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ARTICLE

X-ray crystal structures and anti breast cancer property of 3-*tert*-butoxycarbonyl-2-arylthiazolidine-4-carboxylic acids

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The diastereomeric '*2RS,4R*'-2-aryl thiazolidine-4-carboxylic acids (ATCAs) were synthesized and attempted their resolution to chiraly pure N-BOC derivatives by column chromatography. The absolute stereochemistry of the resolved compounds was ascertained by x-ray single crystal structures. Further application of synthesized compounds was studied for their *in vitro* anti breast cancer activity against MCF7 cell lines using DOX as a standard by MTT assay method. The cell morphology analysis was carried out by fluorescence microscopy. The compounds containing '*2S*' absolute configuration in thiazolidine ring and presence of 2-NO_2, 2,6-Cl groups on '*2R*'-aryl substituent showed significant anti breast cancer activity where some of the compounds were found to be more active than DOX by induced apoptosis mode of MCF7 cell death.

1. Introduction

The thiazolidones and thiazolidines are significant scaffolds to develop the newer and versatile multi-target drugs due to their broad spectrum of biological activities. Especially thiazolidine-2,4-dione nucleus has been widely explored by researchers for their plethora of activities like anti-hyperglycaemics, aldose reductase inhibitors, anti-cancer, anti-inflammatory, antiarthritics, anti-microbials, etc.[1] The 1,3-thiazolidine-4-carboxylic acid (**TCA**) and its derivatives are equally noteworthy because of their tremendous biological potential such as anti acetaminophen hepatotoxicity,[2] HIV protease inhibitor,[3] EDG3 Antagonists,[4] antiproliferative,[5] Antiasthma,[6] antibacterial,[7] influenza neuraminidase inhibitors,[8] serotonin metabolite,[9] tyrosinase inhibitors,[10] metallo-β-lactamases inhibitor.[11]

The cancer stood second following cardiovascular diseases in human mortality. The discovery and development of small molecule drug (**SMD**) for cancer treatment has resulted in to newer additions to the successful cancer chemotherapeutic drugs. The SMDs with molecular targeting capability have been always more effective as they exploit the particular genetic additions, dependencies and vulnerabilities of cancer cells.[12] Considering the SMD application of TCAs, the diastereomeric 2-arylthiazolidine-4-carboxylic acids (**ATCAs**) and their amide derivatives (**ATCAAs**) are focused worldwide by various research groups for *in vitro* anticancer activities against several human cancer cell lines. Miller et. al. have studied these compounds against different prostate cancer cell lines, melonema[13] and have also explored their structure activity relationship where 3,4,5-trimethoxyphenyl group linked by ketone carbonyl to thiazole or thiazolidine ring plays a vital role to enhance the anticancer activity.[14] In extension to these earlier contributions, Miller et. al. have also demonstrated that the N-BOC and N-fmoc protected ATCAAs exhibits excellent anticancer activity *in vitro* against melanoma, prostate cancer cells as well as *in vivo* on mice bearing A375 melanoma tumors.[15] Li et. al. have also studied ATCAAs as selective and excellent antiproliferative agents for melanoma where some of the compounds showed activity similar to sorafenib.[16] Lithium thiazolidine-4-carboxylate have been studied by Corbi et. al.[17] for preliminary *in vitro* cytotoxic studies against human HeLa cells. The recent focus of the anticancer studies revealed a great attention towards the breast cancer causing MCF7 cells. The potential of thiazolidine skeleton against MCF7 has been explored by Gomez-Monterrey et. al. as Indoline-3,2′-thiazolidine based p53 Modulators,[18] Pinho e Melo et. al. as chiral 6,7-bis(hydroxymethyl)-1H,3H-pyrrolo[1,2-c]thiazoles[19] and Serra et. al. as 2-galactosylthiazolidine-4-carboxylic acid amides.[20] The TCA-Cu (II) coordination complexes have been found to be significantly cytotoxic towards colon cancer HCT116 cell line.[21] In current literature, Onen-Bayram et. al[22-23] have revealed that the 3-propionyl-thiazolidine-4-carboxylic acid ethyl esters are significantly active against HUH7 and MV hepatocellular carcinoma cell lines which are capable to induce caspase-9 dependent apoptosis in HUH7 cells.

In order to ascertain the mode of antiproliferative activity exhibited by ATCA amides, Miller et. al.[13] have investigated LPL receptor mRNA expression using a quantitative sandwich ELISA method which measures DNA-histone complex released during apoptosis to demonstrate induced apoptosis in LNCaP, PC-3, and RH7777 cancer cells. In another significant contributions, Miller et. al.[15] and Botelho et. al.[20] have proved dose dependant induced apoptosis at sub-G1 phase by ATCA amides using flow cytometric analysis in LNCaP and MCF-7 cancer cells respectively. Moreover, the docking

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studies by Gomez-Monterrey et. al.[18] has established apoptotic cell death mechanism of MCF-7 cells by significantly induced accumulation of p53 protein on treatment of spiro thiazolidine moieties. The investigation of cytotoxic effect mechanism by Cetin-Atalay et. al.[23] using fluorescence-activated cell sorting (FACS) analysis using propidium iodide stain revealed that the thiazolidine derivative led apoptotic property which triggers caspase-9 activity and arrested Huh7 hepatocellular carcinoma (HCC) cell lines at SubG1/G1phase. All these efforts recommend that the thiazolide based moieties are highly effective anticancer agents with induced apoptosis mechanism.

In cognizance of tremendous anti influenza potential, the thiazolidine class has been also subjected to computational studies to establish their structural activity relation.[24-25]Besides of versatile biological activities, the compounds of thiazolidine class are imperative as spacers to built metal-organic frameworks pertinent in the fields of luminescence, magnetism, CO_2 storage and heterogeneous catalysis.[26] The x-ray crystallography is perhaps an unambiguous, state of art tool for confirmation of the stereochemistry of chiral centers within a bioactive chiral molecule. By taking in to account the enormous applications, it is widely employed by crystallographers to ascertain the chirality in prominent thiazolidine class of organosulfur compounds [27-30] and their transition metal complexes.[31-33]

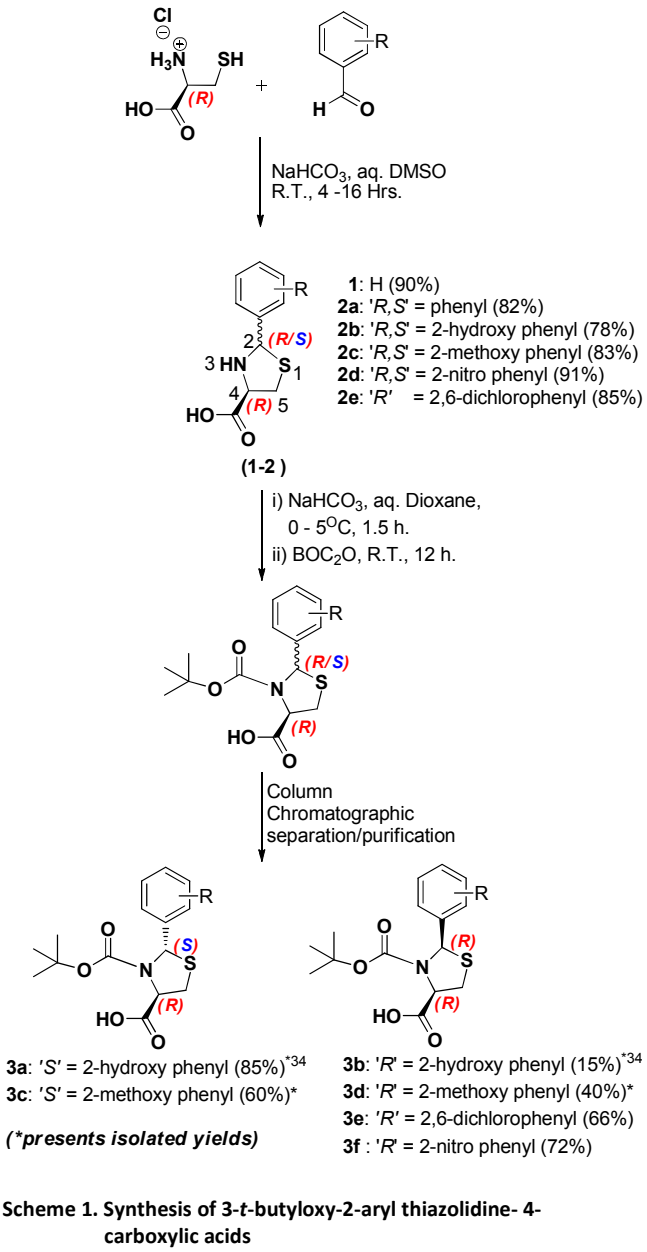
In present article, we report the synthesis of diastereomeric ATCAs and their chirally pure N-BOC derivatives towards their anti breast cancer activity using MCF7 cell lines by MTT assay method. Also the morphological analysis of cells is reported using fluorescence microscopy. The activity results of the compounds have been correlated with their absolute structures.

2. Results and discussion

2.1 Chemistry

The syntheses of TCA, ATCAs (**1, 2b-2f**) were accomplished by using our previously reported protocol[34] using commercially available aldehydes and L-Cysteine.HCl with $NaHCO_3$ as an inexpensive and readily available base in aqueous 20% DMSO solvent medium with excellent yields as shown in **Scheme 1.**

Since the parent diastereomeric amino acids (**2b-2e**) were only soluble in DMSO, their resolution was not feasible by conventional column chromatographic separation technique. The tert-butoxycarbonyl (BOC) is known to be a wonderful protecting group; besides this, it can also build up a variety of weak intermolecular forces of attraction essential for packing of molecules during the crystal growth.[35] In order to achieve the resolution of diastereomers by making them soluble in silica column compatible volatile organic solvents and considering the x-ray crystallographic stereo structure correlated anticancer study approach, we aimed BOC derivatization of the diastereomeric amino acids (**2b-2e**). The compounds **2b-2e** were subjected for BOC derivatization in 50% aqueous 1, 4-Dioxane where we used $NaHCO_3$ as a mild base instead of any strong base like NaOH. The crude oily products obtained after BOC protection were either separated or purified by using silica column chromatography to give the fine solids of **3a-3f** with significant yields. Zhu et. al.[36] have very recently reported the synthesis of similar N-BOC analogues as exclusively single '***2R,4R***' diastereomers on the basis of kinetic dynamic resolution having significant antibacterial activities. In our attempt, the '***2R/S,4R***' precursor **2d** gave exclusively ***'2R,4R'*** diastereomer **3f** on N-BOC protection and it is in agreement with Zhu et. al. On contrary to the propositions of Zhu et. al., on N-BOC protection of **2b, 2c**; we could afford and resolve the mixture of '***2S,4R***' and '***2R,4R***' diastereomers with reversed stereoselectivity which reveals the merits of our synthetic protocol to generate both stereoisomers in a single attempt. More interestingly, the compound **2e** was itself obtained with '***2R,4R***' stereochemistry and also these absolute configurations of chiral centres identified to be retained on BOC derivatization in **3e**. The all functionalized derivatives were completely characterized by IR, ^{1}H NMR, ^{13}C NMR, MS spectroscopic techniques and the spectroscopic data found to be in agreement with their structures. The representative ^{1}H and ^{13}C NMR spectra of compound **3e** are shown in **Fig. 1** and **Fig. 2** respectively.



Scheme 1. Synthesis of 3-*t*-butyloxy-2-aryl thiazolidine- 4-carboxylic acids

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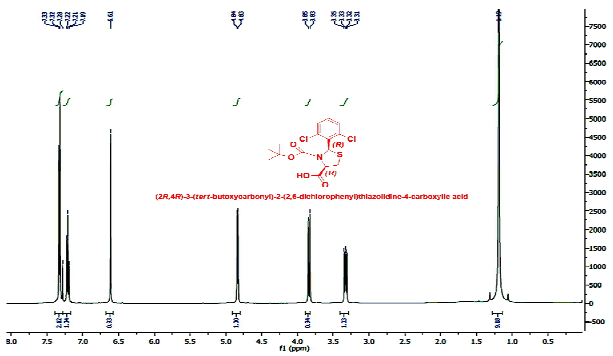


Fig. 1 ^{1}H NMR spectra of compound **3e**

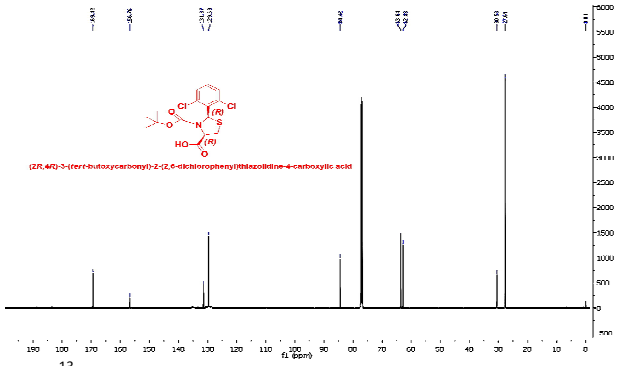
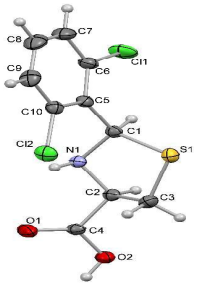


Fig. 2 ^{13}C NMR spectra of compound **3e**

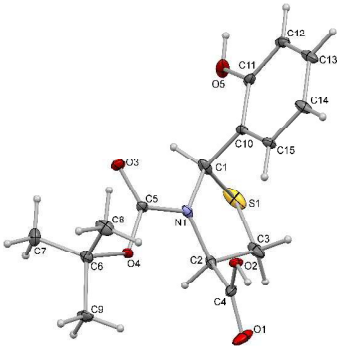
2.2 X-ray crystallographic structures of compound (2e, 3b, 3c, 3e and 3f)

In order to confirm the absolute structures of synthesized moieties by X-ray single crystal studies, all the ATCA and N-BOC-ATCAs were crystallized by vapor deposition technique. Providentially compounds **2e** and **3a-3f** were crystallized with appropriate quality to collect the diffraction data. Out of the total seven diffracted molecules, we have very recently elaborated the crystal structure of compound **3a** (CCDC-977400)[34] in support to stereoselectivity and mechanistic investigations in the formation of ATCAs. The x-ray crystal structure of compound **3d** (CCDC-703635) has been lately studied by Zhu et. al.[36]and thus not incorporated here. However, to the best of our knowledge, we are first time reporting the x-ray crystal structures for rest five moieties (**2e, 3b, 3c, 3e** and **3f**).

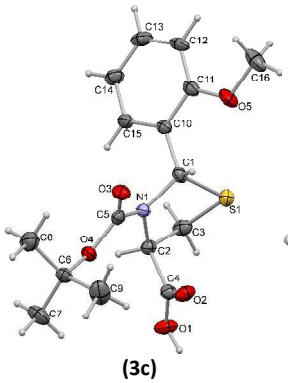
The crystal data of the compounds **2e, 3b, 3c, 3e** and **3f** are elaborated in **Table 1** and their thermal ellipsoids at 50 % probability level are shown in Fig.3. The compound **2e, 3b, 3c, 3e** and **3f** are crystallized in Orthorhombic, Tetragonal, Monoclinic, Orthorhombic and Monoclinic crystal systems respectively. The observed space groups are as P2(1)2(1)2(1), I4, C2, P2(1)2(1)2(1) and P2(1) respectively. A perspective view of the compounds **2e, 3b, 3c, 3e** and **3f** through ORTEP diagram at 50 % probability are shown in **Figure 3** and the detailed crystallographic characteristics are given in supplementary data. Each asymmetric unit of **2e, 3b, 3c, 3e** and **3f** contains a chiral molecule with substituted phenyl ring and functionalized thiazolidine ring oriented orthogonal to each other. All the compounds show the retained absolute configuration '***R***' at COOH substituted chiral carbon (C_4 as per scheme 1) of thiazolidine ring as that of precursor L-Cysteine. Whereas, the suggested absolute configuration at aromatic ring substituted chiral carbon (C_2 as per scheme 1) of thiazolidine ring is '***S***' for compound **3c** and '***R***' for compounds **2e, 3b, 3d, 3e,3f** respectively.



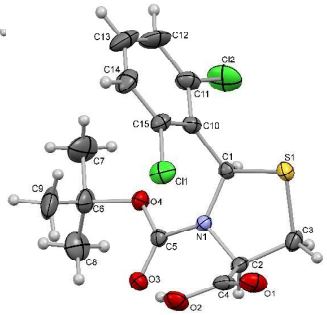
(2e)



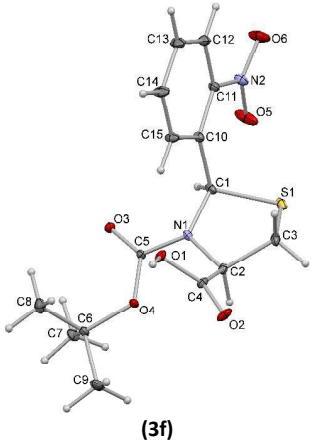
(3b)



(3c)



(3e)



(3f)

Fig. 3 Crystal structures (thermal ellipsoids at the 50% probability level) of compounds 2e, 3b, 3c, 3e and 3f.

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Table 1: Crystal structure data of compounds 2e, 3b, 3c, 3e and 3f

Identification code	2e	3b	3c	3e	3f
Empirical formula	C10 H9 Cl2 N O2 S	C15 H19 N O5 S	C16 H21 N O5 S	C15 H17 Cl2 N O4 S	C15 H18 N2 O6 S
Formula weight	278.14	325.37	339.40	378.26	354.37
Temperature	296(2) K	100(2) K	296(2) K	296(2) K	100(2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Orthorhombic	Tetragonal	Monoclinic	Orthorhombic	Monoclinic
Space group	P2(1)2(1)2(1)	I4	C2	P2(1)2(1)2(1)	P2(1)
Unit cell dimensions	a = 6.9790(7) Å	a = 22.5029(5) Å	a = 31.2006(17) Å	a = 9.4352(11) Å	a = 10.0776(4) Å
	α= 90°.	α = 90°.	α = 90°.	α = 90°.	α = 90°.
	b = 8.4645(9) Å	b = 22.5029(5) Å	b = 10.1101(5) Å	b = 10.4526(12) Å	b = 6.3731(3) Å
	β= 90°.	β = 90°.	β = 93.505(4)°	β = 90°.	β = 90.4180°.
	c = 19.895(2) Å	c = 6.4423(2) Å	c = 5.9607(4) Å	c = 18.336(2) Å	c = 13.2240(6) Å
	γ = 90°.	γ = 90°.	γ = 90°.	γ = 90°.	γ = 90°.
Volume	1175.2(2) $Å^3$	3262.26(18) $Å^3$	1876.73(19) $Å^3$	1808.3(4) $Å^3$	849.30(6) $Å^3$
Z	4	8	4	4	2
Density (calculated)	1.572 Mg/m^3	1.325 Mg/m^3	1.201 Mg/m^3	1.389 Mg/m^3	1.386 Mg/m^3
Absorption coefficient	0.712 mm^{-1}	0.220 mm^{-1}	0.194 mm^{-1}	0.491 mm^{-1}	0.224 mm^{-1}
F(000)	568	1376	720	784	372
Crystal size	0.42 x 0.32 x 0.28 mm^3	0.40 x 0.30 x 0.25 m^3	0.46 x 0.36 x 0.27 mm^3	0.42 x 0.36 x 0.30 mm^3	0.36 x 0.30 x 0.27 mm^3
Theta range for data collection	2.047 to 24.998°	3.622 to 34.372°	2.118 to 24.991°	2.221 to 24.86°	6.073 to 40.150.
Index ranges	-8/8, -9/10, -22/23	-32/29, -27/35, -10/10	-36/36, -12/12, -7/7	-11/11, -12/12, -21/21	-16/17, 11/11, -20/23
Reflections collected	7473	27810	13496	18873	34554
Independent reflections	2058 [R(int) = 0.0243]	5796 [R(int) = 0.0391]	3287 [R(int) = 0.0414]	3186 [R(int) = 0.0270]	9531 [R(int) = 0.0197]
Absorption correction	multi-scan	multi-scan	multi-scan	multi-scan	multi-scan
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	2058 / 0 / 151	5796 / 1 / 204	3287 / 1 / 213	3186 / 0 / 212	9531 / 1 / 221
Goodness-of-fit on F^2	1.083	1.111	1.097	1.094	1.010
Final R indices [I>2sigma(I)]	R1 = 0.0239, wR2 = 0.0642	R1 = 0.0596, wR2 = 0.1567	R1 = 0.0492, wR2 = 0.1182	R1 = 0.0372, wR2 = 0.0933	R1 = 0.0237, wR2 = 0.0646
R indices (all data)	R1 = 0.0245, wR2 = 0.0648	R1 = 0.0681, wR2 = 0.1608	R1 = 0.0537, wR2 = 0.1210	R1 = 0.0387, wR2 = 0.0945	R1 = 0.0239, wR2 = 0.0648
Absolute structure parameter	0.02(2)	0.03(2)	-0.01(4)	0.032(16)	0.024(6)
Largest diff. peak and hole	0.240 and -0.236 e.$Å^{-3}$	1.456 and -0.451 e.$Å^{-3}$	0.663 and -0.194 e.$Å^{-3}$	0.274 and -0.231 e.$Å^{-3}$	0.313 and -0.328 e.Å-3

2.3 *In vitro* anticancer activity[41]

The anticancer activities of ATCA and N-BOC ATCAs moieties viz. compounds **1** to **3f** were tested against breast cancer cell lines: MCF7. Doxorubicin (**DOX**) was selected as a standard drug for comparison. The IC_{50} data for 48, 72 and 96 hrs along with their absolute stereochemistry are summarized in **Table 2.** The compounds **1-3f** exhibited good antiproliferative activity in micro molar concentration. Especially the activity of parent ATCAs was observed to be enhanced on N-BOC derivatization. This outcome may be due to locking of C-2 chiral centre in a fix stereochemistry that can avoid ring opening tautomerism which leads to the formation of conformational isomers[34].

The observed higher IC_{50} values for compound **1, 2a** as 76.4, 66.2 at 96 hrs is attributed to absence of the aromatic ring and the substitution on aromatic ring respectively. Among the parent diastereomeric ATCAs **2b** to **2e,** the observed trend of IC_{50} values at 96 hrs was **2e** (2,6-Cl) < **2c** (2-OCH_3) < **2d** (2-NO_2) < **2b** (2-OH) respectively. The established trend reveals that the +R effect offered by *ortho position* group in aromatic substituents plays a vital role to enhance the anticancer potential of the tested compounds. Although, **2b** contains *ortho*-OH which can give strong +R effect showed surprising higher IC_{50} values (even higher than **2d** with *ortho*-NO_2) perhaps due to ring opening tautomerism in DMSO under the influence of –OH group, generating *E-Z* imines[34] during the activity studies.

The activity results were more interesting for N-BOC derivatized compounds. The diastereomeric pair **3a** with ***(2S,4R)*** stereocentres and **3b** with ***(2R,4R)*** stereocentres having 2-OH phenyl group, the IC_{50} values at 96 hrs were found as 51.3 and 53.8 respectively. Whereas, for another pair **3c** with ***(2S,4R)*** stereocentres and **3d** with ***(2R,4R)*** stereocentres having 2-OCH_3 phenyl group as 36.5 and 38.3 respectively. These observations are very promising proof towards the necessity of '**S**' stereochemistry at C-2 chiral centre in **3a, 3c** than '***R***' stereochemistry in **3b, 3d** to have better anticancer properties. The *trans* geometry of the C-2 and C-4 substituents having lower IC_{50} values is may be corroborated to possible strong

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directional interactions. Among the **3e** (IC_{50} 16.7, 96 hrs) and **3f** (IC_{50} 18.6, 96 hrs); the later showed the higher activity which may be ascribed to the strong H bonding capacity of two –Cl groups. Thus six of total twelve studied moieties (**2b-2e, 3e, 3f**) exhibited significant anticancer activity; even marginally higher than DOX drug molecule (IC_{50} 33.4, 96 hrs) against MCF cell line.

Table 2. Anticancer activity of all synthesized compounds (**1-3f**)

Compound No.	48 hrs IC_{50} (μM)	72 hrs IC_{50} (μM)	96 hrs IC_{50} (μM)	C-2,C-4 Absolute Configuration
1	90.7	81.3	76.4	***4R***
2a	83.9	77.4	66.2	***2R/S,4R***
2b	46.7	40.0	26.9	***2R/S,4R***
2c	42.6	32.1	24.9	***2R/S,4R***
2d	49.3	37.7	24.6	***2R/S,4R***
2e	37.2	29.0	21.3	***2R,4R***
3a	67.0	61.4	51.3	***2S,4R***
3b	72.2	66.6	53.8	***2R,4R***
3c	55.4	49.4	36.5	***2S,4R***
3d	62.7	46.7	38.3	***2R,4R***
3e	39.7	26.9	16.7	***2S,4R***
3f	37.2	27.2	18.6	***2R,4R***
DOX (control)	58.6	46.1	33.4	--

**Cells were incubated with DMSO solution of the selected compounds, washed and then cell proliferation was evaluated by MTT assay; IC_{50} was determined from dose-response curves by sigmoid decay fitting using Origin 8.0 software.*

2.4 Cell morphology studies by Fluorescence Microscopy

In view of the high toxicity exhibited by compounds **1-3e** against MCF7 cell lines, the morphological analysis of treated cells were done by fluorescence microscopy as shown in **Figure 4**.

The cells were stained blue with 1-Chloro-9,10-bis(phenylethynyl)anthracene. The studies were performed with IC_{50} values of these compounds at 48 h to determine the viable cell mass. All the compounds exhibited a decrease in viable cell population. This was in corroboration with the data obtained by the MTT assay. When compared to the control cells, there was no significant change in the morphology of the stained cells indicating induced apoptosis mode of cell death.

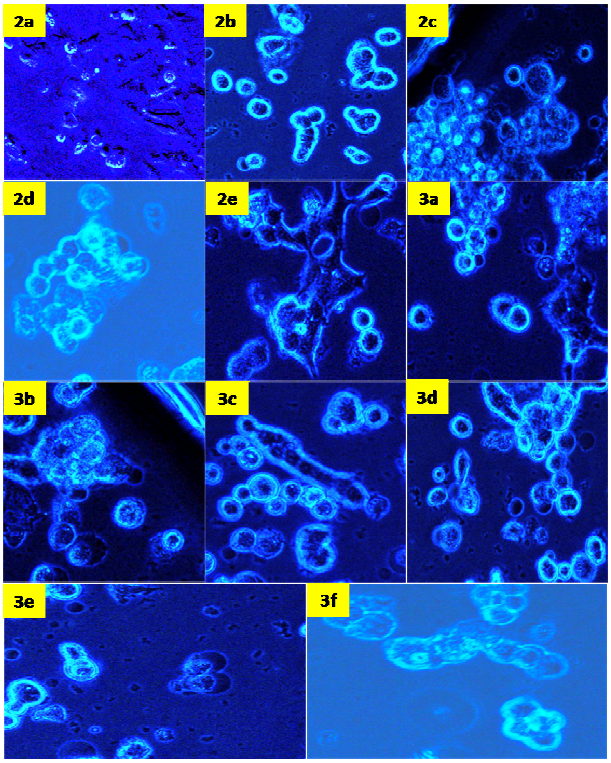

Fig. 4 Fluorescence microscopic images of MCF7 cancer cells at 48 hrs.

3. Experimental Section

3.1 Materials and methods

The TLC monitoring of the reactions was done using Merck DC precoated TLC plates coated by 0.25 mm Kieselgel 60 F_{254} and visualization was done using 254 nm UV lamp. The spectroscopic characterization of compounds was done by ^{1}H and ^{13}C NMR spectroscopy in δ ppm scale (400 and 100 MHz NMR spectrometers, VARIAN Mercury and Bruker instruments) and IR spectroscopy in cm^{-1} (FTIR-8400 spectrometer, SHIMADZU). The LCMS analysis was done using Ultra Fast Mass Spectrometer (LCMS-8030, SHIMADZU) with Chiracel OD column and isobutane as an eluting solvent. Bruker SMART APEX II single-crystal X-ray CCD diffractometer was used for X-ray crystal structure studies.

3.2 Chemistry

3.2.1 Synthesis of compounds 1, 2a-2e[34]

5.0 mM L-Cysteine hydrochloride (0.875 g), 5.0 mM $NaHCO_3$ (0.42 g) and 4.75 mM of respective aldehyde were stirred at R.T. in 150 ml of 20 % aq. DMSO. The reactions were monitored by TLC in terms of consumption of aldehyde and finally the reaction mixture was quenched in crushed ice. The precipitated solid products were filtered under suction and dried to get the compound **1** and diastereomeric (*2R/2S,4R*)-2-aryl thiazolidine-4-carboxylic acids (**1,2a-2e)** with fair purity and significant yields. As per the x-ray crystal structure investigation, the compound **2e** is obtained as a single diastereomer with ***2R,4R*** stereochemistry.

3.2.2 Synthesis and separation/purification of compounds 3a-3f

The 2.0 mM **2b-2e** and 5.0 mM $NaHCO_3$ (0.224 g) were taken in a 100 ml two neck flask and stirred in 50 ml 50 % aqueous 1,4-dioxane for 1.5 hrs at $0^{O}C$. To the resultant yellowish suspensions

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2.8 mM (0.610 g) of di-*t*-butyloxy carbonyl was added drop wise with constant stirring and it was continued to stir by natural heating to R.T. for 12 h. During workup, the reaction mixtures were acidified by addition of ice cold 2N HCl up to pH 4.0 and extracted in ethyl acetate. The ethyl acetate extract were washed by cold saturated $NaHCO_3$ up to alkaline pH 9. The resultant aqueous layers were further acidified up to pH 4.0 by cold 2N HCl. The acidic medium solutions were extracted finally in DCM, which on evaporation gave the oily solids of N-BOC derivatized compounds.

The mixture **3a+3b** was separated on silica column using 40 % ethyl acetate in hexane gave white solids **3a** (*2S,4R* diastereomer) and **3b** (*2R,4R* diastereomer) with isolated yields 85 % and 15 % respectively. Whereas, the mixture of **3c+3d** was separated on silica column using 20 % ethyl acetate in hexane which afforded the **3c** (*2S,4R* diastereomer) and **3d** (*2R,4R* diastereomer) with isolated yields 60 % and 40 % respectively. The crude products **3f** (*2R,4R* diastereomer) and **3e** (*2S,4R* diastereomer) were purified on silica column using 10 % ethyl acetate in hexane with 66 % and 72 % yields respectively.

3.3 Characterization data

3.3.1(4R)-thiazolidine-4-carboxylic acid (1).[34]

$C_4H_7NO_2S$, White solid, yield 0.598 g (90%), IR (KBr,υ,cm^{-1}) 3377(COOH), 3049(N-H), 1627(C=O), 1383(C-S), 1340(C-O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 4.22 (d, 1H, H-2), 4.02 (d, 1H, H-2), 3.84 (t, 1H, H-4), 3.09 (dd, 1H, H-5), 2.82 (dd, 1H, H-5). ^{13}C NMR (75 MHz, DMSO-d_6+TMS) δ 172.45 (C=O), 65.51 (C-2), 54.27 (C-4), 36.34 (C-5). MS (APCI) *m/z* 134 (M + 1).

3.3.2(2R/2S,4R)-2-phenyl thiazolidine-4-carboxylic acid (2a).[34]

$C_{10}H_{11}NO_2S$, White solid, yield 0.861 g (82%). IR (KBr,υ,cm^{-1}) 3431(COOH), 1577(C=O), 1435(aromatic C-C), 1381(C-S), 1305(C-N), 1236(C-O), 1024, 895, 808 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 7.35-7.50 (m, 5H, H Ar), 7.20-7.33 (m, 5H, H Ar), 5.64 (s, 1H, H-2), 5.47 (s, 1H, H-2), 4.22 (t, 1H, H-4), 3.90 (t, 1H, H-4), 3.39–3.31 (m, 2H, H-5), 3.25–3.01 (m, 2H, H-5). ^{13}C NMR (75 MHz, DMSO-d_6+TMS) δ 172.92(C=O), 172.24(C=O), 141.25, 138.88, 128.42, 128.23, 128.15, 127.49, 127.20,126.85 (C Ar), 71.72(C-2), 71.02(C-2), 65.57(C-4), 64.91(C-4), 38.98(C-5), 38.50(C-5). MS (APCI) *m/z* 210.0 (M + 1), 210.0 (M + 1).

3.3.3(2R/2S,4R)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid (2b).[34]

$C_{10}H_{11}NO_3S$, White solid, yield 0.877 g (78%). IR (KBr,υ,cm^{-1}) 3097(COOH), 3010(N-H), 1627(C=O), 1383(C-S), 1332(C-N), 1280(C-O), 1238(C-O), 1097, 1093, 761 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 7.10–7.33 (m, 4H, H Ar), 6.73–6.82 (m, 4H, H Ar), 5.83 (s, 1H, H-2), 5.64 (s, 1H, H-2), 4.21 (t, 1H, H-4), 3.83 (t, 1H, H-4), 3.4 (q, 2H, H-5), 3.33 (q, 1H, H-5). ^{13}C NMR (75 MHz, DMSO-d_6+TMS) δ 172.92(C=O), 172.24(C=O), 155.13, 154.54, 128.99, 128.60, 127.84, 127.49, 126.04, 124.14, 118.99, 118.70, 115.62, 115.00 (C Ar), 67.62(C-2), 65.54(C-2), 65.13(C-4), 64.72(C-4), 38.00(C-5), 36.00(C-5). MS (APCI) *m/z* 226.0 (M + 1), 226.0 (M + 1).

3.3.4(2R/2S,4R)-2-(2-methoxyphenyl) thiazolidine-4-carboxylic acid (2c).[34]

$C_{11}H_{13}NO_3S$, White solid, yield 0.934 g (83%). IR (KBr,υ,cm^{-1}) 3500(broad) 1642(C=O), 1490(aromatic C-C), 1334(C-N), 1244(C-O) cm^{-1}.^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 7.51–7.20 (m, 4H, H Ar), 7.05–6.85 (m, 4H, H Ar), 5.82 (s, 1H, H-2), 5.62 (s, 1H, H-2), 4.22 (t, 1H, H-4), 3.86 (t, 1H, H-4), 3.82 (s, 3H, H CH_3), 3.72 (s, 3H, H CH_3), 3.18–3.05 (m, 2H, H-5), 3.04–2.85(m, 2H, H-5). ^{13}C NMR (75 MHz, DMSO-d_6+TMS) δ 172.75(C=O), 172.24(C=O), 156.5, 156.1, 130.1,129.2, 128.1, 127.2, 126.4, 125.2, 120.4, 120.1, 111.2, 110.70 (C Ar), 66.20 (C-2), 65.30 (C-2), 64.98(C-4), 64.91(C-4), 55.43(C CH_3), 55.39(C CH_3), 37.50(C-5), 37.81(C-5). MS (APCI) *m/z* 239.90 (M+1), 239.90 (M+1).

3.3.5(2R/2S,4R)-2-(2-nitrophenyl) thiazolidine-4-carboxylic acid (2d).[34]

$C_{10}H_{10}N_2O_4S$, Yellow solid, yield 1.151 g (91%). IR (KBr,υ,cm^{-1}) 2797(broad, COOH), 1633(C=O), 1433(N-O), 1348(C-N), 1195(C-O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 8.10–8.03 (m, 2H, H Ar), 7.97–7.91 (m, 2H, H Ar), 7.85–7.81 (m, 2H, H Ar), 7.76–7.66 (m, 2H, H Ar), 6.76 (s, 1H, H-2), 6.33 (s, 1H, H-2), 4.02 (t, 1H, H-4), 3.19 (q, 1H, H-4), 3.03 (m, 2H, H-5), 2.72(m, 2H, H-5). ^{13}C NMR (100MHz, DMSO-*d*6) δ 172.97(C=O), 172.80(C=O), 148.80, 147.90, 139.22, 135.86, 134.22, 133.79, 129.59, 128.92, 127.85, 125.77, 124.65(C Ar), 66.77(C-2), 65.96(C-2), 65.67(C-4), 65.32(C-4), 37.31(C-5), 36.99(C-5). MS (APCI) *m/z* 254.00 (M+1), 254.00 (M+1).

3.3.6(2R,4R)-2-(2,6-dichlorophenyl)thiazolidine-4-carboxylic acid (2e).[34]

$C_{10}H_9Cl_2NO_2S$, White solid, yield 1.18 g (84%). IR (KBr,υ,cm^{-1}) 3454(COOH), 3298(N-H), 1726(C=O), 1330(C-S), 1271(C-O), 912, 781(aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6 +TMS) δ 7.50 (dd, 1H, H Ar), 7.39 (t, 2H, H Ar), 6.42(s, 1H, H-2), 3.88 (dd, 1H, H-4), 3.46 (dd, 1H, H-5), 2.96 (t, 1H, H-5). ^{13}C NMR (75 MHz, DMSO-d_6+TMS) δ 173.00 (C=O), 134.00, 131.00, 130.00, 129.00, 128.60, 128.00 (C Ar), 70.00 (C-2), 63.41 (C-4), 38.58 (C-5). MS (APCI) *m/z* 278 (M + 1).

3.3.7(2S,4R)-3-(tert-butoxycarbonyl)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid (3a).[34]

$C_{15}H_{19}NO_5S$, white solid, isolated yield 1.27 g (85%), IR (KBr,υ,cm^{-1}) 3089 (COOH), 1718(C=O), 1672 (C=O), 1396 (C-S), 1280, 1282 (C-O), 1003, 754 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$ + TMS) δ 10.31 (s, 1H, H COOH), 7.83 (d, 1H, H Ar), 7.17 (t, 1H, H Ar), 6.83 (m, 2H, H Ar), 5.93 (s, 1H, H-2), 4.86 (dd, 1H, H-4), 3.43 (dd, 1H, H-5), 3.43 (3, 3H,trapped MeOH), 2.73 (dd, 1H, H-5), 1.38 + 1.25 (2s, 9H, H $C(CH_3)_3$). ^{13}C NMR (100 MHz, DMSO-d_6 + TMS) δ 172.69, 172.24(C=O), 154.60, 130.29, 127.50, 120.47, 119.90, 115.13 (C Ar), 83.35($C(CH_3)_3$), 60.89 (C-2), 49.43 (C-4) ,(C-5,*merged to solvent*), 28.10 ($C(CH_3)_3$). MS (APCI) *m/z* 326 (M + 1).

3.3.8(2R,4R)-3-(tert-butoxycarbonyl)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid (3b).[34]

$C_{15}H_{19}NO_5S$, White solid, isolated yield 0.22 g (15%). IR (KBr,υ,cm^{-1}) 3309 (COOH), 1722 (C=O),1683(C=O), 1390 (C-S), 1390, 1242 (C-O), 756 (aromatic C-H) $cm.^{-1}$ ^{1}H NMR (400 MHz, DMSO d_6 +TMS) δ 9.66 (s, 1H,H COOH), 7.81 (d, 1H, H Ar), 7.03 (m, 1H, H Ar), 6.73 (m, 2H, H Ar), 6.11 (s, 1H, H-2), 4.45-4.41 (m,1H, H-4), 3.40-3.35 (m, 1H, H-5), 3.30 (s, 3H,trapped MeOH), 3.03 – 2.91 (m, 1H, H-5), 1.32 +1.10 (2s, 9H, H $C(CH_3)_3$). ^{13}C NMR (100 MHz, DMSO d_6 +TMS) δ 172.20, 171.82 (C=O), 153.58, 152.52, 127.97, 118.43, 114.51(C Ar), 80.42($C(CH_3)_3$), 64.60(C-2), 61.06(C-4), (C-5,*merged to solvent*), 27.65($C(CH_3)_3$). MS (APCI) *m/z* 324 (M - 1).

3.3.9(2S,4R)-3-(tert-butoxycarbonyl)-2-(2-methoxyphenyl) thiazolidine-4-carboxylic acid (3c).

$C_{16}H_{21}NO_5S$, White solid, isolated yield 1.02 g (60 %). IR (KBr,υ,cm^{-1}) 3365 (COOH), 1718 (C=O),1691(C=O), 1359 (C-S), 1240 (C-O), 758 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 – 7.18 (m, 1H, H Ar), 7.05-714 (m, 1H, H Ar), 6.98-6.94 (m, 1H, H Ar), 6.90-6.84 (m, 1H, H Ar), 6.09+6.05 (s, 1H, H-2), 4.89 (dd, 1H, H-4), 3.79+3.77 (s, 3H, H CH_3), 3.42-3.32 (m, 1H, H-5), 3.29 (s, 3H,trapped MeOH), 3.18 – 2.08 (m, 1H, H-5), 1.30 +1.03 (2s, 9H,H $C(CH_3)_3$). ^{13}C NMR (100 MHz, DMSO-d_6 +TMS) δ 172.29, 171.90(C=O), 155.79, 155.68, 153.00, 152.77, 132.65, 131.60, 128.75, 124.98, 124.21, 120.62, 120.57, 111.23(C Ar), 80.50, 80.07, 79.58 ($C(CH_3)_3$),63.68, 59.79(C-2), 58.38, 56.07(C-4), 56.00 (C-OCH_3), 32.49,31.47(C-5), 28.22+27.95($C(CH_3)_3$). [The replications of ^{13}C signals for **3c** may be

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attributed to formation of *cis/trans* imines by thiazolidine ring opening and their coexistence with unopened **3d in** DMSO-d_6**]**.
MS (APCI) m/z 338 (M - 1).

3.3.10(2R,4R)-3-(tert-butoxycarbonyl)-2-(2-methoxyphenyl) thiazolidine-4-carboxylic acid (3d).

$C_{16}H_{21}NO_5S$, White solid, isolated yield 0.69 g (40 %). IR (KBr,υ,cm^{-1}) 2974 (COOH), 1732 (C=O),1637(C=O), 1406 (C-S), 1246 (C-O), 758 (aromatic C-H) cm^{-1}.^{1}H NMR (400 MHz, $CDCl_3$+ TMS) δ 7.94 (m, 1H, H Ar), 7.21 (m, 1H, H Ar), 6.91 (m, 2H, H Ar), 6.12 (s, 1H, H-2), 4.52(m, 1H, H-4), 3.79 (s, 3H, H $C\underline{H}_3$), 3.41–3.39 (m, 1H,H-5), 3.29 (s, 3H,trapped MeOH), 3.10-2.89 (s, 1H,H-5), 1.32 + 1.09 (2s, 9H,H $C(C\underline{H}_3)_3$).^{13}C NMR (100 MHz, DMSO-d_6 +TMS) δ 174.17(C=O), 156.05,128.79, 126.69, 120.59, 110.31(C Ar), 82.23($\underline{C}(CH_3)_3$), 64.40(C-2), 61.14(C-4), 55.46(C -O$\underline{C}H_3$), 31.83(C-5), 29.72+ 27.97($C(\underline{C}H_3)_3$). MS (APCI) m/z 338 (M -1).

3.3.11(2R,4R)-3-(tert-butoxycarbonyl)-2-(2,6-dichlorophenyl) thiazolidine-4-carboxylic acid (3e).

$C_{15}H_{17}Cl_2NO_4S$, White solid, yield: 1.36 g (72 %). IR (neat, υ,cm^{-1}) 2981 (COOH), 1760 (C=O),1610(C=O), 1406 (C-S), 1132 (C-O), 783 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$+TMS) δ 7.39-7.29 (m, 2H, H Ar), 7.20 (t, 1H, H Ar), 6.61 (s, 1H, H-2), 4.84 (d, 1 H, H-4), 3.84 (d, 1 H, H-5), 3.33 (dd, 1 H, H-5), 1.19 (s, 9 H,H $C(C\underline{H}_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$ +TMS) δ 169.42(C=O), 156.76, 131.37, 129.69 (C Ar), 84.42 ($\underline{C}(CH_3)_3$), 63.64(C-2), 62.83(C-4), 30.53(C-5), 27.69 ($C(\underline{C}H_3)_3$). MS (APCI) *m/z* 376 (M-1).

3.3.12(2R,4R)-3-(tert-butoxycarbonyl)-2-(2-nitrophenyl) thiazolidine-4-carboxylic acid (3f).

$C_{15}H_{18}N_2O_6S$, Yellowish solid, yield 1.17 g (66 %). IR (KBr, υ,cm^{-1}) 2977 (COOH), 1734 (C=O),1645(C=O), 1401 (C-S), 1249 (C-O), 733 (aromatic C-H) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$+TMS) δ 8.38 – 8.21 (m, 1H, H Ar), 8.18-8.03 (m, 1H, H Ar), 7.70 (m, 1H, H Ar), 7.49 (m, 1H, H Ar), 6.89+6.60 (s, 1H, H-2), 4.94-4.75 (m, 1H, H-4), 3.41-3.11 (m, 2H, H-5), 1.27 +1.25 (2s, 9H,H $C(C\underline{H}_3)_3$). ^{13}C NMR ($CDCl_3$ +TMS) δ 173.52(C=O), 133.91, 128.60, 128.02, 125.05 (C Ar), 82.94($\underline{C}(CH_3)_3$), 64.61(C-2), 62.62(C-4), 31.82(C-5), 29.72+27.97 ($C(\underline{C}H_3)_3$). MS (APCI) *m/z* 354 (M + 1).

3.4 Crystallographic studies (2e, 3b, 3c, 3e and 3f)

The single crystals of chiraly pure compounds **2e** and **3b, 3c, 3e, 3f** were crystallized in DMF-MeOH and DCM-MeOH co-solvents respectively using Hexane vapour deposition technique.

The single-crystal structure of compounds **2e, 3c and 3e** were determined by measuring X-ray intensity data on a Bruker SMART APEX II single-crystal X-ray CCD diffractometer equipped with graphite-monochromatized (Mo-*K*" = 0.71073 A°) radiation at 296 K. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from total 36 frames. The optimized strategy used for data collection consisted different sets of ϕ and ω scans with 0.5° steps in ϕ/ω. Data were collected with a time frame of 10 s keeping the sample-to-detector distance fixed at 5.00 cm. X-ray intensity data measurements of **3b** and **3f** were carried out on a Bruker D8 VENTURE Kappa Duo PHOTON II CPAD diffractometer equipped with Incoatech multilayer mirrors optics. The intensity measurements were carried out with Mo micro-focus sealed tube diffraction source (Mo-Kα = 0.71073 Å) at 100(2) K temperature. The X-ray generator was operated at 50 kV and 1.4 mA. A preliminary set of cell constants and an orientation matrix were calculated from three sets of 12 frames. Data were collected with ω scan width of 0.5° at different settings of ϕ and ω with a frame time of 40 secs keeping the sample-to-detector distance fixed at 5.00 cm.

The X-ray data acquisition was monitored by APEX2 program suit[37] All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX2 package[37] The structures were solved by direct method and refined by full matrix least squares, based on F^2, using ShelXL-2014/7.[38] Molecular diagrams were generated using Mercury programs.[39] Geometrical calculations were performed using SHELXTL39 and PLATON.[40] Crystal data for the structures have been deposited in the Cambridge Crystallographic Data Center (CCDC) with numbers (compound numbers) 1565464 (2e), 1565470 (3b), 1565479 (3c), 1565478 (3e) and 1565476 (3f).

3.5 In vitro anticancer activity studies[41]

3.5.1 Cell viability assay (MTT assay)

The MCF-7 cell lines were obtained from National Centre for Cell Sciences Repository, Savitribai Phule Pune University, Pune 411007. The cells were maintained in RPMI 1640 w/ 10% Fetal bovine serum (FBS), 2% P/S, 1.25% L-glut, and 1% sodium pyruvate at 37^{o}C (5% CO_2) in the steri-cycle CO_2 incubator with HEPA Class 100 filters, Thermo Electron Corporation.

The number of viable cells remaining after appropriate treatment was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) assay.[i] Briefly, cells were plated (4000 cells/well per 0.2 mL medium) in 96-well microtiter plates and incubated overnight. The compounds **1**-**3f** was then added at indicated concentrations to quadruplicate wells. After 24 hrs MTT was added to each well at a final volume of 0.5 mg/mL and the microplates were incubated at 37^{o}C for 3hrs. After the supernatant was removed, the formazan salt resulting from the reduction of MTT was solubilized in dimethyl sulfoxide (DMSO, Sigma Chemical Co.) and the absorbance was read at 570 nm using an automatic plate reader (Thermo Corporation). The cell viability was extrapolated from optical density (OD) values and expressed as percent survival. The percent cell death was calculated considering the untreated cells as 100 percent viable. The results allowed establishing dose-response curves to calculate IC_{50} values, the concentration required to inhibit cell proliferation by 50%.

3.5.2 Fluorescence microscopy

MCF7 cells were seeded on to glass cover slips at a density of 5×10^4 cells to achieve ~ 80 confluences. The cells were treated with compounds **1-3f** for 48 hrs at 37 ^{o}C. After 48 hrs of incubation the cells were stained with **1-Chloro-9,10-bis(phenylethynyl)anthracene** from Sigma Aldrich staining dye reagents, at a dilution of 1:20 for 30 min at 37 °C. The excess staining solution was removed and fixed in 4% para-formaldehyde (PFA). The microscopic images were observed under Carl Zeiss (Magnification 40x) with a filter set excitation/emission at 536 nm.

Conclusions

In summary, ATCA **2e**, N-BOC ATCA's **3b, 3c, 3e** and **3f** were obtained in chirally pure forms in excellent yields and diffracted by X-ray single crystal technique. The broad spectrum of completely characterized compounds (1-3f) was studied for their *in vitro* anti breast cancer activity by MTTS assay using MCF7 cell lines. Among the resolved diastereomers (**3a-3d**), the '*2S'* stereochemistry is essential for marginally higher anticancer activity over '*2R*' stereocenters on

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thiazolidine skeleton. The IC_{50} value for 2-NO_2 phenyl substituted ATCA was found to be reduced on N-BOC protection. Among the all compounds the presence of 2,6-dichlorophenyl substituent is imperative to give more significant anti breast cancer activity.

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Graphical Abstract & Highlighting Statement

X-ray crystal structures and anti breast cancer property of 3-*tert*-butoxycarbonyl-2-arylthiazolidine-4-carboxylic acids

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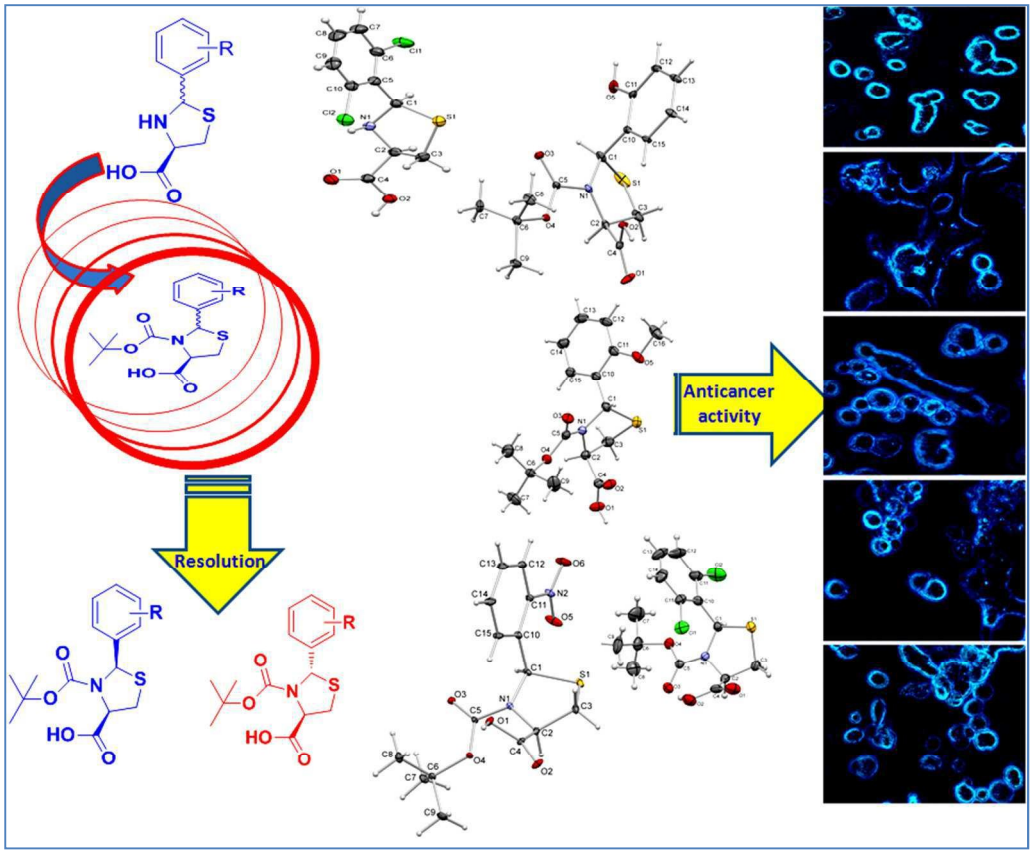

Highlighting Statement:

The present article encompass, resolution and X-ray crystallographically confirmed absolute stereochemistry correlated anticancer activity of diastereomeric 3-(tert-butoxycarbonyl)-2-(2-aryl) thiazolidine-4-carboxylic acids against MCF7 breast cancer cells.