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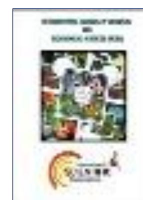

Design and synthesis of a new homologues series of 2-oxo-benzoazepinone derivatives as potent estrogen receptor alpha inhibitors

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Abstract

Selective estrogen receptor modulators (SERMs) bind to estrogen receptors and prevent estrogen from acting on breast cancer cells. A SERM prevents estrogen from attaching to the cancer cell and preventing the cancer cell from receiving signals from estrogen that would otherwise cause it to grow and multiply. The objective of this study is to create innovative Benzoazepinone derivatives that replicate the SERMs' capacity to attach to estrogen receptor protein in the form of tiny, drug-like molecules. (S)-1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one undergoes amidation with (tert-butoxycarbonyl)-L-alanine, gives tert-butyl ((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)carbamate (Inter-1), which is further undergoing deprotection gives (S)-2-amino-N-((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)propanamide (Inter-2) (Figure 1). Inter-2 on reaction with different substituted aromatic carboxylic acids and yields N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl) substituted benzamide derivatives (T16-T30) with the formation of amide bond. All the compounds were screened for their *in-vitro* cytotoxicity activity using Vero and MDA MB 231 cell lines by MTT assay. IC50 values from Cytotoxicity studies by MTT assay ranges from 15.105µg/ml to 155µg/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 T22 (15.105 µg/ml), T24 (37.206 µg/ml), T18 (41.200 µg/ml), T20 (58.302 µg/ml). 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 2-oxo-benzoazepinone derivatives family.

Keywords: ER alpha inhibitors, Raloxifene, *In vitro*, MDA MB-231, Bezoazepenine.

Full length article **Corresponding Author*, e-mail: sangamjagadeesh@gmail.com

1. Introduction

The most fatal disease is cancer, and efforts are being made to find possible anticancer medications. One of the most prevalent cancers afflicting women worldwide, both in industrialised and developing nations, is breast cancer [1]. 14% of all female malignancies in India are breast cancers. Breast cancer was recorded as 1,62,468 new cases and 87, 090 fatalities in India in 2021, according to Globocan statistics [2]. High mortality is influenced by a lack of accurate diagnosis and expensive medical care. Estrogen Receptor (ER) positivity accounts for two-thirds of all breast cancers, hence efforts are being made to identify patients who would benefit from antiestrogen medication. Breast cancer's dependence on oestrogen is a distinctive trait of the illness that can be targeted to successfully restrict growth and/or halt tumour formation. The current approach for treating hormone-dependent breast cancer is, in fact, to stop oestrogen from acting on tumour cells in one of three ways: (a) by preventing oestrogen from binding to its primary target ER with an antiestrogen like tamoxifen/raloxifen [3-8]; (b) by preventing oestrogen synthesis with an aromatase inhibitor [9–15]; or (c) by reducing ER protein levels with a pure antiestrogen like fulvestrant. The emergence of resistance, however, frequently limits the efficacy of these modern endocrine therapies.

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

Example of SERMs (Selective Estrogen Receptor Modulators) The effects of oestrogen on breast tissue are blocked by the drugs Tamoxifen (1) and Raloxifene (2). The oestrogen receptors in breast cells are where SERMs do their action. Estrogen cannot connect to the cell if a SERM is present in the oestrogen receptor because there is no place for it. The signals from oestrogen to grow and multiply are not received by a breast cell if oestrogen is not present. Other bodily tissues, such as the uterus and bones, include cells with oestrogen receptors as well. But based on the type of cell it is in, each oestrogen receptor has a somewhat different shape. As a result, oestrogen receptors on breast cells are distinct from oestrogen receptors on bone cells, and both oestrogen receptors are distinct from oestrogen receptors on uterine cells. Because SERMs are "selective," as their name implies, they can enhance the activity of oestrogen in other cells, including bone, liver, and uterine cells, even while they suppress estrogen's effect in breast cells. Serious adverse reactions from SERMs can include endometrial cancer, stroke, and blood clots. Fulvestrant (3), for instance, is a kind of medication that binds to the ER and, in the course of doing so, causes the ER to be destroyed and downregulated (a selective oestrogen receptor degrader or down regulator, or SERD) [21–22]. Along with older medications like selective oestrogen receptor modulators (SERMs) and aromatase inhibitors, they are used to treat oestrogen receptor-sensitive or progesterone receptor-sensitive breast cancer [23–24]. Candidates for antiestrogen treatment are found since ER positive breast cancer accounts for two thirds of all cases. Breast cancer is a rare illness that can be efficiently managed to reduce growth and/or prevent tumour formation due to its dependence on oestrogen. In fact, the current approach for treating hormone-dependent breast cancer is to prevent oestrogen from acting on tumour cells in one of three ways: (a) by preventing oestrogen from binding to its primary target ER with an antiestrogen like tamoxifen/raloxifene [25]; (b) by preventing oestrogen synthesis with an aromatase inhibitor [23,26-29]; or (c) by lowering ER protein levels with a pure antiestrogen like ful The emergence of resistance, however, frequently limits the efficacy of these modern endocrine medications. The objective of this study is to create innovative Benzoazepinone derivatives that replicate the SERMs' capacity to attach to oestrogen receptor protein in the form of tiny, drug-like molecules. These Benzoazepinone derivatives may help in breast cancer therapy and/or prevention.

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).

2.2 Step 1: Preparation of tert-butyl ((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)carbamate (Inter-1)

To a mixture of N-Boc Alanine, HOBt hydrate, MC07JC1876 in THF under nitrogen atmosphere was added N,N Diisopropyl ethyl amine and EDC. The reaction mixture was stirred for 16 h at RT. After completion of the reaction, the solvent was removed under vacuum, the residue taken in ethyl acetate and water, washed with saturated $NaHCO_3$ solution, 1N HCl, brine solution, dried over sodium sulfate, filtered and concentrated under vacuum to afford pure 10 g of inter-1 in 68% yield. TLC System, 50% Ethyl acetate in hexane Rf Value-0.4.

2.3 Step 2: Preparation of (S)-2-amino-N-((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1 yl) propenamide (Inter-2)

2.3.1 Procedure

A stream of anhydrous HCl gas was passed through a stirred solution of inter-1 in dioxane, chilled in ice bath to about 10°C under nitrogen for 10-15 minutes. The solution was capped, then cooling bath removed, it was allowed to warm to RT with stirring for 2-8 h. After completion of the reaction, it was concentrated to afford 6 g of inter-2 in 83.3% yield. TLC System 10% Methanol in chloroform. Rf Value is 0.2.

2.4 Step 3: Preparation of Final Derivatives N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl) substituted benzamide derivatives (T16-T30)

To a mixture of inter-2, HOBt hydrate, (S)-2-hydroxy-3-methyl butyric acid in THF under nitrogen atmosphere was added N,N-diisopropylethylamine, followed by EDC and stirred for 16 h at RT. After completion of the reaction, the solvent was removed under vacuum. The residue was taken in ethyl acetate and water, washed with saturated $NaHCO_3$ solution, 1 N HCl, brine, dried over sodium sulfate and removed the solvent under vacuum to afford the 4.5 g of MC07JC1990 in 54.8% yield. TLC System, 50% Ethyl acetate in hexane. Rf Value is 0.3. to afford benzoazepinone derivatives T1-T15 in 60-70% yield.

2.5 Pharmacology

2.5.1 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].

$$\left[\% \text{ Cell Viability} = \frac{(\text{Mean OD of Individual Sample})}{(\text{Mean OD of Control})}\right] * 100$$

3. Results and Discussion

3.1 Synthesis

3.1.1 N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl) substituted benzamide derivatives (T16-T30)

(S)-1-amino-3-methyl-1,3,4,5-tetrahydro-2H-benzo[d]azepin-2-one undergoes amidation with (tert-butoxycarbonyl)-L-alanine, gives tert-butyl ((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)carbamate (Inter-1), which is further undergoing deprotection gives (S)-2-amino-N-((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)propanamide (Inter-2) (Figure 1). Inter-2 on reaction with different substituted aromatic carboxylic acids and yields N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl) substituted benzamide derivatives (T16-T30) with the formation of amide bond. Inter-2 undergoes nucleophilic addition reactions with different substituted benzoic acids and yields final derivatives T16-T30 after 12-16 hr reflux in THF solvent. Derivatives yielded 60% to 70% and were recrystallized with ethanol (Figure 2).

3.2 Spectral Interpretation of Synthesized Compounds

3.2.1 Tert-butyl ((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)carbamate (Inter-1)

68% yield; ^{1}H NMR: δ 1.33-1.48 (12H, d), 1.43 (s)), 2.81-2.97 (2H, ddd), 3.08 (3H, s), 3.47-3.71 (2H, ddd), 4.47 (1H, q), 5.65 (1H, s), 7.06-7.31 (4H, td), 7.21 (ddd), 7.22 (ddd), 7.24 (ddd); MASS: 361.20 M/Z.

3.2.2(S)-2-amino-N-((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl) propanamide (Inter-2)

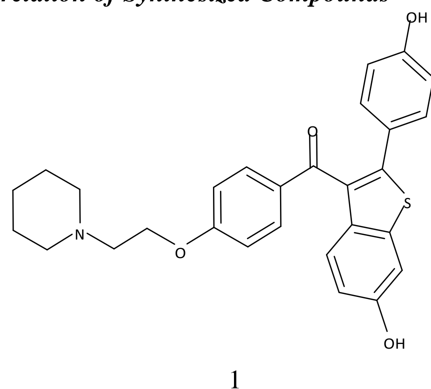
64.85% yield; ^{1}H NMR: δ 1.32 (3H, d), 2.81-2.97 (2H, ddd), 3.08 (3H, s), 3.47-3.71 (3H, ddd), 3.66, q), 5.69 (1H, s), 7.06-7.31 (4H, td); MASS: 261.15M/Z.

3.2.3 N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)substituted benzamide (T16-T30)

62-75% yield; ^{1}H NMR: δ 1.39 (3H, d), 2.81-2.97 (2H, ddd), 2.88 (ddd), 3.08 (3H, s), 3.47-3.71 (2H, ddd), 3.62 (ddd), 4.45 (1H, q), 5.72 (1H, s), 6.72 (2H, dd), 7.06-7.31 (4H, td), 7.21 (ddd), 7.22 (ddd), 7.24 (ddd), 7.40 (1H, t).

3.3 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 T22 (15.105 µg/ml), T24 (37.206 µg/ml), T18 (41.200 µg/ml), T20 (58.302 µg/ml).



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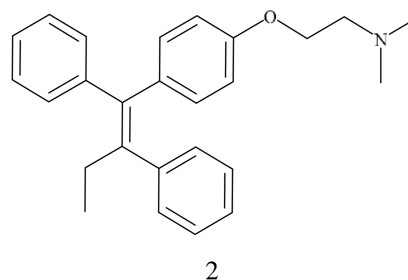

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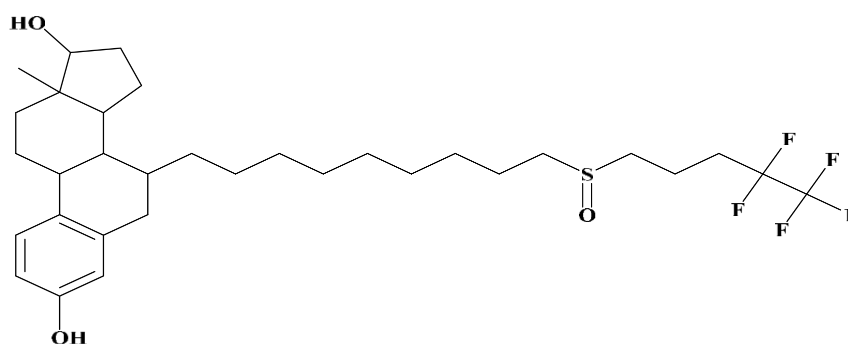

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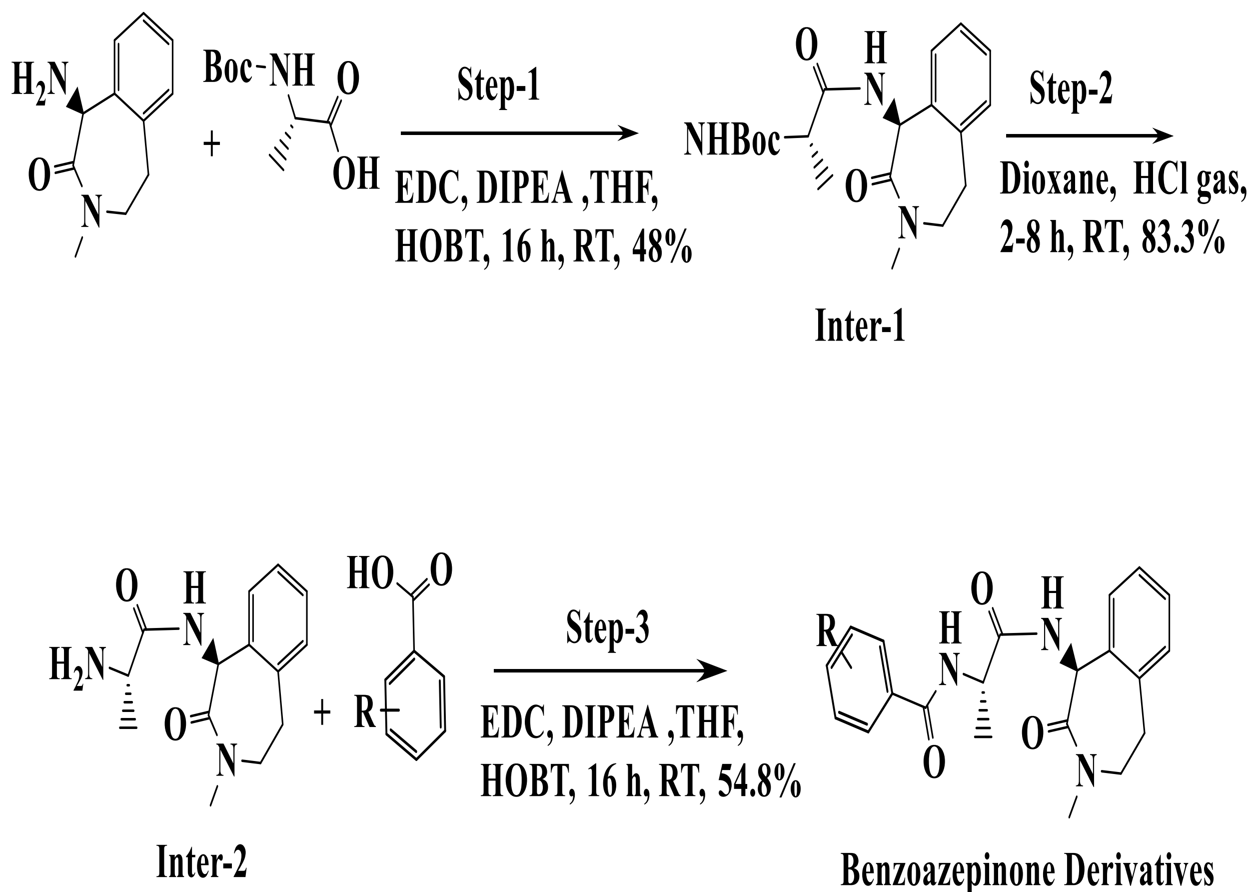

Figure 1: Synthetic scheme for the synthesis of N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)substituted benzamide derivatives (T16-T30)

Table 2: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M.Wt.	mM.	Eq.	Qty.	Unit
1	MC07JC1876	190	57.9	1.0	11	g
2	N-Boc Alanine	189	58.2	1.0	11	g
3	HOBt hydrate	153	64.05	1.1	9.8	g
4	EDC.HCL	191	64.0	1.1	12.3	g
5	N,N Diisopropyl ethyl amine	129	65.1	1.12	8.4	g
6	THF	-	-	-	200	mL

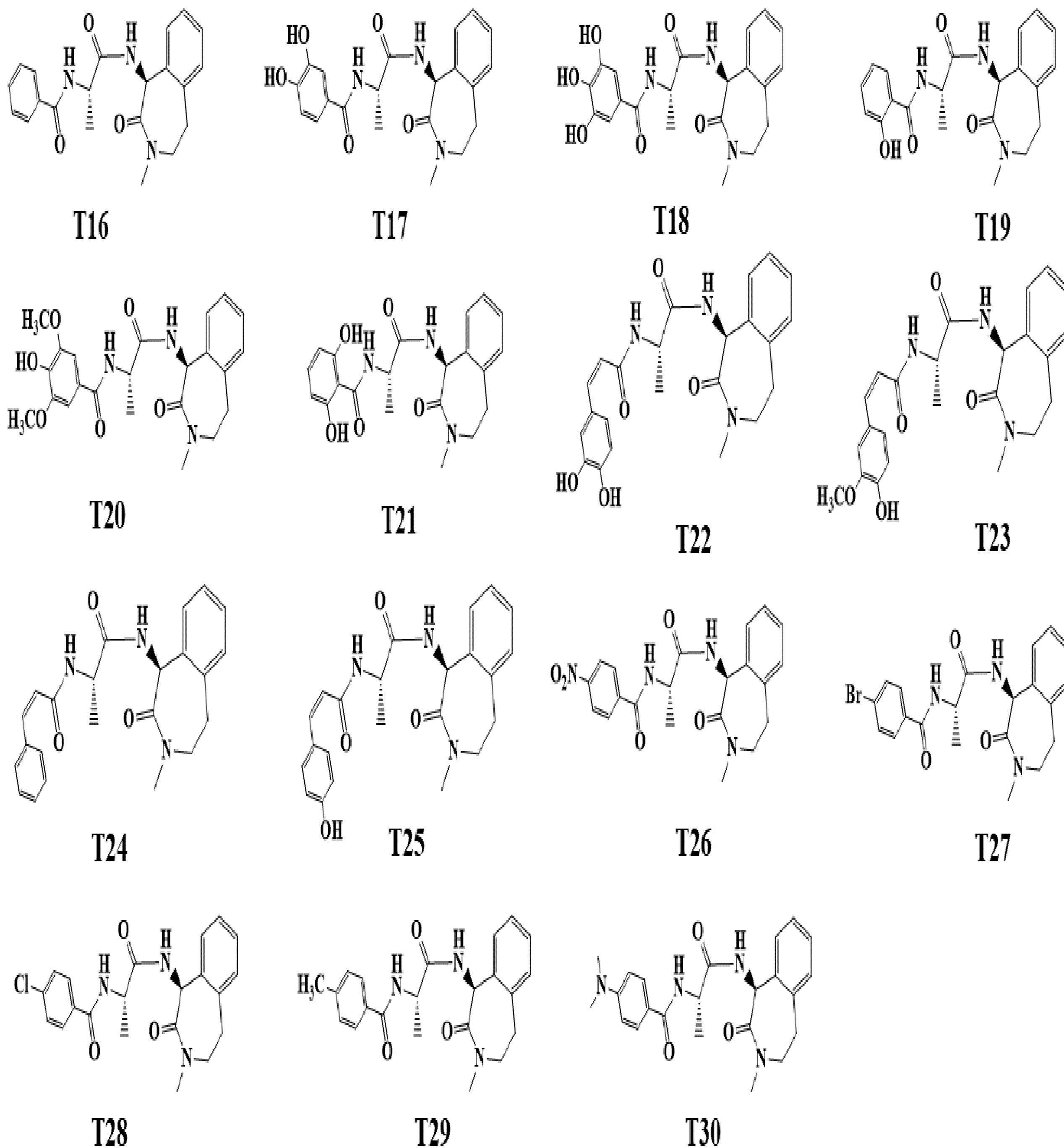

Figure 2: Chemical Structures of synthesized compounds (T16-T30)

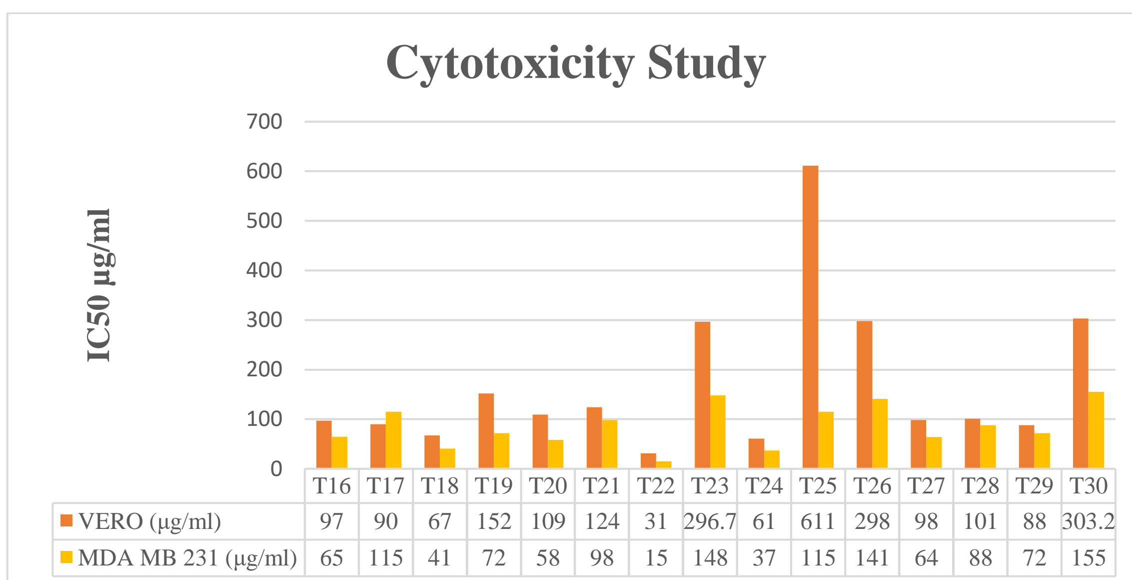

Figure 3: Cytotoxicity results of synthesized small molecules on both Vero and MDA MB 231 cell lines

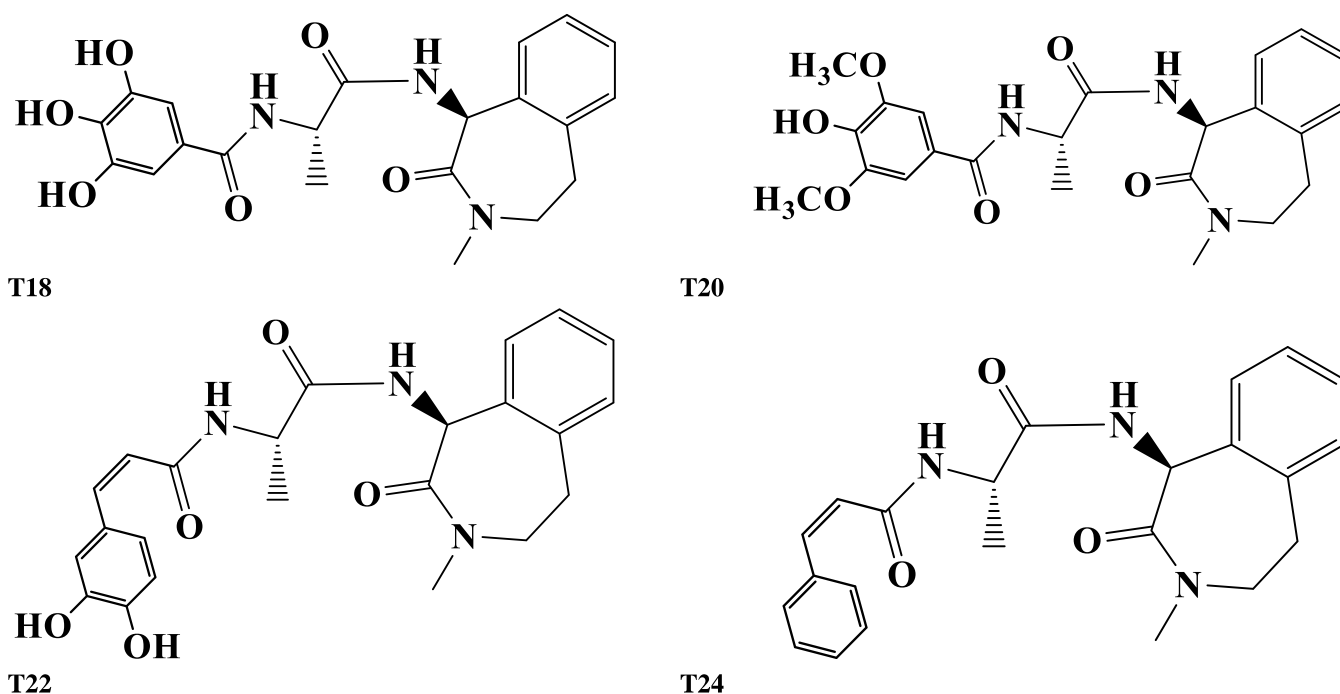

Fig 4: Chemical structures of T18, T20, T22 and T24

Table 3: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M.Wt.	mM.	Eq.	Qty.	Unit
1	Inter-1	362	27.62	1.0	10	g
2	Dioxane	-	-	-	-	mL
3	HCl gas	-	-	-	-	

Table 4: Quantities of Chemicals and Solvents

S. No.	Chemicals and Solvents	M.Wt.	mM.	Eq.	Qty.	Unit
1	Inter-2	262	22.90	1.0	6	g
2	(S)-2-hydroxy-3-methyl butyric acid	118	22.88	1.0	2.7	g
3	HOBt hydrate	153	25.16	1.1	3.85	g
4	EDC.HCl	191	25.36	1.1	4.84	g
5	N,N-diisopropylethylamine	129	32.17	1.4	4.15	g
6	THF	-	-	-	60	mL

Table 5: R Group Substitutions of compounds synthesized (T16-T30)

S. No	Compound	Substitution (R)
1	T16	H
2	T17	3,4-dihydroxy
3	T18	3,4,5-trihydroxy
4	T19	2-hydroxy
5	T20	4-Hydroxy, 3,5-di Methoxy
6	T21	2,6- diHydroxy
7	T22	(Z)-3-(3,4-dihydroxyphenyl)acrylic acid
8	T23	(Z)-3-(3-methoxy, 4-hydroxyphenyl)acrylic acid
9	T24	(Z)-3-(phenyl)acrylic acid
10	T25	(Z)-3-(4-hydroxyphenyl)acrylic acid
11	T26	4-Nitro
12	T27	4-Bromo
13	T28	4-Chloro
14	T29	4-Methyl
15	T30	4-N,N-dimethyl

Table 6: Physical Properties of synthesized compounds (T16-T30)

S. No	Compounds	Mol. Formula	Mol. weight	Melting point (˚C)	**Rf values
1	T16	$C_{21}H_{23}N_3O_3$	365.43	102-105	0.57
2	T17	$C_{21}H_{23}N_3O_5$	397.43	110-104	0.62
3	T18	$C_{21}H_{23}N_3O_6$	413.43	120-123	0.49
4	T19	$C_{21}H_{23}N_3O_4$	381.43	99-102	0.55
5	T20	$C_{23}H_{27}N_3O_6$	441.48	98-101	0.62
6	T21	$C_{21}H_{23}N_3O_5$	397.43	103-106	0.36
7	T22	$C_{24}H_{27}N_3O_5$	437.50	110-113	0.10
8	T23	$C_{23}H_{25}N_3O_3$	391.47	192-195	0.78
9	T24	$C_{23}H_{25}N_3O_4$	407.47	191-194	0.12
10	T25	$C_{21}H_{22}N_4O_5$	410.16	98-100	0.52
11	T26	$C_{21}H_{22}N_4O_5$	410.43	182-183	0.12
12	T27	$C_{21}H_{22}BrN_3O_3$	444.33	175-178	0.86
13	T28	$C_{21}H_{22}ClN_3O_3$	399.88	105-107	0.53
14	T29	$C_{22}H_{25}N_3O_3$	379.46	102-105	0.57
15	T30	$C_{23}H_{28}N_4O_3$	408.50	192-195	0.78

Table 7: Cytotoxicity results of synthesized benzoazepinone derivatives

S. No	Compound	VERO (µg/ml)	MDA MB 231 (µg/ml)
1	T16	97	65
2	T17	90	115
3	T18	67	41
4	T19	152	72
5	T20	109	58
6	T21	124	98
7	T22	31	15
8	T23	296.74	148
9	T24	61	37
10	T25	611	115
11	T26	298	141
12	T27	98	64
13	T28	101	88
14	T29	88	72
15	T30	303.15	155
Standard	Raloxifene	15	6

4. Conclusions

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 T18, T20, T22 and T24 show considerable *in vitro* activity.
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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