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Research paper

Triphenylethylene analogues: Design, synthesis and evaluation of antitumor activity and topoisomerase inhibitors

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ABSTRACT

To structurally relate anticancer drug tamoxifen used in the treatment of breast cancer, a sequence of compounds is designed and synthesized as potential drug candidates. McMurry coupling reaction is used as the key synthetic step in the preparation of these analogues and the ratios of E/Z-isomers are determined on the basis of NMR and HPLC experiments. The new compounds are found to be cytotoxic in the micromolar range with 60 human tumor cell lines at one dose and five dose concentration levels. Detailed studies on the most active compounds **11**–**13** show these compounds are capable to inhibit the growth of cancer cells. Finally, with the aim to correlate the antiproliferative activity with an intracellular target(s), the effect on relaxation activity of DNA topoisomerase-II is assayed. The relevance of interaction of most active compounds with topoisomerase-II is demonstrated which is also supported by docking studies.

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1. Introduction

The incidence of cancer continues to increase and remains the second leading cause of mortality through worldwide [1]. Among women, breast cancer is the one of the most common causes of death [2]. According to American Cancer Society Statistics estimated in 2019, over 1,762,450 cases of cancer were diagnosed in United States alone and that over 606,880 people died from this disease [3]. In recent years, researchers have made considerable progress in the field of drug discovery but still drug resistance in cancer treatment remains a frontline intervention [4]. In pharmaceuticals, a pioneering drug tamoxifen (I, Fig. 1) has attracted the attention of researchers over many years [5]. Tamoxifen (I, Fig. 1) is a triphenylethylene substituted selective estrogen receptor modulator (SERM) which is employed as a chemotherapeutic agent for breast cancer [6]. It is the first line chemopreventive which is effective at every stage of breast cancer in premenopausal and postmenopausal women [7]. These days, tamoxifen (I) (Z-isomer) has been sold as brand name Nolvadex and considered as one of the best selling medicine for breast cancer [8]. In addition to breast cancer, tamoxifen (I) (triphenylethylene derivative) also exhibited immense pharmacological properties such as antibacterial [9],

antioxidant [10,11], antifungal [9], antiangiogenesis [12] and antiviral [9] properties. Moreover, it reduces the risk of coronary artery disease and bone fractures at some sites [13]. In recent years, tamoxifen analogues have made a significant impact on the pharmacotherapy of estrogen receptor (ER) positive cancer cell lines.

Despite its many advantages, the most challenging issue is acquired resistance which often emerges with long term administration of tamoxifen (I, Fig. 1) in the victim [14]. Tamoxifen (I) acts as partial agonistic, thus, increasing the risk of endometrium cancer and venous thromboembolism [15]. It is also associated with numerous side effects such as menstrual abnormalities, vaginal dryness, hot flashes and hepatocytotoxicity [16–18].

Primarily, tamoxifen (I) ceases the tumor growth either by blocking estrogen receptor or inhibiting estrogen receptor mediated signaling pathways which prevents the interaction of transcription factor-ER [19]. As tamoxifen (I) has many sites of action, several non-ER mediated mechanisms of cytotoxcity have been documented such as PKC inhibition [20], calmodulin inhibition [21] and by suppressing formation of L-glutamate [22] etc. Besides these, tamoxifen (I) targets cellular DNA through intercalation [23] or by inhibiting the enzymes which promote DNA replication such as topoisomerase [24,25]. It is a cationic drug and inhibits both β oxidation and respiration in hepatic mitochondrial cells [25]. This causes cell cycle arrest and leads to programmed cell death called apoptosis.

Through structural-activity relationship studies, it has been







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Fig. 1. Structure of drug tamoxifen (I), toremifene (II), ospemifene (III); and tamoxifen derivative in clinical trial droloxifene (IV); and bioactive lead (V).

observed that the triarylethylene core (as in tamoxifen (I)) forms the backbone which mimics the effect of natural estrogen. It was observed that this core acts as an estrogen agonist and side-chain attached to the core is responsible for its antagonistic (full/partial) behavior [26]. Over the past years, some of the triphenylethylene analogues such as toremifene (II), ospemifene (III) and droloxifene (IV) (Fig. 1) have been studied extensively in clinical trials [27]. The effect of varying O-substituted aminoalkyl side chain has been demonstrated in the case of tamoxifen (I) [28], ospemifene (III).

[29] and bioactive lead (V) [30]. Recently, endoxifen, one of the metabolites of tamoxifen has been evaluated in phase I clinical trials for bipolar disorder [31]. Additionally, it has also been reported that substituting one of *N*-methyl group of tamoxifen side chain by a *N*-(2,2,2-trifluoroethyl) group loses its potency to inhibit MCF-7 cells [32]. This profound effect shows that the decrease in basicity of side chain reduces its potency towards cancer cell lines. Altogether, to develop structure-activity relationships towards the novel anticancer active triarylethylenes, variation in side-chain attached to its core has gained substantial attention in literature (Fig. 2).

In our present work, we have synthesized a new series of triphenylethylene analogues *via* McMurry coupling reaction with variations in amine side chain and characterized by various spectroscopic techniques. We described the synthesis of fifteen novel compounds and evaluated their anti-proliferative effect on a panel of 60 human cancer cell lines. It has been revealed that by the replacement of ethyl group with methyl group and introduction of amino-substituted isopropanol chain in tamoxifen (I) is considered as significant structural changes to enhance the anticancer activity. To study the molecular targets for cytotoxcity, the effect on topoisomerase-II relaxation is reported for the most cytotoxic derivatives. Furthermore, colorimetric analysis was also performed to investigate the toxicity towards normal cell lines. Docking studies also suggested a plausible explanation of the biological experimental evidences.

2. Results and discussion

2.1. Chemistry

To synthesize triphenylethylene scaffolds, a chemical approach was depicted including McMurry coupling and nucleophilic substitution reaction as shown in Scheme 1. Cross-coupled product 4-(1,2-diphenylprop-1-en-yl)phenol (3) was easily prepared by the reaction of equimolar amount of 4-hydroxy benzophenone (1) with acetophenone (2) *via* McMurry reaction [33], in a very good yield (80%). Subsequently, the desired intermediate **4** in 3.4:1 ratio of *E*and Z-isomers was furnished in good yield (65%) by the treatment of compound **3** with epichlorohydrin in the presence of potassium carbonate in acetonitrile. Finally, epoxide **4** was treated with various primary and secondary amines in acetonitrile at reflux temperature. The epoxy ring was opened up to afford corresponding derivatives 5-19 in quantitative yield 65-85% with varying ratio of E- and Z-isomers (Scheme 1). The statistical mixture of E- and Z-stereoisomers of triphenylethylene derivatives was obtained and attempts were made to isolate the E- and Z-isomers but these were unsuccessful due to same R_f value in different solvent systems. So, without any isolation of E- and Z-stereoisomers, the synthesized analogues were characterized by ¹H NMR, ¹³C NMR, and mass spectrometry (Schemes S1–S33). Further, the E/Z ratio of triphenylethylene derivatives was confirmed by ¹H NMR from the integral of respective protons [34] as well as HPLC methodology [35]. Moreover, the stereochemical assignments of the E- and Zisomers were determined on the basis of chemical shifts in ¹H NMR as well as 2D-NOESY and COSY experiments (Figs. S34 and S35)



Fig. 2. Design of novel triphenylethylene analogs.



Scheme 1. Synthesis of triphenylethylene analogues with variation of amines (5-19).

where methyl group (deshielded protons at δ 2.14 ppm) of the *E*isomer showed NOE with one of the aromatic ring and that NOE is absent in case of other isomer (Z-isomer, at δ 2.10 ppm). The side chain at aromatic ring also showed similar behavior. This relationship is also found to be true for tamoxifen as all the protons of E-tamoxifen resonate at down-field as compared to Z-tamoxifen [36,37]. The *E*/*Z* ratio of derivatives was confirmed by reverse phase HPLC methodology by opening of the epoxy ring with piperidine (in case of compound 5). Chromatogram of compound 5 spiked with Eand Z-isomers is shown in Fig. S36. The retention times of E- and Ztriphenylethylene have been found to be 10.14 and 12.41 min, respectively. Thus, the ¹H NMR spectrum of compound **5** contained CH₃ singlet, centered at δ 2.14 ppm and δ 2.10 ppm was assigned E configuration (retention time of 10.14) and Z configuration (retention time of 12.14), respectively. It has been revealed that the ratio of *E*/*Z*-isomers determined from ¹H NMR is corroborated with HPLC as both have same ratio of isomers i.e. 2.2:1. The E/Z ratios of other derivatives were also determined in the same methodologies.

2.2. In vitro antiproliferative activity

The *E*/*Z* derivatives of triphenylethylene series **5**–**19** were chosen by the National Cancer Institute, USA on account of computer modeling techniques for anticancer activity [38]. These compounds were evaluated *in vitro* for antiproliferative assay against a panel of 60-human cancer cell lines at a single dose of 10 μ M concentration, and the percentages of growth inhibition (% GI) over these cell lines were determined (Tables 1 and 2). Further the obtained results were also compared with known drug tamoxifen.

Most of the synthesized triphenylethylene analogues show an interesting antiproliferative activity with negative % growth inhibition with a higher cytotoxic activity towards most of the cancer cell lines. An opposite behavior, as expected, was found for tamoxifen, which shows cytotoxicity towards few cancer cell lines. Moreover, the comparison between the obtained results and the chemical structures of the new derivatives 5-19, allowed some interesting structure-activity relationships to be discovered. The analogous with secondary amine substituents piperidine (5), Nethylpiperazine (8) and *N*-benzylpiperazine (10) and primary amine substituents allyl amine (15) and 2-aminopyrazine (19) show a scarce antiproliferative activity and were considered to be least effective in the series. Increase in lipophilicity in case of analogous of triphenylethylene with substituents morpholine (6), *N*-methylpiperazine (7), *N*-phenylpiperazine (9), butyl amine (16), benzyl amine (17) and 4-flouroaniline (18) increased the antiproliferative activity to some extent. Interestingly, derivatives bearing primary amines 4-(2-aminoethyl)morpholine (11), cyclohexylamine (12), N,N-diethylethylenediamine (13) and N,N-dimethylethylenediamine (14) render the compounds more active, suggesting that hydrophilicity and/or hydrogen bond donor played an important role in the cytotoxic effect (Tables S1-S3).

Preliminary *in vitro* activity revealed that compound **6** inhibited the growth of leukemia cancer cell line K-562 and colon cancer cell line HT29 with growth inhibition of 80.30% and 86.88%, respectively. Compound **7** displayed selectivity towards leukemia cancer cell lines (K-562; GI = 95.76%, SR; GI = 96.19%) and colon cancer cell

Table 1

Percentage growth inhibition (GI%) of *in vitro* subpanel tumor cell lines at 10 μ M concentration of compounds 5–12.

Panel	Cell line	5	6	7	8	9	10	11	12
Leukemia	CCRF-CEM	8.64	19.56	24.80	_	29.97	4.15	43.49	-33.44
		4.77	20.20	21.20	7 10	20.05	10.04		
	HL-60(1B)	4.//	39.28	21.39	7.12	38.95	10.84	70.99	-57.06
	K-562	5 24	80.30	95.76	-	43.74	6.25	97.60	98.44
	MOL1-4	5.24	38.51	38.42	-	40.97	-	59.01	-41.90
	RPMI-8226	4.61	24.96	25.00	-	42.42	6.98	79.80	-43.71
	SK	24.68	52.84	96.19	2.33	39.72	-	99.31	-11.36
Lung	A549	-	12.84	27.94	-	21.29	-	29.23	-03.05
	EKVX	4.16	3.36	10.98	-	21.20	-	1/.1/	-32.61
	HOP-62	-	9.90	18.15	-	-	-	25.13	-69.72
	HOP-92	6.16	19.//	35.35	2.85	26.06	-	40.88	-89.52
	NCI-H226	0.76	3.44	-	-	12.46	-	3.88	22.74
	NCI-H23	-	-	5.03	-	25.05	-	16.//	-80.19
	NCI-H322M	-	4.48	9.54	-	6.94	-	27.32	-90.41
	NCI-H460	-	/.41	29.03	11.59	19./1	-	82.40	-92.05
Calar	NCI-H522	4.90	20.75	27.46	-	7.80	-	55.45	-54./1
Colon	UCC 2008	-	30.04	57.40	-	/.80	-	-04.50	-01.83
	HCC-2998	-	-	9.11	-	-	-	-59.62	-93.32
	HC1-116	-	53.05	-	3.68	25.67	-	-	/.30
	HC1-15	9.85	46.16	23.27	-	38.82	-	95.53	-88.15
	H129 KM12	29.64	86.88	91.//	-	05.8/	-	96.81	-42.20
	KM12	-	30.37	28.81	-	26.60	-	45.09	-82.85
CNE	SW-020	3.8/	11.43	17.74	-	20.09	-	30.95	-85.90
CNS	SF-295	-	-	2.85	-	-	-	28.83	-95.44
	SF-339	-	23.15	15.04	-	8.07	-	27.10	-9/.10
	SIND-19 SND 75	- 0 6 1	- 0 61	-	5.50	24.60	-	24.22	-50.55
	5IND-75	0.01 2.50	0.01	25.20	-	24.09	-	54.22	-93.70
Malanoma		3.39	12.51	17.62	-	7 80	-	07.39 81 20	-30.37
wicianoma	MALME 2M	-	24.75	28.55	-	10.00	-	71 70	-77.24
	MI4	2 68	-45.08	3 59		10.69		7 31	7 51
	MDA-MB-435	2.00	9.29	61.61	_	13.34	8 99	-63.83	-93 78
	SK-MEL-2	_	1.12	-	_	10.39	-	5.09	-60.26
	SKMEL-28	-	10.04	17 42	3 39	-	-	-86.2	-96.36
	SK-MEL-5	1.04	12.88	9.87	-	17.79	-	70.97	-96.13
	UAAC-257	-	6.64	16.76	-	8.47	-	-27.51	-62.76
	UAAC-62	-	3.00	37.33	-	12.30	-	37.33	-72.18
Ovarian	IGROV1	-	3.82	36.65	-	9.02	-	36.65	-93.28
	OVCAR-3	-	9.60	22.11	-	15.31	-	22.11	-88.29
	OVCAR-4	1.77	10.69	29.77	-	25.16	-	29.77	-86.38
	OVCAR-5	-	12.81	24.76	-	2.30	3.01	24.76	-91.75
	OVCAR-8	-	11.48	21.84	1.40	11.59	-	21.84	-37.30
	NCI-RES	-	6.78	19.81	-	18.18	-	19.81	-74.71
	SK-OV 3	-	8.68	4.57	-	-	-	4.57	-31.44
Renal	786-0	-	32.87	-	5.97	17.63	-	2.44	-
	A-498	13.49	8.44	13.96	-	-	-	20.05	31.68
	ACHN	-	1.16	1.00	-	2.30	4.23	18.56	-100.0
	CAKI-1	10.98	19.87	11.20	14.26	17.75	-	32.14	-95.99
	SN12C	2.91	11.63	10.98	-	22.36	-	23.93	-91.03
	TK-10	-	5.68	5.31	-	2.44	-	26.48	-66.91
	UO-31	17.37	26.33	22.40	-	63.15	-	30.83	-99.83
Prostate	PC-3	3.23	28.17	16.19	-	22.44	-	34.55	-79.44
	DU-145	-	1.88	11.04	-	8.36	30.12	32.25	-89.14
Breast	MCF7	14.84	15.80	30.20	-	52.99	-	55.39	-89.78
	MDA-MB-231	-	-	-	-	10.24	-	70.63	-90.39
	HS 578T	6.96	9.19	16.16	-	9.59	-	29.51	-58.46
	BT-549	-	6.69	-	-	5.70	10.74	-	-
	T-47D	10.78	39.68	5.73	-	45.18	-	10.65	-60.46
	MDA-MB-468	1.28	14.87	6.56	-	26.24	-	46.86	-79.45

- indicated inactive, **bold** - %GI \geq 50%, **red bold** – cytotoxic

Table 2

Percentage growth inhibition (GI%) of *in vitro* subpanel tumor cell lines at 10 μ M concentration of compounds **13–19**.

Panel	Cell Lines	13	14	15	16	17	18	19	ТАМ
Leukemia	CCRF-CEM	-18.04	69.22	-	31.00	6.47	32.91	-	65.4
	HL-60(TB)	-49.05	-13.70	-	37.01	6.20	69.53	-	60.4
	K-562	-22.96	89.95	2.90	88.82	14.85	29.60	17.73	-
	MOLT-4	-18.48	51.97	-	31.72	19.11	37.40	-	66.7
	RPMI-8226	-34.85	61.26	-	-17.72	-	37.41	-	-28.3
	SR	-5.03	-1.12	4.07	23.56	14.57	30.62	-	-18.9
Lung	A549	-32.08	28.60	1.77	11.03	13.99	24.93	-	50.2
C	EKVX	82.31	19.27	1.36	-	17.19	18.78	-	40.0
	HOP-62	-41.83	22.52	-	4.26	9.26	-	-	56.9
	HOP-92	-9.64	50.20	-	5.49	28.56	-	-	
	NCI-H226	17.14	_	-	3.96	18.51	-	-	7.50
	NCI-H23	65.34	9.83	-	12.29	13.99	18 43	14.06	39.7
	NCI-H322M	-1 25	17.64	-	-45 45	4 28	-	-	34.0
	NCL H460	-91.04	78.33		14 22	5.01	7 71		97.6
	NCI 11522	-01.04	27.80	11.94	26.15	20.68	27.75	22.02	52.4
Calan	COLO 205	-24.05	47.30	11.04	-20.15	20.08	31.15	23.02	55.4 90.0
Cololi	UCC 2008	-50.71	-47.32	-	-37.79	-	- 7.20	-	-09.0
	HCC-2998	-85.53	50.59	-	91.00	-	7.30	-	83.7
	HCI-116	33.21	21.93	28.79	82.10	15.85	25.04	7.02	73.8
	HCT-15	-24.27	69.64	-	89.24	5.69	25.66	3.04	92.4
	HT29	-28.60	90.25	-	60.24	7.92	12.05	-	-11.2
	KM12	-92.32	29.56	-	85.31	6.54	23.17	6.05	75.0
	SW-620	-69.07	28.94	-	1.48	3.38	16.62	-	95.1
CNS	SF-268	-	-	-	-	-	-	-	43.3
	SF-295	-83.19	25.42	-	11.27	5.41	-	-	59.8
	SF-539	-83.56	25.29	6.54	5.35	-	4.09	-	-
	SNB-19	91.33	16.34	1.10	19.29	7.77	-	-	47.4
	SNB-75	-87.43	12.04	-	27.58	20.39	3.70	-	60.1
	U251	-50.84	72.76	-	-81.49	4.71	-	-	95.2
Melanoma	LOX IMVI	-83.53	72.53	-	15.78	13.40	13.73	12.06	-43.9
	MALME-3M	-88.83	37.77	4.11	23.78	12.32	7.44	-	-67.5
	M14	9.43	17.64	-	-	11.49	8.93	-	-75.0
	MDA-MB-435	87.96	92.57	-	-	8.49	3.48	-	62.2
	SK-MEL-2	-41.46	2.14	-	2.72	5.85	-	-	31.5
	SKMEL-28	-87.09	39.73	-	6.69	-	-	-	88.7
	SK-MEL-5	-96 96	24.63	-	-	6 4 1	2.29	-	-95.2
	UAAC-257	-55.80	25.43	-	34 62	-	-	-	-64 3
	UAAC-62	-82 75	-	-	23.22	36 50	11 35	25 70	-
Overien	IGROV1	-32.02	23 75		22.22	16.24	3 55	12.24	58.8
Ovarian	OVCAP 2	-52.02	10.91	-	22.21	7.40	0.21	12.24	47.1
	OVCAR-J	-70.70	27.02	-	-	20.62	17.20	17.15	527
	OVCAR-4	-52.57	12.02	-	40.08	20.02	17.59	17.15	55.7
	OVCAR-5	84.32	12.95	4.33	17.04	2.67	-	-	-
	OVCAR-8	91.29	22.12	-	-	6.12	4.04	-	39.5
	NCI-RES	59.36	7.12	-	18.77	8.36	9.09	-	56.8
	SK-OV 3	-52.71	-	-	9.09	-		-	33.2
Renal	786-0	9.79	9.16	1.87	2.91	-	7.44	-	-41.0
	A-498	-76.21	17.09	4.04	20.97	-	-	20.67	35.9
	ACHN	-33.48	20.65	-	23.63	14.80	10.58	3.74	39.4
	CAKI-1	-56.47	24.18	4.39	-	35.13	72.10	20.07	70.9
	SN12C	68.67	12.96	-	43.55	12.95	21.12	-	52.3
	TK-10	-28.94	11.56	2.21	26.21	-	-	-	48.1
	UO-31	-83.59	30.46	8.86	24.08	42.40	57.23	30.24	59.7
Prostate	PC-3	-22.53	24.19	3.41	62.21	19.26	26.44	6.19	65.6
	DU-145	94 58	22.09	-	-	5.95	3 4 5	-	34.6
Breast	MCF7	_46.00	22.07	_	6.81	-	13 42	_	_10 2
-10031	MDA_MR_231	-43.67	31.70	_	0.01	26.04	28 08	11.66	54.9
	HS 578T	-43.04	22.55	-	-	20.94	20.90	11.00	394.0
	ПЭ J/01 DT 540	-4/.83	42.33	-	96 50	20.62	-	-	39.3
	B1-349 T 47D	0.02	0.3/	-	80.59	10 07	-	-	30.2
	1-4/D	-3.36	10 10	-	-	18.87	25.68	18.42	-
	MDA-MB-468	-05.15	42.18	-	-	8.72	8.97	11.49	-13.3

- indicated inactive, **bold** - %GI \geq 50%, **red bold** – cytotoxic

line (HT29; GI = 91.77%), melanoma cancer cell line (MDA-MB-435; GI = 61.61%) while compound **16** showed cytostatic activity towards leukemia cancer cell line K-562, and colon cancer cell lines HCC-2998, HCT-116, HCT-15, HT29 and KM12, and cytotoxic activity towards RPMI-8226, NCI–H322 M, NCI–H522, COLO 205 and U251 cell lines. As it is clear from the data given in tables, due to an increase in basicity, compounds 11–13 exhibited excellent cytostatic activity as well as cytotoxicity towards most of the cancer cell lines and therefore selected to test their anticancer activity at 5-dose concentration levels. Moreover, for most of the cancer cell lines, compounds **12** and **13** had more cytotoxicity than tamoxifen, indicating these compounds exhibited high selectivity and activity than tamoxifen and may also act *via* non-estrogen mechanism.

Compounds 11–13 were selected by the NCI for further testing for in vitro antitumor activity against 60 human tumor cell lines. The GI₅₀, total growth inhibition (TGI) and half maximal lethal concentration (LC₅₀) were measured in micromolar range for each cell line. For K562 human leukemia cancer cell line, compounds 12 and **13** had GI₅₀ values of 0.35 μ M and 0.52 μ M, respectively; values represent an activity higher than that for tamoxifen (TAM). Compound 13 also showed very good activity towards colon cancer cell line HT29 and melanoma cancer cell line LOXIMVI with GI₅₀ values of 0.56 μM and 0.43 μM , respectively. The MG MID GI_{50} values of these three compounds on the 60 tumor cell lines are shown in comparison with known drug TAM as positive control in Table 3. Compound 11 showed comparable activity as tamoxifen while compound 12 having cyclohexylamine in side-chain with GI₅₀ value of 1.69 µM and compound 13 containing N,N-diethyl ethylenediamine as side chain with GI_{50} value of 1.53 μ M showed enhanced activity as compared to tamoxifen ($GI_{50} = 3.76 \mu M$). It is concluded that compounds 12 and 13 were found to be 2–3 folds more active than tamoxifen.

2.3. MTT assay

To evaluate the cytotoxic effect of compounds **11**, **12** and **13** on human normal cell line Hek293, a colorimetric assay (MTT assay) was performed at five different concentrations of 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} M. It has been observed that derivative **11** showed only 15.82%, 13.73%, 7.04%, 5.43% and 4.74% cytotoxicity to Hek293 cells whereas compound **12** exhibited 18.89%, 15.13%, 9.13%, 6.34% and 5.29%; and compound **13** displayed 20.08%, 16.52%, 14.92%, 14.57%, 8.08% cytotoxicity to Hek293 cells at above said concentrations (Fig. 3). It revealed that the potent compounds **11**, **12** and **13** showed low cytotoxicity against normal cells and selectively kill the cancer cells. These compounds showed only 20% toxicity to Hek293 cells even at 100 μ M concentration.



Fig. 3. Cytotoxic effect of compounds 11, 12 and 13 to human normal cell line (Hek293).

2.4. DNA topoisomerase-II α mediated relaxation activity

To account for possible mechanism of cytotoxicity, the effect of the most active compounds **11**, **12** and **13** on relaxation activity of plasmid DNA catalyzed TOPO-II α was analyzed. As topoisomerase is a class of nucleoenzyme, the enzymatic action of topoisomerase-II α converts the supercoiled DNA into relaxed form. The inhibition of topoisomerase-II action impairs DNA replication, thus prevents the cell growth [39]. Supercoiled pHOT1 plasmid DNA was incubated with TOPO-II α and 50 μ L of compounds **11**, **12** and **13** in different wells on 1% agarose gel and DNA relaxation product was resolved by gel electrophoresis (BIO-RAD). A well-known drug etoposide (DNA intercalator, TOPO-II inhibitor) was used as a positive control. Fig. 4 showed the effect of representative compounds (**11**, **12**, **13** and positive control) on the relaxation of plasmid pHOT1 DNA, mediated by topoisomerase-II α . Among these compounds, compounds



Fig. 4. Effect on relaxation activity of supercoiled pHOT1 plasmid DNA by topoisomerase-II α . Supercoiled plasmid DNA was incubated with topoisomerase-II α , in the absence (TOPO-II) and in the presence of compounds **11**, **12**, **13** and etoposide positive control (PC) at 50 μ M concentrations.

Table 3

Compounds **11–13** and TAM with median growth-inhibitory (GI_{50} , μM), total growth-inhibitory (TGI, μM) and median lethal concentrations (LC_{50} , μM) of *in vitro* subpanel cancer cell lines.

Compound	Activity (µM)	Ι	II	III	IV	V	VI	VII	VIII	IX	MIG-MID ^a
11	GI ₅₀	2.19	4.19	2.06	2.81	1.87	5.20	2.40	3.57	3.69	3.10
	TGI	5.80	12.21	5.51	9.90	3.74	16.32	7.75	12.95	9.08	9.25
	LC ₅₀	23.9	36.33	13.75	25.21	8.55	45.84	22.03	38.45	29.07	26.7
12	GI ₅₀	1.54	1.70	1.56	1.69	1.81	1.96	1.62	1.67	1.73	1.69
	TGI	3.81	3.37	3.30	3.22	3.38	3.43	2.99	3.90	3.58	3.36
	LC ₅₀	38.61	5.85	5.64	5.87	6.11	24.00	5.71	6.11	6.73	11.62
13	GI ₅₀	1.29	1.50	1.23	1.47	1.28	1.65	1.40	2.40	1.59	1.53
	TGI	3.70	3.11	2.85	2.93	2.95	3.27	2.79	4.81	3.54	3.32
	LC ₅₀	1.50	5.87	5.93	5.45	5.46	6.04	5.47	6.39	37.25	8.81
TAM	GI ₅₀	2.08	4.01	1.88	4.04	2.03	4.70	7.71	4.40	3.05	3.76
	TGI	6.42	9.20	5.96	10.6	5.49	11.86	1.19	11.13	10.38	9.01
	LC ₅₀	2.90	2.60	1.77	44.14	1.70	2.78	2.59	233	2.83	24.4

I, leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer. ^a Full panel mean-graph midpoint (μM). TAM = Tamoxifen. **12** and **13** exerted significant but partial inhibitory effect on topoisomerase-II relaxation at 50 μ M concentration, as demonstrated by the appearance of supercoiled DNA. This data revealed that triphenylethylene analogues showed antiproliferative activity through TOPO-II inhibition. Taking into account that compound **11** exerts the low antiproliferative capacity. These data could also suggest the contribution of the inhibition on topoisomerase-II to the cytotoxicity.

2.5. Physicochemical properties

ADMET predicts the pharmacokinetic behavior of a molecule and structural modification in molecule alters its pharmacokinetic and distribution of a drug [40]. To determine the properties and drug-like characteristics of compounds 5-19, we have carried out the theoretical prediction of ADMET parameters (log P, molecular weight, TPSA, number of hydrogen donors and acceptors, and Lipinski rule violation) using MedChem_designer 5.5 software [41]. If a compound has log P \leq 5, TPSA (topological polar surface area) \leq 140, molecular weight \leq 500, number of H acceptor (nNO) \leq 10, number of hydrogen bond donor (nNHOH) \leq 5, then it is considered to be a member of biologically active drug family. $S + \log P$ predict the solubility of the compound in aqueous media and higher $S + \log P$ value lesser the solubility in an aqueous medium. Compounds violating ADMET and Lipinski's rule of five [42] have poor solubility and low absorption. As shown in Table 4, it is concluded that compounds selected for in vitro anticancer activity displayed good physiological properties except 9, 10, 17 and 18 where violation is due to either higher molecular weight or log P > 5. The antitumor activity of compounds **11–13** also supported the physicochemical parameters where no violation exists.

2.6. Molecular docking

Molecular docking studies were performed to understand the intermolecular interactions between the topoisomerase-II (PDB code: 1ZXM) and the ligands (**11**, **12** and **13**) using AutoDock vina software 4.0 and compare with etoposide [43]. Docked structure of compound **12** showed different binding sites than compounds **11** and **13** which share the same binding site. The binding energy scores for ligands **11**, **12**, **13** and etoposide (reference drug) were calculated to be -7.2, -6.0, -6.8 and -9.1 kcal/mol, respectively; suggesting a strong binding affinity of tamoxifen derivatives for the proposed binding sites, although it was not possible to effectively rank the compounds, due to the small binding energy differences

Physicochemical properties of triphonylethylong apalogue 5, 10 and reference drug tam	
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observed among them, that are in general lower than the suggested significant threshold (2.5 kcal/mol). These compounds were stabilized in the active site of TOPO-II through hydrogen bonding, π - π stacking and hydrophobic interactions (Fig. 5). Compound 11 interacted with residues of TOPO-II via Lvs306 (d = 2.59 Å). HIS354 (d = 3.79 Å) and ASN358 (d = 3.62 Å) through hydrogen bonding. and ILE311 (d = 3.60 Å) and MET61 (d = 5.11 Å) with van der Waal's forces (Fig. 5A). Compound 12 is surrounded by PHE308 and LYS357 showing only hydrophobic interaction with TOPO-II (Fig. 5B). Compound **13** binds with TOPO-II MET 273 (d = 2.35 Å) and TYR274 (d = 2.49 Å) residues by hydrogen bonds; GLU305 (d = 3.92 Å)residue by electrostatic- π anionic interactions; and TRP62, TRP361, LYS278 and LYS306 by hydrophobic interactions (Fig. 5C). Reference drug etoposide fits in the binding pocket of TOPO-II (ARG324, TRP62, ARG241, SER320, GLN59, GLN310, THR319 residues) through hydrogen bonding interactions (Fig. 5D).

3. Conclusion

We have identified a novel series of aminopropan-2-ol based E/Z-tamoxifen and characterized by NMR and HPLC spectroscopy. The biological data on 60 different human cancer cell lines at one dose and five dose concentration levels showed interesting activity for most of the described compounds. The presence of primary amines viz., (2-aminoethyl)morpholine (11), cyclohexylamine (12) and N,Ndiethylethylenediamine (13) at the side chain seems to improve the cytotoxic activity due to increase in the ability of hydrogen donor while most of the secondary amines of the tamoxifen skeleton don't affect positively on the cytotoxicity of the compound. MTT assay towards normal cell line Hek293 suggests that these compounds could kill selectively cancer cells and not normal cells. Further biological experiments point out TOPO-II as a possible intracellular target responsible for the cytotoxicity of these compounds. Furthermore, a docking study was performed and predicted the binding of compounds with TOPO-II by forming H-bond, van der Waal's interaction and hydrophobic interaction. In conclusion, interestingly, notwithstanding the common general triarylskeleton, here reported tamoxifen analogues show a different biological profile with respect to the reference drug, being more cytotoxic on non-estrogenic cell lines and affecting the catalvtic activity of the topoisomerase-II. Altogether, these results show various aminopropan-2-ol as an interesting side chain for the obtainment of new anticancer compounds. Altogether, the biological screening results showed triphenylethylene derivatives as an interesting scaffold and gives new insight into the development of

Entry	Diff. Coeff.	MlogP	S + logP	TPSA	Mol. Wt.	nNO	nNHOH	nviolation
5	0.574	4.568	5.750	32.70	427.59	3	1	none
6	0.583	3.564	5.061	41.93	429.56	4	1	none
7	0.564	3.760	4.981	35.94	442.60	4	1	none
8	0.551	3.953	5.224	35.94	456.63	4	1	none
9	0.527	4.754	6.584	35.94	504.67	4	1	1
10	0.516	4.660	6.277	35.94	518.70	4	1	1
11	0.547	3.165	4.847	53.96	472.63	5	2	none
12	0.560	4.761	6.247	41.49	441.62	3	2	none
13	0.539	4.345	5.519	44.73	458.65	4	2	none
14	0.564	3.956	5.109	44.73	430.59	4	2	none
15	0.594	4.494	5.081	41.49	399.54	3	2	none
16	0.574	4.764	5.954	41.49	415.57	3	2	none
17	0.560	5.129	6.136	41.49	449.59	3	2	1
18	0.571	5.571	6.797	41.49	453.56	3	2	1
19	0.583	3.237	4.949	67.27	437.54	5	2	none
TAM	0.610	5.201	6.642	12.47	371.52	2	0	1

TAM = Tamoxifen.

Table 4



Fig. 5. Binding poses showing interactions of compounds 11 (A), 12 (B), 13 (C) and etoposide (D) with TOPO-II (PDB code: 1ZXM).

novel anticancer drugs.

4. Experimental section

4.1. General procedure

All substrates, reagents, and solvents were purchased from Aldrich, Spectrochem, TCI and used without further purification. Reactions were carried out using dry solvent under nitrogen atmosphere. Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin-layer chromatography using silica gel GF-254 and visualized by UV light. Column chromatography was performed using silica gel of mesh size 60–120.¹H and ¹³C NMR were recorded on Jeol spectrometer (400 MHz for ¹H and 100 MHz for ¹³C) using CDCl₃ as a solvent. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference. Mass spectra of the synthesized compounds were observed at Water Micromass-Q-T of Micro.

4.2. Synthesis

4.2.1. 4-(1,2-Diphenylprop-1-en-1-yl)phenol (3)

To a two-necked oven-dried round bottom flask, a suspension of zinc (1.61 g. 25.2 mmol) in 20 mL of dry THF was stirred at 0-5 °C for 15 min. To the stirred suspension, the slow addition of TiCl₄ (1.38 mL, 12.6 mmol) was done carefully with the help of a syringe under the inert atmosphere of nitrogen. After the complete addition of TiCl₄, the resulting black mixture was stirred at room temperature for 30 min and then allowed to reflux for 3 h. The reaction mixture was gradually cooled to 0 °C and was charged with pyridine (0.5 mL, 6.3 mmol), and further stirred for 15 min. A solution of 4-hydroxy benzophenone (0.5 g, 2.5 mmol) and acetophenone (0.29 mL, 2.5 mmol) in dried THF was added slowly to the reaction mixture. The reaction was refluxed for 2.5 h. The reaction was monitored by TLC. After the completion of the reaction, it was quenched by the addition of a 10% aqueous solution of K₂CO₃ till neutralization. Then, the quenched solution was filtered off. From the filtrate, the crude product was extracted with chloroform. The organic layer was dried over sodium sulfate and the solvent was evaporated. A yellow colored oily product was collected. The crude was purified by column chromatography using eluents hexaneethyl acetate (97:3) to obtain the desired product. Finally, the chromatographed product was washed with hexane to afford white-colored precipitates of *E/Z* diastereomers in 1:1; 80% yield; mp. 145–148 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.31 (m, 2H, ArH), 7.23–7.21 (m, 3H, ArH), 7.18–7.05 (m, 12H, ArH), 7.03–6.96 (m, 3H, ArH), 6.89–6.84 (dd, 2H, ArH), 6.81–6.77 (m, 2H, ArH), 6.75–6.71 (m, 2H, ArH), 6.49–6.45 (m, 2H, ArH), 4.81 (s, 1H, OH), 4.59 (s, 1H, OH), 2.14 (s, 3H, CH₃), 2.10 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 154.22, 153.54, 144.32, 144.27, 143.81, 143.42, 138.84, 138.73, 136.21, 135.85, 135.41, 134.95, 132.28, 131.53, 131.02, 130.12, 129.42, 129.40, 128.19, 128.01, 127.93, 127.46, 126.64, 126.19, 126.18, 125.86, 115.00, 114.43 (Ar–C), 23.52 (CH₃), 23.44(CH₃); MS: 287.1 [M+1]⁺.

4.2.2. 2-((4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)methyl)oxirane (4)

4-(1,2-Diphenylprop-1-en-1-yl)phenol (1.0 g, 3.5 mmol) 3 was refluxed with epichlorohydrin (2.73 mL, 34.9 mmol) in the presence of potassium carbonate (0.58 g, 4.1 mmol) in acetonitrile for 16 h. After the completion of reaction, the solvent was evaporated under reduced pressure. Then the product was extracted from aqueous laver using CHCl₃. On removal of chloroform, the white colored semi-solid product was obtained. The crude was further purified by column chromatography by employing hexaneethylacetate (98:2) as solvent system to obtain white solid of E/Z3.4:1; 65% yield; mp. 58-62 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35-7.28 (m, 1H), 7.24-7.19 (m, 1H), 7.17-7.08 (m, 10H), 6.90-6.76 (m, 5H), 6.79-6.76 (m, 1H), 6.59-6.54 (m, 1H), 4.22 (dd, $1H, J = 11.0, J = 3.24 Hz, OCH_2$, 4.18 (dd, 1H, J = 11.04, J = 3.24 Hz, J = 3.24 Hz OCH_2), 3.97 (dd, 1H, J = 10.60, J = 5.64 Hz, OCH_2), 3.82 (dd, 1H, J = 10.92, J = 5.64 Hz, OCH₂), 3.38–3.33 (m, 1H, CH-epoxy), 3.29-3.25 (m, 1H, CH-epoxy), 2.92-2.87 (m, 1H, CH₂-epoxy), 2.86-2.83 (m, 1H, CH₂-epoxy), 2.77-2.73 (m, 1H, CH₂-epoxy), 2.69-2.67 (m, 1H, CH₂-epoxy), 2.14 (s, 3H, CH₃), 2.10 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.28, 156.61, 144.33, 144.30, 143.86, 143.45, 138.92, 138.80, 136.65, 136.64, 136.23, 135.53, 135.10, 132.17, 131.43, 131.07, 130.17, 129.47, 129.44, 128.26, 128.10, 128.00, 127.54, 126.71, 126.27, 125.94, 114.28, 113.62 (Ar–C), 68.88 (OCH₂), 68.61 (OCH₂), 50.30 (epoxy OCH), 50.20 (epoxy OCH), 44.89 (epoxy OCH₂), 44.86 (epoxy OCH₂), 23.59 (CH₃), 23.53 (CH₃); MS: 343.1 [M+1]⁺.

4.2.3. General procedure for the synthesis of triarylethylene analogous **5–19**

To a solution of 2-2-((4-(1,2-diphenylprop-1-en-1-yl)phenoxy) methyl)oxirane **4** (0.10 g, 0.2 mmol) in acetonitrile, K_2CO_3 (0.048 g, 0.35 mmol) was added and the solution was refluxed with various primary and secondary amines (1.4 mmol) for 3–4 h until the completion of reaction. After removal of solvent under reduced pressure, water was added to the reaction mixture and extracted with chloroform. The obtained residues were purified by column chromatography using different ratios of hexane-ethyl acetate as eluents to give compounds **5–19**.

4.2.3.1. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-(piperidin-1-yl)propan-2-ol (**5**). White solid,*E/Z* $: 2.2:1; Yield 75%; mp: 125–127 °C; ¹H NMR (400 MHz, CDCl₃) <math>\delta$ 7.35–7.31 (m, 1H), 7.24–7.20 (m, 1H), 7.15–7.07 (m, 9H), 7.02–6.97 (m, 3H), 6.90–6.84 (m, 4H), 6.77–6.74 (m, 1H), 6.58–6.54 (m, 1H), 4.12–4.06 (m, 1H, OCH₃), 4.01–3.99 (m, 1H, OCH₃), 3.98–3.97 (m, 1H, OCH) 3.96–3.94 (m, 1H, OCH), 3.85–3.79 (m, 1H, OCH₂), 3.49–3.44 (m, 1H, OCH₂), 2.61 (br, s, 4H, pip-NCH₂), 2.51 (m, 1H, OCH₂), 2.48–2.29 (m, 5H), 2.13 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 1.53–1.64 (m, 6H, pip-CH₂), 1.46–1.41 (m, 3H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 157.50, 144.29, 143.43, 138.91, 136.27, 135.36, 131.20, 131.00, 129.40, 127.90, 127.42, 126.15, 125.82, 114.10 (Ar–C), 70.41 (CH₂–O), 65.35 (C–OH), 61.31 (C–N), 54.81 (pip-NCH₂), 26.07 (CH₂-pip), 24.25 (CH₂-pip), 23.51 (CH₃); MS: 428.2 [M+1]⁺.

4.2.3.2. 1 - (4 - (1,2 - Diphenylprop - 1 - en - 1 - yl)phenoxy) - 3 - morpholinopropan - 2 - ol (**6**). White solid,*E/Z* $: 3:1; Yield 77%; mp: 124–127 °C; ¹H NMR (400 MHz, CDCl₃) <math>\delta$ 7.91 (br, s, 1H), 7.42–7.25 (m, 5H), 7.13–6.58 (m, 5H), 4.13–3.86 (m, 8H), 4.01 (s, 2H, OCH), 3.74 (s, 8H, morph O–CH₂), 3.06–2.86 (s, 4H), 2.67–2.32 (m, 14H), 2.15 (s, 3H, CH₃), 2.11 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.36, 156.68, 144.29, 144.24, 143.82, 143.39, 138.84, 138.71, 136.41, 136.00, 135.42, 134.96, 132.06, 131.32, 130.99, 130.09, 129.39, 128.16, 128.00, 127.91, 127.44, 126.60, 126.18, 126.15, 125.84, 114.08, 113.45 (Ar–C), 70.13 (OCH₂), 69.88 (OCH₂), 67.10 (morph-OCH₂), 67.08 (morph-OCH₂), 65.43 (OCH), 65.37 (OCH), 61.13 (N–CH₂), 61.05 (N–CH₂), 53.80 (morph-NCH₂), 23.50 (CH₃), 23.44 (CH₃); MS: 430.2 [M+1]⁺.

4.2.3.3. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-(4-methylpiperazin-1-yl)propan-2-ol (7). White solid,*E/Z* $: 1.75:1; Yield 78%; mp: 132–135 °C; ¹H NMR (400 MHz, CDCl₃) <math>\delta$ 7.34–7.30 (m, 1H), 7.23–7.20 (m, 2H), 7.15–7.06 (m, 9H), 7.02–6.95 (m, 3H), 6.90–6.84 (m, 4H), 6.74–6.78 (m, 1H), 6.57–6.54 (m, 1H), 4.12–4.06 (m, 1H, OCH₂), 4.03–4.00 (m, 1H, OCH₂), 3.98 (br, s, 1H, CH), 3.97 (br, s, 1H, CH), 3.84–3.82 (m, 1H, OCH), 2.70–2.40 (m, 14H), 2.28 (s, 3H, N–CH₃), 2.27 (s, 3H, N–CH₃), 2.13 (s, CH₃), 2.09 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.42, 156.74, 144.29, 144.26, 143.83, 143.40, 138.87, 138.74, 136.35, 135.93, 135.39, 134.92, 132.04, 131.30, 130.99, 130.09, 129.39, 128.16, 128.00, 127.90, 127.43, 126.59, 126.16, 125.82, 114.09, 113.46 (Ar–C), 70.24 (OCH₂), 70.00 (OCH₂), 65.55 (C–OH), 65.48 (C–OH), 60.51 (C–N), 60.44 (C–N), 55.19 (pip-NCH₂), 46.06 (pip-NCH₃), 23.50 (CH₃), 23.43 (CH₃); MS: 443.2 [M+1]⁺.

4.2.3.4. 1-(4-(1,2-Dipheny|prop-1-en-1-y|)phenoxy)-3-(4-ethy|piperazin-1-y|)propan-2-ol (**8**). White solid,*E/Z* $: 1.6:1; Yield 72%; mp: 136–138 °C; ¹H NMR (400 MHz, CDCl₃) <math>\delta$ 7.35–7.31 (m,

1H), 7.24–7.22 (m, 1H), 7.16–7.07 (m, 9H), 7.02–6.98 (m, 3H), 6.90–6.86 (m, 4H), 6.78–6.76 (m, 1H), 6.58–6.55 (m, 1H), 4.14–4.08 (m, 1H, OCH₂), 4.05–4.01 (m, 1H, OCH₂), 4.00 (s, 1H, CH), 3.98 (s, 1H, CH), 3.85–3.83 (m, 1H, OCH₂), 2.74–2.39 (m, 17H), 2.14 (s, 3H, CH₃), 2.11 (s, 2H, CH₃), 1.11–1.08 (m, 5H, N–CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.43, 156.73, 144.27, 143.84, 143.41, 138.89, 138.76, 136.35, 135.93, 132.04, 131.00, 130.99, 130.09, 129.39, 128.16, 128.00, 127.90, 127.43, 126.59, 126.16, 125.83, 114.59, 114.10, 113.47 (Ar–C), 70.27 (OCH₂), 70.03 (OCH₂), 65.55 (C–OH), 65.49 (C–OH), 60.55 (NCH₂), 60.48 (NCH₂), 52.82 (pip-NCH₂), 52.33 (pipalkyl-NCH₂), 23.50 (CH₃), 23.44 (CH₃), 11.97 (pipalkyl-CH₃); MS: 457.3 [M+1]⁺.

4.2.3.5. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-(4phenylpiperazin-1-yl)propan-2-ol (9). White solid, E/Z: 2.3:1; Yield 75%; mp:141–143 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.31 (m, 1H), 7.28-7.26 (m, 2H), 7.23-7.21 (m, 1H), 7.16-7.06 (m, 10H), 7.03-6.97 (m, 3H), 6.94-6.83 (m, 9H), 6.79-6.75 (m, 1H), 6.59-6.55 (m, 1H), 4.77 (s, 2H, OH), 4.18-4.11 (m, 1H, OCH₂), 4.10-4.04 (m, 1H, OCH₂), 4.02 (s, 1H, OCH), 4.01 (s, 1H, OCH), 3.97-3.96 (m, 1H, OCH₂), 3.87-3.86 (m, 1H, OCH₂), 3.26-3.17 (m, 7H, pip-CH₂), 2.87–2.76 (m, 4H, pip-CH₂), 2.68–2.48 (m, 7H), 2.14 (s, 3H, CH₃), 2.10 (s, 2H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.28, 156.60, 151.12, 144.14, 143.29, 138.74, 138.61, 136.29, 135.90, 135.31, 134.90, 131.96, 131.22, 130.89, 129.99, 129.87, 129.29, 129.13, 128.79, 128.72, 128.42, 128.34, 128.06, 127.90, 127.80, 127.33, 126.50, 126.07, 125.98, 125.73, 119.91, 116.13, 114.47, 113.98, 113.35 (Ar-C), 70.06 (OCH₂), 69.83 (OCH₂), 65.54 (C-OH), 65.48 (C-OH), 60.52 (NCH₂), 60.45 (NCH₂), 53.28 (pip-NCH₂), 53.25 (pip-NCH₂), 49.26 (pip-NCH₂), 49.23 (pip-NCH₂), 23.40 (CH₃), 23.34 (CH₃); MS: 505.3 $[M+1]^+$.

4.2.3.6. 1-(4-Benzylpiperazin-1-yl)-3-(4-(1,2-diphenylprop-1-en-1yl)phenoxy)propan-2-ol (10). White solid, *E*/*Z*: 2:1; Yield 74%; mp: 142–144 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.30 (m, 8H), 7.27-7.24 (m, 2H), 7.23-7.22 (m, 1H), 7.18-7.06 (m, 10H), 7.03-6.98 (m, 3H), 6.90–6.86 (m, 4H), 6.78–6.75 (m, 1H), 6.57–6.55 (m, 1H), 5.02 (br, s, 2H, OH), 4.15-4.02 (m, 2H, OCH₂), 3.99 (s, 1H, OCH), 3.98 (s, 1H, OCH), 3.53 (s, 2H, benzyl-CH₂), 3.51 (s, 2H, benzyl-CH₂), 2.82–2.39 (m, 1H), 2.14 (s, 3H, CH₃), 2.11 (s, 2H, CH₃); ¹³C NMR (100 MHz, CDCl₃) § 157.42, 156.74, 144.29, 144.28, 143.85, 143.42, 138.89, 138.76, 137.83, 136.34, 135.93, 135.39, 134.92, 132.07, 131.32, 131.02, 130.12, 129.97, 129.42, 129.37, 129.27, 128.91, 128.39, 128.18, 128.02, 127.93, 127.45, 127.29, 126.62, 126.18, 125.85, 114.59, 114.10, 113.47 (Ar-C), 70.26 (OCH2), 70.01 (OCH2), 65.43 (C-OH), 65.36 (C-OH), 62.98 (NCH₂), 60.60 (benzyl-C), 60.53 (benzyl-C), 53.27 (pip-NCH₂), 52.86 (pip-NCH₂), 23.53 (CH₃), 23.47 (CH₃); MS: 519.3 $[M+1]^+$.

4.2.3.7. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-((2morpholinoethyl)amino)propan-2-ol (11). White solid, E/Z: 1.33:1; Yield 76%; mp: 137–140 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.31 (m, 2H), 7.24-7.19 (m, 2H), 7.17-7.06 (m, 11H), 7.00-6.97 (m, 3H), 6.89-6.84 (m, 4H), 6.78-6.74 (m, 2H), 6.56-6.52 (m, 2H), 4.16-4.10 (m, 1H, OCH₂), 4.06-4.01 (m, 1H, OCH₂), 4.00-3.95 (m, 2H, OCH), 3.87-3.80 (m, 2H, OCH₂), 3.70-3.65 (m, 7H, morpho-CH₂), 3.15-2.73 (m, 12H), 2.56-2.49 (m, 4H), 2.46-2.43 (m, 7H, morpho-CH₂), 2.13 (s, 3H, CH₃), 2.09 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.36, 156.68, 144.29, 144.23, 143.82, 143.39, 138.84, 138.70, 136.39, 135.97, 135.41, 134.96, 132.08, 131.33, 130.99, 130.09, 129.98, 129.39, 128.88, 128.53, 128.17, 128.01, 127.91, 127.44, 126.61, 126.10, 125.85, 114.56, 114.07, 113.44 (Ar-C), 70.38 (OCH₂), 70.16 (OCH₂), 68.50 (C-OH), 68.44 (C-OH), 67.09 (morph-OC), 67.00 (morph-OC), 58.26 (ethylene-NC), 53.78 (morph-NC), 53.72 (morph-NC), 51.86 (NCH₂), 51.80 (CH₂), 45.82 (ethylene-NHC), 23.50 (CH₃), 23.46

(CH₃); MS: 473.29 [M+1]⁺.

4.2.3.8. 1-(Cyclohexylamino)-3-(4-(1,2-diphenylprop-1-en-1-yl)phenoxy)propan-2-ol (12). White solid, E/Z: 2.3:1; Yield 80%; mp: 135–138 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.30 (m, 1H), 7.22-7.20 (m, 1H), 7.16-7.05 (m, 10H), 7.02-6.96 (m, 3H), 6.89-6.83 (m, 4H), 6.78-6.74 (m, 1H), 6.57-6.53 (m, 1H), 4.01-3.87 (m, 4H), 3.83-3.81 (m, 1H, OCH₂), 2.93-2.89 (m, 1H, CH₂), 2.85-2.81 (m, 1H, CH₂), 2.76-2.71 (m, 1H, CH₂), 2.68-2.63 (m, 1H, CH₂), 2.45–2.35 (m, 2H, cyclohexyl-CH), 2.13 (s, 3H, CH₃), 2.03 (s, 3H, CH₃), 1.91–1.84 (m, 4H, cyclohexyl-NCH₂), 1.74–1.68 (m, 4H, CH₂), 1.61–1.57 (m, 4H, cyclohexyl-NCH₂), 1.29–1.19 (m, 4H, CH₂), 1.16–1.00 (m, 4H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 157.34, 156.71, 144.29, 144.25, 143.40, 138.86, 138.72, 136.36, 136.29 135.98, 135.88, 135.39, 135.32, 134.99, 134.93, 132.06, 131.31, 130.99, 130.09, 129.39, 128.16, 128.00, 127.90, 127.43, 126.59, 126.16, 125.83, 114.09, 113.45 (Ar-C), 70.46 (OCH₂), 70.24 (OCH₂), 68.53 (C-OH), 68.48 (C-OH), 56.84 (NCH₂), 56.80 (NCH₂), 48.83 (cyclohexyl-NCH), 48.76 (cyclohexyl-NCH), 34.03 (cyclohexyl-C), 33.98 (cyclohexyl-C), 33.79 (cyclohexyl-C), 33.75 (cyclohexyl-C), 26.15 (cyclohexyl-C), 25.11 (cyclohexyl-C), 23.49 (CH₃), 23.44 (CH₃); MS: 442.2 [M+1]⁺.

4.2.3.9. 1-(Diethylamino)-3-(4-(1,2-diphenylprop-1-en-1-yl)phenoxy)propan-2-ol (13). White solid, E/Z: 2.5:1; Yield 78%; mp: 130–132 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.31 (m, 1H), 7.24-7.22 (m, 1H), 7.16-7.08 (m, 9H), 7.01-6.96 (m, 3H), 6.90-6.88 (m, 4H), 6.79–6.77 (m, 1H), 6.58–6.56 (m, 1H), 4.09–4.05 (m, 1H, OCH₂), 4.02–3.91 (m, 3H), 3.86–3.79 (m, 1H, OCH₂), 3.32 (br, s, 2H, NH), 2.90-2.69 (m, 6H), 2.58-2.49 (m, 9H), 2.15 (s, 3H, CH₃), 2.12 (s, 1H, CH₃), 1.04–0.98 (m, 9H, N,N- diethyl CH₃); ¹³C NMR (100 MHz, CDCl₃) § 157.42, 156.74, 144.31, 144.28, 143.86, 143.42, 138.91, 138.77, 136.32, 135.91, 135.39, 134.92, 132.06, 131.39, 131.31, 131.00, 130.10, 129.41, 129.38, 128.17, 128.02, 127.92, 127.45, 126.68, 126.18, 125.84, 114.12, 113.49 (Ar-C), 70.42 (OCH₂), 70.19 (OCH₂), 68.03 (C-OH), 67.96 (C-OH), 52.31 (NHCH₂), 52.26 (NHCH₂), 52.00 (ethylene-NCH₂), 51.92 (ethylene-NCH₂), 46.93 (ethylene-NHCH₂), 46.85 (N,N-diethyl CH₂), 23.52 (CH₃), 23.46 (CH₃), 11.42 (N,Ndiethyl CH₃); MS: 459.3 [M+1]⁺.

4.2.3.10. 1-(Dimethylamino)-3-(4-(1,2-diphenylprop-1-en-1-yl)phenoxy)propan-2-ol (**14**). White solid, *E/*2: 3.5:1; Yield 75%; mp: 127–129 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.30 (m, 1H), 7.22–7.20 (m, 1H), 7.14–7.06 (m, 14H), 7.00–6.95 (m, 3H), 6.88–6.85 (m, 4H), 6.77–6.72 (m, 1H), 6.56–6.54 (m, 1H), 4.06–4.00 (m, 2H, OCH₂), 3.98–3.93 (m, 2H, OCH), 3.82–3.81 (m, 1H, OCH₂), 2.89–2.66 (m, 6H), 2.47–2.29 (m, 5H), 2.22 (s, 6H, dimethyl-NCH₃), 2.20 (s, 2H, dimethyl-NCH₃), 2.13 (s, 3H, CH₃), 2.09 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.43, 156.73, 144.27, 138.88, 138.75, 136.29, 135.88, 135.37, 134.90, 132.05, 131.30, 131.00, 130.10, 129.95, 129.40, 128.16, 128.01, 127.90, 127.43, 126.59, 126.16, 126.07, 125.82, 114.09, 113.46 (Ar–C), 70.39 (OCH₂), 70.17 (OCH₂), 68.53 (C–OH), 68.47 (C–OH), 59.21 (ethylene-NCH₂), 51.96 (NCH₂), 51.89 (NCH₂), 47.02 (ethylene-NHC), 45.50 (ethylene-NCH₃), 23.50 (CH₃), 23.44 (NCH₃); MS: 431.2 [M+1]⁺.

4.2.3.11. 1-(Allylamino)-3-(4-(1,2-diphenylprop-1-en-1-yl)phenoxy) propan-2-ol (**15**). White solid, *E*/*Z*: 1.7:1; Yield 68%; mp: 120–123 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.31 (m, 1H), 7.23–7.20 (m, 1H), 7.17–7.05 (m, 10H), 7.02–6.95 (m, 3H), 6.89–6.85 (m, 4H), 6.78–6.75 (m, 1H), 6.56–6.52 (m, 1H), 5.91–5.77 (m, 1H, allyl-CH), 5.24–5.12 (m, 2H, allyl CH₂), 4.16–4.07 (m, 1H, OCH₂), 4.05–4.01 (m, 1H, OCH₂), 3.99–3.94 (m, 2H, OCH), 3.87–3.79 (m, 1H, OCH₂), 3.49–3.44 (m, 1H, OCH₂), 3.40–3.17 (m, 2H, allyl-NCH₂), 2.88–2.58 (m, 3H, CH₂), 2.13 (s, 3H, CH₃), 2.10 (s, 2H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.28, 157.24, 156.59,

156.55, 144.28, 144.24, 143.81, 143.75, 143.39, 138.84, 138.71, 136.47, 136.07, 135.43, 134.99, 134.65, 134.55, 132.23, 132.10, 131.36, 131.00, 130.11, 130.02, 129.40, 128.89, 128.53, 128.18, 128.02, 127.92, 127.45, 126.62, 126.19, 125.85, 118.77 (allyl-CH₂), 114.58, 114.48, 114.10, 113.47 (Ar–C), 70.17 (OCH₂), 70.14 (OCH₂), 68.12 (C–OH), 68.05 (C–OH), 52.31 (NCH₂), 52.26 (NCH₂), 41.10 (allyl-NCH₂), 23.52 (CH₃), 23.46 (CH₃); MS: 400.2 [M+1]⁺.

4.2.3.12. 1-(Butylamino)-3-(4-(1,2-diphenylprop-1-en-1-yl)phenoxy) *propan-2-ol* (**16**). White solid, *E*/*Z*: 2.4:1; Yield 85%; mp: 119–121 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.30 (m, 1H), 7.22-7.20 (m, 1H), 7.15-7.07 (m, 9H), 7.00-6.98 (m, 3H), 6.88-6.86 (m, 4H), 6.71–6.69 (m, 1H), 6.55–6.45 (m, 1H), 4.06 (s, 1H, OCH₂), 3.97-3.96 (m, 2H, OCH), 3.92-3.81 (m, 1H, OCH₂), 3.25-3.16 (m, 1H, OCH₂), 2.86–2.58 (m, 4H, OCH), 2.33–2.29 (m, 3H, butyl-NCH₂), 2.13 (s, 3H, CH₃), 2.09 (s, 2H, CH₃), 1.57–1.43 (m, 3H, butyl-CH₂), 1.36–1.31 (m, 3H, butyl-CH₂), 0.92–0.87 (m, 4H, butyl-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.15, 156.78, 144.24, 144.09, 143.24, 138.69, 138.55, 136.27, 135.31, 135.26, 132.05, 131.93, 131.29, 131.18, 130.84, 129.95, 129.24, 128.01, 127.98, 127.86, 127.81, 127.76, 127.29, 126.46, 126.40, 126.03, 125.94, 125.69, 125.62, 114.98, 114.41, 113.93, 113.29 (Ar-C), 70.28 (OCH2), 70.06 (OCH2), 68.00 (C-OH), 67.94 (C-OH), 51.66 (NCH₂), 51.60 (NCH₂), 49.41 (butyl-NCH), 31.90 (butyl-C), 31.54 (butyl-C), 23.35 (CH₃), 20.30 (butyl-C), 14.10 (butyl-C), 13.94 (butyl-C); MS: 416.2 [M+1]⁺.

4.2.3.13. 1-(*Benzylamino*)-3-(4-(1,2-*diphenylprop*-1-*en*-1-*yl*)*phenoxy*)*propan*-2-*ol* (**17**). White solid, *E*/*Z*: 2:1; Yield 67%; mp: 133–135 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.26 (m, 9H), 7.22–7.20 (m, 2H), 7.02–6.98 (m, 3H), 6.89–6.81 (m, 4H), 6.77–6.74 (m, 1H), 6.56–6.53 (m, 1H), 4.10–4.05 (m, 2H, OCH₂), 3.99–3.97 (m, 2H, OCH), 3.84–3.78 (m, 2H, OCH₂), 3.51 (s, 2H, benzyl-CH₂), 3.49 (s, 1H, benzyl-CH₂), 2.77–2.69 (m, 2H, CH), 2.53–2.47 (m, 2H, CH), 2.13 (s, 3H, CH₃), 2.09 (s, 2H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.44, 156.70, 144.28, 143.85, 143.43, 138.90, 138.77, 137.97, 136.34, 135.93, 135.39, 134.92, 132.06, 131.32, 131.02, 130.12, 129.97, 129.42, 129.36, 129.20, 128.53, 128.49, 128.37, 128.18, 128.02, 127.93, 127.45, 127.25, 126.60, 126.18, 126.09, 125.85, 114.60, 114.11, 113.48 (Ar–C), 70.27 (OCH₂), 70.03 (OCH₂), 65.50 (C–OH), 65.45 (C–OH), 60.72 (benzyl-C), 60.49 (benzyl-C), 53.45 (NCH₂), 53.14 (NCH₂), 23.53 (CH₃), 23.47 (CH₃); MS: 450.2 [M+1]⁺.

4.2.3.14. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-((4-fluorophenyl)amino)propan-2-ol (**18**). White solid,*E/Z* $: 12.5:1; Yield 70%; mp: 134–137 °C; ¹H NMR (400 MHz, CDCl₃) <math>\delta$ 7.35–726 (m, 1H), 7.17–7.05 (m, 8H), 7.03–6.96 (m, 3H), 6.92–6.84 (m, 6H), 6.63–6.59 (m, 2H), 4.25–4.24 (m, 1H, OCH₂), 4.10–4.02 (m, 2H, OCH), 3.98–3.91 (m, 1H, OCH₂), 3.49–3.43 (m, 1H, CH), 3.40–3.36 (m, 1H, CH), 3.29–3.22 (m, 1H, CH), 2.13 (s, 3H, CH₃), 2.10 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.04, 144.48, 144.17, 143.34, 138.74, 136.79, 135.56, 131.45, 130.99, 129.40, 127.95, 127.49, 126.25, 125.91, 115.97, 115.75, 114.34, 114.26, 114.10 (Ar–C), 70.06 (OCH₂), 68.83 (C–OH), 47.34 (NCH₂), 23.51 (CH₃); MS: 453.2 [M+1]⁺.

4.2.3.15. 1-(4-(1,2-Diphenylprop-1-en-1-yl)phenoxy)-3-(pyrazin-2ylamino)propan-2-ol (**19**). White solid, *E/Z*: 1.5:1; Yield 65%; mp: 148–150 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.31 (m, 2H), 7.22 (m, 1H), 7.14–7.09 (m, 12H), 7.01–6.99 (m, 4H), 6.90–6.86 (m, 3H), 6.80–6.78 (m, 2H), 6.72–6.74 (m, 2H), 4.78 (br, s, 1H, NH), 4.55 (br, s, 1H, NH), 4.22–3.95 (m, 3H), 2.54–2.40 (m, 2H, CH₂), 2.14 (s, 1H, CH₃), 2.10 (s, 1H, CH₃); MS: 438.2 [M+1]⁺.

4.3. HPLC chromatography

To analyze the ratio of *E*- and *Z*-isomers of the triphenylethylene

derivatives, reversed-phase high-performance liquid chromatography (RP-HPLC) has been performed using Ultimate 3000 HPLC (Thermofisher Scientific) system, equipped with C-18 column $(250 \times 4.6 \text{ mm}, 5 \text{ mm})$ and UV-vis detector. The wavelength used was 276 nm and the mobile phase was comprised of 0.01 M tetrabutylammonium hydrogen sulfate: methanol: acetonitrile (5:10:85: v/v/v) with flow rate 1.0 mL/min. The stock solution of E/Ztriphenvlethylene derivative i.e. 5 (1 mM) was prepared in acetonitrile and protected from direct light. The stock solution was then diluted to produce a solution of compound in acetonitrile $(2.3 \times 10^{-4} \text{ M})$. First, the prepared sample was filtrated using 0.22 μ m cellulose filtrate. Then, 20 μ l of the filtered sample was injected into the column with an injection loop.

4.4. Biological evaluation

4.4.1. MTT assav

The cytotoxicity of triphenylethylene derivatives 11-13 was determined using MTT assay. Hek293 human embryonic kidney cells were seeded in 96 well plates at the density of 1×10^{-5} cells/ well in low glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 50 mM glutamine, 100 U/ ml penicillin and 100 mg/mL streptomycin. Cells were incubated at 37 °C in an atmosphere of 5% CO₂ incubator. Cells were treated with compounds **11**, **12** and **13** at five concentrations $(10^{-4}, 10^{-5}, 10^{-6}, 1$ 10^{-7} , 10^{-8} M) for 24 h at 37 °C 10 μ l of MTT (prepared in PBS buffer) from 5 mg/mL stock was added in each well and incubated at 37 °C for 4 h in dark. The formazan crystals were dissolved using 100 μ l of DMSO. Further, the amount of formazan crystal formation was measured as the difference in absorbance by Bio-Red ELISA plate reader at 570 nm reference wavelength. The experiments were performed three times in triplicates. The relative cell toxicity (%) related to control wells containing culture medium without test material was calculated by using the formula:

% cell toxicity =
$$\frac{OD (compound treated wells)}{OD (untreated wells)} \times 100$$

4.4.2. Topoisomerase-II assay

Relaxation of supercoiled plasmid DNA by TOPO-IIa was assayed in 20 µL of buffer (0.5 M Tris-HCl (pH 8.0), 1.5 M NaCl, 100 mM MgCl₂, 20 mM ATP, 5 mM dithiothreitol, 300 μ g/mL BSA) containing drug solution, 4 U of DNA topoisomerase-IIa and 500 ng of supercoiled pHOT1 plasmid DNA and incubated at 37 °C for 30 min. Reactions were terminated by the addition of 2 μ L of a solution containing 10% sodium dodecyl sulfate (SDS) and 0.5 mg/mL of proteinase K and incubated at 37 °C for 30 min. Then, 1 µL of loading buffer (50% glycerol and 0.25% bromophenol blue) was added. The samples were electrophoresed in 1% agarose gel with TAE buffer (100 mL of 10 stock solution-4.8 g of tris base, 1.14 mL acetic acid, and 0.37 g EDTA, pH 8.1) for 4 h, then the DNA was stained with ethidium bromide and photographed under UV illuminator.

4.4.3. Molecular modeling

Molecular docking studies were employed to authenticate the interaction between ligand and TOPO-II using software AutoDock 4.0. The three-dimensional structure of topoisomerase-II was obtained from the protein data bank (PDB ID: 1ZXM). To setup ligand TOPO-II interaction, AutoDock tools 1.5.6rc3 were performed. The tools removed water molecules and added hydrogen atoms from the protein. A cubic grid dimension 126 Å \times 100 Å \times 126 Å with the grid points along the x, y and z axes and a grid spacing of 0.375 Å was used. The optimized structures of ligand and TOPO-II interactions were converted to the required pdbgt format.

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.2020.112775.

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