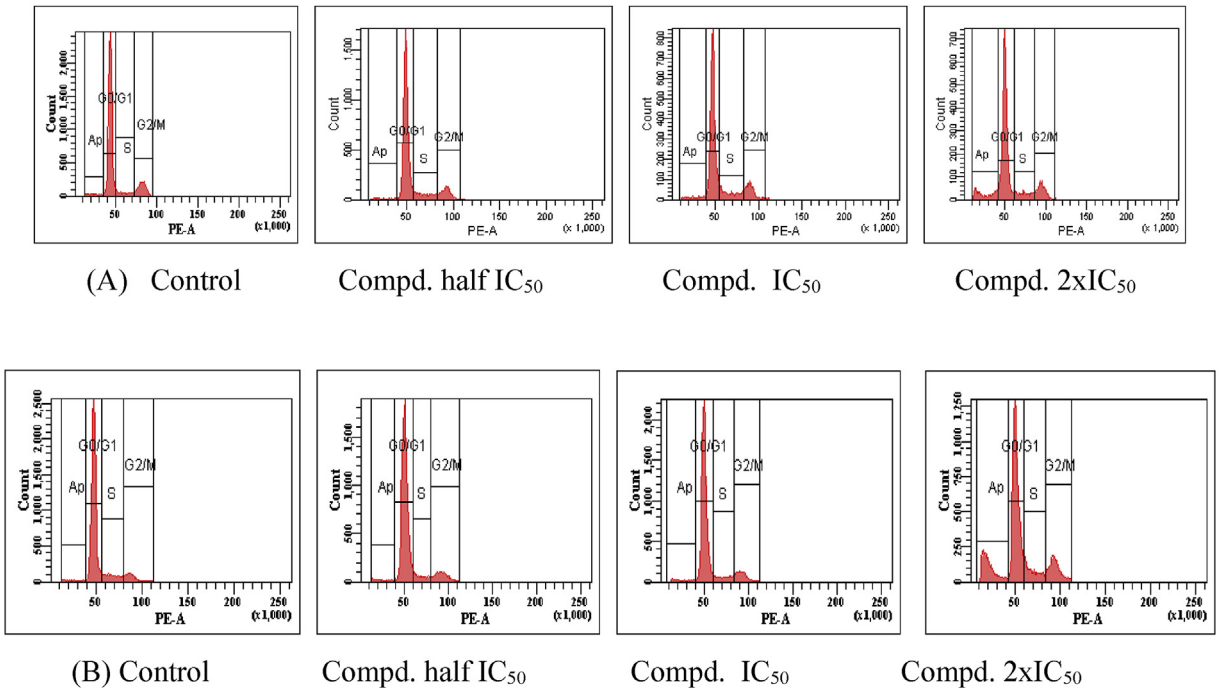


Fig. 3. Effect of compound **50** on cell division cycle; (A) in MCF-7 cells; (B) in MDA-MB-231 cells.

in our all the three pharmacophores (Fig. 2) which are supposed to have quality interactions with β-tubulin and induces antitubulin effect [14,24]. Further, triarylmethanes show affinity for estrogen receptor (ER). It is well established that ERα is over-expressed in hormone dependent breast cancers [25–27]. Due to higher level of estrogenicity in breast tumour cells, there is excessive proliferation. Introduction of an aminoalkyl chain at an appropriate position of estrogen receptor ligands, exhibits estrogen antagonistic activity, which might suppress the proliferation of MCF-7 cells [28–30]. Therefore, compound **50** possessed a pyrrolidinoethyl chain at phenolic hydroxyl (see Fig. 3, Fig. 4a and 4b).

Compound **50** exhibited potential cytotoxicity against both

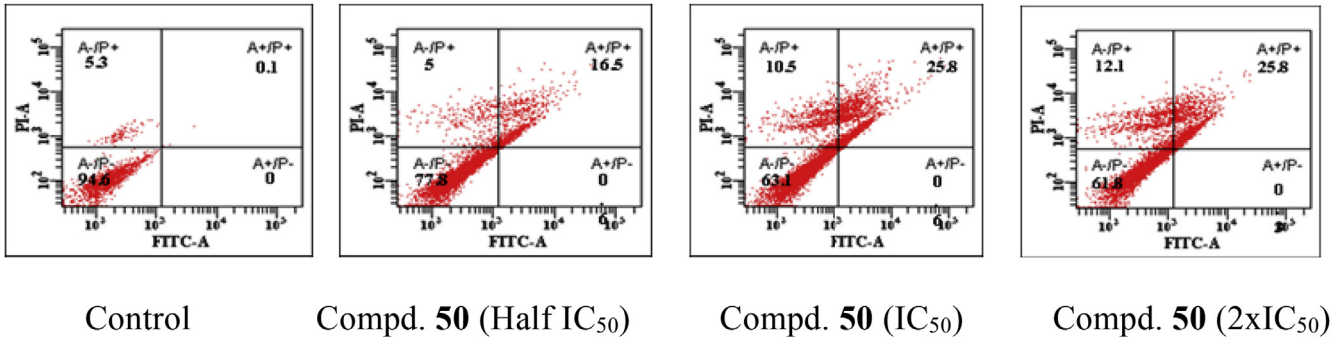


Fig. 4a. Induction of late apoptosis and necrosis by compound **50** in MCF-7 cells by Annexin V-FITC assay.

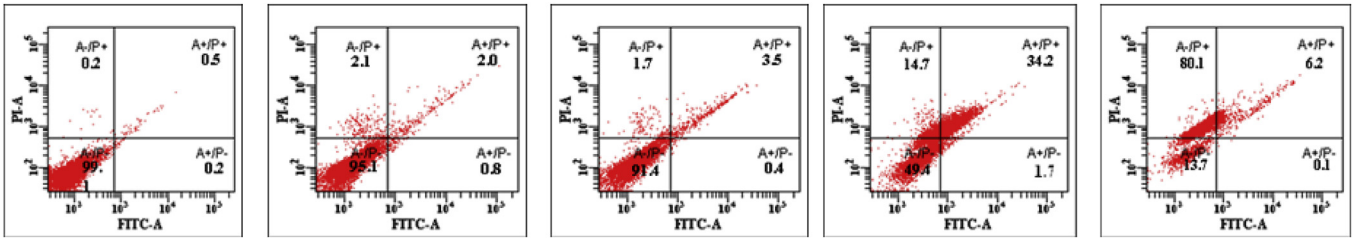


Fig. 4b. Induction of late apoptosis and necrosis by compound **50** in MDA-MB-231 cells by Annexin V-FITC assay.

Table 4
Interaction studies of compounds **50R** and **50S**, colchicine and podophyllotoxin with β-tubulin (PDB ID: 402B).

S. No.	Compounds	Docking Energy	Interacting Amino Acids with in region of 4 Å
1.	Compound **50R**	−8.0	VAL B: 238, CYS B:241, LEU B: 242, LEU B:248, ASN B: 249, ALA B:250, ASP B: 251, LEU B:252, LYS B: 254, LEU B:255, ASN B:258, MET B:259, THR B:314, VAL B:315, ALA B:316,ALA B: 317,ILE B:318, **LYS B:352**,THR B:353, ALA B:354,
2.	Compound **50S**	−8.8	CYS B:241, GLN B:247, LEU B:248, ALA B:250, ASP B:251, LYS B:254, LEU B:255, ASN B:258, MET B:259, THR B:314, VAL B:315, ALA B:316, ALA B:317, ILE B:318, ASN B:349, ASN B:350, **LYS B:352,** ALA B:354
3.	Colchicine	−8.5	VAL B:238, CYS B:241, LEU B:242, LEU B:248, ALA B:250, ASP B:251, LYS B:254, LEU B:255, ASN B:258, MET B:259, THR B:314, VAL B:315, ALA B:316, ALA B:317, ILE B:318, ASN B:350, LYS B:352, ALA B:354, ILE B:378,
4.	Podophyllotoxin	−7.2	CYS B:241, LEU B:242, **LEU B:248**, ALA B:250, ASP B:251, LYS B:254, LEU B:255, ASN B:258, MET B:259, ALA B:316, ILE B:318, LYS B:352, THR B:353, ALA B:354

Table 5
DNA-Topoisomerase-II inhibition activity of compound **50**.

S. No.	Compound	Concentration (μM)	% Inhibition of Topo-II
1.	Compound **50**	4.33 (IC_{50})	0.22
2.	Compound **50**	8.66 (2*IC_{50})	13.68
3.	Compound **50**	17.32 (4*IC_{50})	25.89
4.	Podophyllotoxin[a]	120	26.86
5.	Etoposide	10.76 (4*IC_{50})	47.66

[a] Podophyllotoxin is strong antitubulin but weak topo-II inhibitor.

MCF-7 and MDA-MB-231 cells. Interestingly, it had distinct action on these breast cancer cell lines. In cell cycle analysis, it induced apoptosis in both the cell lines. However, in MCF-7 it induced S phase arrest while, in MDA-MB-231 cells it caused G2/M phase arrest. During the S-phase the entire DNA content of the nucleus is replicated completely and precisely [31]. Induction of cell cycle arrest in MCF-7 cells at S phase indicates the inhibition of any of the cell cycle regulator or enzyme involved in DNA duplication at S phase. Compound **50** exhibited DNA-Topoisomerase-II inhibition activity which is actively involved in S phase of cell cycle process. Topoisomerase-II alters DNA topology and plays roles in the replication, transcription and segregation [32]. Inhibition of this enzyme by several anticancer drugs like etoposide and teniposide etc. lead to Topo-II mediated chromosome DNA breakage and cancer cell death.

Contrarily, in MDA-MB-231 cells, G2/M phase was arrested which might be due to antitubulin effect of compound **50**. Microtubule dynamics plays a crucial role in mitosis process during the G2/M phase of cell division cycle. Modulation of tubulin-microtubules dynamics is one of the most effective targets for cancer chemotherapeutics [10,14].

Molecular docking studies showed that both the enantiomers i.e. compounds **50R**, **50S**, podophyllotoxin, and colchicine occupied the same binding pocket at β-tubulin. There were seven residual amino acids common to these three ligands. Both the enantiomers differed slightly for their binding energies with β-tubulin. Isomer **50S** had better affinity as compared to **50R**. However, both the isomers possessed ALA β:316 and ILE β:318 amino acids, which are considered to be crucial for inducing antitubulin effect by the 3,4,5-trimethoxyphenyl fragment [14].

Compound **50** was safe and well tolerated by Swiss albino mice up to 300 mg/kg dose in acute oral toxicity. However, there were some non-significant changes in some of the parameters like increase in bilirubin and cholesterol levels and lowering in total WBC count in the group of animals treated with the test drug at 300 mg/kg. It is quite possible that a potent cytotoxic compound given through oral route might have such effects. However, sub-acute and chronic experiments with the compound **50** need to be carried out to look for any adverse effect on prolonged exposure of it. Notably, safety is an essential aspect in the development of a drug candidate for a particular pharmacological activity [33]. Nowadays, it is denoted with a unique terminology, 'Pharmacovigilance' [34]. Acute oral and sub-acute oral toxicity are most frequent experiments used to assess the preliminary toxicity of the compounds [35].

4. Conclusion

In summary, we synthesized several compounds based on three designed pharmacophores. Eight analogues exhibited potential antiproliferative activity against breast cancer cell lines. Compound **50** exhibited potential anticancer activity against both the cell lines. It exerted cell cycle arrest in cancer cells and induced apoptosis. In mechanistic studies, it was found to be potent DNA topoisomerase-II inhibitor and moderate tubulin polymerisation inhibitor. Compound **50** was non-toxic and safe up to 300 mg/kg dose in experimental mice. Compound **50**, a triarylmethane is a potent anti-

Table 6
Acute oral toxicity of compound **50** at 5, 50 and 300 mg/kg in Swiss albino mice. (Mean ± SE; n = 6; *, $P < 0.05$ compared to control).

Parameters		Dose of compd. 50 at mg/kg body weight as a single oral dose			
		Control	5 mg/kg	50 mg/kg	300 mg/kg
Body weight (gm)		31.62 ± 1.64	30.58 ± 1.67	30.44 ± 0.94	31.03 ± 0.97
Haematological Profile	Haemoglobin (gm/dL)	12.05 ± 0.88	11.35 ± 0.97	9.96 ± 0.88	10.13 ± 0.44
	RBC (million/mm^3)	10.56 ± 1.22	13.06 ± 1.58	9.84 ± 0.89	11.64 ± 1.67
	WBC(thousands/mm^3)	17.06 ± 1.33	11.11 ± 1.09	13.46 ± 1.59	7.19 ± 1.14*
Liver Function Test	ALP (U/L)	195.80 ± 12.06	198.57 ± 28.63	182.40 ± 21.32	137.70 ± 11.23
	SGOT (U/L)	22.12 ± 1.91	27.32 ± 2.95	25.42 ± 2.56	25.61 ± 3.95
	SGPT (U/L)	21.81 ± 2.10	21.18 ± 1.21	19.12 ± 1.96	22.34 ± 1.33
	Albumin (g/dL)	0.56 ± 0.02	0.65 ± 0.04	0.57 ± 0.05	0.54 ± 0.04
	Total Bilirubin (mg/dL)	0.62 ± 0.05	0.66 ± 0.08	0.62 ± 0.07	1.01 ± 0.08*
	Serum Protein (mg/ml)	2.83 ± 0.09	2.27 ± 0.08	2.52 ± 0.15	2.87 ± 0.16
Kidney Function Test	Creatinine (mg/dL)	0.27 ± 0.02	0.22 ± 0.05	0.34 ± 0.02	0.20 ± 0.05
Lipid Profile	Triglycerides (mg/dL)	78.34 ± 2.99	77.24 ± 6.56	94.42 ± 9.56	106.09 ± 8.86
	Cholesterol (mg/dL)	103.62 ± 7.45	111.17 ± 5.02	100.11 ± 12.23	120.54 ± 6.33

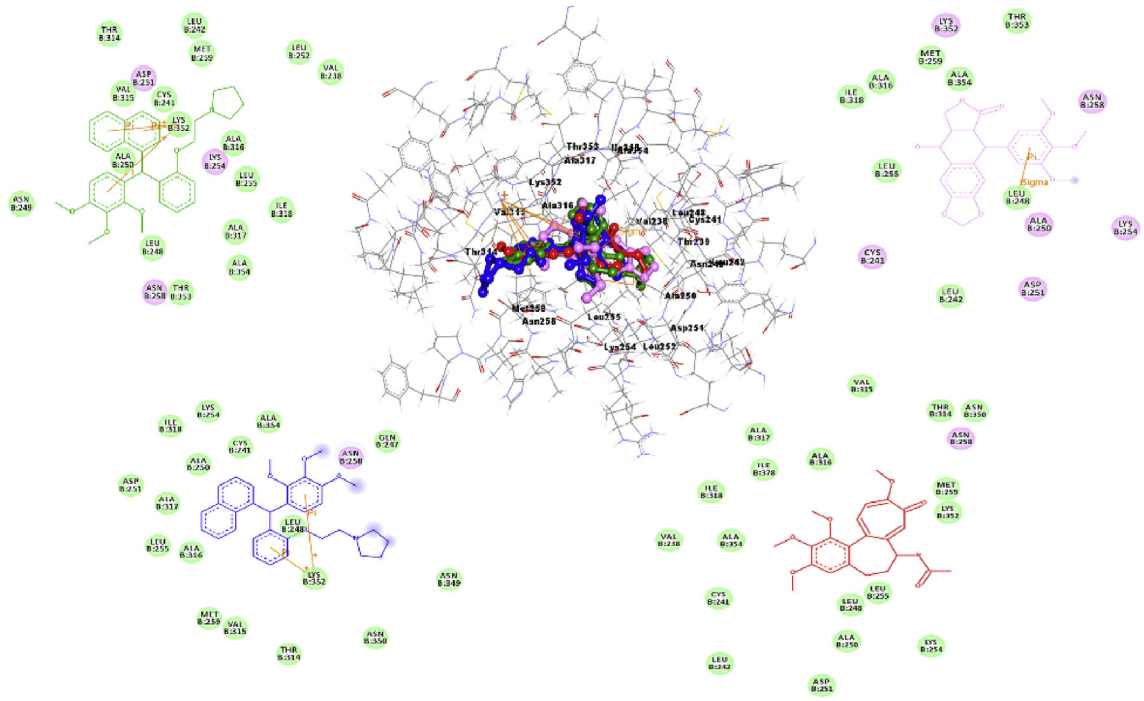


Fig. 5. The molecular docking pose showing the binding of compounds represented in different colours as mentioned (colchicine (red), Compd. **50R** (blue),Compd. **50S** (green) and Podophyllotoxin (pink)) with the Tubulin-Colchicine complex PDB ID: 4O2B. The 2D representation showing the interacting residues within the docking region of 4 Å. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

breast cancer agent. Its further optimization is underway for better efficacy.

5. Experimental

5.1. Chemical synthesis

5.1.1. Synthesis of 2,3,4-trimethoxyphenyl-naphthalen-1-yl-methanone (*3a*)

1-Naphthoic acid (2.06g, 12 mmol) and 1,2,3-trimethoxybenzene (1.68g, 10 mmol) were mixed well with polyphosphoric acid (6g). The reaction mixture was heated at 80 °C for 3h. Reaction mixture was cooled to room temperature and poured into crushed ice, extracted with ethyl acetate (60mLx3). The organic layer was washed with water (30mLx2), dried over anhydrous sodium sulphate and dried *in-vacuo*. The residue obtained was purified through column chromatography over silica gel eluting with hexane-ethyl acetate to get compound **3a** as amorphous solid. Further elution yielded a mono demethylated product of **3a** i.e. 3,4-dimethoxy-2-hydroxynaphthophenone (**3b**) as minor side product.

3a:Yield = 67%. White crystalline Mp = 75–77 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.49 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.71–6.73 (d, 1H, CH, aromatic, J = 9.0 Hz), 7.35–7.36 (d, 1H, CH, aromatic, J = 9.0 Hz), 7.42–7.56 (m, 4H, 4xCH, aromatic), 7.88–7.95 (m, 2H, 2xCH, aromatic), 8.36–8.37 (dd, 1H, CH, aromatic, J = 2.5, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ56.15, 60.86, 61.39, 106.80, 124.32, 125.68, 126.30, 126.39, 127.37, 127.71, 128.09, 128.36, 130.68, 131.52, 133.73, 138.20, 142.24, 153.84, 157.22, 196.86; HRMS (MeOH): m/z $[M+H]^+$ calcd for $C_{20}H_{19}O_4$, 323.1283, found 323.1283.

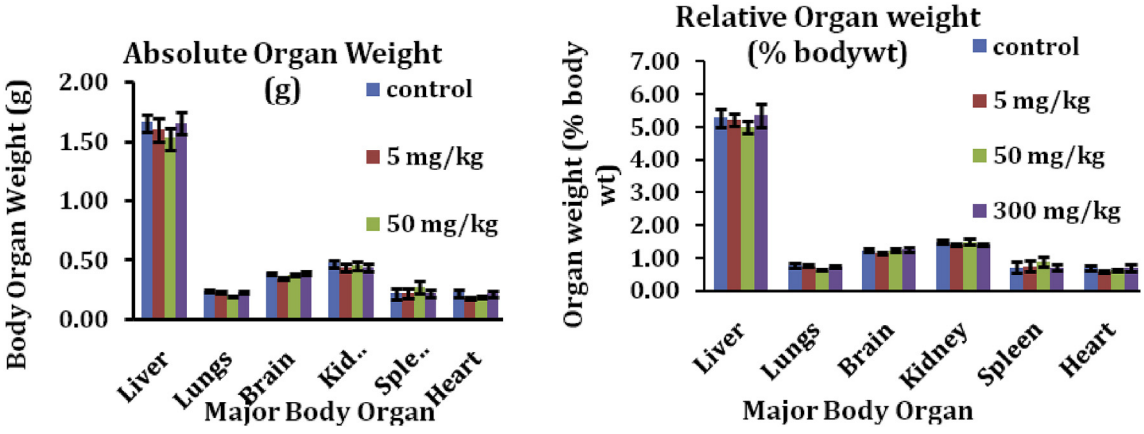


Fig. 6. Effect of compound **50** as a single acute oral dose at 5, 50, 300 and 1000 mg/kg on absolute and relative organ weight in Swiss albino mice.

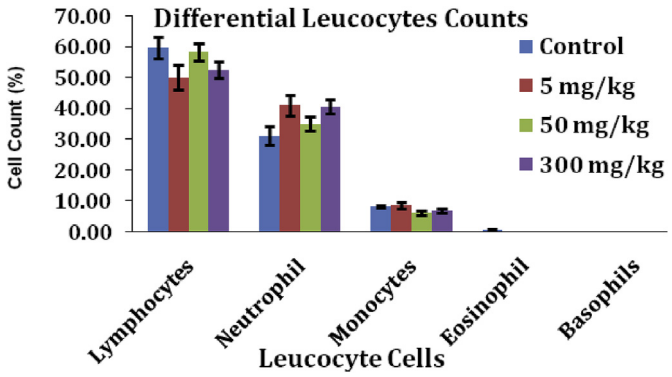


Fig. 7. Effect of compd. **50** as a single acute oral dose at 5, 50, 300 and 1000 mg/kg body weight on differential leucocytes counts in Swiss albino mice.

2-Hydroxy-3,4-dimethoxyphenyl-naphthlen-1-yl-*methanone* (***3b***)¶*:* Yield = 14%. Yellowish viscous,^{1}H NMR ($CDCl_3$, 500 MHz): δ3.89 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.32–6.34 (d, 1H, CH, aromatic, *J* = 9.5 Hz), 7.05–7.07 (d, 1H, CH, aromatic, *J* = 9.0 Hz), 7.47–7.54 (m, 4H, 4xCH, aromatic), 7.89–7.92 (m, 2H, 2xCH, aromatic), 7.97–7.99 (d, 1H, CH, aromatic, *J* = 8.0 Hz), 12.66 (s, 1H, OH, exchangeable); ^{13}C NMR ($CDCl_3$, 125 MHz): δ56.17, 60.80, 102.98, 116.04, 125.39, 126.16, 126.56, 127.19, 128.42, 128.63, 130.40, 130.57, 130.65, 133.58, 135.84, 136.59, 157.93, 159.08, 202.37; Electrospray mass for $C_{19}H_{16}O_4$ (MeOH)- 309 $[M+H]^+$, 331 $[M+Na]^+$; 347 $[M+K]^+$, 307 $[M-H]^-$.

*5.1.2. Alternative synthesis of compound **3a***

In a cool mixture of dry pyridine and dichloromethane (2:1, 15 mL), chromium trioxide (300 mg, 3 mmol) was stirred. To this compound **8** (200 mg, 0.61 mmol) was added and reaction mixture was stirred for an hour with cooling after that at RT for 8h. On completion 1 mL methanol was added and stirred for 10 min to decompose excess of CrO_3. Reaction mixture was evaporated and residue was taken in ethyl acetate (20 mL), washed with water (20 mL), organic layer dried over anhydrous sodium sulphate and evaporated *in vacuo*. Residue thus obtained was purified through silica gel column and eluted with ethyl acetate-hexane to get desired product **3a** at 12% EA-hexane as amorphous solid.

*5.1.3. Synthesis of 2,3,4-trimethoxyphenyl-1-naphthylmethane (**8**)*

To a stirred solution of compound **7** (241 mg, 1.4 mmol) in DMF-H_2O (3:2, 10 mL), bis triphenylphosphine palladium chloride (35 mg, 0.05 mmol) and potassium carbonate (345 mg, 2.5 mmol) were added. To this reaction mixture 2,3,4-trimethoxyphenylboronic acid (212 mg, 1 mmol) was added and the reaction mixture was refluxed for 4h. Reaction mixture was diluted with water (10 mL), extracted with ethyl acetate (10mLx3), and washed with water. Organic layer was dried over anhydrous sodium sulphate, evaporated *in-vacuo* to get a residue which was charged over silica gel column and eluted with ethyl acetate-hexane to get the desired product **8**.

8: Yield = 51%; Mp = 106–108 °C, white amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ3.86–3.95(s, 9H, 3xOCH_3), 4.42 (s, 2H, CH_2), 6.52–6.55 (d, 1H, CH, aromatic, *J* = 8.7Hz), 6.61–6.64 (d, 1H, CH, aromatic, *J* = 8.4Hz), 7.25–7.17 (d, 1H, CH, aromatic, *J* = 6.6Hz), 7.37–7.51 (m, 3H, 3xCH, aromatic), 7.75–7.78 (d, 1H, CH, aromatic, *J* = 8.1Hz), 7.87–7.90 (dd, 1H, CH, aromatic, *J* = 7.5, 3.3 Hz), 8.06–8.09 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ32.70, 56.38, 61.23, 61.29, 107.72, 124.65, 125.60, 126.30, 127.31, 127.33, 128.29, 129.04, 132.65, 134.30, 137.41, 142.76, 152.17, 152.67; Electrospray mass for $C_{20}H_{20}O_3$ (MeOH)- 309$[M+H]^+$, 331$[M+Na]^+$; 347 $[M+K]^+$.

*5.1.4. General procedure for the syntheses of compounds **9**–**16** and **29** by Grignard reaction*

*Synthesis of 1-Naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-ethanol (**9**):* Activated and dry magnesium turning (150g, 6.3 mmol) and methyl iodide (0.4 mL, 912 mg, 6.42 mmol) were stirred in dry diethyl ether (15 mL). A small crystal of iodine was added to initial the reaction. On disappearance of magnesium, compound **3a** (200 mg, 0.63 mmol), dissolved in 2 mL THF) was added dropwise and stirred at RT for 30 min. Solvents were evaporated and residue was diluted with water (10 mL), and extracted with ethyl acetate (3x15mL). Organic layer was dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to get a residue. Residue was charged on a silica gel column and eluted with hexane-ethyl acetate to get desired product at 3% ethyl acetate-hexane as viscous oil.

9: Yield = 87%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.08 (s, 3H, CH_3), 3.09 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.41 (s, 1H, exchangeable, OH), 6.67–6.69 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 7.18–7.19 (d, 1H, CH, aromatic, *J* = 9.0 Hz), 7.28–7.44 (m, 3H, 3xCH, aromatic), 7.64–7.65 (d, 1H, CH, aromatic, *J* = 7.0 Hz), 7.74–7.76 (d, 1H, CH, aromatic, *J* = 8.0 Hz), 7.08–7.82 (d, 1H, CH, aromatic, *J* = 8.0 Hz), 8.36–8.38 (d, 1H, CH, aromatic, *J* = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ29.52, 55.93, 60.03, 60.51, 106.53, 121.13, 123.70, 124.84, 124.92, 125.08, 126.83, 128.28, 128.77, 130.66, 134.24, 134.70, 142.73, 144.36, 151.52, 153.23; Electrospray mass (MeOH)- 361$[M+Na]^+$, 377$[M+K]^+$.

2-Methyl-1-naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-propan-1-ol (**10**): Yield = 78%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ0.75–0.76 (d, 3H, CH_3, *J* = 7.0 Hz), 1.17–1.18 (d, 3H, CH_3, *J* = 6.5 Hz), 3.04 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.85 (s, 1H, CH), 3.88 (s, 3H, OCH_3), 5.08 (s, 1H, exchangeable, OH), 6.72–6.74 (d, 1H, CH, aromatic, *J* = 9.0 Hz), 7.28–7.44 (m, 4H, 4xCH, aromatic), 7.69–7.70 (d, 1H, CH, aromatic, *J* = 9.0 Hz), 7.76–7.77 (d, 1H, CH, aromatic, *J* = 7.5 Hz), 7.80–7.81 (d, 1H, CH, aromatic, *J* = 8.0 Hz), 7.87 (s, br, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ17.43, 18.19, 35.09, 55.93, 60.31, 60.53, 80.48, 106.25, 121.57, 124.07, 124.80, 125.42, 125.81, 127.13, 127.42, 128.10, 132.03, 132.43, 133.00, 142.77, 146.17, 152.82; Electrospray mass (MeOH)- 389 $[M+Na]^+$, 365 $[M-H]^-$.

1-Naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-but-3-en-1-ol (**11**): Yield = 73%; Yellowish gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.00 (s, 3H, OCH_3, *J* = 7.0 Hz), 3.29–3.31 (d, 2H, CH_2, *J* = 6.0 Hz, olefinic), 3.77 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.24 (s, 1H, exchangeable, OH), 5.01–5.11 (m, 2H, CH_2, olefinic), 5.78–5.84 (m, 1H, CH, olefinic), 6.68–6.69 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 7.28–7.29 (t, 2H, 2xCH, aromatic, *J* = 3.5 Hz), 7.34–7.37 (m, 1H, CH, aromatic), 7.43–7.46 (d, 1H, CH, aromatic, *J* = 7.5 Hz), 7.67–7.69 (d, 1H, CH, aromatic, *J* = 7.0 Hz), 7.74–7.76 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 7.79–7.80 (d, 1H, CH, aromatic, *J* = 7.0 Hz), 8.36–8.38 (d, 1H, CH, aromatic, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ45.23, 55.86, 59.91, 60.46, 78.00, 106.36, 117.88, 121.67, 124.58, 124.66, 124.84, 125.03, 126.74, 128.34, 128.75, 130.87, 132.92, 134.42, 134.70, 142.50, 151.59, 153.06; Electrospray mass (MeOH)- 365 $[M+H]^+$, 387 $[M+Na]^+$, 403 $[M+K]^+$.

Ethyl-3-Hydroxy-3-naphthalen-1-yl-3-(2,3,4-trimethoxyphenyl)-2-methyl-propionate (**12**): Yield = 68%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.15–1.17 (d, 3H, CH_3, *J* = 7.0Hz), 1.29–1.32 (t, 3H, CH_3, *J* = 7.5Hz), 2.65 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.01–4.03 (q, 1H, CH of CH_2, *J* = 7.0 Hz,14.0Hz), 4.14–4.18 (m, 1H, CH), 4.25–4.27 (m, 1H, CH of CH_2), 5.44 (s, 1H, exchangeable, OH), 6.77–6.79 (d, 1H, CH, aromatic, *J* = 9.0Hz), 7.25–7.33 (m, 2H, 2xCH, aromatic), 7.40–7.43 (t, 1H, CH, aromatic, *J* = 8.0Hz), 7.69–7.74 (m, 3H, 3xCH, aromatic), 7.90–7.92 (d, 1H, CH, aromatic,

J = 8.5Hz), 8.61–8.63 (d, 1H, CH, aromatic, J = 8.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ13.96, 14.17, 44.80, 55.81, 59.27, 60.44, 60.88, 78.60, 106.24, 122.83, 124.07, 124.11, 124.85, 128.02, 128.41, 128.48, 129.73, 131.89, 134.86, 141.64, 142.64, 150.24, 152.82, 178.91; Electrospray mass (MeOH)- 447 $[M+Na]^+$.

1-Naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-4-phenyl-but-3-en-1-ol (**13**): Yield = 74%; Yellowish viscous.^{1}H NMR (CD_3OD, 500 MHz): δ2.61 (s, br, 3H, OCH_3), 3.67 (s, 6H, 2xOCH_3), 5.10–5.20 (dd, 2H, CH_2, J = 10.5 Hz, 12.0 Hz), 6.36–6.37 (d, 1H, CH, olefinic, J = 9.0 Hz), 6.65–6.68 (m, 1H, CH, aromatic), 6.98–7.22 (m, 8H, 8xCH, aromatic), 7.25–7.28 (t, 1H, CH, aromatic, J = 8.0 Hz), 7.48–7.51 (t, 1H, CH, aromatic, J = 8.0 Hz), 7.74–7.77 (m, 2H, 2xCH, aromatic), 8.10 (bs, 1H, CH, aromatic), 8.54–8.55 (d, 1H, CH, olefinic, J = 8.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz): δ56.42, 60.74, 79.58, 107.54, 117.69, 124.31, 125.40, 125.62, 125.77, 126.78, 126.89, 127.19, 127.50, 128.08, 128.29, 128.51, 128.65, 129.23, 129.42, 129.68, 131.34, 133.64, 136.53, 142.15, 142.38, 143.44, 144.84, 153.43, 153.95; Electrospray mass (MeOH)- 463$[M+Na]^+$.

1-Naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-2-phenylethanol (**14**): Yield = 90%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.89 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.82 (s, 2H, CH_2, benzylic), 3.86 (s, 3H, OCH_3), 4.42 (s, 1H, exchangeable, OH), 6.63–6.65 (d, 1H, CH, aromatic, J = 9Hz), 6.80–6.81 (d, 1H, CH, aromatic, J = 7Hz), 7.10–7.14 (m, 4H, CH, aromatic), 7.28–7.43 (m, 4H, 4xCH, aromatic), 7.41–7.43 (d, 1H, CH, aromatic, J = 8Hz), 7.67–7.69 (d, 1H, CH, aromatic, J = 7.5Hz), 7.76–7.77 (d, 1H, CH, aromatic, J = 8.5Hz), 8.20–8.22 (d, 1H, CH, aromatic, J = 8.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.89, 59.78, 60.46, 78.43, 106.62, 121.70, 124.47, 124.59, 124.77, 125.05, 126.17, 126.56, 127.45, 128.37, 128.77, 130.93, 131.14, 132.56, 134.64, 136.87, 142.37, 142.53, 151.62, 152.87; Electrospray mass (MeOH)- 437 $[M+Na]^+$, 453 $[M+K]^+$.

1-Naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-2-(4-tolyl)-ethanol (**15**): Yield = 84%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.25 (s, 3H, CH_3), 2.86 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2, benzylic), 3.84 (s, 3H, OCH_3), 4.32 (s, 1H, exchangeable, OH), 6.61–6.66 (m, 3H, 3xCH, aromatic), 6.89–6.91 (d, 2H, 2xCH, aromatic, J = 8Hz), 7.12–7.13 (d, 1H, CH, aromatic, J = 9Hz), 7.21–7.24 (m, 1H, CH, aromatic), 7.32–7.42 (m, 2H, 2xCH, aromatic), 7.66–7.68 (d, 1H, CH, aromatic, J = 7.5Hz), 7.74–7.75 (d, 1H, CH, aromatic, J = 8Hz), 7.78–7.80 (d, 1H, CH, aromatic, J = 8Hz), 8.19–8.21 (d, 1H, CH, aromatic, J = 9Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ21.03, 46.71, 55.88, 59.75, 60.46, 78.27, 106.53, 121.75, 124.54, 124.62, 124.74, 125.02, 126.60, 128.15, 128.22, 128.31, 128.74, 130.96, 132.64, 133.56, 134.62, 135.64, 142.38, 142.51, 151.61, 152.83; Electrospray mass (MeOH)- 451 $[M+Na]^+$, 467 $[M+K]^+$.

2-(4-Fluorophenyl)-1-naphthalen-1-yl-1-(2,3,4-trimethoxyhenyl)-ethanol (**16**): Yield = 77%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.86 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.78 (s, 2H, CH_2, benzylic), 3.85 (s, 3H, OCH_3), 4.31 (s, 1H, exchangeable, OH), 6.63–6.65 (d, 1H, CH, aromatic, J = 9Hz), 6.69–6.98 (m, 4H, 4xCH, aromatic), 7.15–7.25 (m, 2H, 2xCH, aromatic), 7.33–7.39 (m, 2H, 2xCH, aromatic), 7.59–7.61 (d, 1H, CH, aromatic, J = 7.5Hz), 7.73–7.74 (d, 1H, CH, aromatic, J = 8Hz), 7.79–7.80 (d, 1H, CH, aromatic, J = 8Hz), 8.14–8.16 (d, 1H, CH, aromatic, J = 9Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ46.04, 55.90, 59.79, 60.46, 78.17, 106.65, 114.02, 114.19, 121.47, 124.52, 124.63, 124.80, 125.14, 126.26, 130.54, 130.74, 132.35, 132.41, 132.49, 132.66, 134.57, 142.18, 142.61, 151.60, 152.99, 160.68, 162.62 Electrospray mass (MeOH)- 455 $[M+Na]^+$, 471 $[M+K]^+$.

Naphthalen-1-yl-*(2,3,4-trimethoxy-phenyl)-methanol* (**29**): Yield = 86%; Mp = 108–110 °C, White amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.80 (s, 3H, OCH_3), 3.90–3.92 (s, 6H, 2xOCH_3), 6.51–6.53 (d, 1H, CH, aromatic, J = 9.0Hz), 6.70–6.71 (d, 1H, CH, aromatic, J = 8.50Hz), 6.79 (s, 1H, CH), 7.42–7.51 (m, 3H, 3xCH, aromatic), 7.67–7.68 (d, 1H, CH, aromatic, J = 7.0 Hz), 7.79–7.81 (d, 1H, CH, aromatic, J = 8.5 Hz), 7.85–7.87 (dd, 1H, CH, aromatic, J = 2.5Hz, 6.5Hz), 7.99–8.01 (dd, 1H, CH, aromatic, J = 2.5Hz, 6.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.90, 60.77, 61.24, 68.35, 107.07, 122.63, 123.96, 124.11, 125.29, 125.46, 125.98, 128.09, 128.63, 129.17, 130.78, 133.74, 138.55, 142.01, 151.50, 153.48; Electrospray mass (MeOH)- 347 $[M+Na]^+$; 363 $[M+K]^+$.

5.1.5. General procedure for the synthesis of compound **21**–**22**

*Synthesis of (Z/E)1-[2-(4-Fluorophenyl)-1-(2,3,4-trimethoxyphenyl)-vinyl]-naphthalene (**21** & **22**):* Alcohol **16** (150 mg, 0.36 mmol) was taken in methanol (5 mL). To this stirred solution 2–3 drops Conc. hydrochloric acid was added and reaction mixture was refluxed for 2h. Solvent was evaporated and water (10 mL) was added to it. It was extracted with ethyl acetate (10mLx3), washed with water, dried over anhydrous sodium sulphate and evaporated *in vacuo*. The crude mass was charged on a silica gel column and carefully eluted with ethyl acetate-hexane to get compound **21** and **22** as pure isomers.

*(Z)1-[2-(4-Fluorophenyl)-1-(2,3,4-trimethoxyphenyl)-vinyl]-naphthalene (**21**):* Yield = 46%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.43 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.74–6.80 (m, 3H, 3xCH, aromatic), 6.93–6.95 (m, 2H, 2xCH, aromatic), 7.12–7.13 (d, 1H, CH, aromatic, J = 8.5Hz), 7.40 (s, 1H, CH, olefinic, Cis-diphenyl), 7.42–7.48 (m, 2H, 2xCH, aromatic), 7.53–7.58 (m, 2H, 2xCH, aromatic), 7.93–7.95 (d, 1H, CH, aromatic, J = 8.5Hz), 7.96–7.98 (d, 1H, CH, aromatic, J = 8.5Hz), 8.08–8.10 (d, 1H, CH, aromatic, J = 8.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.95, 60.03, 60.68, 106.93, 114.67, 114.84, 124.71, 125.63, 125.70, 125.92, 126.18, 127.62, 128.18, 130.48, 130.54, 131.31, 131.37, 133.52, 133.86, 137.41, 137.42, 138.90, 142.66, 151.98, 153.35, 160.37–162.33 (d, $J_{C,F}$ = 245Hz); Electrospray mass (MeOH): 415$[M+H]^+$.

*(E)1-[2-(4-Fluorophenyl)-1-(2,3,4-trimethoxyphenyl)-vinyl]-naphthalene (**22**):* Yield = 26%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.21 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.59–6.60 (d, 1H, CH, aromatic, J = 8.5Hz), 6.74 (s, 1H, CH, olefinic, Trans diphenyl), 6.86–6.93 (m, 3H, 3xCH, aromatic), 7.09–7.11 (m, 2H, 2xCH, aromatic), 7.32–7.46 (m, 4H, 4xCH, aromatic), 7.74–7.76 (dd, 1H, CH, aromatic, J = 8Hz & 1.5Hz), 7.84–7.86 (dd, 1H, CH, aromatic, J = 8Hz & 2Hz), 8.39–8.41(d, 1H, CH, aromatic, J = 8Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.91, 59.97, 60.74, 107.23, 114.88, 115.05, 125.03, 125.49, 125.55, 125.76, 126.21, 126.30, 127.39, 128.07, 128.26, 130.61, 130.67, 131.76, 133.69, 134.13, 137.24, 142.33, 142.81, 151.85, 153.58, 160.59–162.55 (d, $J_{C,F}$ = 245Hz); Electrospray mass (MeOH): 415 $[M+H]^+$, Negative mode: 413 $[M-H]^-$.

5.1.6. Synthesis of naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methanone oxime (**23**)

Naphthophenone **3a** (200 mg, 0.62 mmol) was taken in ethanol (8 mL). To this stirred solution DMAP (18 mg, 0.15 mmol) and hydroxylamine hydrochloride (207 mg, 3 mmol) were added and reaction mixture was refluxed for 18h. Solvent was evaporated and water (10 mL) was added to it. It was extracted with ethyl acetate (15mLx3), washed with water and dried over anhydrous sodium sulphate. Organic solvent was dried *in vacuo* and residue was charged on a silica gel column and eluted with chloroform-acetone to get oxime derivative **23** as amorphous solid after recrystallization with chloroform-hexane (1:3).

23: Yield = 87%; Mp = 175–178 °C, White solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.13 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.58–6.60 (d, 1H, CH, aromatic, J = 9Hz), 7.16–7.17 (d, 1H, CH, aromatic, J = 9Hz), 7.29–7.30 (d, 1H, CH, aromatic, J = 1Hz), 7.30–7.53 (m, 3H, 3xCH, aromatic), 7.86–7.98 (m, 3H, 3xCH, aromatic), 9.47 (s, 1H, exchangeable, N-OH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.99, 60.18, 60.69, 106.98, 124.27, 124.92, 124.98,

125.48, 125.99, 126.21, 126.95, 128.21, 128.96, 130.18, 133.29, 133.79, 142.60, 152.71, 154.97, 155.97; Electrospray mass (MeOH): 338 $[M+H]^+$, 360 $[M+Na]^+$; HRMS (MeOH): *m/z* $[M+H]^+$calcd for $C_{20}H_{20}NO_4$, 338.1392, found 338.1389.

*5.1.7. Synthesis of naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methanone oxime acetate (**24**)*

Oxime **23** was acetylated using acetic anhydride, DMAP in dry chloroform at RT as per reported method:**24:** Yield = 86%. Mp = 104–105 °C, White crystalline solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.79 (s, 3H, CH_3), 3.05 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.69–6.71 (d, 1H, CH, aromatic, *J* = 9Hz), 7.21–7.22 (d, 1H, CH, aromatic, *J* = 7Hz), 7.38–7.51 (m, 4H, 4xCH, aromatic), 7.82–7.86 (m, 3H, 3xCH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ19.46, 56.07, 60.28, 60.67, 106.97, 123.09, 124.62, 125.08, 125.58, 126.09, 126.13, 126.56, 128.25, 129.46, 129.82, 133.10, 133.50, 142.60, 153.15, 156.10, 163.74, 168.58; Electrospray mass (MeOH)-380 $[M+H]^+$, 402 $[M+Na]^+$, 418 $[M+K]^+$; HRMS (MeOH): *m/z* $[M+H]^+$calcd for $C_{22}H_{22}NO_5$, 380.1497, found 380.1539.

*5.1.8. General procedure for the synthesis of compounds **25**–**27** and **47***

*Synthesis of Ethyl-naphthalen-*1-yl-*(2,3,4-trimethoxyphenyl)-methyleneaminooxy]-acetate (**25**):* Ketoxime **23** (100 mg, 0.30 mmol) was stirred in dry THF (10 mL). To this sodium hydride (60 mg, 2.5 mmol) and ethylbromoacetate (100 mg, 0.6 mmol) were added and reaction mixture was refluxed for 2h. On completion, reaction mixture was acidified with dil. HCl (5%, 5 mL), extracted with ethyl acetate (10mLx3) and washed with water (15mLx2). The organic layer was dried over anhydrous sodium sulphate and evaporated *in vacuo*. The crude mass thus obtained was purified through column chromatography over silica gel using chloroform-hexane to get ester **25** at 80% chloroform-hexane as amorphous solid.

25: Yield = 92%; Mp = 84–85 °C, White crystalline Solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.28–1.31 (t, 3H, CH_3, *J* = 7.0 Hz), 3.12 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.20–4.24 (q, 2H, CH_2, *J* = 7.0 Hz, 14Hz), 4.62 (s, 2H, CH_2), 6.66–6.68 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 7.24–7.53 (m, 5H, 5xCH, aromatic), 7.83–7.86 (m, 2H, 2xCH, aromatic), 8.08–8.09 (d, 1H, CH, aromatic, *J* = 8.0 Hz,). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.24, 56.04, 60.30, 60.68, 60.79, 107.06, 124.35, 124.83, 124.96, 125.40, 125.93, 126.08, 127.19, 128.02, 130.13, 133.21, 133.92, 142.72, 152.78, 155.14, 156.64, 170.05; Electrospray mass (MeOH)- 424 $[M+H]^+$, 446 $[M+Na]^+$, 418 $[M+K]^+$; HRMS (MeOH): *m/z* $[M+H]^+$calcd for $C_{24}H_{26}NO_6$, 424.1760, found 424.1757.

*Ethyl-3-[Naphthalen-*1-yl-*(2,3,4-trimethoxyphenyl)-methyl-eneaminooxy]-propionate* (**26**): Yield = 87%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.12–1.52 (t, 3H, CH_3, *J* = 7.5Hz), 2.65–2.67 (t, 2H, CH_2, *J* = 6.5Hz), 3.08 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.01–4.02 (t, 2H, CH_2, *J* = 7Hz), 4.36–4.39 (q, 2H, CH_2, *J* = 7Hz), 6.67–6.69 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.20–7.24 (m, 2H, 2xCH, aromatic), 7.40–7.48 (m, 3H, 3xCH, aromatic), 7.84–7.89 (m, 3H, 3xCH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ14.08, 34.86, 56.04, 60.19, 60.38, 60.64, 69.72, 107.02, 124.74, 124.80, 125.18, 125.78, 125.97, 126.90, 128.05, 128.71, 130.09, 133.17, 134.36, 142.68, 152.73, 155.02, 155.64, 171.30; Electrospray mass for $C_{25}H_{27}NO_6$, (MeOH) 460 $[M+Na]^+$, 476 $[M+K]^+$.

*Ethyl 4-[naphthalen-*1-yl-*(2,3,4-trimethoxyphenyl)-methyl-eneaminooxy]-but-2-enoate* (**27**): Yield = 86%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.27–1.29 (t, 3H, CH_3, *J* = 7.0Hz), 3.12 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.15–4.20 (q, 2H, CH_2, *J* = 7.17 Hz), 4.75–4.77 (dd, 2H, CH_2, *J* = 2.0Hz, 5.0Hz), 5.82–5.85 (dd, 1H, CH, olefinic, *J* = 14.0Hz, 16.0Hz), 6.67–6.68 (d, 1H, CH, aromatic, *J* = 8.5Hz), 6.96–6.99 (d, 1H, CH, olefinic, *J* = 16.0 Hz), 7.22–7.85 (m, 8H, 8xCH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ14.19, 55.94, 60.23, 60.27, 60.60, 72.74, 107.05, 121.93, 124.33, 124.79, 124.81, 125.09, 125.88, 126.10, 126.72, 128.11, 128.80, 130.00, 133.16, 134.14, 142.71, 143.92, 152.69, 155.08, 156.24, 166.13; Electrospray mass (MeOH)- 450 $[M+H]^+$, 472 $[M+Na]^+$, 488 $[M+K]^+$; HRMS (MeOH): *m/z* $[M+H]^+$, calcd for $C_{26}H_{28}NO_6$, 450.1916, found 450.1911.

*Ethyl-2-[4-(naphthalen-*1-yl-*(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-ethanoate* (**47**): Yield = 84%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.29–1.30 (t, 3H, CH_3, *J* = 3.5Hz), 3.47(s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.24–4.28 (q, 2H, OCH_2, *J* = 7.17Hz), 4.58 (s, 2H, 2H, OCH_2), 6.43–6.44 (d, 3H, 2xCH, aromatic, *J* = 8.5Hz), 6.50–6.51 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 6.50 (s, 1H, 1H, CH), 6.81–6.83 (d, 2H, 2xCH, aromatic, *J* = 9.0Hz), 6.94–6.96 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.03–7.05 (d, 2H, 2xCH, aromatic, *J* = 8.50Hz), 7.33–7.44 (m, 3H, 3xCH, aromatic), 7.71–7.73 (d, 1H, CH, aromatic, *J* = 8.0Hz), 7.82–7.84 (d, 1H, CH, aromatic, *J* = 7.5Hz), 7.98–7.99 (d, 1H, CH, aromatic, *J* = 8.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ14.16, 45.38, 55.84, 60.64, 60.73, 61.32, 65.57, 106.66, 114.51, 124.34, 124.45, 125.08, 125.41, 126.03, 127.03, 127.13, 128.58, 130.50, 130.62, 131.84, 133.94, 137.13, 140.41, 142.40, 151.40, 152.40, 156.33, 169.03; ESI-MS (MeOH) for $C_{30}H_{30}O_6$: 487 $[M+H]^+$, 509 $[M+Na]^+$, 525 $[M+K]^+$.

*5.1.9. General procedure for the synthesis of compounds **28** and **49***

*Synthesis of [Naphthalen-1-yl-(2,3,4-trimethoxy-phenyl)-methyl-eneaminooxy]-acetic acid (**28**)*:Ester **25** (100 mg, 0.24 mmol) was taken in 6% KOH in MeOH:Water (9:1) (5 mL). It was refluxed for 2h. Reaction mixture was acidified with dil. HCl (5%, 5 mL), extracted with ethyl acetate (10mLx3), washed with water (15mLx2) and dried over anhydrous sodium sulphate. The organic layer was evaporated under vacuum and residue was purified through chromatography eluting with chloroform-acetone to get acid **28** at 5% acetone-chloroform and recrystallized with chloroform:pentane (1:3) as amorphous solid.

28: Yield = 66%; Mp = 103–104 °C, White solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.96 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.47 (s, 2H, CH_2), 6.64–6.66 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.10–7.12 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.15–7.16 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.31–7.37(m, 3H, 3xCH, aromatic), 7.73–7.77 (m, 2H, 2xCH, aromatic), 7.90–7.92 (d, 1H, CH, aromatic, *J* = 8.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ59.15, 63.47, 63.57, 74.09, 111.05, 128.16, 128.39, 128.82, 129.19, 129.44, 129.47, 130.88, 131.54, 132.36, 134.10, 137.32, 137.94, 146.43, 156.32, 159.12, 160.36; Electrospray mass (MeOH): Positive mode: 396 $[M+H]^+$, 418 $[M+Na]^+$, negative mode: 394 $[M-H]^-$; HRMS (MeOH): *m/z* $[M+H]^+$, calcd for $C_{22}H_{22}NO_6$, 396.1447, found 396.1441.

*2-[4-(naphthalen-*1-yl-*(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-ethanoic acid* (**49**): Yield = 72%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.48 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.62 (s, 2H, OCH_2), 6.43–6.51 (m, 3H, 3xCH, aromatic), 6.50–6.51 (d, 2H, 2xCH, aromatic, *J* = 4.5Hz), 6.82–6.84 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 6.94–6.95 (d, 1H, CH, aromatic, *J* = 7.0 Hz), 7.04–7.06 (d, 1H, CH, aromatic, *J* = 8.5 Hz), 7.32–7.44 (m, 3H, 3xCH, aromatic), 7.71–7.73 (d, 1H, CH, aromatic, *J* = 8.0 Hz), 7.83–7.84 (d, 1H, CH, aromatic, *J* = 7.5 Hz), 7.97–7.99 (d, 1H, 1xCH, aromatic, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ45.36, 55.85, 60.68, 60.75, 65.05, 106.71, 114.51, 124.30, 124.47, 125.10, 125.45, 126.07, 127.02, 127.18, 128.61, 130.41, 130.73, 131.81, 133.95, 137.47, 140.31, 142.36, 151.35, 152.40, 155.94, 173.28; Electrospray mass for $C_{28}H_{27}O_6$ (MeOH): 481$[M+Na]^+$; Negative mode: 457$[M-H]^-$.

*5.1.10. Synthesis of 4-[Naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl]-phenol (**30**) and 2-[Naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl]-phenol (**31**)*

Alcohol **29** (200 mg, 0.62 mmol) was taken in a solution of dry benzene (12 mL) and Conc. HCl (0.2 mL). To this stirred reaction mixture phenol (71 mg, 0.75 mmol) was added and further stirred at RT for 6h. Water (20 mL) was added to it, extracted with ethyl acetate (20mLx3), and washed with water. Organic layer was dried over anhydrous sodium sulphate and dried *in vacuo*. The residue thus obtained was purified through column chromatography over silica gel eluting with ethyl acetate-hexane to get phenolic derivatives **30** and **31** at 6% and 8% ethyl acetate-hexane respectively as amorphous solids.

30: Yield = 48%; Mp = 168–170 °C, White crystalline solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.48 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.17 (s, 1H, OH, exchangeable), 6.44–6.52 (m, 3H, 3xCH, aromatic), 6.73–6.74 (d, 2H, 2xCH, aromatic, J = 8.5Hz), 6.95 (s, 1H, CH), 6.97–6.99 (d, 1H, CH, aromatic, J = 8.50Hz), 7.33–7.44 (m, 3H, 3xCH, aromatic), 7.71–7.73 (d, 1H, CH, aromatic, J = 9.0Hz), 7.82–7.84 (d, 1H, CH, aromatic, J = 7.5Hz), 7.98–8.00 (d, 1H, CH, aromatic, J = 8.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 45.38, 55.85, 60.70, 60.77, 106.71, 115.20, 120.65, 124.37, 124.51, 125.09, 125.42, 126.03, 127.00, 127.11, 128.58, 129.65, 130.69, 130.72, 131.85, 133.94, 135.97, 140.54, 142.35, 151.35, 152.32, 154.04; Electrospray mass for $C_{26}H_{24}O_4$ (MeOH)- 423[M+Na]$^+$; 439[M+K]$^+$.

31: Yield = 26%; Mp = 119–120 °C, White amorphous solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.53 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.21 (s, 1H, OH, exchangeable), 6.55 (s, 2H, 2.xCH, aromatic), 6.73 (s, 1H, CH, aromatic), 6.77–6.84 (m, 3H, 3xCH, aromatic), 7.04–7.06 (d, 1H, CH, aromatic, J = 7.5Hz), 7.11–7.14 (m, 1H, CH, aromatic), 7.35–7.45 (m, 3H, 3xCH, aromatic), 7.75–7.77 (d, 1H, CH, aromatic, J = 8.0Hz), 7.84–7.86 (d, 1H, CH, aromatic, J = 8.0Hz), 7.99–8.00 (d, 1H, CH, aromatic, J = 3.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 40.12, 55.86, 60.74, 60.80, 106.93, 115.96, 120.61, 124.30, 124.45, 125.18, 125.57, 126.22, 126.58, 127.53, 127.80, 128.85, 129.63, 130.08, 130.48, 131.87, 134.09, 138.90, 142.52, 151.54, 152.65, 153.36; Electrospray mass (MeOH)- 423[M+Na]$^+$; 439[M+K]$^+$, 399 [M-H]$^-$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{26}H_{25}O_4$, 401.1752, found 401.1743.

*5.1.11. General procedure for the synthesis of compounds **32**–**40***

*Synthesis of methoxy-1-[naphthalen-1-yl- 1-(2,3,4-trimethoxyphenyl)-methane (**32**)*: Alcohol **29** (100 mg, 0.31 mmol) was stirred in dry THF (10 mL) and sodium hydride (40 mg, 1.67 mmol) was added to it. After 10 min methyl iodide (0.1 mL, 228 mg, 1.61 mmol) was added and reaction mixture was refluxed for an hour. It was acidified with dil. HCl (5%, 5 mL), extracted with ethyl acetate (10mLx3), and washed with water (10mLx2). Organic layer was dried over anhydrous sodium sulphate and evaporated in rotavapour. The residue was purified through a filter column eluting with ethyl acetate-hexane to get compound **32** as amorphous solid.

32: Yield = 77%; yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.34 (s, 1H, CH), 6.56–6.57(d, 1H, CH, aromatic, J = 9.0Hz), 6.85–6.87 (d, 1H, CH, aromatic, J = 8.5Hz), 7.43–7.52 (m, 3H, 3xCH, aromatic), 7.64–7.66 (d, 1H, CH, aromatic, J = 7.0 Hz), 7.78–7.80 (d, 1H, CH, aromatic, J = 13.5Hz), 7.83–7.86 (m, 1H, CH, aromatic), 8.02–8.04 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.92, 57.50, 60.78, 61.17, 76.79, 107.27, 122.84, 123.76, 124.30, 125.31, 125.44, 125.99, 127.42, 127.99, 128.66, 131.33, 133.87, 136.96, 142.05, 151.85, 153.35; Electrospray mass (MeOH)- 361 [M+Na]$^+$; 377[M+K]$^+$.

*Ethoxy-1-[naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-methane (**33**)*:Yield = 76%; Yellow viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.31–1.33 (t, 3H, CH_3, J = 7.0Hz), 3.66–3.71 (q, 2H, O-CH_2, J = 7.0, 14.0Hz), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.47 (s, 1H, CH), 6.55–6.57 (d, 1H, CH, aromatic, J = 9.0Hz), 6.86–6.87 (d, 1H, CH, aromatic, J = 9.0Hz), 7.43–7.52 (m, 3H, 3xCH, aromatic), 7.67–7.69 (d, 1H, CH, aromatic, J = 7.0Hz), 7.79–7.80 (d, 1H, CH, aromatic, J = 8.5Hz), 7.83–7.86 (m, 1H, CH, aromatic), 8.04–8.06 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.60, 55.92, 60.79, 61.16, 65.18, 74.47, 107.30, 122.98, 123.83, 124.42, 125.34, 125.42, 125.94, 127.86, 127.94, 128.65, 131.37, 133.89, 137.43, 142.09, 151.82, 153.30; Electrospray mass (MeOH)- 375 [M+Na]$^+$; 391[M+K]$^+$.

*Propyloxy-1-[naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-methane (**34**)*:Yield = 81%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ0.95–0.98 (t, 3H, CH_3, J = 7.5Hz), 1.70–1.74 (m, 2H, CH_2), 3.56–3.58 (t, 2H, OCH_2, J = 6.5Hz), 3.81 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.44 (s, 1H, CH), 6.57–6.58 (d, 1H, CH, aromatic, J = 8.5Hz), 6.89–6.91(d, 1H, CH, aromatic, J = 8.5Hz), 7.43–7.51 (m, 3H, 3xCH, aromatic), 7.65–7.67 (d, 1H, CH, aromatic, J = 7.0Hz), 7.78–7.79 (d, 1H, CH, aromatic, J = 8.0Hz), 7.84–7.86 (m, 1H, CH, aromatic), 8.06–8.08 (d, 1H, CH, aromatic, J = 8.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.87, 23.33, 55.91, 60.76, 61.11, 71.61, 74.58, 107.29, 122.99, 123.92, 124.54, 125.30, 125.39, 125.89, 127.86, 127.90, 128.62, 131.42, 133.88, 137.63, 142.06, 151.81, 153.24; Electrospray mass (MeOH)- 389[M+Na]$^+$; 405[M+K]$^+$.

*Allyloxy-1-[naphthalen-1-yl-1-(2,3,4-trimethoxyphenyl)-methane (**35**)*: Yield = 86%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.80 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90(s, 3H, OCH_3), 4.13–4.16 (m, 2H, CH_2), 5.19–5.21 (dd, 1H, CH of = CH_2, J = 1.5, 10.5Hz), 5.29–5.33 (dd, 1H, CH of = CH_2, J = 1.5, 17.5Hz), 6.05–6.06 (m, 1H, CH, olefinic), 6.31 (s, 1H, CH), 6.53 (s, 1H, CH), 6.56–6.58 (d, 1H, CH, aromatic, J = 8.5Hz), 6.90–6.92 (d, 1H, CH, aromatic, J = 9.0Hz), 7.44–7.52 (m, 3H, 3xCH, aromatic), 7.70–7.71 (d, 1H, CH, aromatic, J = 7.0Hz), 7.79–7.80 (d, 1H, CH, aromatic, J = 8.5Hz), 7.84–7.86 (m, 1H, CH, aromatic, J = 7.0Hz), 8.06–8.08 (m, 1H, CH, aromatic, J = 7.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.92, 60.75, 61.13, 70.57, 73.92, 107.26, 117.03, 123.05, 123.82, 124.62, 125.34, 125.42, 125.95, 127.58, 127.99, 128.65, 131.33, 133.87, 134.98, 137.20, 142.05, 151.79, 153.34; Electrospray mass for $C_{23}H_{24}O_4$ (MeOH)- 387[M+Na]$^+$; 403 [M+K]$^+$.

2-[(Naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methoxy]-N,N-dimethyethanamine (**36**)*: Yield = 67%; Brown-viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.31 (s, 6H, $N(CH_3)_2$), 2.68–2.70 (t, 2H, N-CH_2, J = 5.5Hz), 3.71–3.73 (t, 2H, OCH_2, J = 5.0Hz), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.43 (s, 1H, CH), 6.54–6.56 (d, 1H, CH, aromatic, J = 8.5Hz), 6.87–6.89(d, 1H, CH, aromatic, J = 9.0Hz), 7.43–7.49 (m, 3H, 3xCH, aromatic), 7.64–7.65 (d, 1H, CH, aromatic, J = 7.0Hz), 7.76–7.78 (d, 1H, CH, aromatic, J = 8.0Hz), 7.83–7.84 (d, 1H, CH, aromatic, J = 6.0Hz), 8.04–8.05 (d, 1H, CH, aromatic, J = 5.0Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ45.62, 55.91, 58.73, 60.72, 61.06, 67.36, 74.97, 107.24, 122.99, 123.79, 124.56, 125.29, 125.72, 125.94, 126.37, 127.61, 127.99, 128.63, 131.26, 133.83, 137.23, 141.95, 151.74, 153.32; Electrospray mass (MeOH)- 396 [M+H]$^+$.

1-Benzyloxy-1-[naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methane (**37**)*: Yield = 83%; Brown-viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.63–4.70 (q, 2H, OCH_2, benzylic, J = 12.2Hz), 6.59–6.60 (d, 2H, 2xCH, J = 7.0Hz), 7.00–7.01(d, 1H, CH, aromatic, J = 9.0Hz), 7.29–7.53 (m, 8H, 8xCH, aromatic), 7.74–7.75 (d, 1H, CH, aromatic, J = 7.0Hz), 7.80–7.81 (d, 1H, CH, aromatic, J = 8.5Hz), 7.85–7.87 (m, 1H, CH, aromatic), 8.04–8.06 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.93, 60.75, 61.02, 71.42, 73.99, 107.29, 123.11, 123.89, 124.80, 125.34, 125.45, 125.96, 127.47, 127.58, 128.06, 128.09, 128.33, 128.64, 131.41, 133.89, 137.27, 138.44, 142.02, 151.81, 153.33; Electrospray mass (MeOH)- 437[M+Na]$^+$; 453[M+K]$^+$.

1-(4-methylbenzyloxy)-1-[naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methane (**38**):*Yield = 83%; Yellow viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.35 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.57–4.65 (m, 2H, OCH_2, benzylic, *J* = 12.5Hz), 6.57–6.59 (m, 2H, 2xCH, aromatic & one CH), 6.98–7.00 (d, 1H, CH, aromatic, *J* = 10Hz), 7.14–7.18 (m, 2H, 2xCH, aromatic), 7.26–7.28 (m, 2H, 2xCH, aromatic), 7.42–7.52 (m, 3H, 3xCH, aromatic), 7.71–7.73 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.79–7.80 (d, 1H, CH, aromatic, *J* = 8.0Hz), 7.84–7.86 (m, 1H, CH, aromatic), 8.02–8.04 (m, 1H, CH, aromatic). ^{13}C NMR ($CDCl_3$, 125 MHz): δ21.17, 55.92, 60.73, 61.01, 71.27, 73.83, 107.27, 123.12, 123.92, 124.82, 125.32, 125.41, 125.91, 127.11, 127.55, 128.00, 128.19, 128.61, 128.98, 129.24, 131.43, 133.88, 135.38, 137.24, 137.34, 142.02, 151.81, 153.28; Electrospray mass (MeOH)- 451$[M+Na]^+$; 467$[M+K]^+$.

1-(3,5-dimethoxybenzyloxy)-1-[naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methane (**39**):* Yield = 81%; Yellow viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.74(s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.89 (s, 6H, 2xOCH_3), 4.58–4.64 (q, 2H, OCH_2, benzylic *J* = 10.8Hz), 6.40–6.41 (m, 1H, CH), 6.55–6.61(m, 4H, 4xCH, aromatic), 7.02–7.04 (d, 1H, CH, aromatic, *J* = 9.0Hz), 7.44–7.52 (m, 3H, 3xCH, aromatic), 7.71–7.73 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.79–7.81 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.85–7.86 (m, 1H, CH, aromatic), 8.06–8.08 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.30, 55.94, 60.73, 60.99, 71.38, 74.06, 99.75, 105.82, 107.27, 123.08, 123.94, 124.92, 125.34, 125.45, 125.94, 127.38, 128.09, 128.65, 131.40, 133.90, 137.28, 140.83, 142.03, 151.80, 153.34, 160.83; Electrospray mass (MeOH)- 497$[M+Na]^+$, 513$[M+K]^+$.

Methyl-4-[naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methoxymethyl]-benzoate (**40**):* Yield = 63%; yellow viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.77 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.88(s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.67–4.74 (q, 2H, OCH_2, benzylic, *J* = 11.7Hz), 6.58 (s, 1H, CH, benzylic), 6.58–6.60 (d, 1H, CH, aromatic, *J* = 9.0Hz), 6.97–6.99 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.44–7.53 (m, 5H, 5xCH, aromatic), 7.72–7.74 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.80–7.82 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.85–7.87 (m, 1H, CH, aromatic), 8.00–8.08 (m, 1H, CH, aromatic). ^{13}C NMR ($CDCl_3$, 125 MHz): δ52.10, 55.92, 60.76, 61.05, 70.75, 74.41, 107.22, 123.03, 123.74, 124.73, 125.36, 125.53, 126.06, 127.11, 127.50, 128.71, 129.26, 129.65, 130.67, 131.29, 133.87, 136.95, 141.95, 143.78, 151.78, 153.43, 167.04; Electrospray mass (MeOH)- 495$[M+Na]^+$, 511 $[M+K]^+$.

5.1.12. General procedure for the synthesis of compounds **41** and **48**

*Synthesis of 4-[Naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methoxymethyl]-phenyl-methanol (**41**):*To a cold stirred solution of ester **40** (50 mg, 0.11 mmol) in dry THF (8 mL), lithium borohydride (20 mg, 0.95 mmol) was added in portions and further stirred at 50 °C for an hour. Reaction mixture was acidified with dil. HCl (5%, 5 mL) and extracted with ethyl acetate (10mLx3), washed with water (10mLx2) and dried over anhydrous sodium sulphate. On evaporation of organic layer a residue was obtained which on recrystallaised from chloroform:pentane (1:3) afforded alcohol **41** as viscous liquid.

41: Yield = 83%; viscous gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.72 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.63–4.69 (m, 4H, 2xCH_2, 2xbenzylic, *J* = 10.8Hz), 6.58 (s, 1H, CH benzylic), 6.58–6.59 (d, 1H, CH, aromatic, *J* = 6Hz), 6.99–7.01(d, 1H, CH, aromatic, *J* = 9.0Hz), 7.31–7.53 (m, 7H, 7xCH, aromatic), 7.73–7.75 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.80–7.82 (d, 1H, CH, aromatic, *J* = 8.0Hz), 7.86–7.87 (d, 1H, CH, aromatic, *J* = 5.0Hz), 8.05–8.06 (m, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ55.93, 60.79, 61.08, 65.06, 71.17, 74.07, 107.27, 123.13, 123.88, 124.82, 125.38, 125.51, 126.02, 127.01, 127.39, 128.13, 128.22, 128.29, 128.39, 128.69, 131.39, 133.90, 137.20, 137.79, 140.33, 141.99, 151.81, 153.34; Electrospray mass (MeOH)- 467$[M+Na]^+$, 483 $[M+K]^+$.

2-[4-(Naphthalen-1-yl-*(2,3,4-trimethoxy-phenyl)-methyl)-phenoxy]-ethanol (**48**):* Yield = 85%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.49 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.94–3.94 (bs, 2H, OCH_2), 4.04–4.05 (bs, 2H, OCH_2), 6.44–6.45 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 6.51–6.52 (bs, 1H, CH), 6.83–6.84 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 6.95–6.96 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.04–7.05 (d, 2H, 2xCH, aromatic, *J* = 8.0Hz), 7.33–7.44 (m, 3H, 3xCH, aromatic), 7.72–7.73 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.83–7.84 (d, 1H, CH, aromatic, *J* = 7.5Hz), 7.99–8.01 (d, 1H, CH, aromatic, *J* = 8.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ45.39, 55.85, 60.69, 60.75, 61.52, 69.10, 106.66, 114.32, 124.36, 124.45, 125.10, 125.43, 126.04, 127.00, 127.12, 128.60, 130.59, 130.62, 131.86, 133.95, 136.49, 140.52, 142.39, 151.40, 152.38, 157.04; Electrospray mass (MeOH)- 467$[M+Na]^+$; 483$[M+K]^+$; HRMS (MeOH): *m/z* $[M+H]^+$, calcd for $C_{28}H_{29}O_5$, 445.2015, found 445.1935.

5.1.13. Synthesis of compounds **42**–**46**

*Synthesis of 2-[4-(naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-N,N-dimethyl-ethnamine (**42**)*:Phenol **30** (50 mg, 0.13 mmol) was taken in dry THF (10 mL) and sodium hydride (20 mg, 0.84 mmol). To this stirred reaction mixture, 2-chloro-N,N-dimethylethanamine hydrochloride (56 mg, 0.39 mmol) was added and refluxed for 2h. Water was added to it and reaction mixture was extracted with ethyl acetate (10mLx3), washed with water and dried *in-vacuo* to get a residue which was charged on a silica gel column, eluted with chloroform-methanol to get desired product **42** at 2% methanol-chloroform as gummy viscous oil.

42: Yield = 77%; Brown viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.95 (s, br, 6H, $N(CH_3)_2$), 3.48 (s, br, 5H, OCH_3, N-CH_2), 3.78 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.45 (s, br, 2H, O-CH_2), 6.41(s, 1H, CH), 6.47 (s, br, 2H, 2xCH, aromatic), 6.81–7.03 (m, br, 5H, 5xCH, aromatic), 7.33–7.39 (m, br, 3H, 3xCH, aromatic), 7.70–7.96 (m, br, 3H, 3xCH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 44.01, 45.32, 55.86, 56.75, 60.73, 62.92, 106.74, 114.40, 124.24, 124.43, 125.11, 125.45, 126.07, 126.96, 127.17, 128.62, 130.23, 130.77, 131.75, 133.92, 137.44, 140.30, 142.35, 151.30, 152.41, 155.65; Electrospray mass (MeOH)-472 $[M+H]^+$; 494$[M+Na]^+$; HRMS (MeOH): *m/z* $[M+H]^+$, calcd for $C_{30}H_{34}NO_4$, 472.2487, found 472.2481.

*2-[4-(naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-N,N-Diethyl-ethnamine (**43**):* Yield = 81%; Brown viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.23–1.26 (t, 6H, 2xCH_3, *J* = 7.0 Hz), 2.96–3.01 (q, 2H, N-CH_2, *J* = 7.0Hz), 3.19–3.21 (t, 2H, N-CH_2, *J* = 5.0Hz), 3.48 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.24–4.26 (t, 2H, OCH_2, *J* = 5.0Hz), 6.42–6.44 (d, 1H, CH, aromatic, *J* = 8.5Hz), 6.49 (s, 1H, CH benzylic), 6.50–6.51 (d, 1H, CH, aromatic, *J* = 9.0Hz), 6.78–6.80 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 6.93–6.94 (d, 1H, CH, aromatic, *J* = 7.0Hz), 7.02–7.04 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 7.32–7.43 (m, 3H, 3xCH, aromatic), 7.71–7.72 (d, 1H, CH, aromatic, *J* = 8.5Hz), 7.82–7.83 (d, 1H, CH, aromatic, *J* = 7.5Hz), 7.97–7.98 (d, 1H, CH, aromatic, *J* = 8.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ9.70, 45.36, 47.03, 50.74, 55.84, 60.68, 60.74, 64.10, 106.68, 114.24, 124.32, 124.44, 125.09, 125.41, 126.99, 127.13, 128.59, 130.47, 130.67, 131.82, 133.94, 136.80, 140.44, 142.38, 151.37, 152.39, 156.35.Electrospray mass (MeOH)- 500$[M+H]^+$; HRMS (MeOH): *m/z* $[M+H]^+$calcd for $C_{32}H_{38}NO_4$, 500.2800, found 500.2790.

*2-[4-(naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-N,N-Diethyl-2-methylpropanamine:(**44**)*:Yield = 82%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.37 (s, 3H, CH_3), 2.48 (s, 6H, $N(CH_3)_2$), 3.16–3.19 (q, 1H, O-CH, 5.5 Hz), 3.47 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.96–3.97 (t, 1H, CH of −N-CH_2, *J* = 5.0 Hz), 4.04–4.05 (t, 1H, CH of −N-CH_2, *J* = 4.0 Hz), 6.43–6.51 (m, 3H, 2xCH, aromatic & 1H, CH), 6.81–6.82 (d, 2H, 2xCH, aromatic, *J* = 8.5Hz), 6.94–7.04 (m, 3H, 3xCH, aromatic), 7.32–7.43 (m, 3H, 3xCH, aromatic), 7.71–7.72 (d, 1H, CH, aromatic,

J = 8.0 Hz), 7.82–7.83 (d, 1H, CH, aromatic, J = 7.5 Hz), 7.98–8.00 (d, 1H, CH, aromatic, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ41.03, 45.37, 45.75, 55.84, 58.77, 60.68, 60.73, 68.89, 106.68, 114.32, 115.76, 124.34, 124.39, 124.45, 125.09, 125.41, 125.99, 126.02, 126.99, 127.11, 128.59, 130.59, 131.87, 133.95, 136.47, 140.53, 142.40, 151.39, 152.34, 152.37, 156.85; Electrospray mass (MeOH)- 486[M+H]$^+$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{31}H_{36}NO_4$, 486.2644, found 486.2641.

2-[4-(naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-pyrrolidine (**45**):* Yield = 81%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ2.07–2.10 (bd, 4H, 2xCH_2, J = 12.0 Hz), 3.23–3.46 (m, 4H, N(CH_2)$_2$), 3.83 (bs, 2H, N-CH_2), 3.46 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.43–4.55 (t, 2H, O-CH_2, J = 6.5 Hz), 6.39–6.48 (m, 2H, 2xCH, aromatic), 6.50 (s, 1H, CH benzylic), 6.78–6.79 (d, 2H, 2xCH, aromatic, J = 8.5 Hz), 6.89–6.91 (t, 1H, CH, aromatic, J = 6.0 Hz), 7.00–7.02 (d, 2H, 2xCH, aromatic, J = 8.5 Hz), 7.30–7.40 (m, 3H, 3xCH, aromatic), 7.69–7.70 (d, 1H, CH, aromatic, J = 8.5 Hz), 7.80–7.81 (d, 1H, CH, aromatic, J = 7.5 Hz), 7.94–7.96 (d, 1H, CH, aromatic, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ23.24, 45.33, 53.76, 54.38, 55.85, 60.70, 60.72, 63.52, 106.75, 114.36, 124.26, 124.45, 125.09, 125.41, 126.04, 126.98, 127.14, 128.59, 130.28, 130.75, 131.78, 133.93, 137.37, 140.33, 142.37, 151.32, 152.41, 155.75; Electrospray mass (MeOH)- 498 [M+H]$^+$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{32}H_{36}NO_4$, 498.2644, found 498.2638.

2-[4-(naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methyl)]-phenoxy]-piperazine (**46**):* Yield = 74%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ0.85–0.88 (t, 2H, CH_2, J = 7.0Hz), 1.24–1.41 (m, 4H, 2xCH_2), 2.01–2.09 (m, 4H, N(CH_2)$_2$), 3.32 (br s, 2H, N-CH_2), 3.63 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.46 (br s, 2H, OCH_2), 6.41–6.48 (m, 2H, 2xCH, aromatic), 6.51 (s, 1H, CH benzylic), 6.78–6.80 (d, 2H, 2xCH, aromatic, J = 9.0Hz), 6.91–6.93 (d, 1H,CH, aromatic, J = 7.0Hz), 7.02–7.04 (d, 2H, 2xCH, aromatic, J = 8.5 Hz), 7.32–7.42 (m, 3H, 3xCH, aromatic), 7.71–7.72 (d, 1H, CH, aromatic, J = 8.0 Hz), 7.82–7.83 (d, 1H, CH, aromatic, J = 8.0 Hz), 7.96–7.97 (d, 1H, CH, aromatic, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ22.07, 22.97, 45.34, 53.97, 54.04, 54.20, 56.45, 60.70, 60.75, 63.00, 106.69, 114.31, 124.28, 124.44, 125.09, 125.43, 126.06, 126.99, 127.17, 128.61, 130.31, 130.76, 131.79, 133.94, 137.32, 140.33, 142.37, 151.35, 152.41, 155.74; Electrospray mass (MeOH)- 512[M+H]$^+$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{33}H_{38}NO_4$, 512.2800, found 512.2790.

5.1.14. General procedure for the synthesis of compounds **50** and **51**

Synthesis of [2-(2-[Naphthalen-1-yl-(2,3,4-trimethoxyphenyl)-methyl]-phenoxy]-ethyl-1-pyrrolidine (50):To a stirred solution of phenol **31** (50 mg, 0.13 mmol) in dry THF (10 mL), sodium hydride (Pre-washed, 20 mg, 0.84 mmol) was added. After 10 min, 2-pyrrolidinoethyl chloride hydrochloride (88 mg, 0.39 mmol) was added to it and reaction mixture was refluxed for 2h. Water was added to it and extracted with ethyl acetate (10 mL x3) and washed with water (20 mL). Organic layer was dried over anhydrous sodium sulphate and evaporated to dryness under vacuum. The residue was charged on a silica gel column and eluted with chloroform-methanol to get desired product **50** at 5% methanol:-chloroform as gummy viscous liquid.

50: Yield = 87%; Yellowish viscous; Specific rotation [α] (EtOH), (20 °C) = +40°; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.23–1.26 (t, 4H, 2xCH_2, J = 6.5Hz), 2.60 (br s, 4H, N(CH_2)$_2$, 3.07 (s, br, 2H, N-CH_2), 3.44 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.32 (s, br, 2H, OCH_2), 6.38–6.44 (m, 2H, 2xCH, aromatic), 6.70 (s, 1H, CH), 6.79–6.91 (m, 4H, 4xCH, aromatic), 7.19–7.40 (m, 4H, 4xCH, aromatic), 7.67–7.69 (d, 1H, CH, aromatic, J = 8.5Hz), 7.80–7.81 (d, 1H, CH, aromatic, J = 7.5Hz), 7.90–7.92 (d, 1H, CH, aromatic, J = 7.0Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ22.79, 39.74, 53.63, 54.05, 55.79, 60.79, 63.98, 106.79, 111.34, 121.23, 124.01, 125.51, 126.20, 126.43, 126.56, 127.05, 127.98, 128.69, 129.24, 130.46, 131.79, 132.03, 133.89, 140.04, 142.34, 151.12, 152.51, 154.94; Electrospray mass (MeOH)- 498 [M+H]$^+$; 520[M+Na]$^+$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{32}H_{36}NO_4$, 498.2644, found 498.2641.

[2-(2-[Naphthalen-1-yl-*(2,3,4-trimethoxyphenyl)-methyl]-phenoxy]-ethyl-1-piperrdine(**51**):* Yield = 81%; Yellowish viscous; ^{1}H NMR ($CDCl_3$, 500 MHz): δ1.26 (s, br, 4H, 2xCH_2), 1.45 (br s, 4H, N(CH_2)$_2$), 2.30 (br s, 2H, CH_2), 2.80 (br s, 2H, N-CH_2), 3.47 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.18–4.24 (m, 2H, OCH_2), 6.40–6.47 (m, 2H, 2xCH, aromatic), 6.76 (s, 1H, CH), 6.80–6.93 (m, 4H, 4xCH, aromatic), 7.18–7.32 (m, 2H, 2xCH, aromatic), 7.39–7.41 (m, 2H, 2xCH, aromatic), 7.67–7.69 (d, 1H, CH, aromatic, J = 8.0Hz), 7.80–7.82 (d, 1H, CH, aromatic, J = 7.0Hz), 7.96–7.97 (d, 1H, CH, aromatic, J = 7.5Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ22.43, 23.73, 24.99, 39.53, 53.65, 54.64, 55.80, 56.99, 60.70, 60.75, 64.19, 106.67, 111.30, 114.57, 120.87, 124.11, 124.42, 125.32, 126.09, 126.47, 126.94, 127.79, 128.61, 129.39, 130.36, 131.91, 132.31, 133.94, 140.24, 142.37, 151.32, 152.42, 155.21; Electrospray mass (MeOH)- 512[M+H]$^+$; 534[M+Na]$^+$; HRMS (MeOH): m/z [M+H]$^+$, calcd for $C_{33}H_{38}NO_4$, 512.2800, found 512.2783.

5.2. Purity profile of compound **50** by UPLC

A reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of purity of compound **50** in the presence of its possible process impurities and storage degradation products. Liquid chromatographic system was consisting ACQUITY- UPLC H-Class Bio System (Waters, USA) equipped with a PDA detector.205 nm). The UPLC column was C-18 (BEH 130 Å, 1.7 × 50 mm, 1.7 μm, Waters, Milford, USA). The binary mobile phases were A (water with0.1% formic acid) and B (acetonitrile). The column was thermostated at 30 ± 0.1 °C. A gradient elution composition of mobile phase was selected 10% to 90% B in 5 min, and held for next 10 min. A constant flow rate was maintained at 0.3 mL/min.The injection volume was 3.0 μL. In absence of reference with defined potency, the purity of the compound was calculated by peak area normalization method.

5.3. Biological evaluation

All the biological evaluations were done as per standard protocols. Detailed descriptions are in Supplementary information (S1 to S9).

5.3.1. Cytotoxicity by Sulphorhodamine assay

As per Akindele et al. [36].

5.3.2. Soft agar colony assay

Soft agar colony formation assay was performed as per the reported method Kakuguchi et al. [37]. Colonies of MCF-7 cells were formed and treated with compound **50** at various concentrations 6.25–50 μg/mL. Tamoxifen was used at various concentrations of 1–27 μg/mL as positive control.

5.3.3. Cell cycle analysis of MCF-7 cells

The effect of compound **50** on cell division cycle of MCF-7 cells as well as MDA-MB-231 cells different concentrations (Half IC_{50}, IC_{50}, and double IC_{50}) was assessed by flow cytometry with PI-stained cellular DNA as per Riccardi et al. [38].

5.3.4. Annexin V-FITC staining assay

To assess the apoptosis Vs necrosis induction by compound **50**, we followed Annexin V-FITC apoptosis assay by Flow cytometry as per Looi et al. [39]. Different concentrations of compound **50** were used *i.e.* half IC_{50}, IC_{50}, and double IC_{50}. The assay was performed

against both MCF-7 and MDA-MB-231 cells separately.

5.3.5. Molecular docking studies

Performed by AutoDockVina [40] to know the binding conformation of ligands in active site of proteins structures. The ligands bound protein 3D crystallographic structure of Tubulin-Colchicine complex PDB ID: 402B to know the binding of enantiomers **50R** and **50S** along with colchicine and podophyllotoxin as control drugs.

5.3.6. Tubulin polymerisation assay

Tubulin Polymerisation assay was performed using 'assay kit' from Cytoskeleton, USA, as per Manufacturer's reported protocol [41,42]. Podophyllotoxin (PDT) was used as standard inhibitor and paclitaxel as standard stabilizer of tubulin polymerase and DMSO as negative control. The IC_{50} value was determined from dose-dependent analysis and is defined as the concentration that inhibits the rate of polymerisation by 50%.

5.3.7. Topoisomerase-II inhibition assay

Topoisomerase-II inhibition assay was performed on ELISA kit from Cloud-Clone Corp., USA (Catalog no. SEA792Hu) as per reported method [43]. Compound **50** was used at multiple IC_{50} concentrations. Etoposide and podophyllotoxin were used as positive controls.

5.3.8. Acute oral toxicity

Safety assessment was done as per reported method [44]. Compound **50** was given as single acute dose at 5 mg/kg, 50 mg/kg, and 300 mg/kg oral doses. The study and number of animals used were approved *via* CIMAP/IAEC/2016-19/32 dated 09-02-2017 by the Institutional Animal Ethics Committee (IAEC) of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

5.3.9. Statistical analysis

All data have been expressed as mean ± standard deviation (SD) were calculated using MS-Excel. Statistical analysis of differences was carried out by ANOVA followed by Tukey's multiple comparison test. Comparisons are made relative to the untreated controls. Differences with a p value < 0.05 were considered significant.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111986.

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