Bioorganic & Medicinal Chemistry 19 (2011) 5409–5419

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and synthesis of 1,3-biarylsulfanyl derivatives as new anti-breast cancer agents

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ARTICLE INFO

Article history:
Received 16 June 2011
Revised 27 July 2011
Accepted 27 July 2011
Available online 2 August 2011

Keywords:
Anti-breast cancer
SERM
Anti-implantation
Raloxifene
Osteoporosis

ABSTRACT

A new series of 1,3-biarylsulfanyl derivatives (homodibenzyl core motif) have been designed and synthesized as new estrogen receptor ligands by chopping benzothiophene core of raloxifene to engender seco-raloxifene scaffold. All the synthesized compounds were screened for anti-proliferative, anti-osteoporotic, and anti-implantation activity. Compounds (**35**, **36**) having basic amino anti-estrogenic side chain were exhibiting potential anti-proliferative activity in MCF-7, MDA-MB-231 and ishikawa cell lines. Some of the synthesized compounds having homodibenzyl motif (**5**, **8**, **10**) have shown moderate anti-osteoporotic activity.

1. Introduction

Breast cancer is the most frequently diagnosed life-threatening disease in women and more than 1 million women are diagnosed with breast cancer every year, accounting for 10% of all new cancers and 23% of all female cancer cases.[1] The disease accounts for 40,000 deaths each year in the United States alone.[2] It is established from earlier studies that, estrogens are mainly responsible for initiation and growth of breast cancer.[3] Estrogen is important regulatory hormone which plays vital role in the development and maintenance of the female reproductive organs, mammary glands, and other sexual characteristics.[4] Recently involvement of estrogens in the growth and/or function of a number of other tissues, such as in skeleton, cardiovascular system, and central nervous system, has also been well recognized.[5] Use of available chemotherapeutics for the treatment of breast cancer is very limited because of undesirable side effect. Hence there is critical need to develop new chemotherapeutic agents for effective treatment of the breast cancer.[6,7]

Early drug discovery efforts were focused on the design and synthesis of non-steroidal estrogen ligands with antagonistic properties at breast and reproductive tissues.[8] Tamoxifen is most widely used estrogen antagonist as it shows anti-estrogenic effect[9] on estrogen receptor (ER) positive breast cancer cells. Tamoxifen stimulates endometrial proliferation in ovariectomized rats via an estrogen receptor-dependent mechanism and its resistance limiting the clinical acceptability.[10] Raloxifene and arzoxifene, are selective estrogen receptor modulators and marketed for the prevention and treatment of osteoporosis. Raloxifene has exhibited better profile at endometrium level than tamoxifen and the difference in proliferative activity in the uterus is the most important difference between the selective estrogen receptor modulator (SERM) profiles of tamoxifen and raloxifene.[11,12] Raloxifene has also shown to prevent bone loss, reduce serum cholesterol, and provide potent anti-proliferative effects.[13] The search for SERMs that would have an ideal profile of tissue selectivity for the treatment of breast cancer and osteoporosis is an active aspect of current pharmaceutical research endeavors.[14,15] As a part of our drug discovery effort toward identifying potential SERMs for the treatment of osteoporosis and breast cancer, we have synthesized 1,3-biarylsulfanyl derivatives and evaluated them for theiranti-osteoporotic, anti-implantation and anti-proliferative activity.

The principal idea for the designing of 1,3-biarylsulfanyl derivatives comes from estradiol, noncyclic structure diethyl stilbestrol and similar structures (Fig. 1).[16,17] It can be argued that the central bond of diethyl stilbestrol (DES) was important factor for the correct orientation of ethyl and phenol for binding to estrogen

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doi:10.1016/j.bmc.2011.07.056

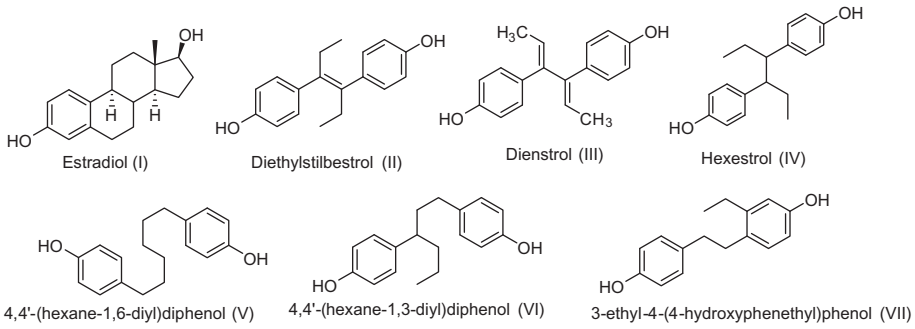


Figure 1. Cyclic versus non-cyclic bioisosteric replacement of estradiol, diethylstilbesterol and similar analogues.

receptor. This observation was further supported by biological activity, as cis isomer of DES is only 1/14 times active as trans isomer.[18] The noncyclic scaffolds such as DES, Dienestrol and hexestrol are equipotent as estradiol, however compounds V, VI, VII did not have significant activity.

Inspired by these finding, we wish to report here design and synthesis of 1,3-biarylsulfanyl derivatives (seco-raloxifene) a noncyclic and nonclassical bioisosteres of known SERMs such as raloxifene, arzoxifene, trioxifene, ERA-923 and EM-652[19] (Fig. 2). It is interesting to note that all these SERMs have a common pattern that the aryl group bearing anti-estrogen side chain (alkylamino side chain) is three carbons (three atoms) away from aryl group present in estrogenic subunit. The basic design of 1,3-biarylsulfanyl derivatives is based on combination of two strategies: (1) noncyclic/seco estradiol retains biological activity; (2) positioning of anti-estrogenic side with respect to estrogenic subunits. These are applied here to generate a new estrogen receptor ligands by chopping benzothiophene core of raloxifene to build 1,3-biarylsulfanyl derivatives and keeping the required positioning of antiestrogenic side chain (Fig. 2).

2. Results and discussion

2.1. Chemistry

Substituted chalcones (**3a–j**) were synthesized by the conden sation of substituted aldehydes and acetophenones via Claisen–Schmidt reaction using zirconium chloride as a catalyst. Initially, the reaction of 1,3-diaryl-2-propenones (**3a–j**) with mercaptans in the presence of a catalytic amount of zirconium(IV) chlorides in anhydrous dichloromethane gave corresponding Michael adducts (**5–26**) in high yields at room temperature (Scheme 1). We have also synthesized compounds **27–32** (Scheme 1.) having alkyl sulfanyl group by the use of $ZrCl_4$ as a catalyst. Compounds **33–38** were synthesized from *para*-hydroxy acetophenone in two steps. *p*-Hydroxy acetophenone reacted with different chloro alkyl amine

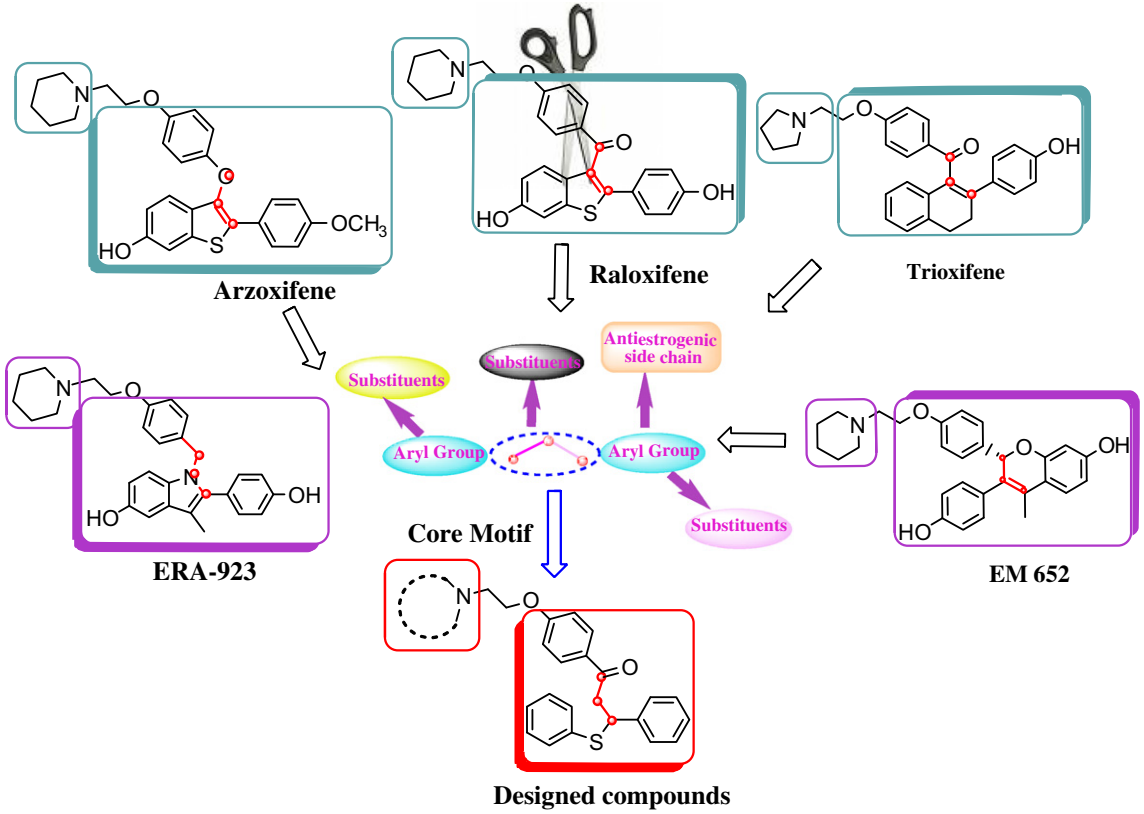
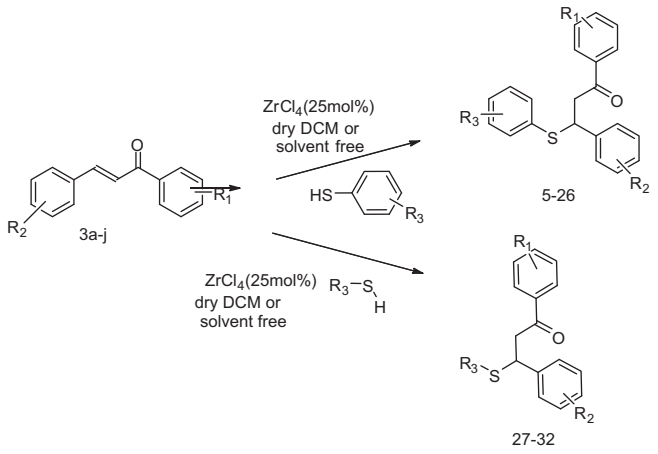


Figure 2. Design of 1,3-biarylsulfanyl derivatives.



Scheme 1. Synthesis of 1,3-biarylsulfanyl derivatives.

hydrochlorides in the presence of anhydrous K_2CO_3 to furnish **4a–c** followed by the thia-Michael reaction to give desired products **33–38** (Scheme 2).

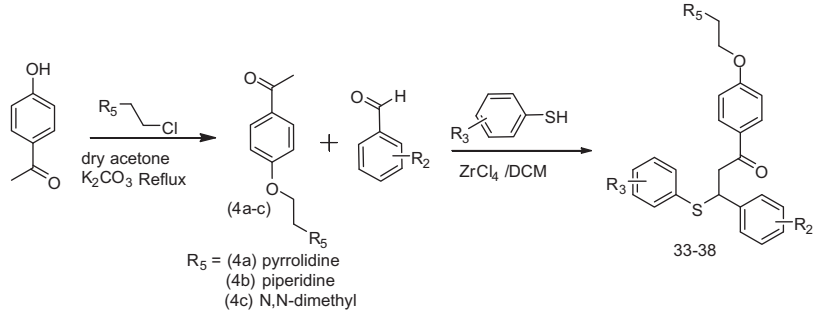
The introduction of antiestrogenic side chain in compound **3g** having the free hydroxyl group at *para* position of the aryl ring was achieved on reaction with different alkyl mercaptan via thia-Michael reaction followed by alkylation of hydroxyl group by various chloro alkyl amine hydrochlorides in the presence of K_2CO_3 and acetone to furnish compound **39–41** in good yields (Scheme 3).

All the products were characterized by the ^1H,^{13}C NMR and Mass spectroscopy. Synthesized compounds were screened for anti-proliferative activity (Tables 1 and 2) antiosteoporotic, receptor binding affinity, estrogenicity, antiestrogenictiy and anti-implantation activity (Table 3). In order to evaluate the molecular docking result (Fig. 3) of β-aryl β-mercapto ketone analogues on the basis of their molecular conformations and interaction profile in the protein binding site, the docking study of the compound **35** was carried out as an instructive step that can provide further insight into the nature of ligand binding of the ER α. The interaction patterns of compounds **35** suggested that it anchor to the binding pocket (amino acids Glu353 and Arg394) with dock score of −27.592 kJ/mol Figure 3.1.

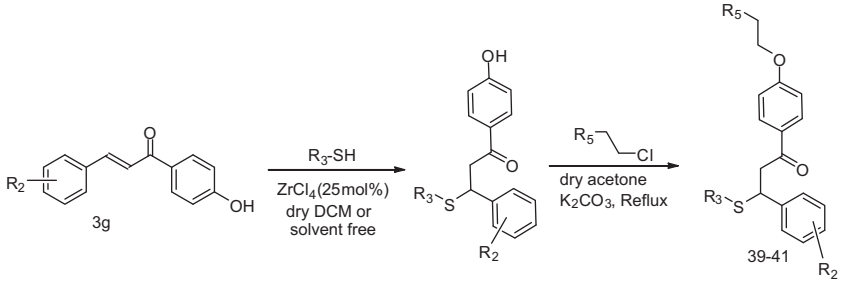
2.2. Biology

All the compounds synthesized were evaluated for their anti-proliferative activity in MCF-7, MDA-MB-231 and Ishikawa cell lines (Tables 1 and 2). Compounds exhibiting promising biological activity were screened for their relative binding affinity, estrogenicity, anti-estrogenicity, anti-osteoporotic and anti-implantation activity (Table 3). Two compounds (**35**, **36**) with anti-estrogenic side chains showed significant anti-proliferative activity similar to that of tamoxifen against MCF-7, MDA-MB-231 cancer cell lines (Table 2). Compounds **35**, **36** have exhibited excellent receptor binding affinity (RBA) (4.467 and 5.272) and may interact with estrogen receptor with good affinity. Interestingly, **35** and **36** were exhibiting low estrogenicity at uterine level (79% and 58%) in comparison to tamoxifen (155%) and showed good anti-estrogenicity (31% and 34%). These compounds have also showed good antiosteoporotic as well as anti-implantation activity (Table 3).

It is evident from Table 3 that hydroxyl group was essential in arylsulfanyl moieties (**35**, **36**) for promising activity as well as receptor binding affinity, whereas corresponding alkyl substitution were found to be less active. Compounds (**35**, **36**) have been shown higher RBA value, possibly due to the presence of hydroxyl groups present at *para* position of the phenyl ring. Compounds having anti-estrogenic alkyl amino side chain with piperidine groups were exhibiting better biological profile in comparison to pyrrolidine and dimethylamino groups. It is noteworthy that the central homodibenzylic core (compounds **5**, **10**) was exhibiting promising antiosteoporotic activity (43.8% and 39.0%), which is comparable to



Scheme 2. Synthesis of substituted 1,3-biarylsulfanyl derivatives via a multicomponent reaction.



Scheme 3. Synthesis of 1,3-biarylsulfanyl derivatives.

Table 1
Antiproliferative activity of 1,3-biarylsulfanyl derivatives (**5**–**32**)

Entry	R^1	R^2	R^3	Yield[b] (%)	Antiproliferative activity			
					MCF-7 [a] IC_{50} (μM)	Iska [a] IC_{50} (μM)	MDA-MB-231 IC_{50}[a] (μM)	HEK-293 IC_{50}[a] (μM)
5	4-H	4-H	4-H	92	24 ± 2.02	20 ± 0.04	57 ± 7.83	—
6	4-H	4-H	4-Me	96	NA	NA	NA	—
7	4-H	4-H	4-Cl	90	NA	NA	NA	—
8	4-H	4-OMe	4-H	95	21 ± 3.21	26 ± 1.13	34 ± 1.32	—
9	4-H	4-OMe	4-Me	94	23 ± 1.33	17 ± 2.31	45 ± 5.02	—
10	4-OMe	4-OMe	4-H	92	17 ± 1.74	16 ± 1.16	31 ± 2.35	38.2
11	4-OMe	4-OMe	4-Me	90	18 ± 1.49	NA	28 ± 4.33	NA
12	4-H	4-OH	4-H	88	19 ± 2.37	NA	33 ± 2.34	31.4
13	4-H	4-OH	3-Me	85	25 ± 1.34	22 ± 2.31	35 ± 3.07	—
14	3-Cl	4-Me	4-Me	96	27 ± 2.03	NA	41 ± 5.14	—
15	4-OH	4-H	4-H	94	24 ± 3.14	NA	38 ± .352	—
16	4-Cl	4-H	4-Cl	98	28 ± 1.07	24 ± 1.13	31 ± 2.46	—
17	4-NO_2	H	4-Cl	85	25 ± 2.35	21 ± 1.66	29 ± 2.35	—
18	4-OMe	4-H	4-Cl	90	NA	22 ± 0.37	NA	—
19	4-Cl	4-OMe	4-H	88	NA	25 ± 2.35	NA	—
20	4-OMe	4-H	4-H	96	29 ± 0.37	NA	41 ± 4.02	—
21	3-NO_2	4-H	4-H	78	25 ± 4.37	21 ± 4.37	38 ± 3.74	—
22	4-H	4-H	4-NO_2	68	NA	23 ± 3.43	44 ± 3.74	—
23	Napthyl[c]	4-H	4-H	78	30 ± 4.65	26 ± 2.61	48 ± 5.67	—
24	4-H	4-OMe	4-Cl	90	25 ± 1.17	22 ± 2.39	36 ± 3.12	—
25	3-Cl	4-Me	4-H	98	25 ± 2.09	28 ± 2.89	36 ± 1.87	—
26	3-Cl	4-H	4-H	98	29 ± 1.98	29 ± 2.41	32 ± 3.36	—
27	4-H	4-H	CH_2CH_2COOH	88	NA	23 ± 1.07	42 ± 4.31	—
28	4-H	4-OMe	CH_2CH_2COOH	86	NA	24 ± 1.43	44 ± 4.25	—
29	4-H	4-OMe	Ethyl	89	24 ± 0.16	NA	NA	—
30	4-H	4-OH	Ethyl	70	NA	NA	NA	—
31	4-OMe	4-OMe	Ethyl	90	27 ± 2.05	NA	NA	—
32	4-H	4-H	Me	90	25 ± 3.34	25 ± 0.32	NA	—

[a] IC_{50} values for anti-breast cancer activity of ligands using MCF-7, Ishikawa and MDA-MB-231 cell lines, using tamoxifen as a positive control. The results are representative of one of three similar experiments each performed in triplicate. Each datum depicts the IC_{50} for each scaffold. The deviation in each case was less than 5% and NA represents ligands that were found to be inactive at 50 μM in MTT assays.
[b] Isolated yield.
[c] In compound **23** phenyl ring containing R^1 is replaced by naphthyl ring.

raloxifene (38%) in anti-resorptive PTH induced resorption of 45Ca chick bone assay. Compounds **5**, 8 and **10** have been showing significant RBA (1.25, 2.67, 2.21) hence it appears that compounds may interact with estrogen receptor.

Compounds (**5, 8, 10**) were also screened for osteoclastogenesis in response to RANK (Receptor activator of nuclear factor-kappa B ligand) and MCSF (macrophage colony-stimulating factor) treatment for 7 days in bone marrow cells (BMCs). These compounds at 1 μM concentrations, significantly inhibited expression of tartrate-resistant acid phosphatise (TRAP), and RANK Fig. 4a and b.

These compounds (**5, 8, 10**) were showing high estrogenicity with moderate antiestrogenicity. HEK-293 cell line is derived from

Table 2
Antiproliferative activity alkyl amino substituted 1,3-biarylsulfanyl derivatives (**33**–**41**)

Entry	R^{2c}	R^3	R^5	Yield[b] (%)	MCF-7 IC_{50}[a] (μM)	Iska. IC_{50}[a] (μM)	MDA-MB-231 IC_{50}[a] (μM)	HEK-293 IC_{50}[a] (μM)
33	H	H		86	>50	20 ± 0.04	27 ± 5.21	NA
34	H	Me		85	>50	NA	28 ± 4.22	NA
35	3-OH	OH		88	12.2 ± 1.22	17 ± 2.12	19 ± 2.44	65.8
36	H	OH		82	9.6 ± 1.24	16.2 ± 1.13	16.5 ± 1.24	75.2
37	OH	OH		82	25 ± 4.37	17.3 ± 2.31	>50	28.7
38	H	OH		85	28.6 ± 3.02	16.0 ± 1.16	27 ± 1.21	27.5
39	H	Et		86	27.0 ± 2.41	NA	38 ± 4.65	32.8
40	H	Et		90	32.8 ± 2.16	28.5 ± 4.60	40.2 ± 2.64	NA
41	H	Et		80	27 ± 1.43	>50	>50	29.2
Tamoxifen					12.48 ± 1.23	22.5 ± 0.8	18.9 ± 1.26	10

[a] IC_{50} values for anti-breast cancer activity of ligands using MCF-7, Ishikawa and MDA-MB-231 cell lines, using tamoxifen as a positive control. The results are representative of one of three similar experiments each performed in triplicate. Each datum depicts the IC_{50} for each scaffold. The deviation in each case was less than 5% and NA represents ligands that were found to be inactive at 50 μM in MTT assays.
[b] Isolated yield.
[c] All the position of R^2, at the *para* position unless otherwise specified.

Table 3
RBA, estrogencity, antiestrogencity, antiosteoporotic (antiresorptive), and antiimplantation activity of some substituted 1,3-biarylsulfanyl derivatives

Entry	RBA[a]	Estrogenicity (% increase in uterine weight)[b]	Antiestrogenicity (% inhibition in uterine Wt. gain)[c]	Anti-resorptive (PTH induced resorption of ^{45}Ca)		Anti-implantation activity (%) (20 mg/kg)[f]
				T/C ratio[d]	(%) Inhibition[e]	
5	1.25	185	16	0.562	43.8	Inactive
8	2.67	192	18	0.784	21.6	Inactive
10	2.21	187	16	0.610	39.0	Inactive
33	0.157	174	12	0.863	13.7	Inactive
34	0.245	76	8	0.845	15.5	Inactive
35	4.467	79	31	0.812	18.8	100
36	5.272	58	34	0.787	21.3	100
37	5.154	111	24	0.934	6.6	Inactive
38	0.670	101	18	0.902	9.8	60
39	0.246	212	4	0.944	5.6	Inactive
40	0.175	134	7	0.947	5.5	Inactive
41	0.191	152	9	0.963	3.7	100
Tamoxifen	2.2	155	49			
Raloxifene				0.620	38	

[a] Percent (%) of estradiol-17β.
[b] Control group of animals received the vehicle alone. The values represent the mean uterine weight and activity expressed as percent increase over that of control used as basal value.
[c] Computed as (E − T) × 100/E where E and T refer to the mean uterine weights from animals treated with 17α-ethynylestradiol.
[d] T/C (Treated/Control).
[e] Denotes the ability of the test compounds to inhibit PTH induced 45Ca resorption, computed as (C − T) × 100/C.
[f] All rats pregnant $n = 6$.

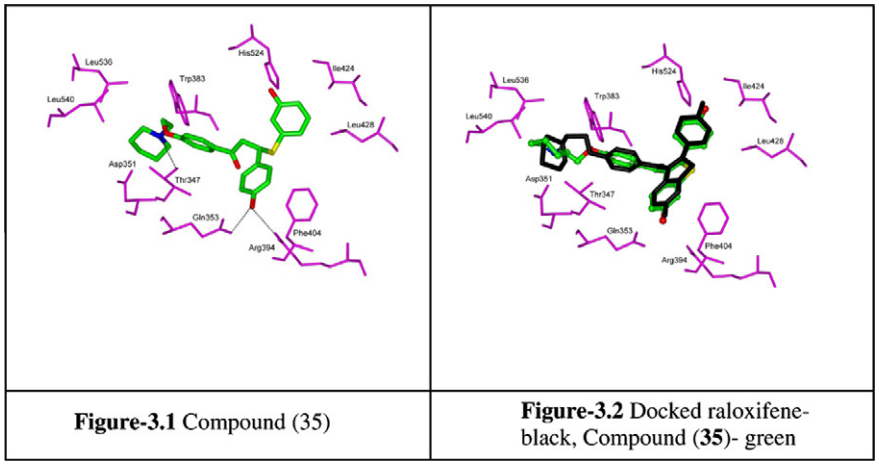


Figure-3.1 Compound (35)

Figure-3.2 Docked raloxifene-black, Compound (**35**)- green

Figure 3. Molecular modeling studies of compound **35** and raloxifene.

normal fetal kidney tissues which of non-cancer origin and hence, commonly considered as in vitro model for evaluating non-specific cytotoxicity. Therefore, compounds (**35**–**39**, **41**) were also tested for their non-cancer specific cytotoxicity in HEK-293 cell line. The two most active compounds **35** and **36** were exhibiting low cytotoxicity than other compounds of the series. All the results are summarized in Table 3.

3. Conclusions

In present study we have designed and synthesized 1,3-biarylsulfanyl derivatives and evaluated them for their SERM behavior in various cancer cell lines as well as for their antiosteoporotic activity. Our finding suggests that β-aryl-β-mercapto ketone analogues (**5–32**) without antiestrogenic side chain have exhibited moderate antiproliferative activity in MCF-7, Ishikawa and MDA-MB-231 cancer cell lines. Whereas, compounds (**33–41**) with basic alkyl amino side chain were showing significant antiproliferative activity. In addition compounds (**5, 8, 10**) were evaluated for their antiosteoporotic activity and showed promising activity similar to that of raloxifene. Most potent compounds **35**, **36** of the series exhibited antiproliferative activity comparable to tamoxifen. Thus, **35**, 36 cause selective inhibition of ER+ve MCF-7 in comparison to ER−ve MDA-MB-231 by acting as ER antagonist and this is well supported by their significant affinity towards ER.

4. Experimental section

4.1. General consideration

Unless otherwise specified all the reagents and catalysts were purchased from Sigma–Aldrich and were used without further any purification. The common solvents were purchased from Ranbaxy. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was accomplished using flash chromatography on 230–400 mesh silica gel. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates visualized under UV light, iodine or $KMnO_4$ staining. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-200 MHz Spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hertz. IR spectra were recorded on a FTIR spectrophotometer

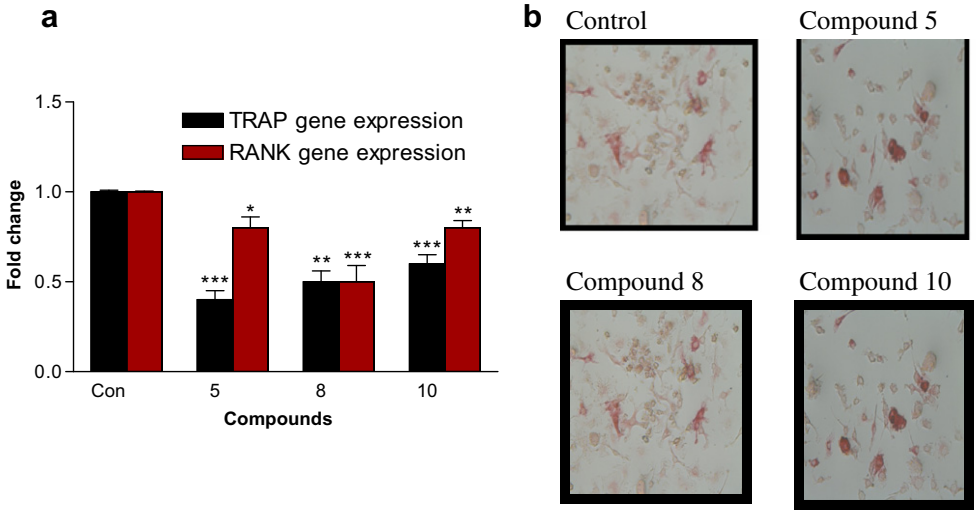


Figure 4. (a) Compounds (**5**, **8**, **10**) were tested for their effects on the induction of TRAP, and RANK, in freshly isolated murine bone marrow cells treated with RANKL and MCSF for 7 days. QPCR determination of TRAP, and RANK, was made and the data expressed as fold been expressed as the number of animals taken in each group ($n = 5$). (b) Representative photomicrograph of TRAP-stained cells that were counted. change over vehicle (control) using quantitative real time PCR. (3.1) Fold change of TRAP positive cells in control and test compounds Data have.

Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra (ESIMS) were obtained by micromass quattro II instrument.

4.2. Procedure for synthesis of chalcones (3a–j)

Chalcones (**3a–j**) were synthesized via Clasien–Schmidt condensation of substituted benzaldehyde and acetophenones. In dry DCM substituted benzaldehyde (1.0 mmol), acetophenone (1.0 mmol) in the presence of zirconium chloride (20 mol %) was taken. Reaction was stirred at room temperature for 2 h leading to generation of chalcone. Progress of reaction was monitored by TLC. After completion of reaction solvent was evaporated under the reduced pressure and residue was extracted with ethyl acetate and water. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator.

4.3. Representative procedure for the zirconium catalyzed 1, 4 conjugate addition of aryl thiols to 1,3-diaryl-2-propenones (5–26)

To a magnetically stirred mixture of 1,3-diaryl-2-propenone (1 mmol) with arylmercaptan (1.5 mmol) in dry DCM (1–2 mL), zirconium chloride (25 mol %) was added. The reaction mixture was stirred at room temperature for 20–30 min. There was formation of orange color transition metal complex with evolution of heat, which gradually converted into the light yellow to ceramic white after the completion of the reaction. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was extracted with EtOAc or DCM. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator.

4.4. Spectroscopic and analytical details of β-aryl-β-mercapto ketones (5–26)

4.4.1. 1,3-Diphenyl-3-phenylsulfanyl-propan-1-one (5)

Yield: 92%; White solid; mp 118–120 °C. IR ν_{max}: 1588, 1677, 2364 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.50–3.73 (m, 2H), 4.91 (t, 1H, $J = 7.6$, Hz), 7.13–7.54 (m, 13H), 7.85 (d, 2H, $J = 7.5$ Hz), ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.1, 48.6, 127.7, 127.9, 128.2, 128.4, 128.8, 129.0, 129.2, 133.1, 133.6, 134.6, 137.1, 141.6, 197.4; MS (*m/z* %): 319 $[M+H]^+$; Anal. Calcd for $C_{21}H_{18}OS$: C, 79.21; H, 5.70. Found: C, 79.26; H, 5.71.

4.4.2. 1,3-Diphenyl-3-p-tolylsulfanyl-propan-1-one (6)

Yield: 96%; White solid; mp 110–112 °C. IR ν_{max}: 1601,1664 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.29 (s, 3H), 3.56–3.63 (m, 2H), 4.88 (t, 1H, $J = 7.0$ Hz), 7.01 (d, 2H, $J = 7.5$ Hz), 7.17–7.54 (m, 10H), 7.85 (d, 2H, $J = 7.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.5, 45.0, 49.0, 127.7, 128.2, 128.4, 128.8, 128.9, 130.0, 130.7, 133.6, 133.8, 137.1, 138.2, 141.7, 197.5; MS (*m/z* %): 333 $[M+H]^+$; Calcd for $C_{22}H_{20}OS$: C, 79.48; H, 6.06. Found: C, 79.51; H, 6.10.

4.4.3. 3-(4-Chloro-phenyl sulfanyl) 1,3-diphenyl-propan-1-one (7)

Yield: 90%; White solid; mp 90–92 °C. IR ν_{max}: 688, 1473, 1685 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.57–3.62 (m, 2H), 4.90 (t, 1H, $J = 7.0$ Hz), 7.14–7.25 (m, 9H), 7.40–7.86 (m, 3H), 7.87–7.90 (d, 2H, $J = 6.9$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.9, 48.7, 127.9, 128.1, 128.4, 128.9, 129.0, 129.3, 133.0, 133.7, 134.2, 134.6, 137.1, 141.4, 197.1; MS (*m/z* %): 353 $[M+H]^+$; Calcd for $C_{21}H_{17}ClOS$: C, 71.48; H, 4.86. Found: C, 71.51; H, 4.82.

4.4.4. 3-(4-Methoxyphenyl)-1-phenyl-3-(phenylthio)propan-1-one (8)

Yield: 95%; White solid; mp 80–82 °C. IR ν_{max}: 1602, 1665, 3429 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.52–3.59 (2H, m) 3.83 (s, 3H), 4.91 (t, 1H, $J = 6.3$ Hz), 6.86 (d, 2H, $J = 8.8$ Hz), 7.34–7.16 (m, 10H), 7.83 (d, 2H, $J = 8.8$ Hz, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.7, 48.7, 55.8, 114.1, 127.7, 127.8, 128.2, 128.8, 129.2, 130.3, 130.7, 133.0, 134.7, 141.7, 164.0, 195.8; MS (*m/z* %): 349 $[M+H]^+$; Calcd for $C_{22}H_{20}O_2S$: C, 75.83; H, 5.79. Found: C, 75.85; H, 5.75.

4.4.5. 3-(4-Methoxyphenyl)-1-phenyl-3-(*p*-tolylthio)propan-1-one (9)

Yield: 94%; White solid; mp 98–100 °C. IR ν_{max}: 1600, 1683, 3420 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.29 (s, 3H), 3.57–3.51 (m, 2H), 3.85 (s, 3H), 4.83 (t, 1H, $J = 6.4$ Hz), 6.86 (d, 2H, $J = 8.8$ Hz), 7.40–7.00 (m, 9H), 7.83 (d, 2H, $J = 8.8$ Hz, Ar-H); ^{13}C

NMR (50 MHz, $CDCl_3$) δ: 21.5, 44.5, 49.1, 55.8, 114.1, 127.6, 128.2, 128.7, 128.9, 130.0, 130.2, 130.7, 133.8, 138.1, 141.8, 163.9, 196.0; MS (*m/z* %): 363 $[M+H]^+$; Calcd for $C_{23}H_{22}O_2S$: C, 76.21; H, 6.12. Found: C, 76.23; H, 6.17.

4.4.6. 1,3-Bis-(4-methoxy-phenyl)-3-phenylsulfanyl-propan-1-one (10)

Yield: 92%; White solid; mp 120–122 °C; IR ν_{max}: 3435, 1670, 1599 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.49–3.57 (m, 2H), 3.74 (s, 3H), 3.84 (s, 3H,), 4.89 (t, 1H, J = 6.4 Hz), 6.75 (d, 2H, J = 8.6 Hz), 6.86 (d, 2H, J = 8.8 Hz), 7.20–7.35 (m, 7H), 7.83 (d, 2H, J = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.7, 48.1, 55.6, 55.8, 114.0, 114.1, 127.7, 129.2, 130.2, 130.8, 132.9, 133.6, 134.9, 138.5, 159.0, 163.9, 196.1; MS (*m/z* %): 379 $[M+H]^+$; Calcd for $C_{23}H_{22}O_3S$: C, 72.99; H, 5.86. Found: C, 72.96; H, 5.87.

4.4.7. 1,3-Bis-(4-methoxy-phenyl)-3-*p*-tolyl-sulfanyl-propan-1-one (11)

Yield: 90%; White solid; mp 130–135 °C. IR ν_{max}: 1167, 1234, 1599, 1670, 2364 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.29 (s, 3H), 3.47–3.55 (m, 2H), 3.74 (s, 3H), 3.84 (s, 3H), 4.88 (t, 1H, J = 6.3 Hz), 6.75 (d, 2H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.8 Hz), 7.05 (d, 2H, J = 8.0 Hz), 7.20–7.25 (m, 4H), 7.87 (d, 2H, J = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.5, 44.7, 48.6, 55.6, 55.8, 114.0, 114.1, 127.7, 129.2, 130.0, 130.3, 130.7, 131.0, 133.7, 138.0, 159.0, 163.9, 196.2; MS (*m/z* %): 393 $[M+H]^+$; Calcd for $C_{24}H_{24}O_3S$: C, 73.44; H, 6.16. Found: C, 73.46; H, 6.13.

4.4.8. 3-(4-Hydroxyphenyl)-1-phenyl-3-(phenylthio) propan-1-one (12)

Yield 88%; White solid; mp 103–105 °C; IR ν_{max}: 1602, 1663, 3420 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.44–3.67 (m, 2H), 4.89 (t, 1H, J = 7.1 Hz), 6.80 (d, 2H, J = 8.7 Hz), 7.16–7.42 (m, 10H), 7.78 (d, 2H, J = 9.4 Hz), 8.12 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.6, 48.8, 115.8, 127.7, 127.9, 128.1, 128.8, 129.2, 131.2, 131.5, 133.1, 134.6, 141.5, 161.1, 196.6; MS (*m/z* %): 335 $[M+H]^+$; Calcd for $C_{21}H_{18}O_2S$: C, 75.42; H, 5.43. Found: C, 75.45; H, 5.47.

4.4.9. 3-(4-Hydroxyphenyl)-1-phenyl-3-(*m*-tolylthio)propan-1-one (13)

Yield: 85%; White solid; mp 98–101 °C. IR ν_{max}: 1604, 1666, 3422 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.31 (s, 3H), 3.51–3.57 (m, 2H), 4.86 (t, 1H, J = 7.2 Hz), 6.80 (m, 3H), 7.02–7.39 (m, 9H), 7.82 (d, 2H, J = 8.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.4, 44.6, 48.8, 115.8, 127.7, 127.9, 128.1, 128.8, 129.2, 131.0, 131.4, 133.0, 134.7, 141.5, 161.1, 196.6; MS (*m/z* %): 349 $[M+H]^+$; Calcd for $C_{22}H_{20}O_2S$: C, 75.83; H, 5.79. Found: C, 75.88; H, 5.81.

4.4.10. 1-(3-Chlorophenyl)-3-*p*-tolyl-3-(*p*-tolylthio)propan-1-one (14)

Yield: 96%; White solid; mp 100–104 °C. IR ν_{max}: 1601, 1674, 3420 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.32 (s, 3H), 2.40 (s, 3H), 3.57 (d, 2H, J = 7.2 Hz), 4.83 (t, 1H, J = 7.2 Hz), 7.11–7.31 (m, 10H), 7.77 (d, 2H, J = 7.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.0, 22.0, 44.7, 47.6, 126.4, 126.8, 127.8, 128.2, 128.3, 128.5, 129.7, 130.7, 129.9, 133.9, 144.0, 141.1, 144.6, 196.6; MS (*m/z* %): 381 $[M+H]^+$; Calcd for $C_{23}H_{21}ClOS$: C, 75.52; H, 5.56. Found: C, 75.48; H, 5.57.

4.4.11. 1-(4-Hydroxyphenyl)-3-phenyl-3-(phenylthio) propan-1-one (15)

Yield: 94%; White solid; mp 103–106 °C. IR ν_{max}: 1682, 3347 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.48–3.71 (m, 2H), 4.50–4.55 (m, 1H), 6.68 (t, 1H, J = 7.5 Hz), 6.97 (d, 1H, J = 7.9 Hz), 7.04 (d, 1H, J = 7.9 Hz), 7.14–7.24 (m, 5H), 7.40–7.45 (m, 2H), 7.50–7.57 (m, 2H), 7.93 (d, 2H, J = 7.2 Hz), 8.14 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.1, 49.3, 115.9, 117.8, 120.8, 128.1, 128.2, 129.2, 132.4, 134.2, 136.9, 137.6, 141.9, 158.7, 197.8; MS (*m/z* %): 335 $[M+H]^+$; Calcd for $C_{21}H_{18}O_2S$: C, 75.42; H, 5.43. Found: C, 75.40; H, 5.39.

4.4.12. 1-(4-Chlorophenyl)-3-(4-chlorophenylsulfanyl)-3-phenyl-propan-1-one (16)

Yield: 98%; White solid; mp 84–86 °C. IR ν_{max}: 688, 1473, 1685, 3061 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.58 (d, 2H, J = 7.1 Hz), 4.88 (t, 1H, J = 7.1 Hz), 7.16–7.58 (m, 11H), 7.88 (d, 2H, J = 7 8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.8, 48.3, 128.4, 128.9, 129.0, 129.1, 129.4, 129.5, 130.8, 133.9, 134.5, 134.8, 137.5, 140.1, 196.8; MS (*m/z* %): 387 $[M+H]^+$; Calcd for $C_{21}H_{16}Cl_2OS$: C, 65.12; H, 4.16. Found: C, 65.10; H, 4.17.

4.4.13. 3-(4-Chlorophenylthio)-1-(4-nitrophenyl)-3-phenylpropan-1-one (17)

Yield: 85%; White solid; mp 94–96 °C. IR ν_{max}: 688, 1346, 1519, 1681, 3058 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.65 (d, 2H, J = 7.1 Hz), 4.94 (t, 1H, J = 7.1 Hz), 7.15–7.61 (d, 2H, J = 7.8 Hz) 7.88–7.91 (m, 9H), 8.09–8.12 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.3, 48.5, 124.2, 128.2, 128.4, 128.8, 128.9, 129.4, 131.6, 132.2, 134.1, 135.1, 136.6, 149.3, 196.2; MS (*m/z* %): 398 $[M+H]^+$; Calcd for $C_{21}H_{16}ClNO_3S$: C, 63.39; H, 4.05; N, 3.52. Found: C, 63.36; H, 4.01; N, 3.54.

4.4.14. 3-(4-Chlorophenylthio)-1-(4-methoxyphenyl)-3-phenylpropan-1-one (18)

Yield: 90%; White solid; mp 86–90 °C. IR ν_{max}: 1610, 1674, 2947 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.53–3.62 (m, 2H), 3.74 (s, 3H), 4.93 (t, 1H, J = 8.5 Hz), 6.79 (d, 2H, J = 8.7 Hz), 7.17–7.54 (m, 9H), 7.89 (d, 2H, J = 7.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 45.1, 48.4, 55.6, 114.3, 123.4, 124.1, 124.8, 125.2, 128.5, 129.0, 129.3, 129.4, 133.4, 133.8, 134.1, 137.1, 159.3, 197.3; MS (*m/z* %): 383 $[M+H]^+$; Calcd for $C_{22}H_{19}ClO_2S$: C, 69.01; H, 5.00. Found: C, 68.98; H, 5.03.

4.4.15. 1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-3-(phenylthio)propan-1-one (19)

Yield: 88%; White solid; mp 84–86 °C. IR ν_{max}: 688, 1473, 1685, 3061 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.58 (m, 2H), 3.72 (s, 3H), 4.88 (t, 1H, J = 7.1 Hz), 7.16–7.58 (m, 11H), 7.88 (d, 2H, J = 7 8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 43.8, 49.3, 55.6, 127.4, 128.6, 129.0, 129.1, 129.2, 129.5, 130.8, 132.9, 134.6, 134.8, 137.5, 142.1, 196.8; MS (*m/z* %): 383 $[M+H]^+$; Calcd for $C_{22}H_{19}ClO_2S$: C, 69.01, H, 5.00. Found: C, 68.97; H, 5.02.

4.4.16. 1-(4-Methoxyphenyl)-3-phenyl-3-(phenylthio) propan-1-one (20)

Yield: 96%; White solid; mp 87–88 °C. IR ν_{max}: 1580, 1683, 3445 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.78 (s, 3H), 4.91 (t, 1H, J = 8.2 Hz), 3.52–3.59 (m, 2H), 6.78 (d, 2H, J = 8.6 Hz), 7.26–7.54 (m, 10H), 7.85 (d, 2H, J = 7.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.7, 48.7, 55.8, 114.1, 127.7, 127.8, 128.2, 128.8, 129.4, 130.3, 130.7, 133.0, 134.7, 141.7, 164.0, 195.8; MS (*m/z* %): 349 $[M+H]^+$; Calcd for $C_{22}H_{20}O_2S$: C, 75.83; H, 5.79. Found: C, 75.81; H, 5.76.

4.4.17. 1-(3-Nitrophenyl)-3-phenyl-3-(phenylthio) propan-1-one (21)

Yield: 78%, White solid; mp 96–98 °C. IR ν_{max}: 688, 1353, 1549, 1683, 3058 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 3.65 (d, 2H, J = 7.1 Hz), 5.00 (t, 1H, J = 7.1 Hz), 7.21–7.60 (m, 11H) 7.91 (d, 2H, J = 7.1 Hz), 8.09–8.15 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.6, 48.2, 123.9, 124.2, 124.7, 125.1, 125.8, 127.6, 128.1, 128.6, 128.9, 129.9, 131.2, 132.0, 134.3, 135.5, 136.2, 149.0, 197.1; MS (*m/z* %): 364 $[M+H]^+$; Calcd for $C_{21}H_{17}O_3NS$: C, 69.40; H, 4.71; N, 3.85. Found: C, 69.38; H, 4.68; N, 3.87.

4.4.18. 3-(4-Nitro-phenylsulfanyl)-1,3-diphenylpropan-1-one (22)

Yield: 68%, White solid; mp 87–89 °C; IR ν_{max}: 1679, 3345 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.61–3.66 (m, 2H), 4.91 (t, 1H J = 7.8 Hz), 7.34–7.37 (m, 4H), 7.42–7.47 (m, 4H), 7.49–7.63 (m, 1H), 7.89–7.91 (m, 2H), 8.03–8.06 (m, 2H), 8.17–8.20 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 45.6, 47.1, 124.4, 124.9, 126.9, 128.2, 128.4, 128.6, 129.2, 129.4, 29.5, 134.1, 140.8, 145.8, 196.7; MS (*m/z* %): 364 $[M+H]^+$; Calcd for $C_{21}H_{17}NO_3S$: C, 69.40; H, 4.71; N, 3.85. Found: C, 69.42; H, 4.76; N, 3.87.

4.4.19. 1-Napthalen-2-yl-3-phenyl-3-phenyl-sulfanyl propan-1-one (23)

Yield 78%, White solid; mp 100–101 °C; IR ν_{max}: 1585, 1687, 3445 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.76 (d, 2H, J = 6.Hz), 5.81 (t, 1H, J = 7.1 Hz), 7.20–7.59 (m, 12H), 7.22 (d, 1H, J = 8.6 Hz), 7.84–7.92 (m, 3H), 8.32 (d, 1H, J = 9.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.3, 48.6, 122.4, 123.2, 123.8, 124.3, 124.6, 125.8, 126.2, 127.1, 127.8, 128.2, 128.7, 129.1, 129.8, 132.6, 132.9, 134.6, 136.3, 138.9, 196.4; MS (*m/z* %): 369 $[M+H]^+$; Calcd for $C_{25}H_{20}OS$: C, 81.49; H, 5.47. Found: C, 81.45; H, 5.48.

4.4.20. 3-(4-Chlorophenylthio)-3-(4-methoxyphenyl)-1-phenylpropan-1-one (24)

Yield: 90%, White solid; mp 100–109 °C. IR ν_{max}: 1601, 1674, 3420 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.51–3.56 (m, 2H), 3.85 (s, 3H), 4.91 (t, 1H, J = 7.0 Hz), 6.91 (d, 2H, J = 7.7 Hz), 7.15–7.28 (m, 9H), 7.86 (d, 2H, J = 7.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 45.0, 48.6, 55.4, 113.9, 128.1, 129.2, 129.0, 129.6, 133.3, 133.9, 132.6, 134.0, 134.2, 137.8, 159.6, 197.4; MS (*m/z* %): 383 $[M+H]^+$; Calcd for $C_{22}H_{19}ClO_2S$: C, 69.01; H, 5.00. Found: C, 68.98; H, 5.02.

4.4.21. 1-(3-Chlorophenyl)-3-(phenylthio)-3-p-tolylpropan-1-one (25)

Yield: 98%, White solid; mp 106–108 °C. IR ν_{max}: 1601, 1674, 3420 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.39 (s, 3H), 3.54–3.52 (m, 2H), 4.89 (t, 1H, J = 7.0 Hz), 7.16–7.31 (m, 11H), 7.80 (d, 2H, J = 7.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 22.8, 44.5, 47.3, 126.4, 126.6, 127.6, 128.0, 128.2, 128.7, 129.0, 129.2, 130.7, 133.7, 141.1, 143.8, 144.0, 196.1; MS (*m/z* %): 367 $[M+H]^+$; Calcd for $C_{22}H_{19}ClOS$: C, 72.02; H, 5.22. Found: C, 72.00; H, 5.19.

4.4.22. 1-(3-Chlorophenyl)-3-phenyl-3-(phenylthio)propan-1-one (26)

Yield: 98%; White solid; mp 106–108 °C; IR ν_{max}: 1601, 1674, 3420 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.54–3.52 (m, 2H), 4.89 (t, 1H, J = 7.0 Hz), 7.16–7.31 (m, 12H), 7.80 (d, 2H, J = 7.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 44.5, 47.3, 126.4, 126.6, 127.6, 128.0, 128.2, 128.7, 129.0, 129.2, 130.7, 133.7, 141.1, 143.8, 144.0, 196.1; MS (*m/z* %): 353 $[M+H]^+$; Calcd for $C_{21}H_{17}ClOS$: C, 71.48; H, 4.86. Found: C, 71.46; H, 4.85.

4.5. Typical experimental procedure for the zirconium catalyzed 1, 4 conjugate addition of mercapto carboxylic acid to 1,3-diaryl-2-propenones (27–28)

To a magnetically stirred mixture of 1,3-diaryl-2-propenone (1 mmol), zirconium chloride (25 mol %) and mercapto carboxylic acid (2.5 mmol) were added at room temperature in solvent free condition. The reaction was stirred for 6–8 h. The precipitate was filtered and washed with water and hexane. The solid or oily residue was crystallized by ethyl acetate and hexane to afford the pure products in high yield.

4.5.1. 3-(3-Oxo-1,3-diphenylpropylthio)propanoic acid (27)

Yield: 88%; Yellow oil; IR ν_{max}: 1598, 1600, 3407 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.52–2.60 (m, 4H), 3.52 (d, 2H, J = 6.9 Hz), 4.59 (t, 1H, J = 6.9 Hz), 7.19–7.59 (m, 8H), 7.88 (d, 2H, J = 7.1 Hz), 11.3 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.4, 34.4, 44.9, 45.6, 127.9, 128.2, 128.5, 129.0, 130.1, 133.7, 137.0, 142.0, 177.3, 197.1; MS (*m/z* %): 315 $[M+H]^+$; Calcd for $C_{18}H_{18}O_3S$: C, 68.76; H, 5.77. Found: C, 68.72; H, 5.74.

4.5.2. 3-(1-(4-Methoxyphenyl)-3-oxo-3-phenylpropylthio)propanoic acid (28)

Yield: 86%; Yellow semi solid; IR ν_{max}: 1502, 1599, 1602, 3407 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.51–2.60 (m, 4H), 3.49 (d, 2H, J = 6.9 Hz), 3.85 (s, 3H), 4.58 (t, 1H, J = 6.9 Hz), 6.92 (d, 2H, J = 8. 8 Hz), 7.26–7.43 (m, 5H), 7.91 (d, 2H, J = 8. 8 Hz) 11.6 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 26.4, 34.7, 45.1, 45.2, 55.9, 114.1, 127.8, 128.2, 129.0, 130.8, 132.7, 142.2, 164.0, 178.0), 195.7; MS (*m/z* %): 345 $[M+H]^+$; Calcd for $C_{19}H_{20}O_4S$: C, 66.26; H, 5.85. Found: C, 66.24; H, 5.86.

4.6. Typical experimental procedure for the zirconium catalyzed 1, 4 conjugate addition of alkyl thiols to 1,3-diaryl-2-propenones (29–32)

In a typical experiment 1,3-diaryl-2-propenone (1 mmol), zirconium chloride (25 mol %) in dry DCM (2–3 mL) were mixed together and then alkyl thiol (2 mmol) was added drop wise with help of syringe. The solution stirred at room temperature under an air atmosphere for appropriate time of period. After completion of the reaction (monitored by TLC), the reaction mixture was worked up by simple extraction with ethyl acetate and water. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator to afford the desired product in high purity. The further purification was performed by crystallization.

4.6.1. 3-(Ethylthio)-3-(4-methoxyphenyl)-1-phenylpropan-1-one (29)

Yield: 89%; White solid; mp 90–92 °C. IR ν_{max}: 1600, 1680, 3428 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.15 (t, 3H, J = 7.3 Hz), 2.28–2.40 (m, 2H), 3.45 (d, 2H, J = 7.0 Hz), 3.82 (s, 3H), 4.54 (1H, J = 7.0 Hz), 6.86 (d, 2H, J = 8.8 Hz), 7.19–7.32 (m, 5H), 7.87 (d, 2H, J = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.7, 25.8, 44.6, 45.3, 55.8, 114.1, 127.5, 128.2, 128.8, 129.2, 133.0, 134.7, 141.7, 164.0, 195.0; MS (*m/z* %): 301 $[M+H]^+$; Calcd for $C_{18}H_{20}O_2S$: C, 71.96; H, 6.71. Found: C, 71.92; H, 6.68.

4.6.2. 3-(Ethylthio)-3-(4-hydroxyphenyl)-1-phenylpropan-1-one (30)

Yield: 70%; White semi solid; IR ν_{max}: 1605, 1673, 3422 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.10 (t, 3H, J = 7.3 Hz), 2.20–2.41 (m, 2H), 3.46 (d, 2H, J = 7.1 Hz), 4.51 (t, 1H, J = 7.1 Hz), 6.84 (d, 2H, J = 7.7 Hz), 7.15–7.81 (m, 5H), 7.85 (d, 2H, J = 9.8 Hz), 8.12 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.7, 25.8, 44.8, 45.3, 116.0, 126.3, 127.6, 128.1, 129.7, 131.3, 142.4, 161.7, 197.3; MS (*m/z* %): 287 $[M+H]^+$. Calcd for $C_{17}H_{18}O_2S$: C, 71.30; H, 6.34. Found: C, 71.34; H, 6.38.

4.6.3. 3-Ethylsulfanyl-1,3-bis-(4-methoxy-phenyl)-propan-1-one (31)

Yield: 90%; White solid; mp 159–161 °C. IR ν_{max}: 1669, 1680, 3430 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.12 (t, 3H, J = 7.3 Hz), 2.28–2.38 (m, 2H), 3.43 (d, 2H, J = 7.1 Hz), 3.77 (s, 3H), 3.85 (s, 3H), 4.51 (t, 1H, J = 7.0 Hz), 6.80–6.88 (m, 4H), 7.30 (d, 2H, J = 8.6 Hz), 7.87 (d, 2H, J = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.7, 25.7, 44.0, 45.5, 55.6, 55.8, 110.0, 114.1, 114.2, 129.2, 130.3,

130.8, 134.6, 158.9, 163.9, 196.0; MS (*m/z* %): 331 $[M+H]^+$; Calcd for $C_{19}H_{22}O_3S$: C, 69.06; H, 6.71. Found: C, 69.10; H, 6.67.

4.6.4. 1,3-Diphenyl-3-(*p*-tolylthio)propan-1-one (32)

Yield: 90%; White solid; mp 155–157 °C IR ν_{max}: 693, 750,1594, 1677 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.39 (s, 3H), 3.51 (d, 2H, J = 7.0 Hz), 4.55(t, 1H, J = 7.0 Hz), 7.16–7.33 (m, 7H), 7.39–7.54 (m, 5H), 7.89 (d, 2H, J = 7.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.7, 25.8, 44.4, 45.7, 127.6, 128.2, 128.5, 128.9, 129.0, 133.6, 137.2, 142.6, 197.3; MS (*m/z* %): 333 $[M+H]^+$; Calcd for $C_{22}H_{20}OS$: C, 79.48; H, 6.06. Found: C, 79.45; H, 6.01.

4.7. Spectroscopic and analytical details of basic alkyl amino side chain analogues β-aryl-β-mercapto ketones (33–41)

In 100 mL of round bottom flask, compound (**4c**) (1 mmol), substituted aldehyde (1 mmol) and arylmercaptan (1.5 mmol) was taken in dry DCM, zirconium chloride (25 mol %) was added as a catalyst. Reaction mixture was stirred at room temperature 60 min. There is a formation of colored complex with evolution of heat, which gradually converts light yellow to ceramic white at the completion of the reaction. The progress of the reaction was monitored by checking TLC. After completion of the reaction, the reaction mixture was extracted with EtOAc or DCM. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator. The crude solid was obtained which was further purified by simple crystallization to obtain product in good yield.

4.7.1. 1-(4-(2-(Dimethylamino)ethoxy)phenyl)-3-phenyl-3-(phenylthio)propan-1-one (33)

Yield: 86%; Creamish solid; mp 119–120 °C. IR ν_{max}: 1679, 3345 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.28 (s, 6H), 2.79 (t, 2H, J = 5.7 Hz), 3.22–3.27 (m, 2H), 4.10 (t, 2H, J = 5.7 Hz), 4.39 (t, 1H, J = 7.1 Hz, SCH), 6.85–7.02 (m, 3H), 7.08–7.16 (m, 5H), 7.18–7.23 (m, 4H), 7.80 (d, 2H, J = 8.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 41.8, 42.2, 47.9, 58.9, 71.9, 114.3, 124.9, 126.6, 126.9, 127.9, 128.2, 128.7, 129.1, 129.7, 135.8, 141.0, 163.8, 196.7; MS (*m/z* %): 406 $[M+H]^+$; Calcd for $C_{25}H_{27}NO_2S$: C, 74.04; H, 6.71; N, 3.45. Found: C, 74.08; H, 6.70; N, 3.48.

4.7.2. 1-(4-(2-(Dimethylamino)ethoxy)phenyl)-3-phenyl-3-(p-tolylthio)propan-1-one (34)

Yield: 85%; Yellow solid; mp 127–129 °C. IR ν_{max}: 1679, 3345 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.26 (s, 6H), 2.29 (s, 3H) 2.81 (t, 2H, J = 5.7 Hz), 3.21–3.28 (m, 2H), 4.12 (t, 2H, J = 5.7 Hz) 4.41(t, 1H, J = 7.1 Hz), 6.82–6.86 (3H, m, Ar-H), 6.97–6.99 (2H, m, Ar-H), 7.04–7.12 (4H, m, Ar-H), 7.23–7.28 (2H, m, Ar-H), 7.78 (2H, d, J = 8.6 Hz, Ar-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 41.8, 42.2, 47.9, 50.2, 58.9, 71.9, 114.3, 124.9, 126.6, 126.9, 127.9, 128.2, 128.7, 129.1, 129.7, 135.8, 141.0, 163.8, 196.7; MS (*m/z* %): 420 $[M+H]^+$; Calcd for $C_{26}H_{29}NO_2S$: C, 74.43; H, 6.97; N, 3.34. Found: C, 74.40; H, 6.92; N, 3.36.

4.7.3. 3-(3-Hydroxyphenyl)-3-(4-hydroxyphenylthio)-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propan-1-one (35)

Yield: 88%; Yellow solid; mp 137–139 °C. IR ν_{max}: 1679, 3345 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.44–2.68 (6H, m, CH_2), 2.81 (t, 4H, J = 5.5 Hz), 2.99 (t, 2H, J = 5.5 Hz), 3.43–3.49 (m, 2H), 4.04 (t, 2H, J = 6.1 Hz), 4.86 (t, 1H, J = 7.0 Hz), 6.78 (d, 2H, J = 8.4 Hz), 7.10–7.22 (m, 6 H), 7.38–7.41 (m, 4H), 7.51 (d, 2H, J = 8.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 23.9, 30.1, 32.6, 44.7, 54.5, 55.1, 67.7, 114.7, 126.3, 127.6, 127.7, 127.9, 128.2, 128.8, 129.2, 129.5, 130.8, 133.0, 134.8, 140.8, 163.3, 195.8; MS (*m/z* %): 478 $[M+H]^+$; Calcd for $C_{28}H_{31}NO_4S$: C, 70.41; H, 6.54; N, 2.93. Found: C, 70.39; H, 6.52; N, 2.95.

4.7.4. 3-(4-Hydroxyphenylthio)-3-phenyl-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propan-1-one (36)

Yield: 82%; White solid; mp 122–123 °C. IR ν_{max}: 689, 1582, 1680, 3336 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 2.46–2.70 (m, 6H), 2.86 (t, 4H, J = 6.0 Hz), 4.89 (t, 2H, J = 6.0 Hz), 3.53–3.59 (2H, m), 4.34 (t, 2H, J = 6.1 Hz), 4.96 (t, 1H, J = 7.0 Hz), 6.98 (d, 2H, J = 8.4 Hz), 7.18–7.28 (m, 6H), 7.31 (d, 2H, J = 8.4 Hz), 7.38–7.41 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.7, 23.9, 30.1, 32.6, 44.7, 48.7, 53.8, 54.6, 55.2, 56.0, 67.7, 114.7, 127.3, 127.6, 127.9, 128.2, 128.8, 129.2, 129.5, 130.8, 133.0, 134.8, 141.0, 163.2, 197.8; MS (*m/z* %): 462 $[M+H]^+$; Calcd for $C_{28}H_{31}NO_3S$: C, 72.85; H, 6.77; N, 3.03. Found: C, 72.84; H, 6.74; N, 3.05.

4.7.5. 3-(4-Hydroxyphenyl)-3-(4-hydroxyphenylthio)-1-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)propan-1-one (37)

Yield: 82%; Yellow solid; mp: 132–135 °C. IR ν_{max}: 688, 1679, 2782, 2932, 2952 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.59–1.6 (m, 4H), 2.25–2.32 (m, 4H), 2.78 (t, 2H, J = 6.0 Hz), 3.18–3.21(m, 2H), 4.08 (t, 2H, J = 6.0 Hz), 4.54 (t, 1H, J = 7.1 Hz), 6.87 (d, 2H, J = 8.2 Hz), 7.03–7.11 (m, 2H), 7.14–7.19 (m, 8H), 7.78 (d, 2H, J = 8.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 24.9, 44.5, 47.7, 52.5, 54.0, 72.9, 114.4, 124.9, 126.3, 126.8, 127.9, 128.5, 128.9, 129.6, 130.3, 135.6, 142.7, 163.2, 195.8; MS (*m/z* %): 464 $[M+H]^+$; Calcd for $C_{27}H_{29}NO_4S$: C, 69.95; H, 6.31; N, 3.02. Found C, 69.93; H, 6.29; N, 3.05.

4.7.6. 3-(4-Hydroxyphenylthio)-3-phenyl-1-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)propan-1-one (38)

Yield 85%; Yellow oil; IR ν_{max}: 687, 1678, 2784, 2942, 2950 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.62–1.64 (m, 4H), 2.28–2.32 (m, 4H), 2.82 (t, 2H, J = 5.8 Hz), 3.20–3.26 (m, 2H), 4.10 (t, 2H, J = 5.8 Hz), 4.56 (t, 1H, J = 7.2 Hz), 5.27 (s, 1H), 6.87–6.98 (m, 5H), 7.07–7.19 (m, 6H), 7.79 (d, 2H, J = 8.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8, 24.9, 43.5, 48.2, 54.0, 72.9, 114.4, 124.8, 126.2, 126.8, 127.9, 128.4, 128.7, 129.3, 130.1, 137.6, 141.9, 163.0, 196.0; MS (*m/z* %): 448 $[M+H]^+$; Calcd for $C_{27}H_{29}NO_3S$: C, 72.45; H, 6.53; N, 3.13. Found: C, 72.48; H, 6.58; N, 3.10.

4.7.7. 1-(4-(2-(Dimethylamino)ethoxy)phenyl)-3-(ethylthio)-3-phenylpropan-1-one (39)

Yield: 86%; yellow semi solid; IR ν_{max}: 688, 1582, 1679, 3345 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.24 (t, 3H, J = 7.0 Hz), 2.30 (s, 6H), 2.48–2.51 (m, 2H), 2.72–2.79 (m, 2H), 3.21–3.28 (m, 2H), 4.12 (t, 2H, J = 5.8 Hz), 4.49 (t, 1H, J = 7.1 Hz), 6.84 (d, 2H, J = 8.6 Hz), 7.08–7.21 (m, 5H), 7.81 (d, 2H, J = 8.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.2, 23.2, 24.8, 41.9, 47.9, 58.2, 71.8, 114.4, 126.6, 128.4, 128.9, 129.0, 129.9, 138.4, 163.2, 196.1; MS (*m/z* %): 358 $[M+H]^+$; Calcd for $C_{21}H_{27}NO_2S$: C, 70.55; H, 7.61; N, 3.92. Found: C, 70.53; H, 7.58; N, 3.95.

4.7.8. 3-(Ethylthio)-3-phenyl-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propan-1-one (40)

Yield: 90%; Yellow solid; mp 142–145 °C. IR ν_{max}: 1689, 3354 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.16 (t, 3H, J = 7.3 Hz), 2.41–2.20 (m, 2H), 2.41–2.44 (m, 6H), 2.68 (t, 4H, J = 5.8 Hz), 2.99 (t, 2H, J = 5.8 Hz), 3.45 (d, 2H, J = 7.1 Hz), 4.04 (t, 2H, J = 6.1 Hz), 4.57 (t, 1H, J = 7.1 Hz), 6.89 (d, 2H, J = 8.4 Hz), 7.10–7.22 (m, 5H), 7.31–7.34 (d, 2H, J = 8.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.7, 24.5, 25.8, 26.3, 26.7, 44.5, 54.5, 58.1, 66.6, 114.6, 127.5, 128.2,

128.9, 130.8, 130.9, 142.7, 163.2, 195.8; MS (*m/z* %): 398 $[M+H]^+$; Calcd for $C_{24}H_{31}NO_2S$: C, 72.50; H, 7.86; N, 3.52. Found: C, 72.44; H, 7.83; N, 3.47.

4.7.9. 3-(Ethylthio)-3-phenyl-1-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)propan-1-one (41)

Yield: 80%; Yellow solid; mp 122–125 °C. IR ν_{max}: 688, 1549, 1682, 2428, 2745 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.26 (t, 3H, J = 7.2 Hz), 1.60–1.66 (m, 4H), 2.31–2.36 (m, 4H), 2.46–2.49 (m, 2H), 2.99 (t, 2H, J = 5.8 Hz), 3.25–3.35 (m, 2H), 4.09 (t, 2H, J = 5.8 Hz), 4.59 (t, 1H, J = 7.0 Hz), 6.85 (d, 2H, J = 8.5 Hz), 7.08–7.12 (m, 3H), 7.21 (m, 2H),7.78 (d, 2H, J = 8.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.7, 23.2, 26.3, 40.5, 48.0, 51.9, 54.2, 72.0, 114.3, 127.0, 128.4, 128.9, 129.1, 129.8 140.1, 163.2, 196.2; MS (*m/z* %): 384 $[M+H]^+$. Calcd for $C_{23}H_{29}NO_2S$: C, 72.02; H, 7.62; N, 3.65. Found: C, 71.98; H, 7.58; N, 3.61.

4.8. Cells and cell culture condition

MCF-7 (Estrogen receptor +ve breast cancer cell line), MDA-MB-231 (estrogen receptor −ve breast cancer cell lines), Ishikawa (estrogen receptor +ve endometrial cancer cell line) and HEK-293 (human embryonic kidney epithelial cell line) cells were obtained from ATCC, USA. Cells were cultured at 37 °C with 5% CO2 in DMEM medium with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 U/ml streptomycin, except in growth inhibition assays, where the FBS supplement was reduced to 2%.

4.9. MTT assay

The antiproliferative activities of the compounds were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay. 1×10^4 cells/well (MCF-7, MDA-MB-231, Ishikawa, and HEK-293) were seeded in 100 μl DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium. After 48 h of incubation, media were removed and to each well 10 μl MTT (5 mg/mL) was added and the plates were further incubated for 4 h. Supernatant from each well was carefully removed, formazon crystals were dissolved in 100 μl of DMSO and absorbance at 540 nm wavelength was recorded.

4.10. Estrogen receptor binding affinity assay

The RBA of the compounds for the estrogen receptor was determined by competition assay, employing radio labeled estradiol (3H-E2) as the reference compound. The test ligands and (3H-E2) were incubated (4 °C) with cytosol estrogen receptors obtained from immature 20 to 21-day-old rat uteri. Aliquots of the uterine cytosol (200 μL concentrated 1 uterus per ml) prepared in TEA buffer (10 mM Tris, 1.5 mM EDTA, and 0.02% sodium azide, pH 7.4) were incubated in triplicate with a fixed concentration of radio labeled estradiol with or without various concentrations of the competitor substance dissolved in 60 μL of the TEA buffer containing DMF as co-solvent (final concentration of DMF in the incubation medium never exceeded 5%) for 18 h at 4 °C. At the end of this period, dextran-coated charcoal (DCC) (5% Norit 0.5% dextran) suspension in 100 μL of TEA buffer was added into each tube, which was briefly vortexed and allowed to stand for 15 min. DCC was precipitated by centrifugation (800*g* 10 min) and the supernatants counted for radioactivity in 10 ml of a dioxane-based scintillation fluid. RBA of the text compound was computed from a graph plotted between percent bound radioactivity versus log concentration of the test substance. At 50% inhibition, log of the competitor concentration relative to that of estradiol gave the affinity of the test compound to estrogen receptor relative to estradiol. This when multiplied with 100 gave the percentage value designated as RBA.

4.11. Cell culture conditions

Primary cultures of mouse osteoclast precursor cells in the form of bone marrow-derived monocytes/macrophages (BMMs) were isolated as described by others. Briefly, bone marrow cells from the long bones of 4 to 6-week-old Balb/c mice were collected and plated overnight in a MEM (Life Technologies, Rockville, MD, USA) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10 ng/ml macrophage-colony stimulating factor (M-CSF). The next day, The nonadherent cells were collected and after centrifugation, cultured on 48-well tissue culture plates in a-MEM with heat-inactivated fetal bovine serum (10%), RANKL (50 ng/ml), and M-CSF (10 ng/ml). Cells were exposed to test agents at 1 μM concentration culture media and indicated supplement(s) were changed every 2 d for a total of 7 d.

4.16. Anti-osteoporotic activity (anti-bone resorption assay)

The in vitro assay of anti-resorptive activity using 45Ca prelabeled rat fetal bone was done according the literature method. Three month old Sprague–Dawley female rats (180–220 g) maintained at standard conditions (22 ± 1 °C) with alternate 12 h light/dark periods and free access to pellet diet and tap water were used throughout the study. Females were mated to males of proven fertility. 250 μCi/200 μL of $45CaCl_2$ was administered subcutaneously to each rat on day 18 of pregnancy and labeled femur and radio-ulna bones were isolated 48 h thereafter under sterile conditions. Bones were cultured in 200 μL of the BGJb medium supplemented with antibiotic, antifungal and buffer (pH 7.3) for 24 h. The bones were washed twice with PBS and transferred to BJGb medium containing PTH (0.4 μM) and these cultured for 96 h in the presence or absence of test compound (100 μM) or the vehicle (0.1% ethanol/DMSO) in 200 μL of BGJb, medium. Contralateral femur of each fetus served as corresponding control. On termination of the culture, bones were transferred to 0.1 N HCl for 24 h. Radioactivity due to 45Ca in the spent medium collected at 48 and 96 h of culture and the HCl extracts at 96 h of culture were quantified by liquid scintillation spectrophotometer. Bone resorbing activity was expressed as percentage of released 45Ca and the effect of test compounds as percent of corresponding control or T/C ratio. All the synthesized compounds were screened for in vitro bone resorption inhibitory activity in 21 days old fetal rat by measuring the inhibition of PTH (Parahyriod hormone) induced bone resorption inhibitory activity.

4.17. Osteoclastogenesis

Primary cultures of mouse osteoclast precursor cells in the form of bone marrow-derived monocytes/macrophages (BMMs) were isolated as described by others. Briefly, bone marrow cells from the long bones of 4 to 6-week-old Balb/c mice were collected and plated overnight in a MEM (Life Technologies, Rockville, MD, USA) supplemented with 10% heat-inactivated FBS 2 mM L-glutamine, 2 mM L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml macrophage-colony stimulating factor (M-CSF). The next day, The nonadherent cells were collected and after centrifugation, cultured on 48-well tissue culture plates in a-MEM with heat-inactivated fetal bovine serum (10%), RANKL (50 ng/ml), and M-CSF (10 ng/ml). Cells were exposed to test agents at 1 μg/ml concentration culture media and indicated supplement(s) were changed every 2 d for a total of 7 d. Compounds (**5, 8** and **10**) were

tested for their effects on the induction of TRAP and RANK in freshly isolated murine bone marrow cells treated with RANKL and MCSF for 7 days. QPCR determination of TRAP and RANK levels was made and the data expressed as fold change over vehicle (control) using quantitative real time PCR (Fig. 4a). Total RNA was extracted from the cultured cells using Trizol (Invitrogen). cDNA was synthesized from 2 μg total RNA with the reverse script reverse transcription kit (FERMENTAS). SYBR green chemistry was used to perform quantitative determinations of the mRNAs for TRAP, RANK, and housekeeping gene, cyclophilin A, following an optimized protocol. Light cycler (Roche) was used. The design of sense and antisense oligonucleotide primers was based on published cDNA sequences using primer express software (version 2.0.0, Applied Biosystems). At 1 μM concentrations, Compounds (**5, 8** and **10**) significantly inhibited expression of TRAP and RANK induced by RANKL and MCSF (Fig. 4b).

4.18. Estrogen agonistic activity

Twenty one day old immature female Spraugue–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention once daily for 3 consecutive days on days 28–30 of age by oral route. A separate group of animals received only the vehicle for similar duration served as control. At autopsy 24 h after the last treatment on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed. Premature opening of vagina, cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen agonistic activity in comparison to rats of vehicle control group. The objective was to evaluate estrogen agonistic effect of the compounds on the uterus and vagina. Compounds exhibiting no or negligible estrogen agonistic activity in this assay were identified for further development as antiimplantation agents.

4.19. Estrogen antagonistic activity

Twenty-one-day-old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for 7 days were randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg kg^{-1} dose of ethynyl estradiol in 10% ethanol-distilled water once daily for **3** consecutive days on days 28–30 of age by oral route. A separate group of animals receiving only ethynylestradiol (0.02 mg/kg) in 10% ethanol distilled water for similar duration were used for comparison. At autopsy on day 31 of age vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed and fixed for histology. Inhibition in ethynylestradiol induced cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen antagonistic effect of the compounds. Compounds exhibiting potent estrogen antagonistic activity in this assay were identified for further development as antiimplantation agents.

4.20. Anti-implantation activity

Anti-implantation activity of the compounds was studied in sperm positive adult (180–220 g) Sprague–Dawley female albino rats mated to coeval males of proven fertility. The compounds were administered orally as a suspension in gum acacia to colony bread adult mated female rats on days 1–5 post-coitum using five to seven animals in each group. The animals were examined by laprotomy on day 10 of pregnancy for the number and status of implantations and corpora lutea. The results were scored as positive only if implantations were totally absent in both the uterine horns of each animal.

Acknowledgments

All the authors would like to acknowledge generous funding by Ministry of Health and Family Welfare, New Delhi. Funding by DST, New Delhi (to RT) is acknowledged. Fellowship grants from the Council of Scientific and Industrial Research, Government of India (V.D.T., P.K., L.P.G., B.C.) and University Grant Commission (JAS) are acknowledged. This manuscript bears CDRI Communication No. 7787. Authors also acknowledge SAIF-CDRI for providing all analytical and spectral data.

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