Design and Synthesis of Novel Benzoazepinone Derivatives as Potent Estrogen Receptor Alpha Inhibitors

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Abstract

Background: Selective estrogen receptor modulators (SERMs) block the effects of estrogen on breast cancer cells by sitting in the estrogen receptors. If a SERM is in the estrogen receptor, estrogen can't attach to the cancer cell and the cell doesn't receive estrogen's signals to grow and multiply. The goal of this research is to develop small drug-like molecules of novel Benzoazepinone derivatives that mimic the ability of the SERM (Tamoxifene and Raloxifene) to binds with estrogen receptor protein.

Methods: 2-Phenylethyl bromide undergoes amino alkylation through mannich reaction with CH_3NH_2 and chloro acetyl chloride, gives 2-chloro-N-methyl-N-phenethylacetamide, which is further undergoing cyclization gives 3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one.2-phenylethyl bromide. 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. di-p-toluoyl-L-tartaric acid and 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. HCl was obtained by treatment with di-p-toluoyl-L-tartaric acid and con. HCl respectively. Finally, this intermediate undergoes nucleophilic addition reactions with different substituted aldehydes. All the compounds were screened for their *in-vitro* cytotoxicity activity using Vero and MDA MB 231 cell lines by MTT assay.

Results: IC50 values from Cytotoxicity studies by MTT assay ranges from 11µg/ml to 153µg/ml. A total of 15 compounds were synthesized by using a diverse scheme and the title compounds have exhibited low to high *in-vitro* anticancer activity with MDA MB 231 cells. Compared to the standard (Raloxifene 6 µg/ml), the developed compounds T2 (35µg/ml), T10 (36µg/ml), T14 (11µg/ml) and T15 (22 µg/ml).

Conclusion: Finally, four compounds might be used as a lead molecule for future development into a therapeutically viable anti-ER positive breast cancer drug from the benzoazepinone derivatives family.

Keywords- ER alpha inhibitors, Raloxifene, *In vitro*, MDA MB-231, Bezoazepenine, ERα, Breast Cancer.

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1. Introduction

Cancer is the deadliest disease and search is in progress to identify the potential anticancer drugs. Breast cancer is one of the most common malignancies affecting women in both the developed and developing world [1]. In India, breast cancer accounts for 14% of all cancers in women. According to the Globocan 2021 data, 1,62,468 new cases and 87, 090 deaths were reported for breast cancer in India [2]. Poor diagnosis and high cost of treatment contribute to high mortality. Two-thirds of all breast cancers are Estrogen Receptor-α (ERα) positive and therefore identification of candidates for antiestrogen therapy is going on. The estrogen dependency of breast cancer represents a unique feature of the disease that can be manipulated to effectively control growth and/or prevent tumor development. Indeed, the current strategy for treatment of hormone-dependent breast cancer is to block the action of estrogen on tumor cells through either of three possibilities: (a) inhibiting estrogen from binding to its main target ERα using an

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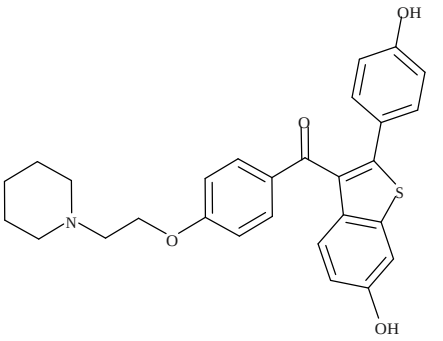
antiestrogen such as tamoxifen/raloxifen [3-8]; (b) preventing estrogen synthesis using an aromatase inhibitor [9-15]; (c) down-regulating ERα protein levels using a pure antiestrogen such as fulvestrant [16-17]. The successes of these current endocrine therapies, however, are often limited by the development of resistance.

1.1. Drugs existing for ER-positive breast cancer therapy and drawbacks

Selective Estrogen Receptor Modulators (SERMs) [18–20] example Tamoxifen (1), Raloxifene (2), block the effects of estrogen in the breast tissue. SERMs work by sitting in the estrogen receptors in breast cells. If a SERM is in the estrogen receptor, there is no room for estrogen, and it can't attach to the cell. If estrogen isn't connected to a breast cell, the cell doesn't receive estrogen's signals to grow and multiply.



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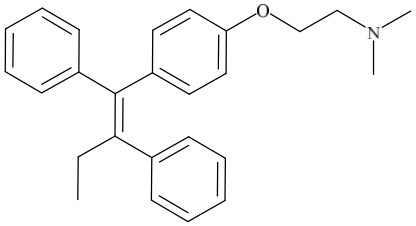

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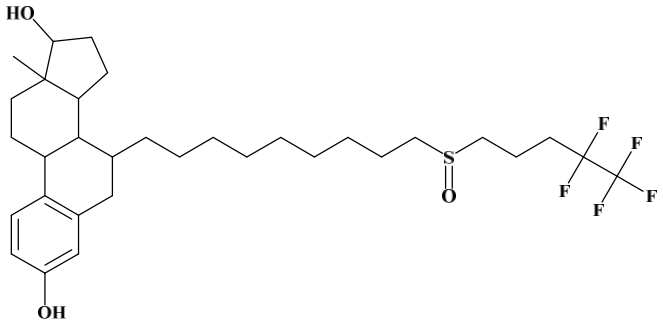

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Cells in other tissues in the body, such as bones and the uterus, also have estrogen receptors. But each estrogen receptor has a slightly different structure, depending on the kind of cell it is in. So breast cell estrogen receptors are other than bone cell estrogen receptors, and both estrogen receptors are different from uterine estrogen receptors. As their name says, SERMs are "selective" this means that a SERM that blocks estrogen's action in breast cells can activate estrogen's activity in other cells, such as bone, liver, and uterine cells. SERMs may cause serious side effects, including blood clots, stroke, and endometrial cancer. A Selective Estrogen Receptor Degrader or down regulator (SERD) [21-22], for example, Fulvestrant (3) is a type of drug which binds to the ERα and, in the process of doing so, causes the ERα to be degraded and thus downregulated. They are used to treat estrogen receptor-sensitive or progesterone receptor-sensitive breast cancer, along with older drugs like selective estrogen receptor modulators (SERMs) and aromatase inhibitors [23-24]. Two-thirds of all breast cancers are ERα positive, so candidates for antiestrogen therapy are identified. The estrogen dependency of breast cancer represents a unique disease that can be manipulated to

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control growth and/or prevent tumor development effectively. Indeed, the current strategy for the treatment of hormone-dependent breast cancer is to block the action of estrogen on tumor cells through either of three possibilities: (a) inhibiting estrogen from binding to its primary target ERα using an antiestrogen such as tamoxifen/raloxifene [25]; (b) preventing estrogen synthesis using an aromatase inhibitor [23,26–29]; (c) downregulating ERα protein levels using a pure antiestrogen such as fulvestrant [22]. However, the successes of these current endocrine therapies are often limited by the development of resistance. The goal of this research is to develop small drug-like molecules of novel Benzoazepinone derivatives that mimic the ability of the SERM (Tamoxifene and Raloxifene) to binds with estrogen receptor protein. These Benzoazepinone derivatives may be useful in the prevention and/or treatment of breast cancer.

2. Experimental Section

2.1. Chemistry

Chemicals used were of the reagent category and purified as needed. Melting points were determined by Lab India digital melting point apparatus. Shimadzu's FTIR spectrometer model was used for recording the IR spectrum of compounds. Bruker DRX-300 spectrometer was used for the determination of NMR spectra in the DMSO solvent with internal standard as a TMS. Shimadzu LCMS 2010A spectrometer was used to examine the MASS spectra of compounds. Responses were checked by thin-layer chromatography (TLC).

Step 1 & 2: Preparation of 2-chloro-N-methyl-N-phenethylacetamide (Inter-2)

To a solution of mono methyl amine in THF was added 2-phenylethyl bromide in THF over a period of 1 h. The reaction mixture was stirred for 4h at RT. It was diluted with Methyl tertiary butyl ether (MTBE) (1000 mL) [30]. Organic layer was separated and the aqueous layer was extracted with MTBE. The organic layer was taken in a RB containing water and the pH was adjusted to 6 with 5N. HCl. The aqueous portion was separated and diluted with MTBE (500 mL), the pH adjusted to 11 with 5N NaOH. The resulting organic layer was separated and the aqueous extracted with MTBE (500 mL) to afford inter-1.

To a solution of inter-1 in MTBE was added a solution of $NaHCO_3$ in water. A solution of chloroacetyl chloride in MTBE was added and stirred for 1 h at 10°C. After completion of the reaction, the reaction mixture was diluted with MTBE, the organic layer separated and the aqueous extracted with MTBE (300 mL) [30]. The combined organic layer was dried over sodium sulfate and concentrated under vacuum to afford 77 g of inter-2 in 67.5% yield.

Step 3: Preparation of 3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-3)

To a solution of inter-2 in 1, 2-dichlorobenzene was added $AlCl_3$. The reaction mixture was stirred for 2 h at 165°C. After completion of the reaction, it was cooled to 0-5°C and quenched with ice and 1N HCl solution. The organic portion was separated [31]. The organic layer was extracted with DCM (2X500 mL), the combined organic layer washed with 1N HCl (200 mL) and then saturated sodium bicarbonate solution (200 mL). It was concentrated under vacuum to afford the crude product which was purified by silica gel column chromatography eluting with DCM and ethyl acetate to afford pure 58 g of inter-3 in 64.85% yield.

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Table 1: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M. Wt.	mM.	Eq.	Qty.	Unit
1	Inter-2	211	511.8	1.0	108	G
2	$AlCl_3$	133.5	1647	3.0	220	G
3	1,2 Dichloro benzene				350	mL

Step 4: Preparation of 1-(hydroxyimino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-4)

To a stirred solution of inter-3 in THF cooled to 0°C was added isoamylnitrite drop wise. 1N solution of LiHMDS in THF was added maintaining the temperature below -10°C. After completion of the addition, the reaction mixture was allowed to warm to RT and stirred for 2-3 h. It was cooled to 0°C and the pH adjusted to 2-3 with 2N HCl. Water (400 mL) and DCM (1000 mL) was added. The organic layer was separated, dried over sodium sulfate and concentrated under vacuum to afford the crude product [32]. It was purified by silica gel column chromatography eluting with DCM and ethyl acetate to afford pure 42 g of inter-4 in 62% yield.

Table 2: Quantities of Chemicals and Solvents

S. No	Chemicals and Solvents	M.Wt.	mM.	Eq.	Qty.	Unit
1	Inter-3	175	331.4	1.0	58	G
2	Isoamylnitrite	117	430.8	1.3	50.4	G
3	LiHMDS	167	431.0	1.3	72	G
4	THF	-	-	-	500	mL

Step 5: Preparation of 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-5)

To a stirred solution of inter-5 crude in DCM was added IPA.HCl drop wise. The resultant solution was stirred for 3 h at RT. It was concentrated under vacuum, the residue was dissolved in water and washed with chloroform (2X200 mL) [33]. The aqueous layer separated was basified with Na_2CO_3 solution to pH10. It was extracted with 1:1 IPA:$CHCl_3$ mixture and the organic part concentrated under vacuum to afford 25 g of inter-5 in 67% yield.

Table 3: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M. Wt.	mM.	Eq	Qty.	Unit
1	Inter-4	191	162.3	1.0	31	G
2	IPA.HCl			-	100	mL
3	DCM			-	100	mL

Step 6: Preparation of 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. Di-p-toluoyl-l-tartaric acid (Inter-6)

A mixture of *R,S*-isomers of inter-5 in methanol was stirred at 50°C for 30 minutes. A solution of di-p-toluoyl-L-tartaric acid in methanol was added to the warm solution of *R, S*-inter-5 and refluxed for 30 min [34]. The solution was allowed to cool to RT and stirred for 16 h. A white solid separated was filtered and rinsed with cold methanol (100 mL) to afford 25 g of inter-6 in 79% yield.

Table 4: Quantities of Chemicals and Solvents

S. No.	Chemicals	M. Wt.	mM.	Eq.	Qty.	Unit
1	Inter-5	190	110.52	1.0	21	G
2	di-p-toluoyl-L-tartaric acid	386.5	110.73	1.0	42.8	g
3	Methanol			-	800	mL

Step 7: Preparation of 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. HCl (Inter-7)

To a solution of inter-6 in ethyl acetate was added con.HCl at RT and stirred for 3-4 h at 50°C [35]. It was cooled to RT and the resulting HCl salt collected by filtration, washed with ethyl acetate (50 mL) and dried under vacuum to afford 4 g of Inter-7 in 41% yield.

Table 5: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M. Wt.	mM.	Eq.	Qty.	Unit
1	Inter-6	576	43.40	1.0	25	g
2	Con.HCl	-	-	-	2.5	mL
3	Ethyl acetate	-	-	-	250	mL

Step 8: Preparation of Final Derivatives (1-(substituted benzylideneamino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one) T1-T15

To a solution of inter-7 in DMSO was added different substituted aromatic aldehydes at RT and stirred for 3-4 h at 80°C. It was cooled to RT and the resulting solid collected by filtration, washed with water (50 mL) and dried under vacuum to afford benzoazepinone derivatives T1-T15 in 60-70% yield.

2.2. Pharmacology

Cytotoxicity Screening

The cell culture was centrifuged and the cell count was adjusted to $1.0*10^5$ cells/ml using DMEM medium containing 10% FBS. To each well of a 96 well flat bottom microtitre plate, 100 µl of the diluted cell suspension (approximately 10,000 cells/well) was added. After 24 hours, when the cell population was found adequate, the cells were suspended with 100 µl of different test sample concentrations prepared in maintenance media. The plates were then incubated at 37 ^{0}C for 48 h in a 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 h. After 48 h, 20 µl of MTT (2mg/ml) in MEM-PR (MEM without Phenol Red) was added. The plates were gently shaken and incubated for 2 h at 37 ^{0}C in a 5% CO_2 atmosphere [36-40]. The 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage cell viability was calculated using the following formula and the concentration of drug or test samples needed to inhibit cell growth by 50% values were granted from the dose-response curve [41].

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3. Results and Discussion

3.1. Synthesis: 1-(substituted benzylideneamino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (T1-T15) derivatives

2-Phenylethyl bromide undergoes amino alkylation through mannich reaction with CH_3NH_2 and chloro acetyl chloride, gives 2-chloro-N-methyl-N-phenethylacetamide, which is further undergoing cyclization gives 3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Figure 1). 3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one on reaction with isoamylnitrite yields 1-(hydroxyimino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one which is on reduction in the presence of IPA, yields 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. Hydroxyl imine group reduced to amine. 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. di-p-toluoyl-L-tartaric acid and 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. HCl was obtained by treatment with di-p-toluoyl-l-tartaric acid and con. HCl respectively. 1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one. HCl undergoes nucleophilic addition reactions with different substituted benzaldehydes and yields final derivatives (1-(substituted benzylideneamino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one) (T1-T15) after 6-12 hr reflux in DMSO solvent. Derivatives yielded 60% to 70% and were recrystallized with ethanol (Figure 2).

Table 6: R Group Substitutions of compounds synthesized (T1-T15)

S. No	Compound	Substitution (R)
1	T1	H
2	T2	3-NO_2
3	T3	3-Chloro
4	T4	4-methyl
5	T5	4-Bromo
6	T6	3,4,5-tri hydroxy
7	T7	2-hydroxy
8	T8	4-chloro
9	T9	3,4,5-tri methoxy
10	T10	2,4,6-tri chloro
11	T11	3-hydroxy
12	T12	4-iso propyl
13	T13	4-di methyl amine
14	T14	4-methoxy
15	T15	2, 4-di chloro

Spectral Interpretation of Synthesized Compounds

2-chloro-N-methyl-N-phenethylacetamide (Inter-2)

67.5%; ^{1}H NMR: δ 2.58 (3H, s), 2.83 (2H, t), 3.35 (2H, t), 4.13 (2H, s), 7.04-7.35 (5H, 7.11 (dddd), 7.20 (tt), 7.28 (tdd)).; MASS: 212.0 M^++1

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3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-3)

64.85% yield; ^{1}H NMR: δ 2.87 (2H, ddd), 3.04 (3H, s), 3.55-3.71 (4H, 3.63 (ddd), 3.63 (d)), 7.03 (1H, ddd), 7.10-7.29 (3H, 7.17 (ddd), 7.18 (ddd), 7.23 (ddd)).; MASS: 176.0M^++1

1-(hydroxyimino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-4)

62% yield; ^{1}H NMR: δ 2.81 (2H, ddd), 3.06 (3H, s), 3.60 (2H, ddd), 7.17 (1H, ddd), 7.25-7.40 (2H, 7.32 (ddd), 7.33 (ddd)), 7.87 (1H, ddd).

1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (Inter-5, 6, & 7)

67% yield; 79% yield; 41% yield; ^{1}H NMR: δ 2.80-2.96 (2H, 2.88 (ddd), 2.87 (ddd)), 3.07 (3H, s), 3.49-3.71 (2H, 3.56 (ddd, 3.63 (ddd)), 4.99 (1H, s), 7.04-7.18 (3H, 7.10 (td), 7.10 (ddd), 7.12 (td)), 7.26 (1H, ddd).

1-(benzylideneamino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one (T1)

65% yield; ^{1}H NMR: δ 2.83-3.02 (2H, 2.90 (ddd), 2.95 (ddd)), 3.08 (3H, s), 3.48-3.72 (2H, 3.56 (ddd), 3.63 (ddd)), 5.24 (1H, s), 7.08-7.34 (4H, 7.15 (ddd), 7.14 (ddd), 7.24 (ddd), 7.28 (ddd)), 7.37-7.52 (3H, 7.43 (tt), 7.45 (dddd)), 8.19 (2H, dddd), 8.42 (1H, s); MASS: 272.7 M^++3.

Table 7: Physical Properties of synthesized compounds (T1-T15)

S. No	Compounds	Mol. Formula	Mol. weight	Melting point (˚C)	**Rf values
1	T1	$C_{18}H_{18}N_2O$	278.36	192-195	0.78
2	T2	$C_{18}H_{17}N_3O_3$	323.35	191-194	0.12
3	T3	$C_{18}H_{17}ClN_2O$	312.80	98-100	0.52
4	T4	$C_{19}H_{20}N_2O$	292.38	182-183	0.12
5	T5	$C_{18}H_{17}BrN_2O$	357.25	175-178	0.86
6	T6	$C_{18}H_{18}N_2O_4$	326.35	105-107	0.53
7	T7	$C_{18}H_{18}N_2O_2$	294.35	102-105	0.57
8	T8	$C_{18}H_{17}ClN_2O$	312.80	110-104	0.62
9	T9	$C_{21}H_{24}N_2O_4$	368.43	120-123	0.49
10	T10	$C_{18}H_{15}Cl_3N_2O$	381.68	99-102	0.55
11	T11	$C_{18}H_{18}N_2O_2$	294.35	98-101	0.62
12	T12	$C_{21}H_{24}N_2O$	320.44	103-106	0.36
13	T13	$C_{20}H_{23}N_3O$	321.42	110-113	0.10
14	T14	$C_{19}H_{20}N_2O_2$	308.38	108-111	0.58
15	T15	$C_{18}H_{16}Cl_2N_2O$	347.24	96-99	0.92

3.2. In Vitro Cytotoxicity Assay

The MTT assay was performed to screen the synthesized compounds on Vero and MDA MB 231 cells, and the results are shown in Table 8, Figure 3. Even at higher concentrations of synthesized derivatives for treatment, no cytotoxicity was observed in normal cells. Title compounds have exhibited low to high *in-vitro* anticancer activity with MDA MB 231 cells. Four of the synthesized compounds have shown good IC_{50} values like T2 (35 µg/ml), T10 (36.606 µg/ml), T14 (11 µg/ml), T15 (22 µg/ml).

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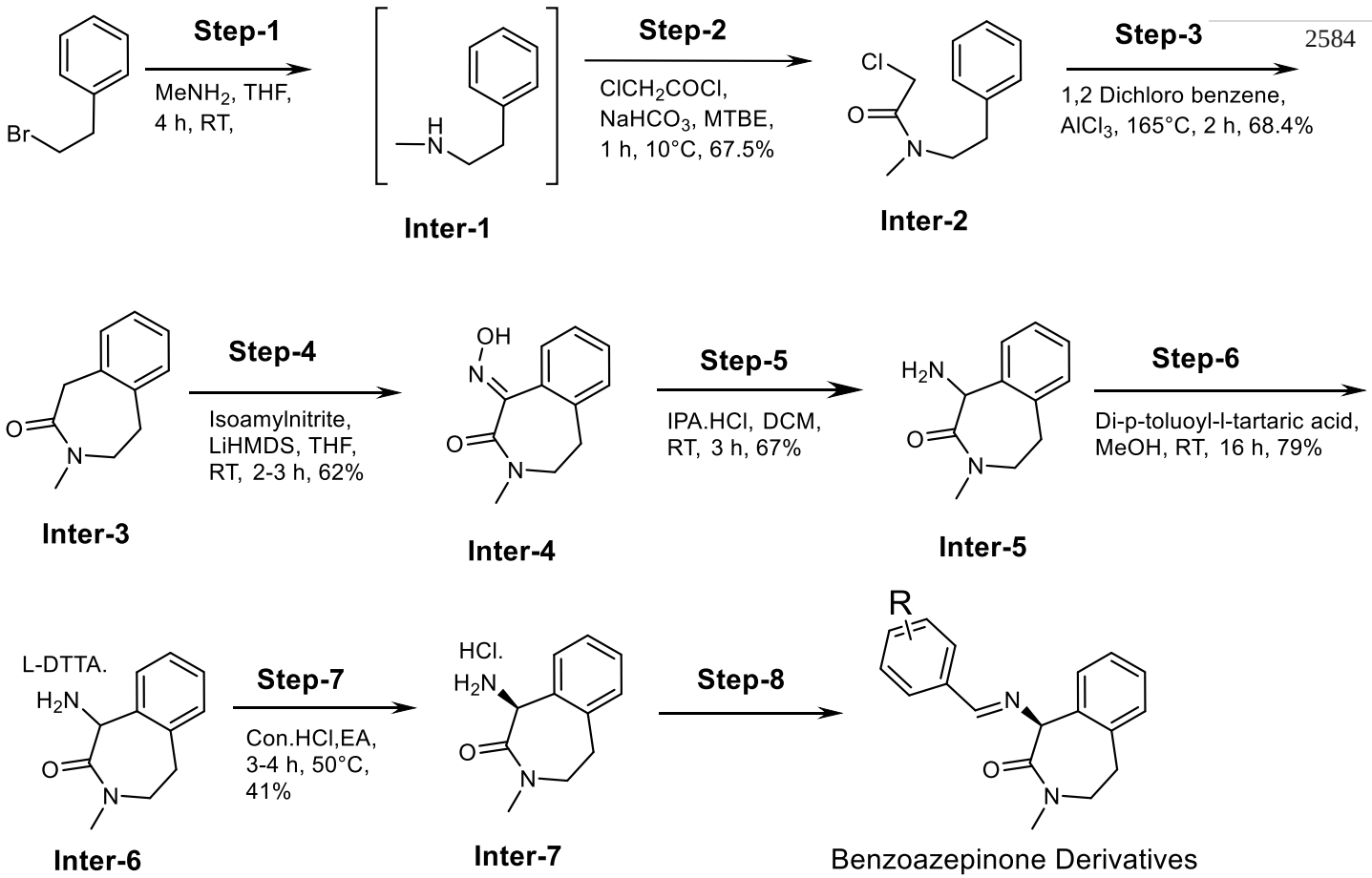

Figure 1: Synthetic scheme for the synthesis of (1-(substituted benzylideneamino)-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one) (T1-T15)

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Chemical Structures of synthesized compounds (T1-T15)

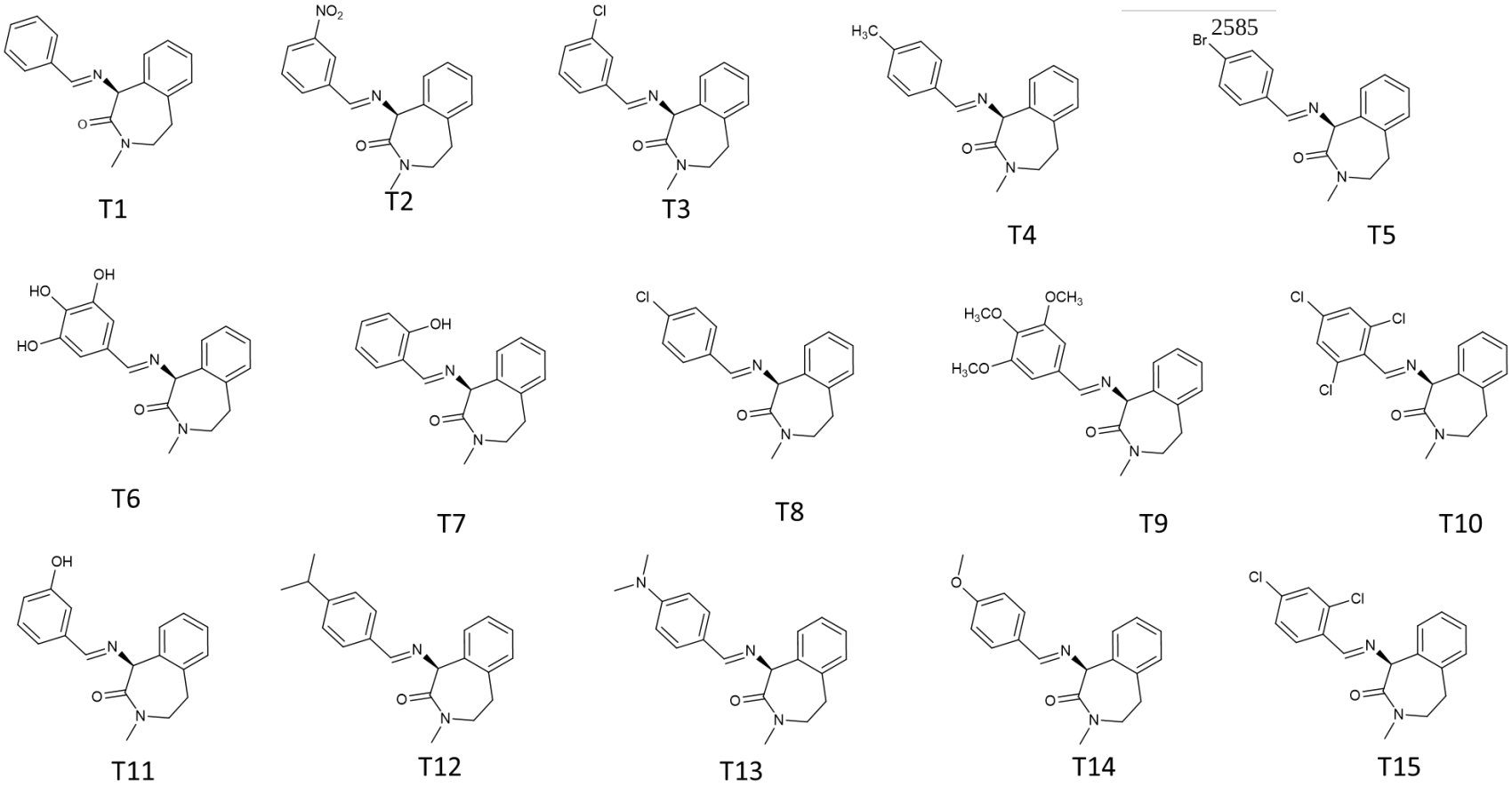

Figure 2: Chemical Structures of synthesized compounds (T1-T15)

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Table 8: Cytotoxicity results of synthesized benzoazepinone derivatives

S. No	Compound	VERO (µg/ml)	MDA MB 231 (µg/ml)
1	T1	314.9	150
2	T2	58	35
3	T3	596	124
4	T4	304	153
5	T5	106	58
6	T6	96	91
7	T7	85	68
8	T8	94	63
9	T9	99	112
10	T10	76	36
11	T11	148	69
12	T12	104	61
13	T13	126	102
14	T14	31	11
15	T15	33	22
Standard	Raloxifene	15	6

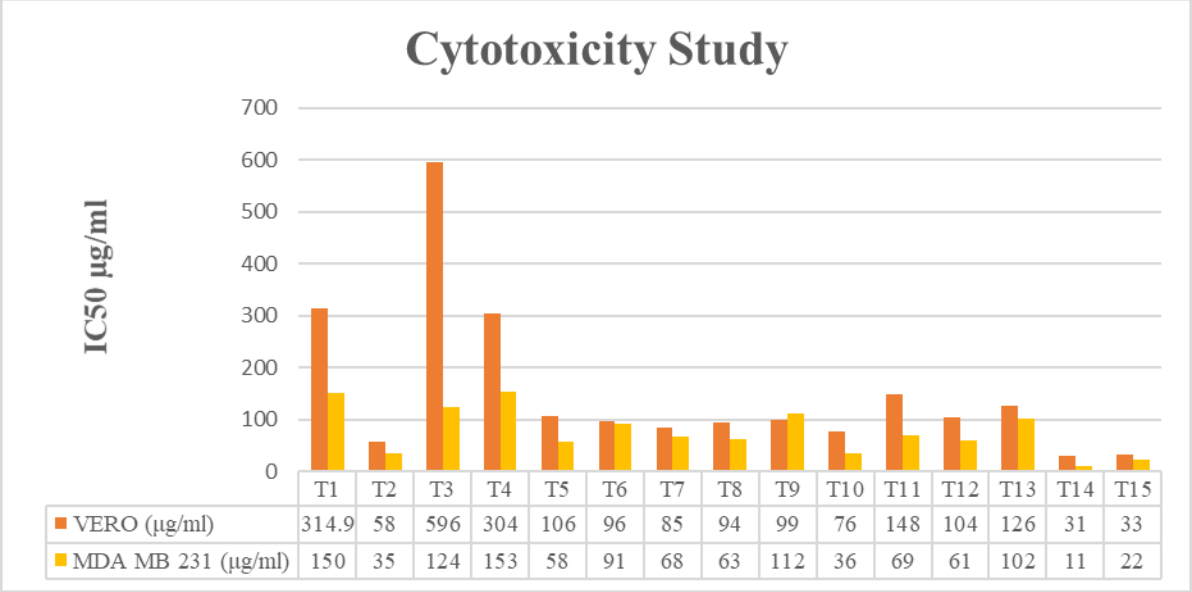

Figure 3. Cytotoxicity results of synthesized small molecules on both Vero and MDA MB 231 cell lines

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4. Conclusion

We have designed, synthesized and characterized a group of ERα antagonists that mimic the manner in which SERM and inserts into a cavity within ERα and inhibits its activity. A total of 15 compounds were synthesized by using a diverse scheme and the title compounds have exhibited low to high *in-vitro* anticancer activity with MDA MB 231 cells. Compared to the standard, the developed compounds T2, T10, T14 and T15 show considerable *in vitro* activity.

T2

T10

T14

T15

Finally, four compounds might be used as a lead molecule for future development into a therapeutically viable anti-ER positive breast cancer drug from the benzoazepinone derivatives family.

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