https://doi.org/ 10.33472/AFJBS.6.9.2024.4463-4477

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Structure based design and characterization of the Highly potent and selective Estrogen receptor(β) targeting the Breast cancer cell line.

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Article History: Volume 6,Issue 9, 2024

Received: 30 Apr 2024

Accepted : 25 May 2024

doi: 10.33472/AFJBS.6.9.2024.4463-4477

Abstract

A novel 2-(((3S,5S,6R)-5-methoxy-6-((E)-(phenylimino)tetrahydro-2H-pyran-3-yl)1 λ^4-diazenyl)phenol (CS-SA) was synthesized to evaluate anticancer activity against MCF-7 breast cancer cell line. The structure of the ligand was confirmed from FT-IR, NMR spectral studies. Estrogen receptor (ER) selective agonists are considered potential therapeutic agents for a variety of pathological conditions, including uterus and breast cancers. Their development is particularly challenging, since differences in the ligand binding cavities of two ER subtypes are minimal. We have carried out rational design of new salicylaldoxime derivatives which display unprecedentedly High-level ER selectivity for this class of compound, both in binding affinity and in cell-based functional assays. An endogenous gene expression assay was used to further characterize the pharmacological action of inhibit proliferation of a breast cancer cell line in-vitro. The new bio-composite is high- quality and high- purity with estrogen receptor ligand binding Domain (PDB ID: 5TOA).

Keyword; FT-IR; Estrogen receptor; SBDD; and invitro-Docking analysis

1.INTRODUCTION

Conjugation of nucleobase with various natural and synthetic biopolymer can form the derivatives with enhanced biological activity. However due to high molecular weight, poor solubility and weak bio activity chitosan is difficult to develop into biomaterials and products that can be directly applied. Therefore, the structure of chitosan can be modified by chemical methods to induce synergistic interaction between the grafted active groups and chitosan.

Direct oxidation of the hydroxyl groups is possible by the introduction of amino (Aniline) groups at C6 and salicylaldoxime in C=N-OH at C2 below were reported C2-Benzaldyhyde C6- aniline double Schiff bases derivatives of chitosan were synthesized with positioning protection method for the first time [1]. Since carbonyl groups, chitosan are regarded as one of the best choices for enhancing chitosan's antibacterial properties. With an imine characteristic group, the Chitosan Schiff basis (-RC=N-) was reported by [3] A chemical molecule called aniline has an amino group linked to a phenyl group and aniline has good antibacterial and anti-microbial activity [4]. the Investigated were impacts of common ions, especially Salicylaldoxime and carbonate as a collector for hydroxyl ion functional group, and structure was identified [5]. Salicylaldoxime is phenol ring bioisosteric replacement, a characteristic operative unit of the majority of estrogen receptors (ER) ligands. Estrogen receptors (ER β) are nuclear transcriptional regulators of the ways in which estrogenic substances work physiologically. Many of the activities carried out by these receptors take place in the nucleus, where they bind to related DNA regulatory sequences and influence the transcription of particular target genes. [5-8] A lot of interest is being paid to Schiff-based ligands because of the diverse biological and pharmacological functions they exhibit. metal DNA interactions, structural variety, and bonding options

Furthermore, the - subtypes are typically thought of as a tumor suppressor in tissues like the breast and uterine because they inhibit ER- β promoted cell hyperproliferation in these tissues. These antiproliferative effects of ER β were also seen in a number of cancerous tissues, including mesothelioma, glioma, breast, prostate, colon, kidney, and pleural. In particular, the fact that women are less likely than males to develop gliomas and that this risk is further decreased by exogenous estrogen use supports the protective role of ER β in gliomas. All of the data points to the possibility of using selective activation of these receptor subtypes to produce an antitumor impact [9-12].

However, the 59 kDa ER β 1 isoform that was first cloned appears to be the sole completely functional ER isoform; this is the variant that is simply referred to as ER β. abundantly dispersed throughout the human body, various organ systems' biochemical processes being modulated. They play crucial roles in the skeletal, circulatory, and nervous systems in addition to regulating the feminine reproductive system.In the uterus and mammary glands, as well as in maintaining bone homeostasis and controlling metabolism, ER β plays a more significant function. The effects of ER β on the immune system and central nervous system (CNS) are more prominent [13]

CS-SA Bio-composite were synthesized by condensation process for the first time. This compound is high- quality and high- purity Bio-composite (CS-SA). A newly synthesized compound (CS-SA) expected to display remarkably high subtype this compound will be proven to behave as a full ER agonist and to initiate transcription as genes, highlighted where there is very high ER β potency-selectivity, which is unprecedented for this chemical class of ligand.

2.EXPERIMENTAL SECTION

2.1 Materials and methods:

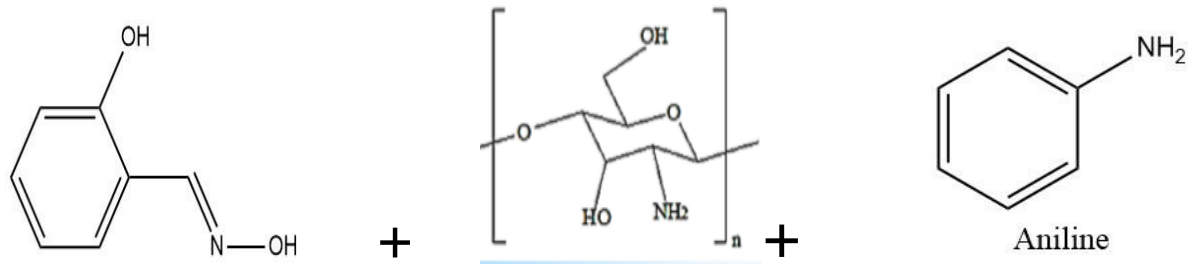
The compound salicylaldoxime,chitosan and aniline with a purity of 99% were from Sigma-Aldrich. The solvent Acetic acid and DMSO for used in the synthesis with 99% purity

of the Analytical Reagent. Without additional purification, they were used after being obtained from Sigma-Aldrich.

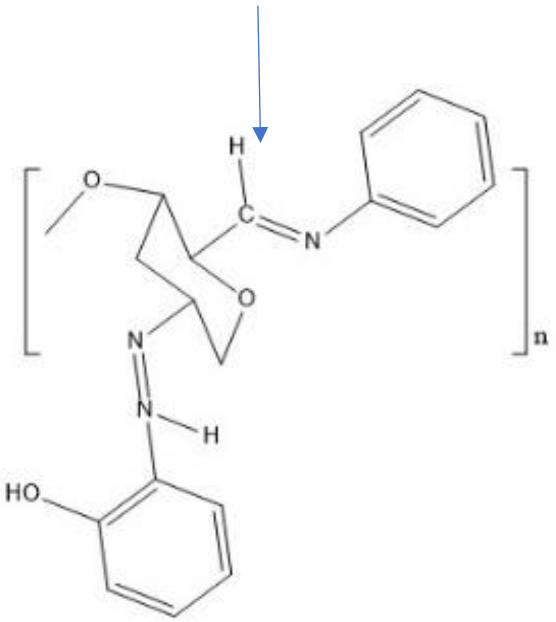
2.2 Synthesis

Chitosan that had previously been purified (1.004g mmol) was dissolved in acetic acid (25ml) and stirred at 45°C for two hours. The viscous solution was then supplemented with salicylaldoxime (1.006g mmol) dissolved in (20ml) DMSO, which was agitated for 12 hours. An exact solution was then obtained after adding (1.005g mmol) of aniline and stirring constantly for 3hrs at 45°C.the development of a Schiff base with a faint brownish hue on the chitosan matrix. Its purity controlled by HPLC analysis, UV-Visible spectroscopy, FT-IR spectroscopy, NMR, and GC-mass spectroscopy. To evaluate the salicylaldoxime, Thermogravimetric analysis (TG/DSC) was utilized to determine the effects of chitosan and aniline oxidation on the thermal durability of CS-SA.

2.3



Salicylaldoxime **Chitosan**



2-(((3*S*,5*S*,6*R*)-5-methoxy-6-((*E*)-(phenylimino)methyl)tetrahydro-2*H*-pyran-3-yl)-1λ⁴-diazenyl)phenol

"**Figure 1": Synthetic Route of CS-SA.**

2.3 Molecular modelling and Design

In order to look for additional beneficial interactions that could increase ER binding affinity, the most effective and selective salicylaldoxime-based ER β selective positions of the hydroxyl and oxime groups of the salicylaldoxime were further analysed with chitosan and aniline complex derived by a docking procedure of the Bio composite into binding cavity. The intramolecular H-bond of these oxime derivatives causes the pseudo cycle to be significantly distorted by the presence of the oxime component.

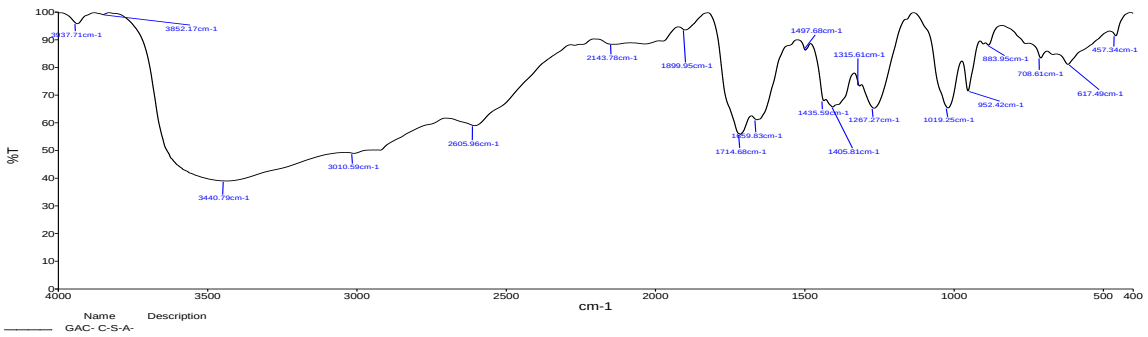
The strong interaction of azo (N=N) group formation in the C2 Position of chitosan due to salicylaldoxime, the N=N group is highly active in drug targeting. This is due to large steric interaction of the phenol group to hydrogen atom with the adjacent C-H bond due to C6 Position of chitosan and aniline the C=N group is highly selective drug derivatives [18]. The oxime OH group participating in a highly energetic H-bond network with residues Arg.346 and GLU 276 in docking analysis. The synthetic route shown in fig 1.

3.RESULT AND DISCUTION

3.1.FT-IR- Structure confirmation

In the FT-IR spectrum of vibrational peaks, the functional group for this new compound has been confirmed by the FT-IR spectrum and the occurrence of the vibration that stretches the imine linkage 3440 (C=NH) at a C6 position of Chitosan is a narrow amide band that was supplanted by a sharp band. This indicates that the band's spectral region is related to the igh-frequency O-H stretching vibrations 3937 and 3852 [7] The formation of the azo (N=N) bond at a C2 position of chitosan and salicylaldoxime was confirmed by the sharp band 1659.The C-N stretching vibrations of aniline is at 1315 and replacement of pigment aromatic ortho C-H and OH out of plane stretching at 709[17].The groups at 883, 617 are due to the C-H wag vibrations and ring out of plane vibrations wag of hydrogen-bonded chitosan. The bands at 1267 ,1019, and 952 involve in-plane movement of the ring carbons of chitosan and the subsequent band at 2605 is O-H elongation is the cause of salicylaldoxime. The band at 2143 is a cumulated double bond due to the as-symmetric stretching of salicylaldoxime and An iline.[11] The bond 3010 is because of the N-H lengthening chitosan and aniline.

FT-IR



"Figure 2". FT-IR spectrum of CS-SA

Table 1.
The FT-IR absorption peak of CS-SA
u, cm^{-1}

Sample	$d_{C=NH}$	$u_{(C=N)}$	d_{CN}	$u_{N=N}$	u_{C-H}	$u_{C=N-OH}$
CS-SA	3440	3015	2605.14	1659	756	883,617

Table 2 .
Wavenumber and assignment of CS-SA

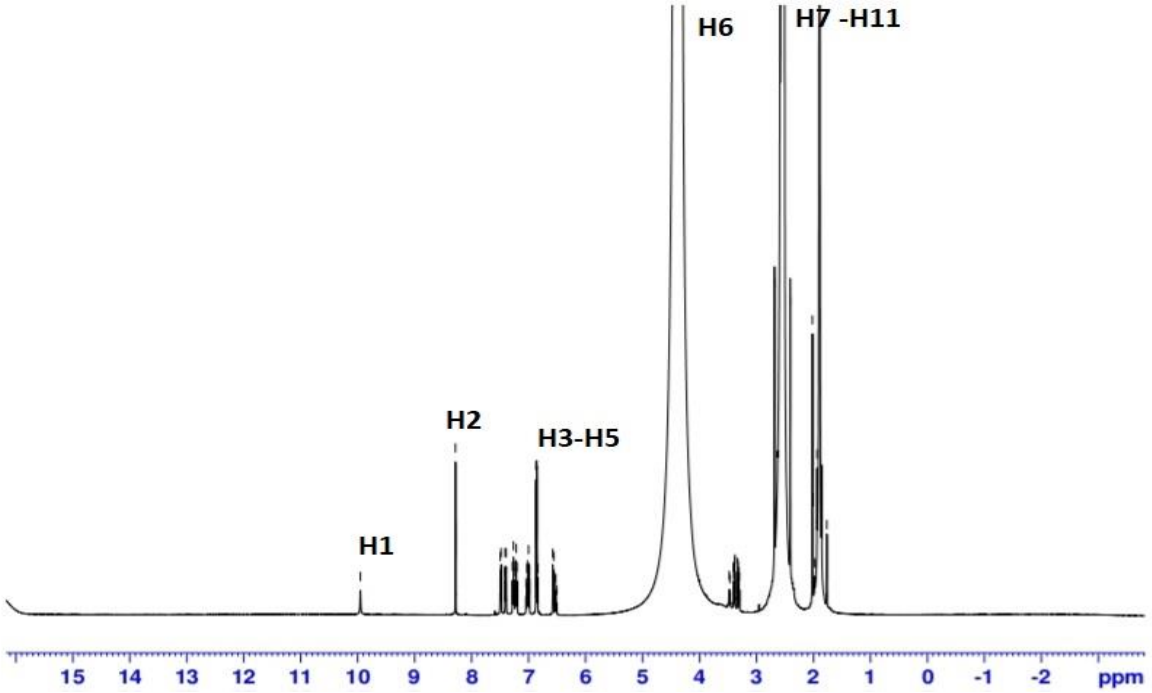
Wavenumber cm^{-1}	**Assignment**
3973.71 and 3852	O-H stretching of vibrational chitosan intra molecular bonding
3440.79	C=NH intermolecular bonding
1659	N=N azo group due to chitosan and salicylaldoxime
1267 ,1019 and 952	Ring carbons of chitosan
883 and 617	C-H wag vibration of hydrogen-bonded chitosan
2605.14	O-H extending of salicylaldoxime and aniline
1315	C-N aniline
3010	N-H chitosan and aniline

3.2 NMR

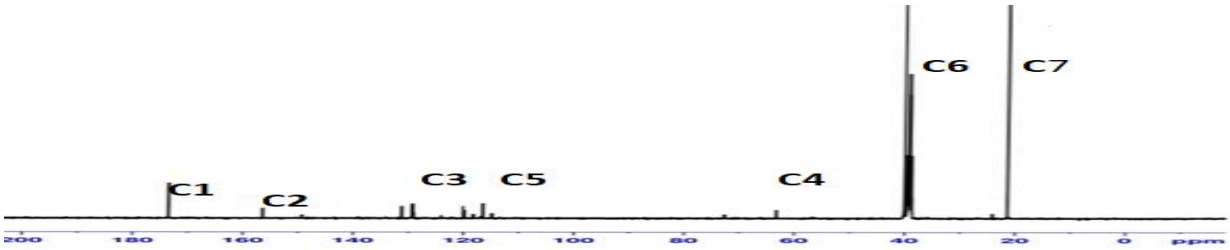
The proton NMR spectrum of CS-SA Bio-composite is displayed in figure 3. solvent indication is displayed at 7.267ppm and is in good agreement with the literature level. The signal established the protonation of NH_2 and CH_2OH to C=NH group and the coordination of anionic moiety with CS-SA [14]. A singlet appeared at 8.285ppm due to the protons of azo group N=N between NH_2 of chitosan and C=N-OH of salicylaldoxime [16]. The DMSO signal appeared at 72.64ppm. The chemical shift at 114.79ppm to 129.35ppm is attributed to carbon at a present aromatic ring of salicylaldoxime and aniline. The resonance peaks of C=NH are observed at 63.22ppm as in pure chitosan the signals for CH_2OH are observed at 34.5ppm and the signal for NH_2 of the peak change downfield from 64.5ppm to 63.22ppm confirms the presence of CH2OH with H+ anions, which in turn evaluates the coordination of H+ anions and NH2 cations with CS-SA bio-composite at aniline at 119.85ppm [9]. The signal for oxime C=N-OH carbon are observed at 156.72ppm in pure salicylaldoxime. The multiplets from 114.79ppm to 149.17ppm due to the aromatic carbons in the new CS-SA bio-composite of

aniline and salicylaldoxime. There are doublet, triplet, and quadruplets 21.35ppm to 40.35ppm due to amine NH_2 in the ring structure [19].

3.2 NMR



"Figure 3". shows ^{1}H-NMR spectrum of CS-SA



"Figure 4". Shows ^{13}C- NMR spectrum of CS-SA

Table 3

Assignments of the ^{1}H-NMR and ^{13}C- NMR signals of CS-SA

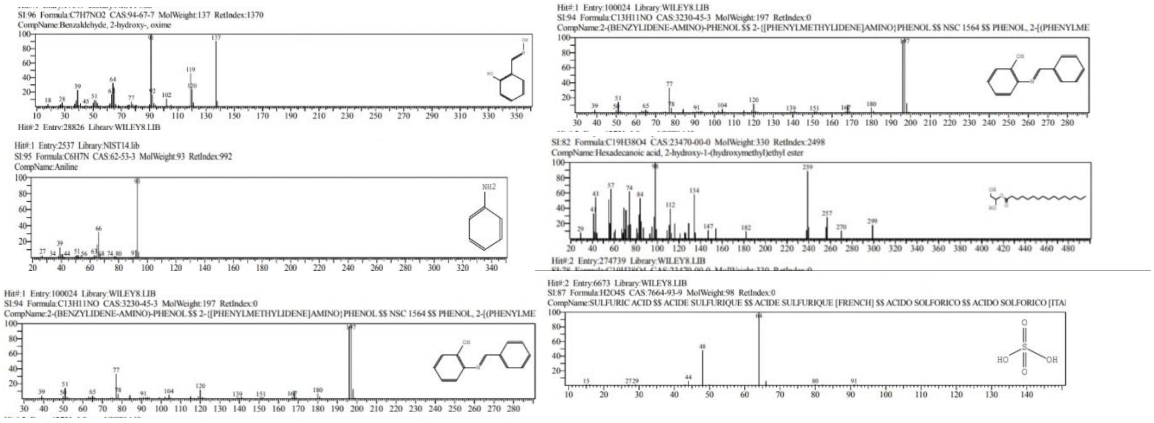
NMR	Chemical shift (ppm)					Group Identification
	CS-SA	Salicylaldoxime	Chitosan	Aniline	DMSO	
	9.956		9.95			C-H
	8.285	8.25				N=N

^{1}H	6.533-7.498				7.26	Solvent peak
	4.381		4.38			OH
	3.300-3.476			3.37		NH_2
	1.766-1.981		1.93			OH
	6.841			6.84		C=NH
^{13}C	156.38	156.38				N=N
	63.22		63.22			C=NH
	149.17	149.17				OH
	72.64				72.64	Solvent peak
	114.79-131.17		119.85			C-H
	38.65-40.05			38.65		OH
	21.35-40.35			21.35		NH_2

3.3 GC-MS Chromatography

The GC-Mass spectrum was originally recorded in order to study the evolved component of CS-SA. The peaks were attributed to a molecular weight of multiple compounds at 200°C to 250°C and the duration of solvent cut time of 3.50min [13]. The main area of use is in the separation and analysis of multiple compound mixtures. The peaks identified the molecular weight of the compounds. The polymer compound of chitosan was Fragmented. The total molecular weight of Bio-composite (CS-SA) is 919g/mol. Figure 5 shown GC-MS Peaks. The degree to which the chitosan's high molecular weight has been polymerized, salicylaldoxime,aniline is 21.37.

4.3 GC-MS



"Figure 5". GC-MS of CS-SA

Physical properties of CS-SA

A given solution has a viscosity of 0.35x10-4 Pa and viscosity in solutions diminishes with temperature**.** calculating the density of the given solution is based on the solution's bulk a nd volume, 2.8kg/m [15]. pH value of CS-SA 4.8 to be acidic. The molecular weight of the given solution is 919g/mol. Confirmed by the viscosity of the solution and these values were verified by GC-MS Spectroscopy.

Table 4

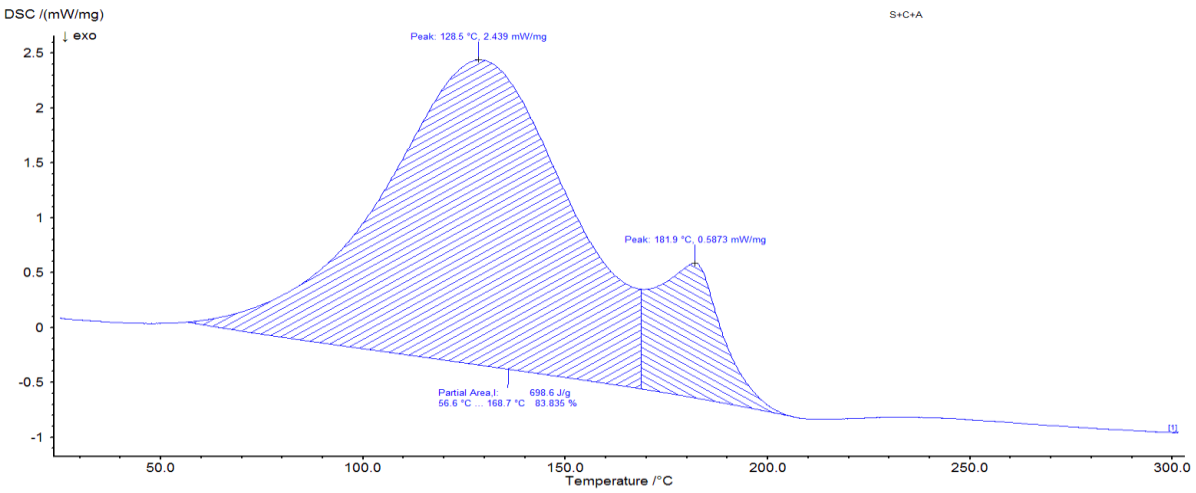
Molecular weight comparison

GC-MS Spectroscopy $M_n = M_1 + M_2 - \cdots$	$M_n = \sum \frac{n_i m_i}{n_i}$
919g/mol	919g/mol

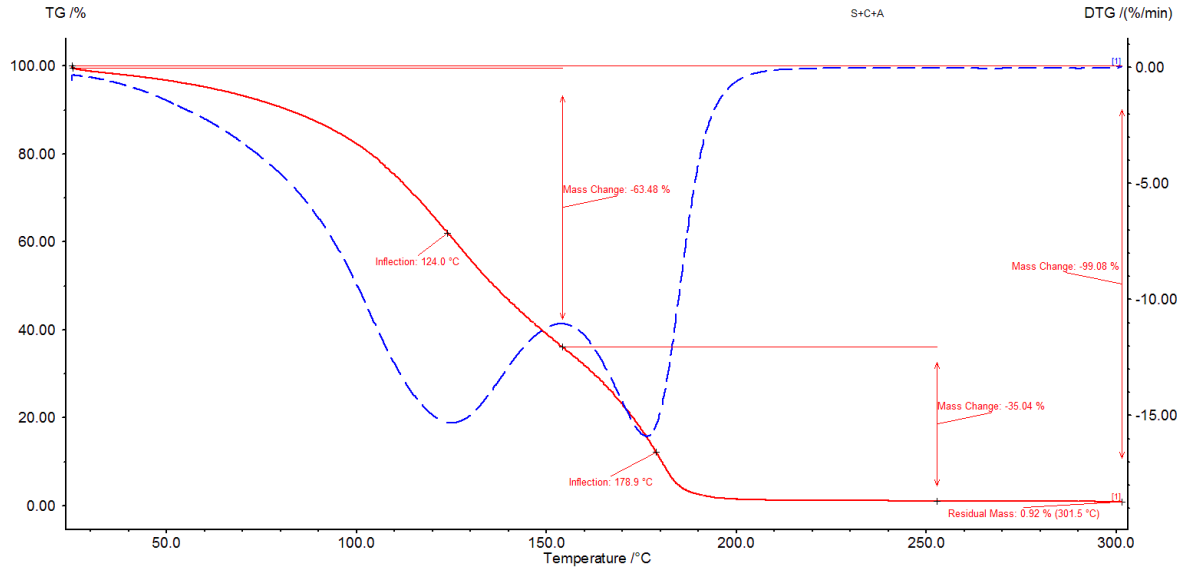
3.4 Thermal study (TG-DSC)

Analysis of the thermal stability and thermal breakdown of CS-SA was done using the thermal study. Three bulk losses were visible on the TG curve. -63.48% is the first mass drop. The second mass loss-36% and third mass loss-99.08%. DSC curve shows two endothermic peaks. The first endothermic peak is the enthalpy (128.5), and the second endothermic peak is enthalpy (181.9).[13] Thermodynamical data is shown in table 5 and the DSC curve in figure 9 and DSC in figure 10 TG-DSC.

3.4 TG-DSC



"Figure 6". DSC curve of CS-SA.



"Figure 7". TG-DSC Curve of CS-SA

Activation energy

$$IN\left[IN\left(\frac{1}{y}\right)\right] = \left(-\frac{E_a}{R}\right)\frac{1}{T} + Constan$$

Table 5

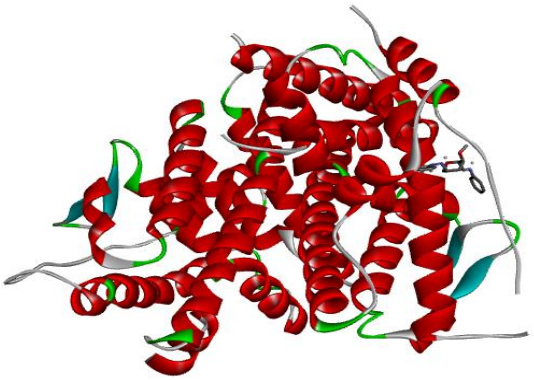
Thermodynamical data of CS-SA

Decomposition Temp (ºC)	181.5°C
Phase Transition	178°C
Enthalpy ΔH_f ($kJmol^{-1}$)	698.6
Entropy ΔS_f ($Jk^{-1}mol^{-1}$)	2.326
Gibbs free energy ΔG_f ($Jk^{-1}mol^{-1}$)	0.8
Activation Energy Ea^c ($kJmol^{-1}$)	165
Heat Capacity (C) J	1228.79
Specific Heat Capacity (Cp) J/g ºC	0.1585
Onset Peak	56.6 °C
Thermal stability	180°C

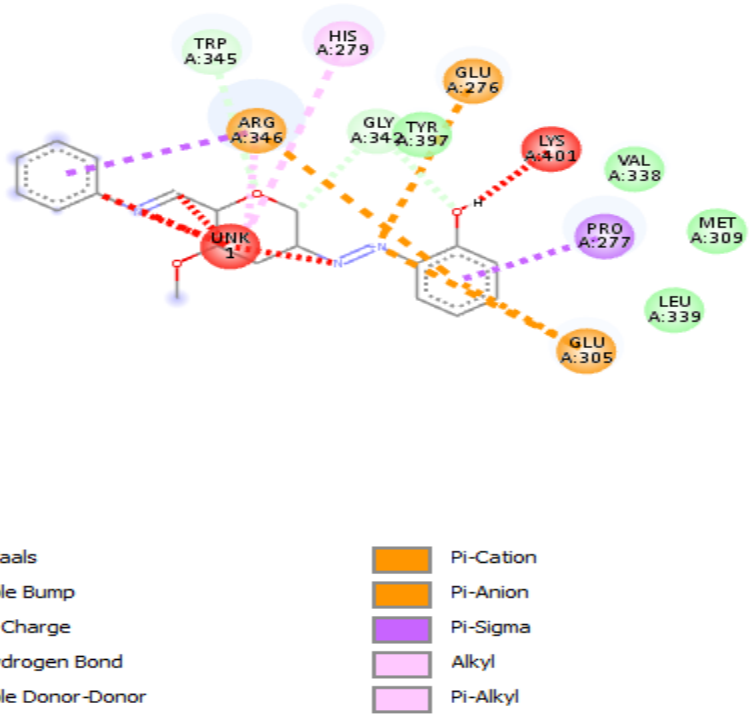
3.5 Molecular docking Studies

The best binding mode of docked ligand (CS-SA) with Estrogen receptor Beta binding Domain (PDB Id:5TOA). The analysis of the molecular docking study helps to identify

different types of interactions. During this interaction, H-Bonding, hydrophobic and electrostatic interactions. Docking interaction of amino acid residues of cell surface receptor LYS A.401 and acidic amino acid (GLU 276) [20]. The two hydrogen bonding with two amino acid TRP A:345 and GLY A.342.Bonds are formed and -7.5Kcal/mol binding energy is released. The molecular docking analysis confirms the CS-SA binds in estrogen receptor beta**.**



"Figure 8". CS-SA against 5TOA



"Figure 9". CS-SA against 5TOA Protein

Table 6
Molecular docking result for CS-SA

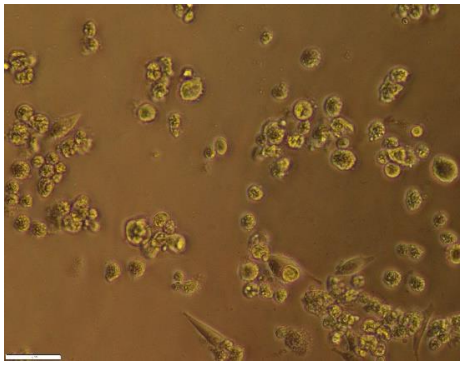
Ligand	Protein	Bending Affinity (keal/mol)
CS-SA	5TOA	-7.5

3.6 In-Vitro studies

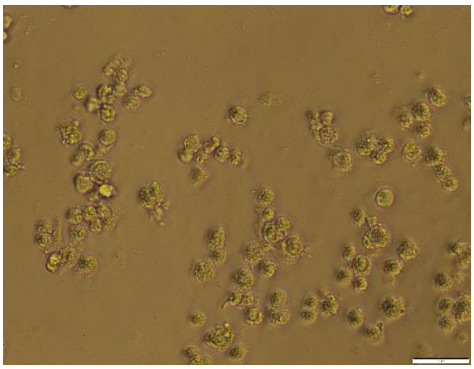
The effects of various CS-SA scaffold concentrations on cytotoxicity was obtained by MCF-7 231 A cell line from human breast cancer [14]. The result showed the MTT assay was used to test the cell viability. The concentration increases the % of cell viability decreases cancer activity tested by in-vitro studies shown in table 5. If dead cells are abundantly seen, the compounds have more active toward cancer cells. The compounds were highly active in cancer cells. It has been reported that structural variation, type of substituent, type of cancer and dose may affect the function of the compound. Overall, these findings indicated that test compounds are selective estrogen receptor Beta in their anticancer effect.

3.6 In-vitro

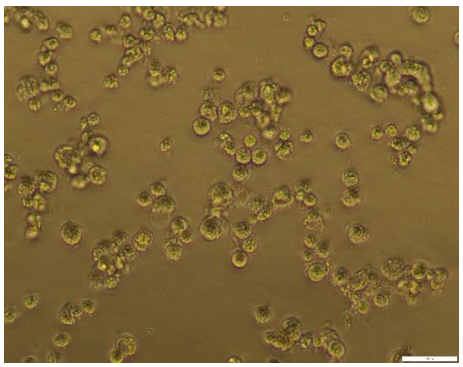
Control



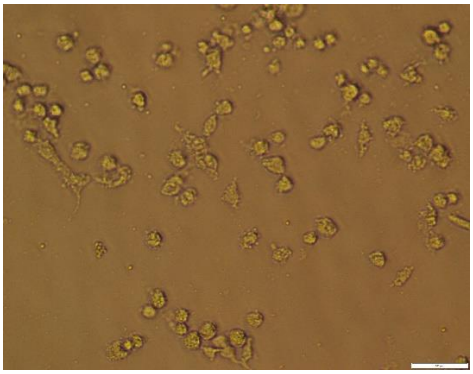
5 mg



25mg

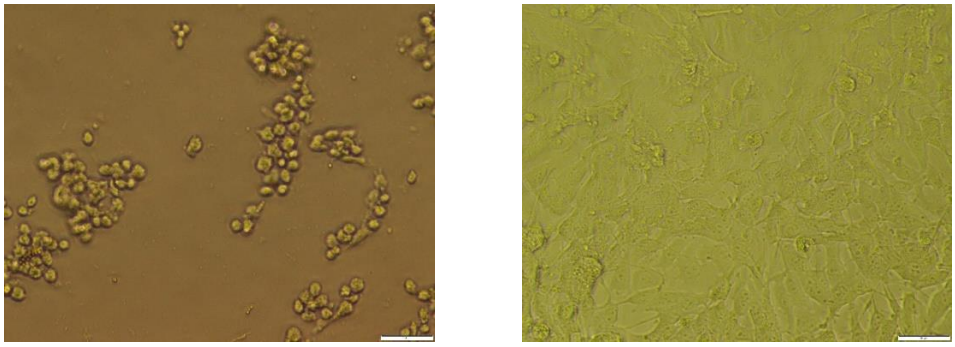


50mg



75mg

100 mg



"Figure 10". CS-SA – control, and compound treated

Table 7

Anticancer activity of CS-SA

CS-SA	Optical density		% of cell viability	
Control	0.827	1.233	100	100
5	0.527	0.601	62.5891	30.2413
25	0.423	0.384	41.8052	21.4178
50	0.263	0.209	26.8409	9.50226
75	0.101	0.074	11.9952	5.58069
100	0.042	0.019	4.98812	1.43288

Conclusion

In This work, new CS-SA was act as Estrogen receptor β. The data reported show that the effect of Variation in the chemical structure on activity with various therapeutic agents such as anti-biotic, anti-Cancer have been incorporated in the bio composite CS-SA. The IR spectra showed mass losses and the and presence of imine groups in the compound CS-SA. The NMR analysis that the C-2 position of N=N and C-6 position of C=N. The C=N-OH presence in 156.72 ppm. TG/DSC investigation in thermal reactions has the potential to the new understanding of the compound. The GC-MS and TG/DSC are identification technique on effect of the polymerization reaction of polymer. Finally, we were able to show that one of these salicylaldoxime compounds effectively showed the growth of ER β expressing human breast cancer cells, in vitro studies. The therapeutic potential of results is increased in ER β selective agonists.

Acknowledgments

Authors would like to thank Sophisticated Analytical Instrument Facility (SAIF) IIT Madras for their FT-IR, UV-Vis spectroscopy, TG/DSC and NMR spectroscopy. In-vitro anticancer study (NCIF) National College, Trichy

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