



New Chitosan Polymer Scaffold Schiff Bases as Potential Cytotoxic Activity: Synthesis, Molecular Docking, and Physicochemical Characterization

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Specialty section:

This article was submitted to
Polymer Chemistry,
a section of the journal
Frontiers in Chemistry

Received: 17 October 2021

Accepted: 06 December 2021

Published: 17 January 2022

Citation:

Packialakshmi P, Gobinath P, Ali D, Alarifi S, Gurusamy R, Idhayadhulla A and Surendrakumar R (2022) New Chitosan Polymer Scaffold Schiff Bases as Potential Cytotoxic Activity: Synthesis, Molecular Docking, and Physicochemical Characterization. *Front. Chem.* 9:796599. doi: 10.3389/fchem.2021.796599

In this work, we synthesize the sulfonated Schiff bases of the chitosan derivatives 2a-2j without the use of a catalyst in two moderately straightforward steps with good yield within a short reaction time. The morphology and chemical structure of chitosan derivatives were investigated using FT-IR, NMR (¹H—¹³C), XRD, and SEM. Furthermore, our chitosan derivatives were tested for their anticancer activity against the MCF-7 cancer cell line, and doxorubicin was used as a standard. In addition, the normal cell lines of the breast cancer cell MCF-10A, and of the lung cell MRC-5 were tested. Compound 2h, with a GI₅₀ value of 0.02 μM for MCF-7, is highly active compared with the standard doxorubicin and other compounds. The synthesized compounds 2a-2j exhibit low cytotoxicity, with IC₅₀ > 100 μg/ml, against normal cell lines MCF-10A, MRC-5. We also provide the results of an *in-silico* study involving the Methoxsalen protein (1Z11). Compound 2h exhibits a higher binding affinity for 1Z11 protein (−5.9 kcal/mol) and a lower binding affinity for Doxorubicin (−5.3 kcal/mol) than certain other compounds. As a result of the aforementioned findings, the use of compound 2h as an anticancer drug will be researched in the future.

Keywords: schiff base, SEM, anticancer activity, MCF-7, *in-silico* molecular docking, structure activity relationship

INTRODUCTION

Cell proliferation becomes abnormal when it expands outside its normal limits, invades adjacent tissues, and/or feeds on other parts of the body, and can cause cancer. Cancer can begin anywhere in the body. A period of growth is the primary trigger for cancer-related death. The World Health Organization (WHO) reports that cancer remains the leading cause of death in most countries. Cancer is the second leading cause of death worldwide, with new cancer diagnoses expected to reach 18.1 million in 2018 and with deaths due to cancer expected to exceed 9.6 million. Both men and women can be affected by cancer. According to a UN DESA assessment from 2014, the world's population, currently at 7.3 billion people, will grow to 8.5 billion by 2030, to 9.7 billion by 2050, and to 11.2 billion by 2,100. The estimate is that 1.5 billion cancer cases will be diagnosed, and that 1.2 billion fatalities will occur (Bray and Møller, 2006). In addition, with an anticipated 8.5 billion people on the planet, global mortality is expected to hit 2.14 billion by 2030 (Ali et al., 2011; Pereira et al., 2012). Cancers of the breast and lung are major causes of death in both women and men (5,22,000 deaths in 2012). In over 140 nations, these two cancers are the most often diagnosed (Ali et al., 2015).

Lung cancer and female breast cancer are the two most life-threatening malignancies, according to research. Breast cancer is one of the most frequent cancers in women and one of the most deadly (Bray et al., 2018). The activities of chitosan in killing cancer cells are known to work via the induction of apoptosis due to the activation of caspases 3, 8, and 9; the modification of the Bax- to Bcl-2 ratio; and the induction of DNA damage (Adhikari and Yadav, 2018), with the features of chitosan protonation present in acidic environments. Chitosan and its derivatives have been shown to selectively permeate cancer cell membranes and exhibit anticancer activity via cellular enzymatic, antiangiogenic, immune-enhancing, antioxidant defense mechanism, and apoptotic mechanisms. This activity can be reduced in oral administration due to protonation of the amine group (Mahmood et al., 2019). Despite this, the clinical options for chitosan in the treatment of a number of human cancers remain limited (Kuppusamy and Karuppaiah, 2013). The therapeutic use of chitosan-based molecules with minimal non-cancer cell damage is vital. Chitosan-based molecules were discovered to have anticancer properties that cause minimal damage to non-cancer cells (Wimardhani et al., 2014), and its effect varies greatly depending on the molecular weight and DDA against different cancer cell lines. Many cancer cells are affected by chemotherapeutic medicines such as cisplatin, 5-fluorouracil (5-FU), docetaxel, procarbazine, methotrexate, etc. according to studies conducted *in vitro* and *in vivo* (Andreadis et al., 2003). The Schiff base approach was used to make the DCMC/SS film, which has a wide range of biological applications including wound treatment, artificial skin, and tissue engineering (Wang et al., 2019). In addition to its mucoadhesive properties, chitosan suppresses drug efflux processes, allowing for greater drug penetration into cells (Taher et al., 2019). Chitosan and its derivatives, notably chitosan-drug nanocomposites as the principal anticancer formulations, can be viable natural choices to overcome these problems due to their selective antitumor effects, nontoxicity, biocompatibility, and biodegradability. Chitosan is a nontoxic, non-allergenic, biocompatible, and biodegradable natural polyaminosaccharide deacetylated from chitin (Imran et al., 2020). Chitosan has three types of reactive groups at C-2, C-3, and C-6: the primary amine group, and the primary and secondary hydroxyl groups. Among the three reactive groups for chitosan's biological activity, glucosamine's main amine at C-2 is the most important group (Aranaz et al., 2010). Chitosan and chitosan compounds with higher DDA and lower MW had better antibacterial, antioxidant, and anticancer properties (Kim, 2018). Chitosan and its derivatives have good biological activity, including anticancer properties, and makes it a good carrier for the preparation of prodrugs, as an accelerator of tissue engineering, and as an excipient for drug delivery and gene delivery (Muanprasat and Chatsudhipong, 2017; Cheung et al., 2015; Zhang et al., 2002; Ahmed and Ikram, 2016; Zhang et al., 2018; Bakar et al., 2017). The biological characteristics of chitosan are influenced by its solubility in water and in other commonly used solvents (Kumirska et al., 2011). Under mild conditions, chitosan includes two types of reactive functional groups (an amino group and a hydroxyl group) that can be used to modify its characteristics and to generate other derivatives (Li et al., 2015). Chitosan has

garnered a lot of interest recently because of its commercial potential in the medical, chemical, and food industries. Chitosan is frequently employed in biomedical applications due to its ability to obtain an extremely adaptable combination of chemical elements and properties as well as due to its ease of extraction, chemical modifications, and low cost (Chen and Gong, 2016). Chitosan and chitosan derivatives have good anticancer effects and decrease the unwanted effects of some medicine due to its controlled administration of doses for cancer treatment and its slow release of the free drug from conjugates (Perni and Prokopovich, 2017). Following that, efforts are required to identify newer, more effective, and less toxic chemotherapeutic drugs for the treatment of cancers (Srivastava et al., 2019). The anticancer active compounds of chitosan analogs are shown in **Figure 1** (Jiang et al., 2011; Adhikari and Yadav, 2018; Li et al., 2020; Saeed et al., 2020). In recent years, CH-based tissue regeneration and drug delivery to implants as well as chitosan's potential for use as a biomaterial in dental applications have been researched (Husain et al., 2017; Qasim et al., 2018). Chitosan is utilized in the pharmaceutical industry for making drug delivery matrixes such as blend films (CS/PAH) as well as antibacterial activities (Ali et al., 2020; Sarwar et al., 2020).

The contemporary work addresses the development of the cytotoxic activity of fresh sulfonated Schiff base of chitosan derivatives can be synthesized and characterized by FT-IR, NMR (^1H NMR, ^{13}C NMR) spectroscopy, and its physical properties were observed. *In-silico* molecular docking analysis and also evaluated the cytotoxic activities against a cancer cell lines.

EXPERIMENTAL

Materials and Methods (Chemistry)

All the substance and reagents such as chitosan (degree of deacetylation - 75%, Mw141 KDa), acetic acid, AEEA, chlorosulfonic acid, aromatic aldehydes, ethanol and DMF are purchased from Merck. The melting points of synthesized substances were evaluated in open capillary tubes, and were uncorrected. The FT-IR spectra were recorded in KBr on a Shimadzu 8201pc (4,000–1,000 cm^{-1}). The ^1H -NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-300 MHz. To obtain NMR spectra, the product was dissolved in DMSO- d_6 . TLC was used to assess the purity of the compounds, with silica-gel and 60F254 aluminium sheets as the adsorbent.

Preparation of Chitosan Analogue (1, 2, 2a–2j)

Chitosan (3 g, deacetylated) was dissolved in 20 ml of 1% CH_3COOH (pH =3.4–3.6) and aminoethylethanolamine (AEEA) (2.0 ml) was reacted at 100°C for 4 h to obtain compound 1. To adjacent the pH of compound 1, 1 M NaOH was added. An electrophilic substitution process in which the proton was replaced by the SO_3H group was the primary mechanism of the reaction (Morrison and Boyd, 1997). Compound 1 was dissolved in dimethylformamide (DMF), chlorosulfonic acid (4.5 ml in 30 ml DMF) was fractionally

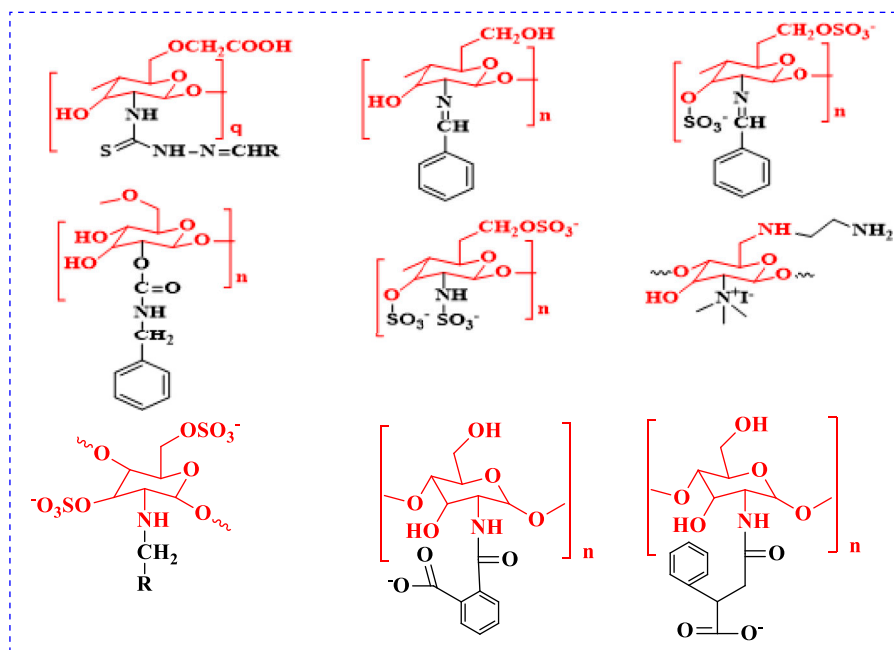


FIGURE 1 | Anticancer active compounds of chitosan derivatives.

added, and the mixture was stirred at room temperature for 5 h. It was neutralized with a 20% NaOH solution, and the precipitate of compound 2 was obtained, and reprecipitated with methanol twice (Martins et al., 2003). Compound 2 (2.5 g dissolved in acetic acid with ethanol) and benzaldehyde (1.0 ml) were mixed with ethanol and stirred for 8 h at 60°C. The white gel obtained indicates the formation of Schiff base compound 2a. A solution of 5% NaOH was used to precipitate the product, filtered, washed with ice-cold water, and add ethanol to remove any unreacted products. The final product was soluble in DMF. TLC was used to track the reaction's progress. In TLC, the eluting solvents were hexane and ethylacetate (4:6). Compounds 2b–2j were synthesized using the technique outlined above.

Synthesis of (2,3,4,6)-3-(benzylideneamino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-yl sulfate (2a)

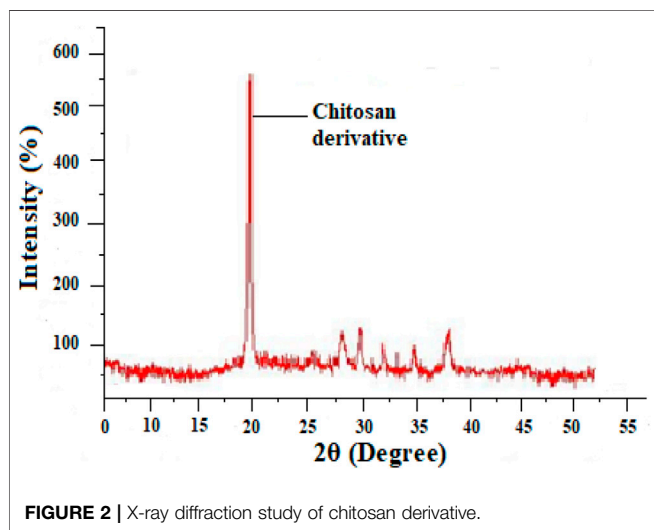
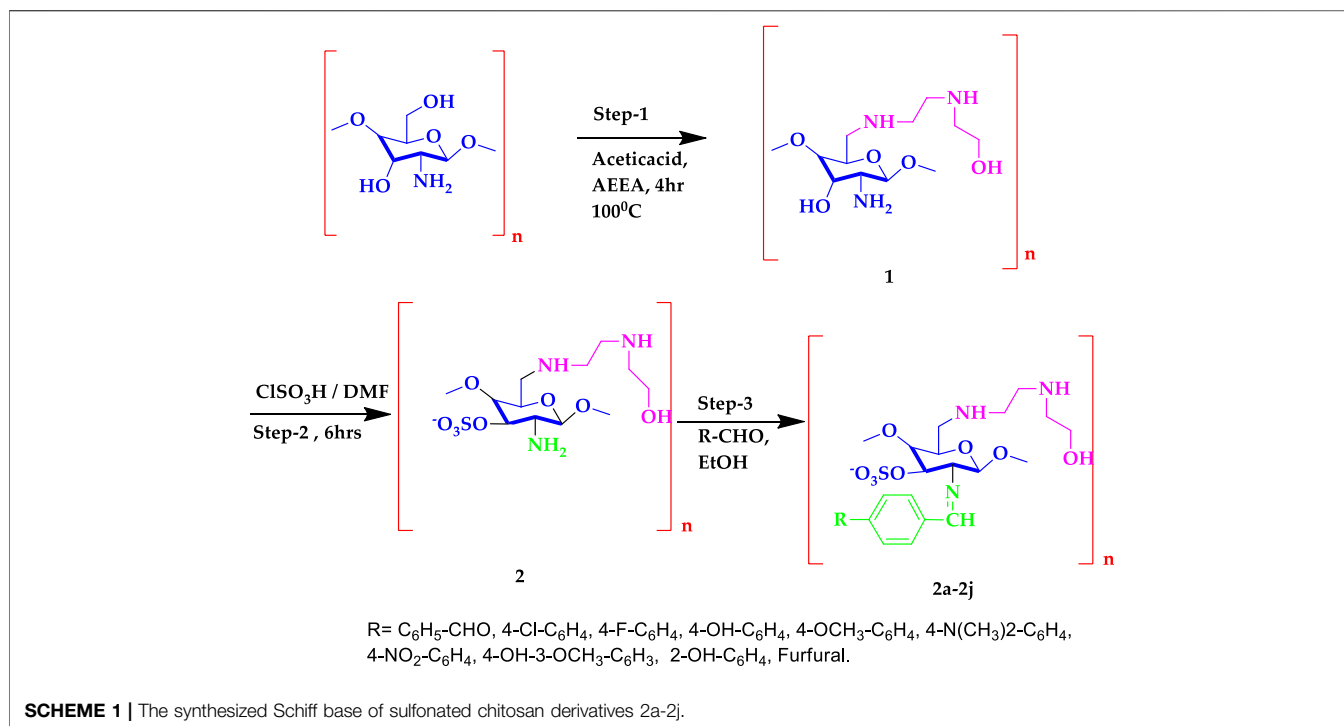
Yield: 65%; IR (KBr) (cm^{-1}): 3,452 (NH, str), 3,280 (OH-str), 2,951 (CH-str Ar ring), 1,645 (N = CH, str), 1,383 (N-C, str), 1,326 (S = O), 972 (C-O-S, str). ^1H NMR (DMSO- d_6), δ (ppm): 8.20 (s, 1H, N = CH), 7.85–7.52 (m, 5H, Ph), 5.21–3.10 (m, 5H, CS-H), 3.68 (s, 1H, OH), 3.52–3.33 (s, 6H, OCH_3 -CS), 3.45–2.70 (m, 4H, OH-(CH_2) $_2$), 2.80–2.54 (m, 2H, CH_2 -NH), 2.68–2.51 (m, 4H, N (CH_2) $_2$), 2.02 (s, 2H, NH); ^{13}C NMR (DMSO- d_6) δ (ppm): 163.8 (1C, N = CH), 136.5, 131.2, 129.3, 128.9 (6C, Ph), 112.3, 87.4, 71.5, 65.2, 60.7 (5C, CS), 61.7, 52.0 (2C, C-OH), 57.6, 55.7 (2C, OCH_3 -CS), 49.6, 49.0 (2C, N-C in amine), 48.4 (1C, N-CH).

Synthesis of (2,3,4,6)-3-(((4-chlorobenzylidene)amino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-yl sulfate (2b)

Yield: 68%; IR (KBr) (cm^{-1}): 3,454 (NH, str), 3,286 (OH-str), 2,954 (CH-str Ar ring), 1,641 (N = CH, str), 1,378 (N-C, str), 1,320 (S = O), 972 (C-O-S, str), 544 (C-Cl). ^1H NMR (DMSO- d_6), δ (ppm): 8.18 (s, 1H, N=CH), 7.80–7.50 (m, 4H, Cl-Ph), 5.23–3.12 (m, 5H, CS-H), 3.66 (s, 1H, OH), 3.54–3.35 (s, 6H, OCH_3 -CS), 3.47–2.72 (m, 4H, OH-(CH_2) $_2$), 2.82–2.56 (m, 2H, CH_2 -NH), 2.65–2.50 (m, 4H, N (CH_2) $_2$), 2.04 (s, 2H, NH); ^{13}C NMR (DMSO- d_6) δ (ppm): 163.4 (1C, N = CH), 136.8, 134.2, 130.3, 129.0 (6C, Cl- Ph), 112.5, 87.6, 71.7, 65.4, 60.9 (5C, CS), 61.9, 52.2 (2C, C-OH), 57.8, 55.9 (2C, OCH_3 -CS), 49.8, 49.2 (2C, N-C in amine), 48.6 (1C, N-CH).

Synthesis of (2,3,4,6)-3-(((4-fluorobenzylidene)amino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-yl sulfate (2c)

Yield: 70%; IR (KBr) (cm^{-1}): 3,450 (NH, str), 3,282 (OH-str), 2,945 (CH-str Ar ring), 1,643 (N = CH, str), 1,384 (N-C, str), 1,322 (S = O), 978 (C-O-S, str). ^1H NMR (DMSO- d_6), δ (ppm): 8.41 (s, 1H, N=CH), 7.85–7.38 (m, 4H, F-Ph), 5.20–3.08 (m, 5H, CS-H), 3.65 (s, 1H, OH), 3.50–3.31 (s, 6H, OCH_3 -CS), 3.43–2.68 (m, 4H, OH-(CH_2) $_2$), 2.81–2.53 (m, 2H, CH_2 -NH), 2.66–2.52 (m, 4H, N (CH_2) $_2$), 2.03 (s, 2H, NH); ^{13}C NMR (DMSO- d_6) δ (ppm): 165.5, 132.2, 130.3, 115.9 (6C, F-Ph), 163.6 (1C, N = CH), 112.1,



87.3, 71.2, 65.0, 60.5 (5C, CS), 61.4, 52.1 (2C, C-OH), 57.4, 55.3 (2C, OCH₃-CS), 49.7, 49.1 (2C, N-C in amine), 48.2 (1C, N-CH).

Synthesis of (2,3,4,6)-3-((4-hydroxybenzylidene)amino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-ylsulfate (2d)

Yield: 62%; IR (KBr) (cm⁻¹): 3,458 (NH, str), 3,285 (OH-str), 2,956 (CH-str Ar ring), 1,643 (N = CH, str), 1,385 (N-C, str), 1,327 (S = O), 980 (C-O-S, str). ¹H NMR (DMSO-d₆), δ (ppm): 10.54 (s, 1H, OH-Ph), 8.61 (s, 1H, N = CH), 7.80–6.82 (m, 4H, OH-Ph), 5.22–3.11 (m, 5H, CS-H), 3.69 (s, 1H, OH), 3.53–3.34 (s, 6H, OCH₃-CS), 3.46–2.71

(m, 4H, OH-(CH₂)₂), 2.81–2.53 (m, 2H, CH₂-NH), 2.69–2.52 (m, 4H, N (CH₂)₂), 2.03 (s, 2H, NH); ¹³C NMR (DMSO-d₆) δ (ppm): 163.9 (1C, N = CH), 160.5, 130.2, 129.3, 116.9 (6C, OH-Ph), 112.2, 87.3, 71.4, 65.3, 60.6 (5C, CS), 61.6, 52.1 (2C, C-OH), 57.5, 55.6 (2C, OCH₃-CS), 49.7, 49.1 (2C, N-C in amine), 48.3 (1C, N-CH).

Synthesis of (2,4,5,6)-2-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-3,6-dimethoxy-5-(((4-methoxybenzylidene)amino)tetrahydro-2H-pyran-4-yl sulfate (2e)

Yield: 67%; IR (KBr) (cm⁻¹): 3,455 (NH, str), 3,287 (OH-str), 2,952 (CH-str Ar ring), 1,646 (N = CH, str), 1,386 (N-C, str), 1,328 (S = O), 982 (C-O-S, str). ¹H NMR (DMSO-d₆), δ (ppm): 8.45 (s, 1H, N=CH), 7.89–7.10 (m, 4H, OCH₃-Ph), 5.25–3.14 (m, 5H, CS-H), 3.86 (s, 3H, OCH₃), 3.70 (s, 1H, OH), 3.55–3.36 (s, 6H, OCH₃-CS), 3.48–2.74 (m, 4H, OH-(CH₂)₂), 2.84–2.57 (m, 2H, CH₂-NH), 2.69–2.55 (m, 4H, N (CH₂)₂), 2.06 (s, 2H, NH); ¹³C NMR (DMSO-d₆) δ (ppm): 163.4 (1C, N = CH), 162.5, 130.4, 128.3, 114.6 (6C, OCH₃-Ph), 112.6, 87.7, 71.8, 65.5, 60.9 (5C, CS), 61.4, 52.3 (2C, C-OH), 57.9, 55.8 (2C, OCH₃-CS), 55.4 (1C, OCH₃-Ph), 49.9, 49.1 (2C, N-C in amine), 48.5 (1C, N-CH).

Synthesis of (2,3,4,6)-3-(((4-(dimethylamino)benzylidene)amino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-ylsulfate (2f)

Yield: 72%; IR (KBr) (cm⁻¹): 3,457 (NH, str), 3,286 (OH-str), 1,944 (CH-str Ar ring), 1,645 (N = CH, str), 1,386 (N-C, str), 1,329

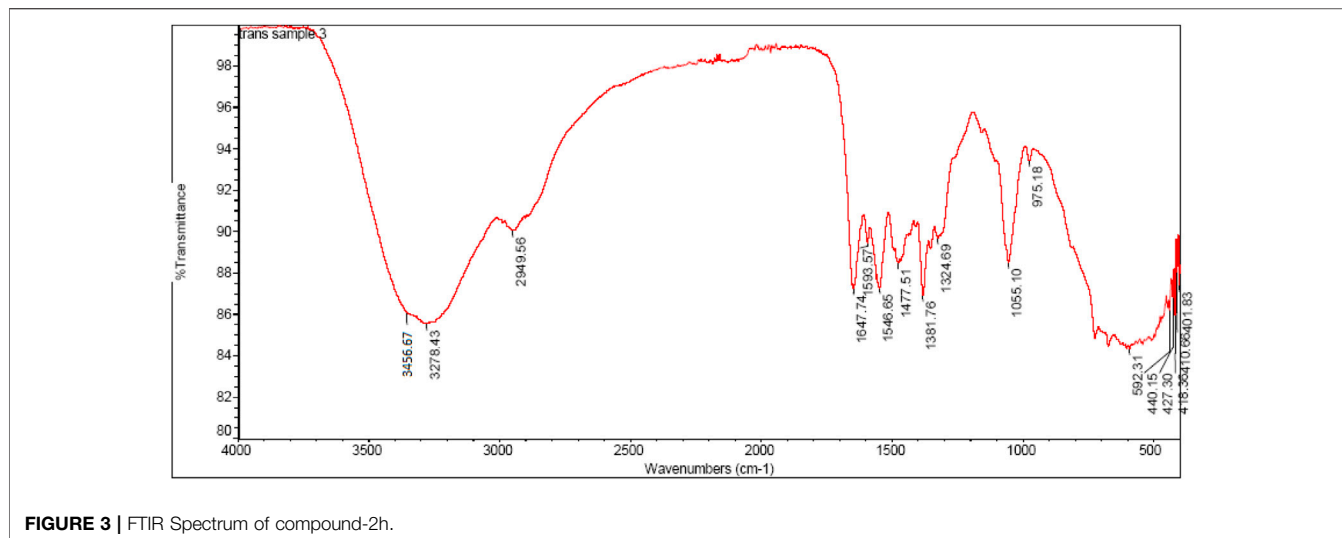


FIGURE 3 | FTIR Spectrum of compound-2h.

(S = O), 980 (C-O-S, str). ^1H NMR (DMSO- d_6), δ (ppm): 8.27 (s, 1H, N=CH), 7.55–6.86 (m, 4H, $\text{N}(\text{CH}_3)_2$ -Ph), 5.25–3.14 (m, 5H, CS-H), 3.70 (s, 1H, OH), 3.56–3.37 (s, 6H, OCH_3 -CS), 3.47–2.73 (m, 4H, OH-(CH_2) $_2$), 3.05 (s, 6H, $\text{N}(\text{CH}_3)_2$), 2.82–2.56 (m, 2H, CH_2 -NH), 2.64–2.50 (m, 4H, N (CH_2) $_2$), 2.06 (s, 2H, NH); ^{13}C NMR (DMSO- d_6) δ (ppm): 163.5 (1C, N = CH), 153.4, 126.0, 124.3, 112.0, 41.5 (8C, $\text{N}(\text{CH}_3)_2$ -Ph), 112.5, 87.6, 71.7, 65.4, 60.3 (5C, CS), 61.8, 52.4 (2C, C-OH), 57.8, 55.4 (2C, OCH_3 -CS), 49.8, 49.1 (2C, N-C in amine), 48.6 (1C, N-CH).

Synthesis of (2,4,5,6)-2-(((2-((2-hydroxy ethyl)amino)ethyl)amino)methyl)-3,6-dimethoxy-5-((4-nitrobenzylidene) amino)tetrahydro-2H-pyran-4-yl sulfate (2g)

Yield: 76%; IR (KBr) (cm^{-1}); 3,459 (NH, str), 3,285 (OH-str), 2,951 (CH-str Ar ring), 1,641 (N = CH, str), 1,474 (N-O, str),

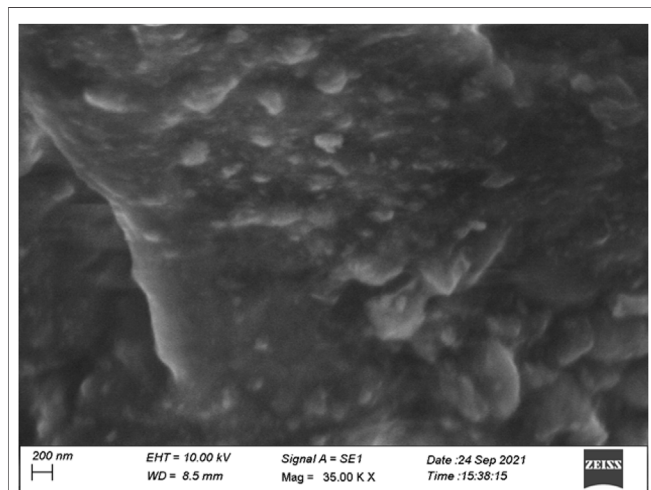


FIGURE 4 | SEM image of chitosan derivative.

1,385 (N-C, str), 1,321 (S = O), 973 (C-O-S, str). ^1H NMR (DMSO- d_6), δ (ppm): 8.26 (s, 1H, N = CH), 8.43–8.12 (m, 4H, NO_2 -Ph), 5.23–3.12 (m, 5H, CS-H), 3.64 (s, 1H, OH), 3.55–3.35 (s, 6H, OCH_3 -CS), 3.47–2.73 (m, 4H, OH-(CH_2) $_2$), 2.82–2.53 (m, 2H, CH_2 -NH), 2.65–2.52 (m, 4H, N (CH_2) $_2$), 2.06 (s, 2H, NH); ^{13}C NMR (DMSO- d_6) δ (ppm): 163.3 (1C, N = CH), 150.5, 142.2, 130.3, 124.9 (6C, NO_2 -Ph), 112.4, 87.6, 71.3, 65.5, 60.4 (5C, CS), 61.3, 52.4 (2C, C-OH), 57.4, 55.3 (2C, OCH_3 -CS), 49.7, 49.2 (2C, N-C in amine), 48.5 (1C, N-CH).

Synthesis of (2,3,4,6)-3-((4-hydroxy-3-methoxybenzylidene)amino)-6-(((2-((2-hydroxyethyl) amino)ethyl)amino) methyl)-2,5-dimethoxytetrahydro-2H-pyran-4-ylsulfate (2h)

Yield: 84%; IR (KBr) (cm^{-1}); 3,456 (NH, str), 3,278 (OH-str), 2,949 (CH-str Ar ring), 1,647 (N = CH, str), 1,381 (N-C, str), 1,324 (S = O, str), 975 (C-O-S, str). ^1H NMR (DMSO- d_6), δ

TABLE 1 | Anticancer activity of compounds (μM) (2a-2j).

Compounds	MCF-7 cell line		
	GI $_{50}$ (μM)	TGI (μM)	LC $_{50}$ (μM)
2a	03.3 \pm 0.23	07.3 \pm 1.15	07.5 \pm 0.03
2b	0.05 \pm 0.15	0.65 \pm 2.45	0.89 \pm 0.28
2c	0.20 \pm 0.82	0.50 \pm 1.50	0.75 \pm 0.35
2d	0.10 \pm 0.74	0.70 \pm 0.68	1.50 \pm 1.40
2e	0.05 \pm 0.33	0.06 \pm 1.32	0.79 \pm 1.25
2f	0.65 \pm 0.10	11.0 \pm 1.62	20.3 \pm 0.24
2g	0.43 \pm 0.90	0.75 \pm 1.53	15.6 \pm 0.72
2h	0.02 \pm 0.24	0.15 \pm 0.19	0.72 \pm 0.31
2i	0.07 \pm 0.56	0.60 \pm 0.41	2.25 \pm 0.31
2j	0.04 \pm 0.50	0.29 \pm 0.72	9.12 \pm 1.21
Doxorubicin	0.04 \pm 0.75	0.25 \pm 0.23	0.80 \pm 0.46

Data represent the mean \pm standard deviation (SD) of the mean values of three separate experiments.

TABLE 2 | *In vitro* cytotoxicity of chitosan derivatives (2a-2j) on normal cells.

Compd. No	MCF-10A	MRC5
	IC50 (μM)	IC50 (μM)
—		
2a	65.62	60.53
2b	72.04	75.35
2c	71.51	73.03
2d	54.16	50.37
2e	62.08	67.59
2f	56.80	51.11
2g	74.62	70.23
2h	86.5	80.84
2i	69.04	58.95
2j	52.46	61.27

Each compound was tested in triplicate. All error bars represent mean \pm SD from three independent experiments.

(ppm): 8.20 (s, 1H, N = CH), 7.55–6.93 (m, 3H, Vanillin), 5.37 (s, 1H, OH-Ph), 5.20–3.13 (m, 5H, CS-H), 3.63 (s, 1H, OH), 3.73 (s, 3H, OCH₃), 3.50–3.31 (s, 6H, OCH₃-CS), 3.42–2.68 (m, 4H, OH-(CH₂)₂), 2.83–2.53 (m, 2H, CH₂-NH), 2.65–2.52 (m, 4H, N(CH₂)₂), 2.07 (s, 2H, NH); ¹³C NMR (DMSO-d₆) δ (ppm): 163.4 (1C, N = CH), 151.5, 149.2, 133.3, 123.0, 117.2, 112.5 (6C, Vanillin), 112.4, 87.1, 71.3, 65.0, 60.4 (5C, CS), 61.6, 52.2 (2C, C-OH), 57.5, 55.4 (2C, OCH₃-CS), 56.5 (1C, OCH₃), 49.7, 49.1 (2C, N-C in amine), 48.2 (1C, N-CH).

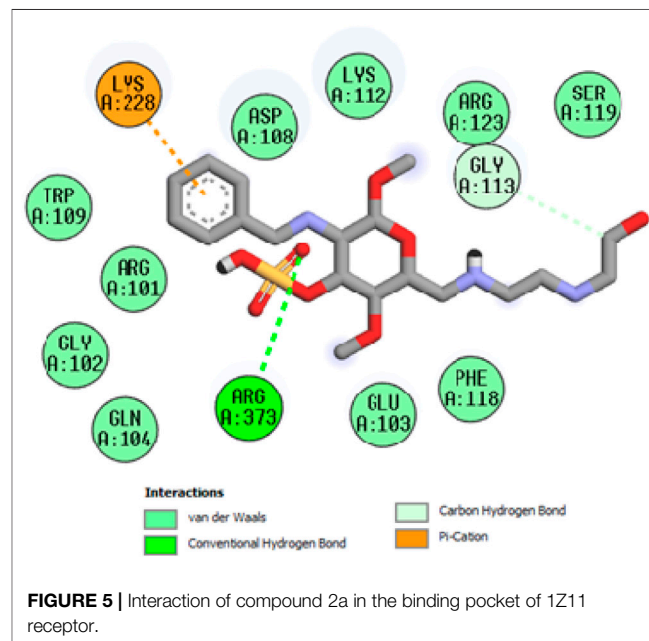
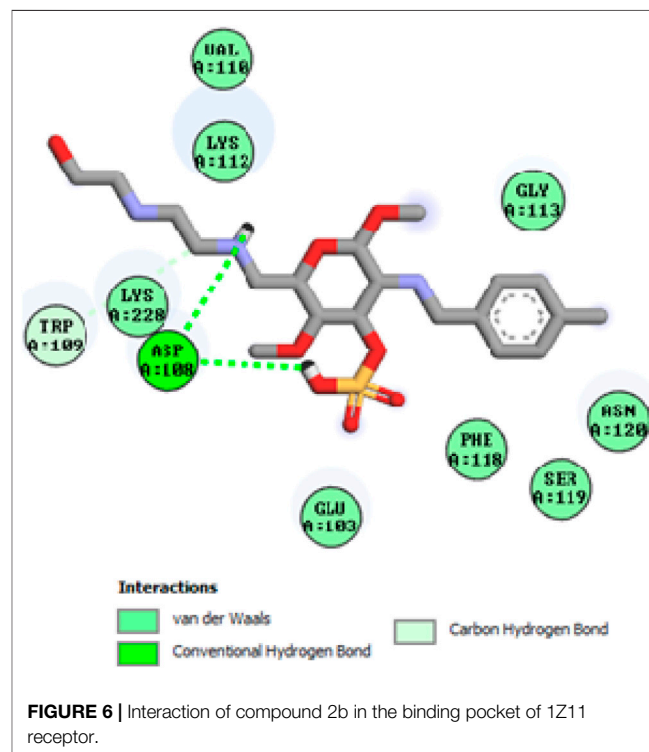
Synthesis of (2,3,4,6)-3-((2-hydroxybenzylidene)amino)-6-(((2-((2-hydroxyethyl)amino)ethyl)amino)methyl)-2,5-dimethoxy tetrahydro-2H-pyran-4-yl sulfate (2i)

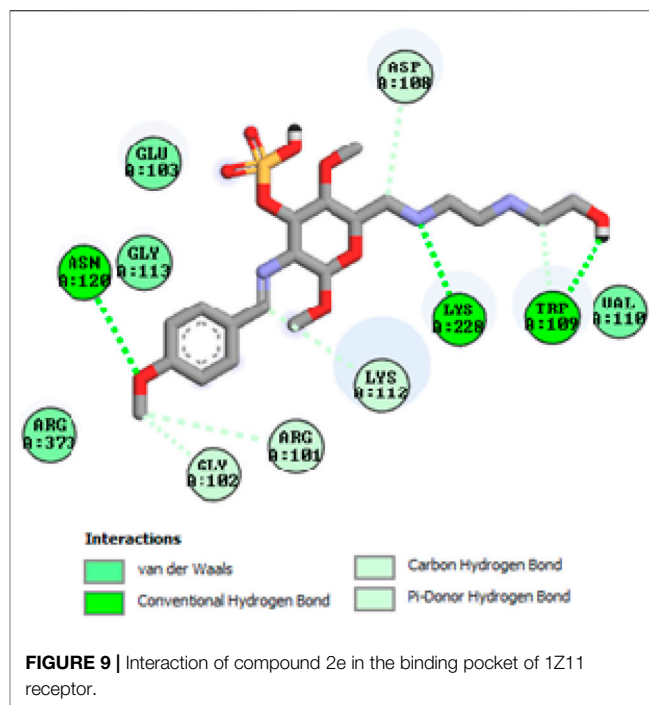
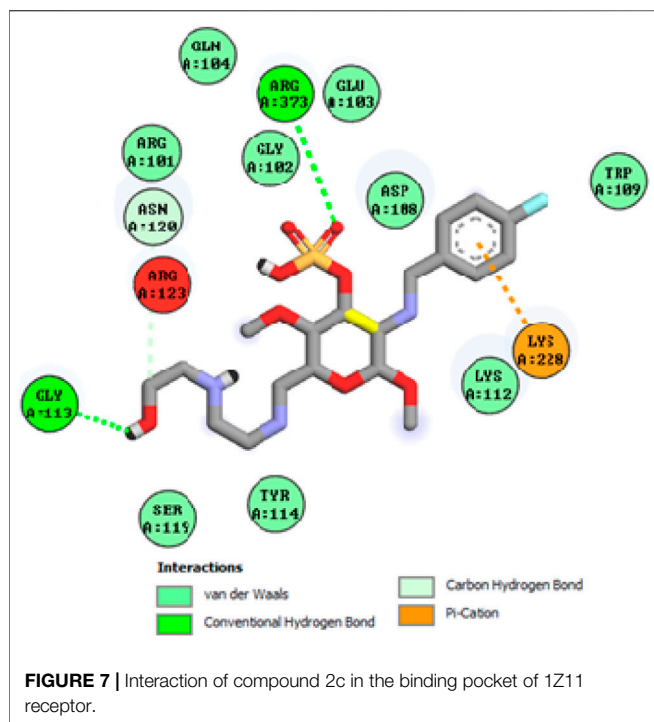
Yield: 73%; IR (KBr) (cm⁻¹): 3,458 (NH, str), 3,284 (OH-str), 2,950 (CH-str Ar ring), 1,642 (N = CH, str), 1,386 (N-C, str), 1,326 (S = O), 989 (C-O-S, str). ¹H NMR (DMSO-d₆) δ (ppm): 8.27 (s, 1H, N = CH), 7.68–7.03 (m, 4H, Ph), 5.45 (s, 1H, OH-Ph), 5.24–3.13 (m, 5H, CS-H), 3.65 (s, 1H, OH), 3.54–3.35 (s, 6H,

TABLE 3 | Molecular docking interaction of compounds 2a-2j and Doxorubicin against protein 1Z11.

Compound	Human microsomal cytochrome P450 2A6 complexed with methoxsalen (PDB ID: 1Z11)		
	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
2a	-5.6	1	Arg373
2b	-5.0	2	Asp108
2c	-5.2	2	Gly113 and Arg373
2d	-4.8	1	Asn120
2e	-4.4	3	Trp109, Asn120 and Lys228
2f	-5.7	2	Phe118 and Asn120
2g	-4.8	2	Phe118 and Asn120
2h	-5.9	2	Phe118 and Asn120
2i	-4.8	2	Phe118 and Asn120
2j	-5.2	3	Asn120 and Arg373
Doxorubicin	-5.3	1	Arg373

OCH₃-CS), 3.43–2.74 (m, 4H, OH-(CH₂)₂), 2.83–2.55 (m, 2H, CH₂-NH), 2.63–2.52 (m, 4H, N(CH₂)₂), 2.06 (s, 2H, NH); ¹³C NMR (DMSO-d₆) δ (ppm): 163.6 (1C, N = CH), 161.5, 132.6, 132.0, 124.9, 124.0 (6C, Ph), 112.1, 87.2, 71.5, 65.3, 60.4 (5C, CS), 61.5, 52.6 (2C, C-OH), 57.7, 55.8 (2C, OCH₃-CS), 49.9, 49.0 (2C, N-C in amine), 48.5 (1C, N-CH).

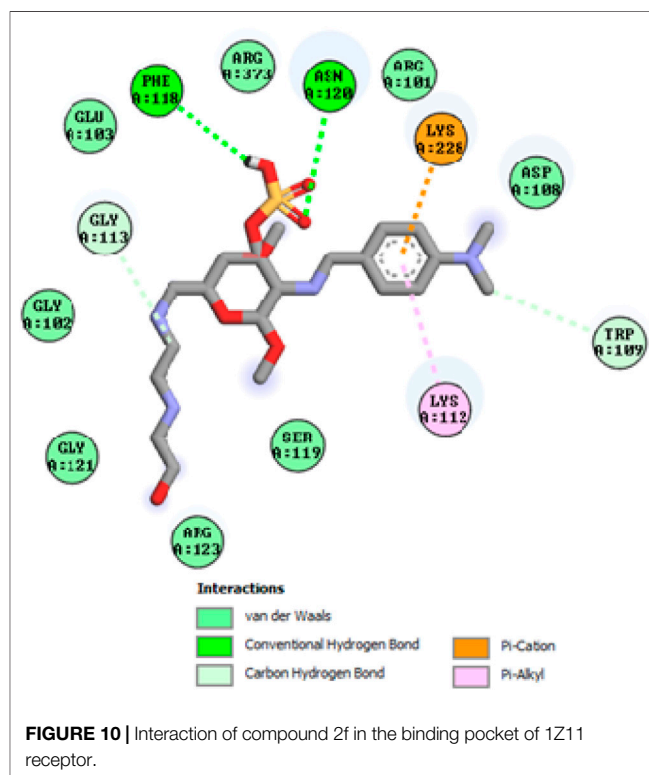
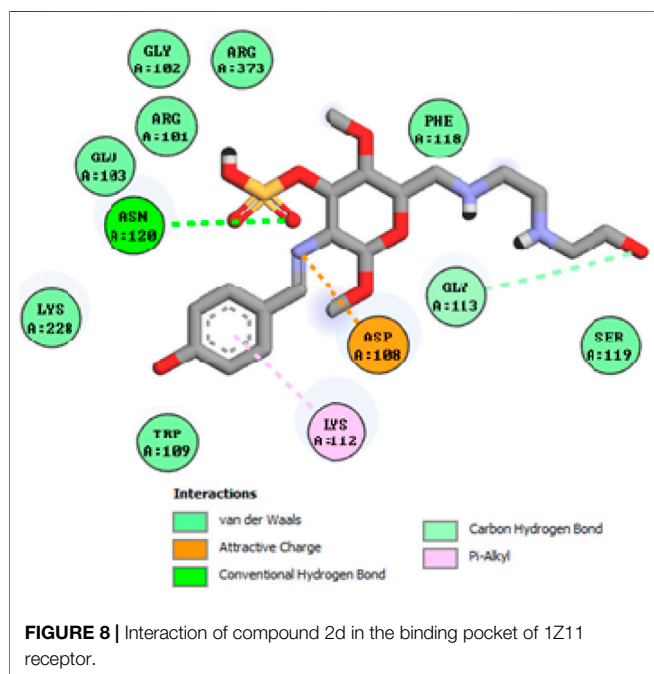
**FIGURE 5** | Interaction of compound 2a in the binding pocket of 1Z11 receptor.**FIGURE 6** | Interaction of compound 2b in the binding pocket of 1Z11 receptor.

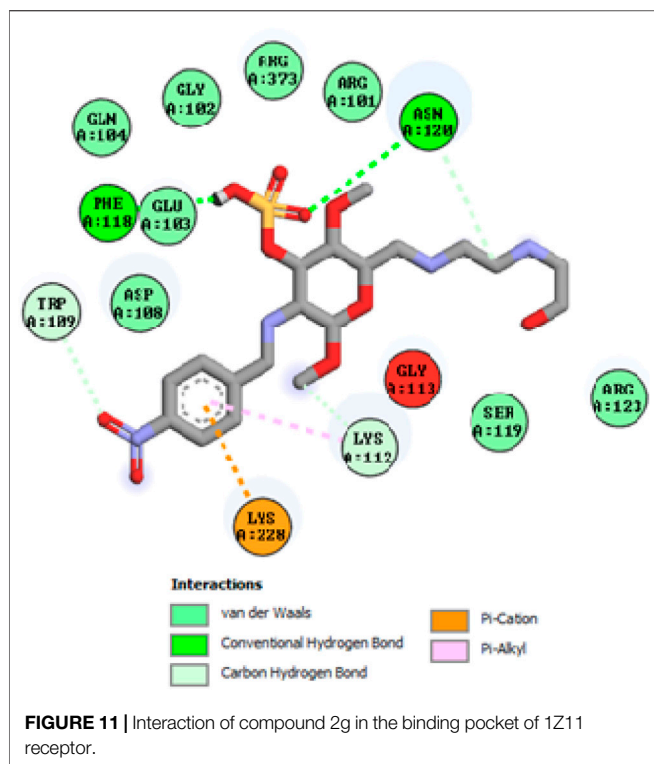


Synthesis of (2,3,4,6)-3-((furan-2-ylmethylene)amino)-6-(((2-(2-hydroxyethyl)amino)ethyl) amino)methyl)-2,5-dimethoxy tetrahydro-2H-pyran-4-yl sulfate (2j)

Yield: 68%; IR (KBr) (cm^{-1}); 3,458 (NH, str), 3,279 (OH-str), 2,957 (CH-str Ar ring), 1,644 (N = CH, str), 1,383 (N-C, str), 1,321 (S = O), 812 (C-O-S, str). ^1H NMR (DMSO- d_6), δ (ppm):

8.25 (s, 1H, N = CH), 7.72–6.50 (m, 3H, furfural), 5.26–3.17 (m, 5H, CS-H), 3.68 (s, 1H, OH), 3.57–3.34 (s, 6H, OCH_3 -CS), 3.43–2.72 (m, 4H, $\text{OH}-(\text{CH}_2)_2$), 2.81–2.55 (m, 2H, CH_2 -NH), 2.62–2.53 (m, 4H, N $(\text{CH}_2)_2$), 2.05 (s, 2H, NH); ^{13}C NMR



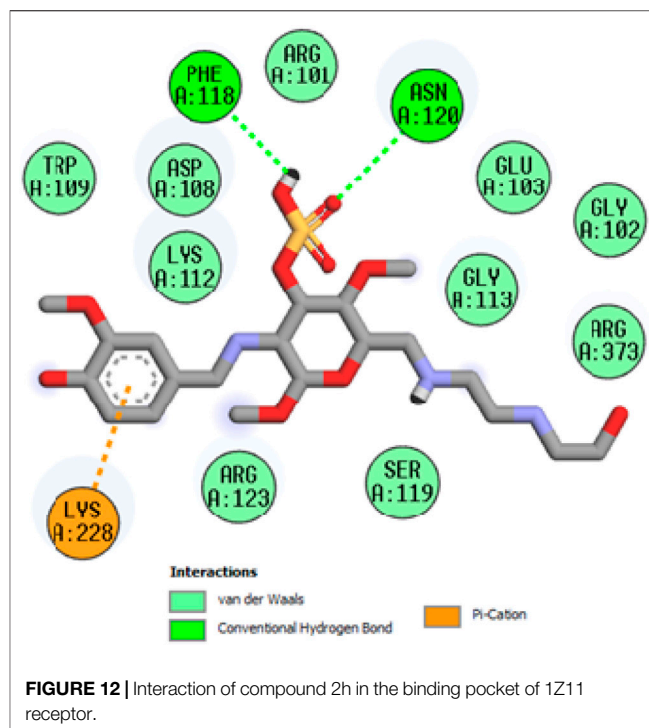


(DMSO- d_6) δ (ppm): 163.2 (1C,N = CH), 149.6, 144.2, 118.3, 112.9 (4C, furfural), 112.9, 87.8, 71.7, 65.6, 60.5 (5C, CS), 61.4, 52.3 (2C, C-OH), 57.2, 55.1 (2C, OCH₃-CS), 49.8, 49.1 (2C, N-C in amine), 48.6 (1C, N-CH).

BIOLOGICAL SCREENING

Cytotoxic Activity

The cytotoxic activity of the newly synthesized compounds (2a-2j) was tested using a previously described method (Scudiere et al., 1988; Surendra Kumar et al., 2017). Compounds 2a-2j was tested in the MCF-7 cell line for 48 h using a single dose of primary anticancer assay at a concentration of 100 μ m (MTT anticancer assay). MCF-7 breast cell line was employed in this study. The MCF-7 cell line was pre-incubated on a micro titer plate in this technique. Each test's results are expressed as a percentage of the treated cells' growth relative to the untreated control cells. Compounds with anticancer activity were those that reduced the proliferation of the cell lines by 30% or less. 0.1 ml of the cell suspension (containing 5×10^6 cells/100 μ L) and 0.1 ml of the test solution (6.25–100 μ g in 1% DMSO such that the final concentration of DMSO in media was less than 1%) were added to the 9 well plates and incubated at 37°C for 72 h in a 5 percent CO₂ incubator. The control wells included 1 percent DMSO and cell suspension, while the blank contained simply cell suspension. After 72 h, 20 μ L of MTT were added and left in the CO₂ incubator for 2 h before adding 100 μ L of propanol. To protect the plate from light, it was wrapped in aluminum foil. The 9 well plates were then shaken for 10–25 min in a rotary shaker. The 9 well plates were



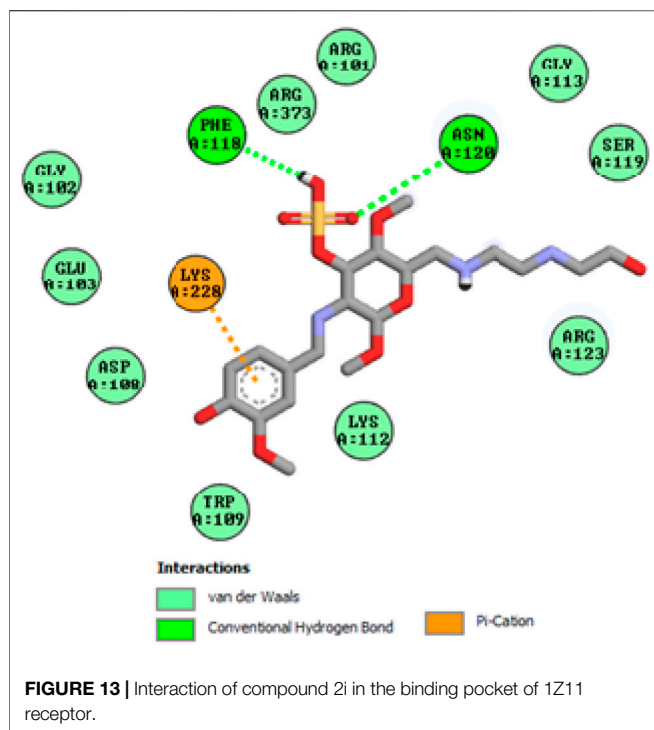
processed on an ELISA reader for absorbance at 562 nm after 10–20 min.

Molecular Docking

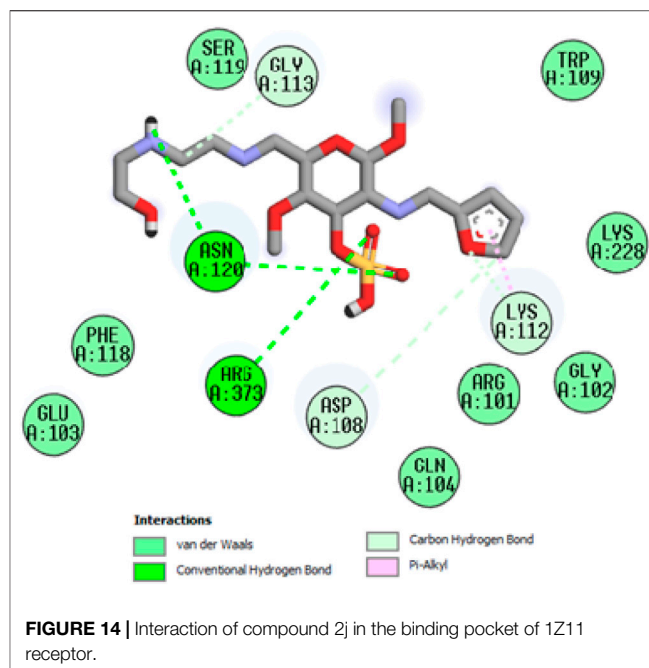
The binding abilities and interaction between compound 2a-2j, Doxorubicin, and the Human microsomal cytochrome P450 2A6 complexed with Methoxsalen (PDB ID: 1Z11) protein were investigated using Autodock vina 1.1.2 (Trott and Olson, 2009). To discover the target protein, the Protein Data Bank (<http://www.rcsb.org>) was used (PDB ID: 1Z11). The 3D structures of the compounds 2a-2j and Doxorubicin were created using Chem3D Pro 12.0 and Chem Draw Ultra 12.0. The AutoDock Tools 1.5.6 application suite was used to build the input files for Autodock Vina. The grid co-ordinates for the target protein were observed to be 56.316, 77.155, 60.326, with size x,y,z: 24, 20, 20 and 1.0 spacing. The interpretation of exhaustiveness has been assigned the number eight. Other Vina docking parameters are enabled by default and are not listed. The compound with the lowest binding affinity value obtained the highest score, which was determined visually using the software Discovery Studio 2019.

RESULT AND DISCUSSION

The novel chitosan compounds were established by ¹H NMR, and ¹³C NMR, as presented in the Supplementary Materials. The FT-IR spectra presented absorption bands (2a-2j) at 3,450–3,458, 3,280–3,289, 3,050–3,057, 2,928, 1,645, 1,592, 1,474, 1,420, 1,242 and 815 cm⁻¹, confirming the NH, OH, Ar-H, CH, N = CH, NH₂, (N-O), N-C, (S = O), and (C-O-S) groups. The absorption bands



of other compounds are summarized in the Experimental section. The ^1H NMR spectra (2a–2j) show shifts in the chemical values at 8.18–8.45, 7.52–7.85, 6.50–7.72, 3.64–3.70, 3.33–3.52, 3.10–5.21, 2.72–3.43, 2.54–2.80, 2.51–2.68 and 2.02–2.07 ppm, confirming the presences of $\text{N}=\text{CH}$, $\text{Ph}-\text{CH}$, furfural, CH_2-OH , OCH_3 , $\text{CS}-\text{H}$, $\text{OH}-(\text{CH}_2)_2$, CH_2-NH , $\text{N}(\text{CH}_2)_2$, and NH protons, respectively. The ^1H NMR spectra of other compounds are summarized in the Experimental section. The ^{13}C NMR (2a–2j) showed signals at 163.2–165.5, 128.9–136.5, 112.9–149.6, 60.7–112.3, 52.0–61.7, 55.7–57.6, and 48.3–49.0 ppm, confirming the presence of carbon atom in $\text{N}=\text{CH}$, Ph , furfural, CS , $\text{C}-\text{OH}$, OCH_3 , $\text{N}-\text{C}$, and $\text{N}-\text{CH}$. The shift in chemical values of other compounds are given in the experimental section. Compound 2 (2.5 g dissolved in acetic acid with ethanol) and 4-chlorobenzaldehyde (1.2 ml) were mixed with ethanol and stirred for 8 h at 60°C . The light-yellow gel obtained indicates the formation of compound 2b. A solution of 5% NaOH was used to precipitate the product, was filtered, and was washed with ice-cold water and ethanol to remove any unreacted products. The final product was soluble in DMF. In this synthesis of chitosan derivatives without using any catalyst, we obtained better yields with in a shorter reaction time. The progress of the reaction was monitored by TLC. The same procedure was followed by the synthesis of other sulfonated chitosan derivatives 2c–2j. The synthesized compounds 2a–2j were assessed for their cytotoxic activities against the MCF-7 cancer cell line and against the normal cell lines breast cancer cell MCF-10A and lung cell MRC-5 using an MTT assay, and doxorubicin was used as a standard. Compound 2h was found to be highly active, with a GI_{50} value of $0.02\ \mu\text{M}$ for MCF-7 compared with other compounds. The synthesized chitosan derivatives showed less cytotoxicity in normal cell lines MCF-



10A and MRC-5, with $\text{IC}_{50} > 100\ \text{g/ml}$, indicating that their use is safe. **Scheme 1** illustrates the synthesis of sulfonated chitosan derivatives.

XRD or X-Ray Diffraction study

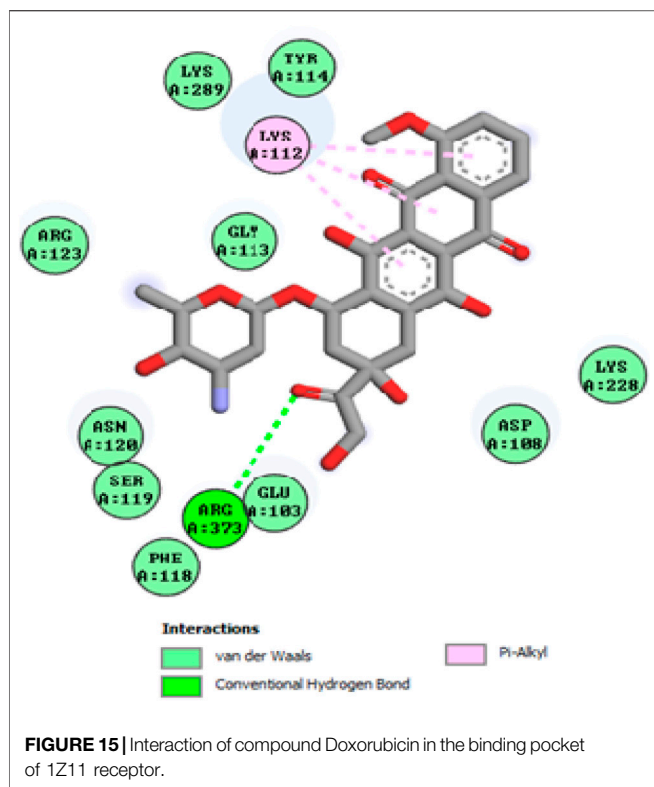
An X-Ray diffraction investigation of chitosan derivatives are shown in **Figure 2** (Kumar et al., 2009) it shows modest peaks for chitosan derivatives at 2θ of 20° . In chitosan derivatives the very sharp peaks at 20° became narrow, it indicates that chitosan derivative has moral compatibility and good development of porous xerogel network. According to the XRD results the chitosan derivative is amorphous in nature.

FTIR spectroscopy of highly Active compound 2h

In FTIR spectra of chitosan NH peak was observed at 3456cm^{-1} which can be assigned to stretching vibration of amino group, another peak observed at $3,278\text{cm}^{-1}$ can be attributed to hydroxyl group (OH , str). Similarly the stretching vibration of carbon and hydrogen in aromatic ring observed at $2,949\text{cm}^{-1}$. The sharp characteristic peak at 1647cm^{-1} can be attributed to stretching vibration of imine linkage ($\text{N}=\text{CH}$, str). In the chitosan-Schiffbase the stretching vibration of $\text{N}-\text{C}$ observed at 1381cm^{-1} can be assigned to ($\text{N}-\text{C}$, str), another sharp peak at 1324cm^{-1} can be attributed to stretching vibration of ($\text{S}=\text{O}$, str), and $\text{C}-\text{O}-\text{S}$ can be observed as a stretching vibration of 975cm^{-1} . The IR spectrum of highly active compound are shown in **Figure 3**.

Scanning Electron Microscopy

One of the most effective techniques for studying surface phenomena of prepared materials is the scanning electron microscope (SEM). The morphology of schiffbase



of sulfonated chitosan derivative 2 h is the particles with sizes of 200 nm. These observations show that the microstructures of chitosan changed after chemical

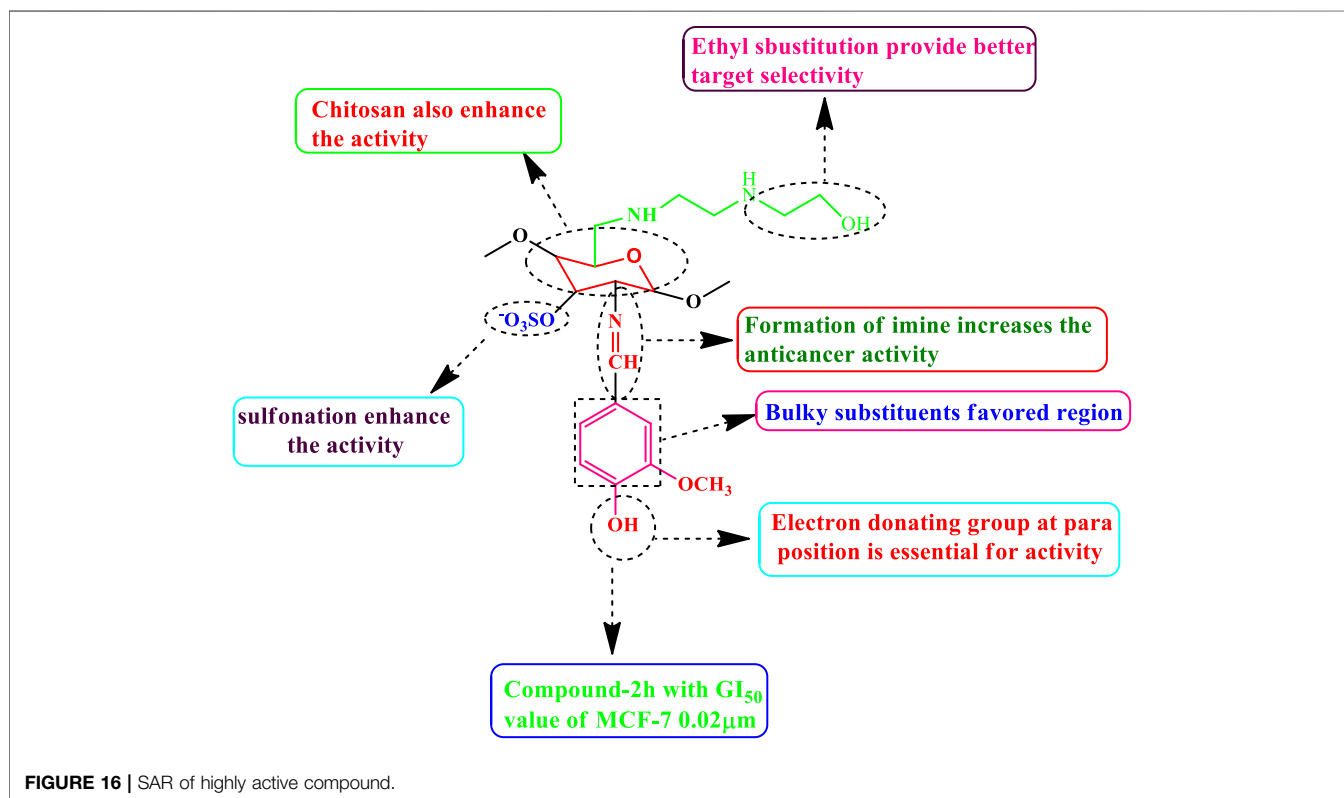
modification. The SEM image of chitosan analogues are shown in **Figure 4**.

Cytotoxic Activity

The newly synthesized Schiff base of sulfonated chitosan derivatives (2a-2j) were screened for their cytotoxic activity using MTT anticancer assay (dose of 100 μ M at 48 h) against MCF-7 cancer cell line, and also evaluated for their possible cytotoxicity in breast cancer cell (MCF-10A), and lung cells (MRC-5) by employing MTT assay. The assay results suggested that these compounds did not significantly affect normal breast, lung cells' growth (As most of the compound's IC₅₀ values are >100). The **Table 1** shows the growth inhibitor concentration (GI₅₀), total growth of inhibition (TGI), and lethal concentration (LC₅₀) values. Moreover the compound 2 h (GI₅₀ = 0.02 μ M) was relatively more active than standard doxorubicin and other compounds. The *in vitro* cytotoxicity of Schiff base of sulfonated chitosan derivatives of normal cell lines are given in **Table 2**. Finally, compound 2 h can be used as a lead compound to further develop more effective drugs for the MCF-7 (breast) cancer cell line.

Docking studies

To acquire a better understanding of the future evolution of biotic behavior, docking mock-ups were added. The docking impact of compounds 2a-2j and Doxorubicin regulation with protein 1Z11 was tested using the Autodock Vina software. Compound 2 h has a higher binding affinity for 1Z11 protein (-5.9 kcal/mol) than other compounds and a lower binding affinity for Doxorubicin (-5.3 kcal/mol). Compound 2 h makes two hydrogen bonds with



the 1Z11 receptor. In the hydrogen bonding interaction, the amino acid residues Phe118 and Asn120 were active. In hydrophobic interaction, the amino acid residues Arg101, Gly102, Glu103, Asp108, Trp109, Lys112, Gly113, Ser119, Arg123, Lys228 and Arg373 were active. Doxorubicin regulates the 1Z11 receptor by forming one hydrogen bonds with it. In the hydrogen bonding interaction, the amino acid residues Arg373 were active. The hydrophobic interactions of Glu103, Asp108, Lys112, Gly113, Tyr114, Phe118, Ser119, Asn120, Arg123, Lys228 and Lys289 were observed. **Figures 6, 7** show the hydrogen bonding and hydrophobic interactions of amino acid residues in the 1Z11 protein with the 2a-2j and Doxorubicin compounds, respectively. The results suggest that compound 2 h has a significant inhibitory effects for Doxorubicin regulation in the target protein. The docking interaction results are present in **Table 3**. All the docking images are given in **Figures 5–15**.

Structure Activity Relationship

The selected functional group plays important role in modifying the cytotoxic activity of compounds within a certain system can be identified via SAR analysis. Preliminary SARs might be analyzed using the cytotoxic activity data of the chitosan derivatives. Compound 2 h is the most effective (MCF-7, $GI_{50} = 0.02 \mu\text{M}$) control doxorubicin among the chitosan derivatives. Owing to the occurrence of chitosan moiety fused to a vanillin, it was revealed that the new compound acquires a strong cytotoxic effect against cancer cell types the presence of electron donating hydroxyl group in para position enhances the activity as well as sulfonation of chitosan also increases the anticancer activity. SAR of highly active compound present in **Figure 16**.

CONCLUSION

In this paper, we synthesized chitosan derivatives of 2a-2j that were characterized via FTIR, NMR, and morphology study was carried out by XRD and SEM. The synthesized compounds 2a-2j were assessed for their cytotoxic activities against the MCF-7 cancer cell line, normal cell lines of breast cancer cell MCF-10A and lung cell MRC-5 using an MTT assay. Doxorubicin was used as a standard. Compound 2 h was

found to be highly active, with a GI_{50} value of $0.02 \mu\text{M}$ for MCF-7 compared with other compounds. The synthesized chitosan derivatives showed less cytotoxicity in normal cell lines MCF-10A and MRC-5, with $IC_{50} > 100 \text{ g/ml}$, indicating that their use is safe. Furthermore *In silico* molecular docking analysis against the 1Z11 receptor has been studied, compound 2 h has a higher binding affinity for 1Z11 protein (-5.9 kcal/mol) compared with other compounds and Doxorubicin (-5.3 kcal/mol). Finally, the compound 2 h has great activity, and therefore it will be researched for further anticancer drug.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

PP: Analysis of organic compound and spectrum analysis; PG: organic compounds preparation; DA: provision of study materials; SA: Financial Support; RG: Manuscript editing; AI: All kinds of spectral analysis and RS: Project administration and all aspects of this manuscript's chemistry and biology have been investigated. The manuscript was writing original draft preparation through the contributions of all authors.

FUNDING

This work was funded by the Researchers Supporting Project number (RSP-2021/27), King Saud University, Riyadh, Saudi Arabia.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2021.796599/full#supplementary-material>

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