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Research Article

Synthesis, characterization of thia-crown ether sugar derivatives and evaluation of their anticancer activity with computational insight

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Abstract

Several novel macrocyclic chiral derivatives were prepared and developed by a simple, practical and efficient synthetic protocol for easy access to sugars-linked crown moiety from cheap and easy existing D-glucose in good yields. The structures of macrocyclic chiral derivatives are confirmed by FT-IR, 1HNMR, 13CNMR and mass spectroscopy. The recently produced macrocyclic derivatives underwent assessment for their in vitro anticancer properties against human cells. The investigation involving breast (MCF-7) cancer cell lines and non-cancer cells, specifically the MCF-12A cell line, demonstrated that macrocycles (8, 9, 10, and 11) exhibit potential as anticancer binders. They achieved this by impeding cell migration and proliferation and modulating the expression of crucial anticancer genes, including those associated with breast cancer (MCF-7), p53, mda7, and trail. The anti-proliferative effect of macrocycles on tumour cells was evident, with no observed cytotoxic impact on normal cells. Computational elucidation of the anticancer effect revealed that the binders exhibited a more promising binding affinity, indicating a level below. (BA; -6 kcal/mol) against mitogen-activated protein kinase 8 (3ELJ) comparable to Xeloda. Hence, these outcomes could encourage the researchers to open a new destination toward the synthesis of effective antitumor drugs.

Keywords: Anticancer, computationally elucidated, Cytotoxicity assays – MTT, Macrocycles, Thia-crown ether, Molecular docking. Template method.

Introduction

Cancer comprises a diverse group of diseases marked by the uncontrolled growth and dissemination of abnormal cells (D'Arcy, 2019; Ikwegbue et al., 2018). It represents a significant public health concern in various global regions and stands as one of the leading causes of mortality, alongside cardiovascular disease.(Turner et al., 2020). Cancer accounts for one in every eight deaths globally.(Dhillon et al., 2018) The World Health Organization predicts that Cancer will cause 15 million deaths worldwide by 2030.(Cao et al., 2018) Over 200 malignancies impacting vital organs such as the kidney, lung, brain, breast, colon, skin, bladder, and stomach have been identified to date, with ongoing discoveries likely to reveal additional types.(Guo et al., 2012) Anticancer chemicals and antibiotics eliminate carcinomas via inhibiting genes or proteins that are necessary for the life of cancerous cells in the body.(Sehrawat et al., 2021) For example, mitogen-activated protein kinase 8 is responsible for regulating gene expression by phosphorylating transcription factors and plays an important role in many cellular and/or physiological functions such as tumor cell apoptosis, cell migration, regulation of obesity and insulin resistance. (Gomez-Pastor et al., 2018; Rezatabar et al., 2019) In this context, Capecitabine is a major antimetabolic and antineoplastic prodrug. It shows many aspects of improving selectivity and reducing the plasma level of 5-fluorouracil by blocking cells production and DNA repair of cell tumors. However, we are now facing the emergence of carboplatin resistant carcinomas.(Avendaño & Menendez, 2015) so designing, developing, and synthesizing novel anti-tumour medicines has always been a difficult task for scientists(DeSantis et al., 2014; Siegel, DeSantis, et al., 2014; Siegel, Ma, et al., 2014). In many countries especially in developing countries because choosing lifestyle decisions, such as smoking, alcohol drink, westernized foods, and insufficient sport exercise all these causes increase the incidence of cancer (Torre et al., 2015). Several anti-tumour medications have negative impacts on human health, so the big challenge for scientists is how to prepare or develop new affective molecules of cancer drugs that are highly chemically stable and cost-effective, with negligible side effects to healthy cells (Demain & Vaishnav, 2011).

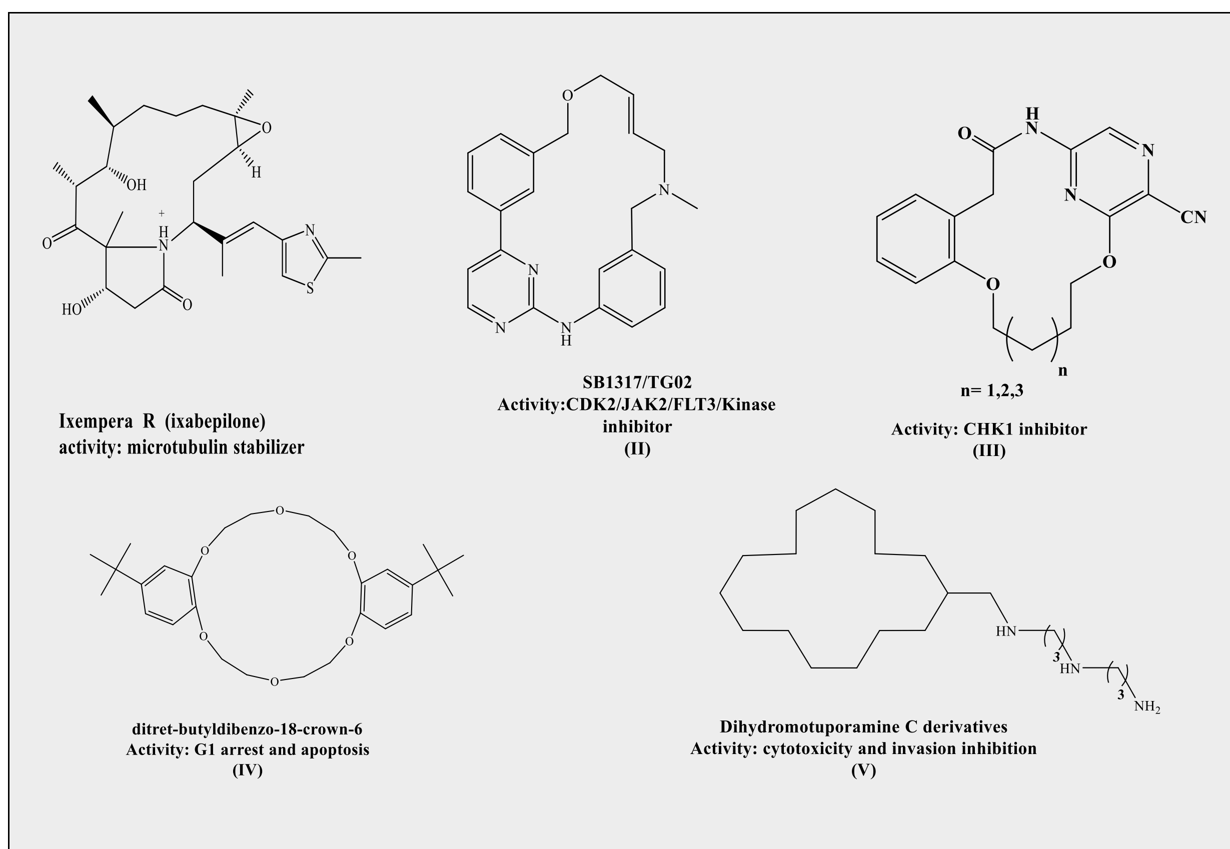


Figure 1. Exemplary instances of biologically active macrocyclic molecules

Many natural products, particularly macrocyclic molecules, have pharmacological significance and have played an essential role in drug (Campos et al., 2019; Zhai et al., 2022) the best alternative among macrocycles because they have unique properties like as (i) The structural rigidity, regioselectivity, complexity, and stereogenicity of macrocyclic molecules(ii) potential hydrogen contribute to their ability to interact with several biomolecules for instance proteins (enzymes), DNA, RNA,(Rose et al., 2015; Veber et al., 2002) (iii) significant degree of chemical structural pre-organization, resulting in contact and attachment of necessary functional groups across the active site of extended proteins(Driggers et al., 2008) (iv) ‘drug-like pharmacokinetic properties in addition to physicochemical (Yudin, 2015). For example, small macrocyclic compounds produced from natural sources (I)(Shuch, Brian; (Lappin, 2003; Rohena & Mooberry, 2014) and numerous synthetic macrocycle apoptosis was observed, (Long et al., 2007) and 15-membered macrocycles had the maximum cytotoxicity (Tuenter et al., 2017) (Figure 1).

Furthermore, macrocyclic compounds displayed anti-tumor, antibacterial, anti-HIV, and antifungal activity.(Peters et al., 2018; Rajakumar et al., 2014; Yan et al., 2018)· Tertiary amines

and alkali metal ions are well-known to form cationic complexes derivatives with crown ethers. Macrocyclic molecules have found application as anticancer agents, drug delivery systems, and key components in DNA-attachment agents and drug molecules. This is attributed to their distinctive complexing capabilities, making them well-suited for biological targets in various therapeutic and biomedical contexts. (Gül et al., 2020). In studies focusing on DNA binding, researchers observed that the positive charge found in cation crown ether complexes improves the affinity of molecules linked to crown ethers for the polyanionic phosphate group backbone of DNA. As a result, medications derived from crown ethers might exhibit an enhanced capability to interact more efficiently with DNA, forming cationic complexes with ions prevalent in cells. This discovery implies potential applications in drug development, where the modulation of these interactions could be utilized for therapeutic purposes.(Mikušová & Mikuš, 2021; Rodríguez-Vázquez et al., 2016). Considering the diverse pharmacological and biomedical applications of macrocycles, along with the challenges associated with their synthesis, as of our last knowledge update, only a limited number of papers have been reported in the literature on employing Thia-Crown Ethers Derivatives Based Methyl α-D-glucose as anticancer drugs with computational insight. The present work aimed to design and synthesize (8,9,10, and 11) from easily available starting materials utilizing simple organic transformations. For the current design and synthesis, the following factors were used as a foundation: (i) The likelihood of macrocyclic thia-ethers being employed in the synthesis of biologically active compounds. (Sukhorukov & Ioffe, 2011) and (ii) access to a variety of topologies(Sreedaran et al., 2008; van Dijk et al., 2018) sulfur atoms in thia-crown ether were shown to be amiable to disrupt protein-protein interactions (Cragg, 2010). Mitogen-activated protein kinase 8 (MAPK8) or (JNK1) is physio-pharmacological importance (Jagodzik et al., 2018).

Experimental part

Chemicals and Instrumentals

Chemicals including Me-α-D-glucopyronside (Sigma Aldrich, USA, 99%), Benzaldehyde dimethyl acetal (Sigma Aldrich, USA, >99.8%), benzyl chloride (99%, Sigma Aldrich, USA, 99%), bis(2-chloroethyl) ether (Sigma Aldrich, USA, 99%), Sodium iodide (Merck , Germany) sodium sulfide non a hydrate (Fluka, Switzerland, 99%), Sodium hydroxide (Merck , Germany, 99.5%), Tetra butyl ammonium bromide (B.D.H, England, 99.4%) Acetone (Fisher, USA, 99.8%) Chloroform (Sigma Aldrich, USA, 99.8%). Ethanol (J.T.Baker, Netherlands, 99.7%) Toluene

(Fisher, USA, 99.9%). Ethyl acetate (Sigma Aldrich, USA, 99%). n-hexane, (J.T.Baker, Netherlands, 99.7%). The chemical structures of the synthesized organic compounds were verified using ^{13}C and ^{1}H NMR analyses conducted on Bruker DRX AVANCE 500 and 600 MHz spectrometers for proton and 150 MHz for carbon in $CDCl_3$ and(DMSO)-*d6* solvents. Without making any adjustments, melting points in open capillaries were found. The fast atom bombardment (FAB) method was used to analyze mass spectra on a VG 70-70EQ mass spectrometer utilizing nitro benzyl alcohol as a matrix and an 8 Kv Xe atomic beam. On 60 F254 plates (Merck), thin-layer chromatography (TLC) was used to track the development of the reactions. Either column chromatography on silica gel (Kieselgel 60, 0.063–0.100 mm, Merck) or crystallization were used for purification. To suggest a possible mechanism or mechanisms accountable for the observed anticancer activity, computational elucidation was utilized.

Chemistry part

Synthesis of compound 2

(20g, 103 mmol) of Methyl α-D-glucopyronside compound1 was dissolved in 100 mL of N,N-Dimethylformamid , then (20g, 131.6 mmol) of benzaldehyde dimethyl acetal Was introduced into the reaction container and the medium was acidified by (0.10 g, 0.585mmol) toly-sulfonic acid. Using a rotary evaporator, the mixture was stirred until thin-layer (TLC) revealed that there was no more starting material. The organic solvent was vacuum-expelled, and the residue was extracted using a mixture of chloroform and water to get the crude, which was then co-evaporating twice with a mixture of toluene and water (50:50) to produce a soft, white powder (compoun2). The Yield (16.3g, 56%). m. p.= (161-163) ºC.

A solution comprising alcohol (10 mmol), alkyl halides (10 mmol), and the catalyst (0.5 mmol) in a toluene and 50% aqueous NaOH (10 mL) mixture was subjected to stirring under varying temperature and duration conditions. The conclusion of the reaction was tracked through TLC (petroleum ether–ethyl acetate 4:1). After catalyst recovery via filtration, 5 mL of toluene was introduced, and the organic layer was separated using a separating funnel. This layer underwent two washes with 10 mL of water, followed by drying with ($MgSO_4$) anhydrous filtration, and subsequent removal of the solvent under vacuum. The resulting crude materials were then purified through column chromatography.

Compound (2): Chemical Formula: $C_{14}H_{18}O_6$, Molecular Weight: 282.29, FT-IR peaks: (3332, 2972, 2885, 1473, 1387, 1242) cm^{-1}, ^{1}H NMR: δ (ppm) 7.48–7.24 (5H; m, Ar-H), 5.53(*J*=3.5 Hz,

1H, d, H-1) 4.86, (1H; s, Ph-CH), 4.29 (*J*=10.9, 4.9 Hz ,1H; dt, H6-ax), 4.00(*J*=9.3 Hz, 1H, dd, H-4), 3.93(*J*=10.9, 4.9 Hz 1H; dd, H6-eq), 3.80(*J*=9.7, 3.6 Hz, 1H; dd, H-3), 3.61(*J*=9.7, 3.6 Hz, 1H; dd, H-2), 3.44 (3H; s, OCH_3), 3.26 (*J*=10.1, 9.1 Hz, 1H; dd, H-5), ^{13}C NMR: δ 138.86 (1C Aromatic, C), 129.48, 128.52, 126.26, (5C Aromatic, CH), 101.82 (1C, C-1), 98.33(1C, PhCHO), 81.77 (1C, C-4), 79.63(1C, C-3), 79.53 (1C, C-2), 68.97 (1C, C-6), 61.99 (1C, C-5), 55.65 (1C, OCH_3).

General procedure of the phase transfer catalysis

A mixture of 20 mmol of alcohol, 25 mmol of alkyl halides, and 10 mmol of tetra butyl ammonium bromide in 100 mL of toluene underwent vigorous stirring with 40 mL of 50% NaOH solution at 25 ºC (Dehmlow, 1977). After phase separation, the organic layer was thoroughly washed with water, dried over magnesium sulfate, and the crude product was obtained by evaporating the solvent.

Synthesis of compound 3

Compound 3 was synthesized according to the general procedure [2.2.2]. (16.3g, 57.7 mmol) of compound 2, (11g, 120 mmol) of benzyl chloride, and (1.61g, 5 mmol) tetra butyl ammonium bromide, Product 3 the yield (20.2g, 43.63 mmol, 75.6%). m. p. = (183-185) ºC.

Compound (3): Chemical Formula: $C_{28}H_{30}O_6$, Molecular Weight: 462.54, FT-IR peaks = (3029, 2917, 2865, 1487, 1363, 1277) cm^{-1}, ^1H NMR: δ (ppm) 7.49–6.88(15H; m, Ar-H), 5.52(*J*=3.5 Hz, 1H, d, H-1), 4.87(*J*=10.9 Hz, 1H; s, Ph-CH), 4.83-4.66(4H; m, 2Ph-CH_2), 4.28(*J*=10.9, 4.9 Hz ,1H; dd, H6-ax), 3.92(*J*=9.3 Hz 1H, dd, H-4), 3.61(*J*=10.9, 4.9 Hz 1H; dd, H6-eq), 3.43-3.37(2H; m, H-3,H-2), 3.50(3H; s, OCH_3), 3.24(*J*=10.1, 9.1 Hz, 1H; dd, H-5), ^{13}C NMR: δ 139.50-137.58 (3C Aromatic, C), 129.06, 128.97, 128.37, 128.31, 127.01, 126.38, 126.18, (15C Aromatic, CH), 101.42 (1C, C-1), 98.59 (1C, Ph-CH), 82.35 (1C, C-4), 80.19 (1C, C-3), 78.38 (1C, C-2), 72.36 (2C, 2Ph-CH_2O), 70.88 (1C, C-6), 69.23 (1C, C-5), 54.3 (1C, OCH_3).

Synthesis of compound 4

A quantity of 20 g (43.63 mmol) of compound 3 was dissolved in a 1:1 mixture of methanol and chloroform (150 mL), and then, 0.10 grams (0.6 mmol) of toluenesulfonic acid monohydrate was added to the solution. For 12 hours, the resultant mixture was agitated. Thin-layer chromatography (TLC) analysis revealed the absence of any remaining starting material [38]. The mixture underwent two washes with saturated aqueous sodium carbonate followed by water, and it was

then dried over ($MgSO_4$). Evaporation of organic solvent (chloroform) yielded the crude product, which was nearly pure. The reaction provided a yield of 13.2 grams (35.3 mmol, 80.8%).

Compound (4): Chemical Formula: $C_{21}H_{26}O_6$, Molecular Weight: 374.43, FT-IR peaks = (3318, 2972, 2885, 1482, 1363, 1179) cm^{-1} , ^1H NMR: δ (ppm) 7.87–7.35(10H; m, Ar-H), 5.51(*J*=3.5 Hz, 1H, d, H–1), 4.63-4.35 (4H; m, 2Ph-CH_2), 4.13-3.97 (2H; m, H-4, H6-ax), 3.79 (*J*=9.7, 3.6 Hz, 1H; dd, H-3), 3.71(*J*=9.7, 3.6 Hz, 1H; dd, H-2), 3.64(*J*=10.9, 4.9 1H; dd, H6-eq), 3.46 (*J*=10.1, 9.1 Hz, dd, 1H; H-5) 3.38(3H; s, OCH_3), ^{13}C NMR: δ 139.50,137.58 (2C, Aromatic, C), 129.06, 128.97,128.37, 128.31, 127.01, 126.38, 126.18(10C, Aromatic, CH), 101.42 (1C, C-1), 82.60(1C, C-3), 83.80(1C, C-5), 81.80(1C, C-2), 74.84(1C, Ph-CH_2O), 74.82(1C, Ph-CH_2O), 69.81(1C, C-4), 61.60 (1C, C-6), 54.20(1C, OCH_3).

Synthesis of compound 5

The compound 5 was prepared based on to the general procedure [3.2], (13.2g, 35.4 mmol) compound 4, (200 mL) of bis(2-chloroethyl) ether and (6.47g, 20 mmol) of tetra butyl ammonium bromide. To furnished compound 5 as yellow the yield (15.4 g, 26.2 mmol, 74.1 %).

Compound (5):Chemical Formula: $C_{29}H_{40}Cl_2O_8$, Molecular Weight: 587.53, FT-IR peaks = (2952, 2867, 1493, 1372, 1130, 1298, 662) cm^{-1}, ^1H NMR: δ (ppm) 7.41–7.29 (10H; m, Ar-H), 5.61 (*J*=3.5 Hz, 1H, d, H-1), 4.96-4.61 (4H; m, 2Ph-CH_2), 3.98(*J*=10.9, 4.9 Hz ,1H; dd, H6-ax),3.93-3.80 (4H; m, 2 CH_2Cl), 3.79–3.73 (4H; m,2OCH_2), 3.74(*J*=9.7, 3.6 Hz, 1H; dd, H-2), 3.72(*J*=10.1, 9.1 Hz, 1H; dd, H-5), 3.71 (*J*=10.1, 9.1 Hz, 1H; dd, H-3), 3.70–3.60(9H; m, H-4, 4OCH_2), 3.51(*J*=10.9, 4.9 Hz, 1H; dd, H6-eq), 3.38(3H; s, OCH_3), ^{13}C NMR: δ 137.22, 137.19 (2C, Aromatic, C), 130.08, 130.06, 130.03, 130.0, 128.63, 128.59, 127.36, 127.33, 127.30, 127.28 (10C, Aromatic, CH), 102.07 (1C, C-1), 82.60 (1C, C-3), 81.15 (1C, C-4), 78.84(1C, C-2), 76.81 (1C, C-6), 73.07 (1C, C-5), 71.73 (1C, PhCH_2O), 71.18 (1C, PhCH_2O), 69.10, 69.05, 68.70, 65.34, 62.54, 62.36(6C, 6CH_2O), 55.47(1C, OCH_3), 39.97, 39.60(2C, 2CH_2Cl).

Synthesis of compound 6

Compound 5 (15.4 g, 26.2 mmol) was dissolved in 150 mL of dry acetone, and then 20 g (133 mmol) of sodium iodide was added to the solution. The mixture was refluxed for 72 hours. Subsequently, the solvent was evaporated, and the residue was dissolved in chloroform (100 mL) and thoroughly washed with water. A crude product was produced by separating the organic layer, drying it on magnesium sulfate, and then evaporating it using a rotary evaporator. This crude

material underwent purification through column chromatography, leading to the formation of compound 6 as a light-yellow syrup (18.8 g, 24.4 mmol, 93.2%).

Compound (6):Chemical Formula: $C_{29}H_{40}I_2O_8$,Molecular Weight: 770.44, FT-IR peaks = (2910, 2865, 1622, 1488, 1357, 1220, 1136, 918, 617) cm^{-1}, ^1H NMR: δ (ppm) 7.41–7.29 (10H; m, Ar-H), 5.64 (*J*=3.5 Hz, 1H, d, H-1), 4.97 (*J*=10.9 Hz, 1H; d, $PhCH_2$), 4.95 (*J*=10.9 Hz, 1H; d, $PhCH_2$), 4.85 (*J*=12.1 Hz, 1H; d, $PhCH_2$), 4.84 (*J*=12.2 Hz, 1H; d, $PhCH_2$), 3.93–3.81 (5H; m, H-5, $2OCH_2$), 3.76- 3.72 (2H; m, H-2, H-3), 3.84 (*J*=10.9, 4.9 Hz ,1H; dd, H6-ax), 3.64–3.54 (8H; m, $4OCH_2$), 3.60 (*J*=9.3 Hz, 1H, t, H-4), 3.54 (*J*=10.9, 4.9 Hz, 1H; dd, H6-eq), 3.40 (3H; s, OCH_3), 3.06–3.02 (4H; m, 2 CH_2), ^{13}C NMR: δ 138.32, 138.19 (2C, Aromatic, C), 128.44, 128.38, 128.13, 127.99, 127.89, 127.57, 126.21(10C, Aromatic, CH), 98.21 (1C, C-1), 81.77 (1C, C-3), 79.68 (1C, C-4), 78.05 (1C, C-2), 75.63 (1C, C-6) 73.39 (1C, C-5), 72.15-71.93 (2C, $2PhCH_2O$), 71.86, 70.90, 70.38, 70.14, 70.02, 69.63 (6C, $6CH_2O$), 55.18 (1C, OCH_3), 3.02,2.90 (2C, CH_2I).

The General procedure of hydrogenation and synthesis compound 7

The compound 6 (18.8 g, 24.4 mmol) The compound was dissolved in dry methanol (100 mL) within a three-necked round-bottom flask. (50 mL) and 5% palladium on charcoal (1 g, 9.4 mmol) and glacial acetic acid (5ml) was added. After flashing out the air with a stream of hydrogen gas in the fume hood. The reaction was stirred under hydrogen pressure using a hydrogen bump reactor for 24 hrs. The palladium catalyst was filtered off, and the methanol was evaporated to obtain compound 7 as a light-yellow syrup (10.1g,17.1 mmol, 70.2%).

Compound (7): Chemical Formula: $C_{15}H_{28}I_2O_8$, Molecular Weight: 590.19, FT-IR peaks = (3392, 2922, 2853, 1357, 1146, 1216, 639) cm^{-1},^1H NMR: δ (ppm), 5.73 (*J*=3.5 Hz, 1H, d, H-1), 4.05–4.00 (5H; m, H-5, $2OCH_2$), 3.95 (*J*=9.7, 3.6 Hz, 1H; dd, H-2), 3.91 (*J*=10.1, 9.1 Hz, 1H; t≈dd, H-3), 3.88 (*J*=10.9, 4.9 Hz, 1H; dt, H6-ax), 3.66 (1H; m, H6-eq), 3.58–3.50 (8H; m, $4OCH_2$), 3.46 (*J*=9.3 Hz, 1H, t, H-4), 3.44 (3H; s, OCH_3), 3.06–3.02 (4H; m, $2CH_2$), ^{13}C NMR: δ, 110.05 (1C, C-1), 86.30 (1C, C-4), 77.40 (1C, C-6), 74.90 (1C, C-5), 74.2 (2C, C-2 and C-3), 72.20, 72.18, 71.75, 70.52, 70.245, 69.95 (6C, $6CH_2O$), 55.14 (1C, OCH_3), 3.12 , 3.10 (2C, $2CH_2I$).

Synthesis of thia-crown ether 8

A quantity of 5 g (6.5 mmol) of compound 6 was dissolved in 150 mL of ethanol, and then 1.56 grams (20 mmol) of sodium sulfide nonahydrate was added. The mixture underwent vigorous

stirring for 4 hours, with Thin-layer chromatography (TLC) indicating the absence of any remaining starting materials. Subsequently, the solvent was evaporated, and the crude product was dissolved in chloroform (100 mL) and washed with saturated sodium carbonate solution, followed by water twice. The solvent was then evaporated under vacuum, and the resulting residue underwent column chromatography, leading to the isolation of thia-crown ether 8 as a yellow semisolid (2 grams, 3.6 mmol, 55.8%). The melting point was recorded as (170-172) ºC.

Compound (8):Chemical Formula: $C_{15}H_{28}O_8S$, Molecular Weight: 368.44, FT-IR peaks = (3334, 2943, 2834, 1373, 1270, 650) cm^{-1},^{1}H NMR: δ (ppm), 5.80 (*J*=3.5 Hz, 1H; d, H-1), 4.10 (*J*=10.1, 9.1 Hz, 1H; dd, H-5), 4.03–3.99 (4H; m, $2OCH_2$), 3.95-3.91(2H; m, H-2, H-3), 3.78 (*J*=10.9, 4.9 Hz ,1H; dd, H6-ax), 3.60 – 3.52 (8H; m, $4OCH_2$), 3.50 (*J*=9.3 Hz, 1H; dd, H-4), 3.44 (*J*=10.9, 4.9 Hz, 1H; dd, H6-eq), 3.28 (3H; s, OCH_3), 2.54 – 2.48 (4H; m, $2CH_2S$), ^{13}C NMR: δ, 109. 30 (1C, C-1), 86.40 (1C, C-4), 74.81 (1C, C-5), 74.64 (1C, C-2), 74.62(1C, C-3), 73.10, 73.08, 72.15, 71.52, 71.30 (5C, $6CH_2O$), 71.00 (1C, C-6), 69.40 (1C, CH_2O), 54.40 (1C, OCH_3), 31.92,31.88 (2C, $2CH_2S$).

Synthesis of compound 9

Compound 8 (1g, 2.7 mmol), ter-butyl bromoacetate (1g, 5.13 mmol) and tetra-butyl ammonium bromide (0.32 g, 1mmol) were subject to the general phase transfer catalyst procedure (2.3.7) to furnish after chromatography the macrocyclic 9 as yellow semisolid (0.45g, 28 %). m. p. = (198-201) ºC.

Compound (9):Chemical Formula: $C_{27}H_{48}O_{12}S$, Molecular Weight: 596.73, FT-IR bands compound 9 (1715, 1361, 1240, 740, 653) cm^{-1}, ^{1}H NMR: δ (ppm), 5.18 (J=3.5 Hz, 1H; d, H-1), 4.82-4.66(4H; m, $2CH_2COOR$) 4.20 (J=10.1, 9.1 Hz, 1H; dd, H-5), 4.06–4.02 (4H; m, $2CH_2O$), 3.84–3.80 (Hz, 1H; m, H-2, H-3), 3.78 (J=10.9, 4.9 Hz ,1H; dd, H6-ax), 3.72–3.58(8H; m, $4CH_2O$), 3.48 (J=9.3 Hz, 1H; dd, H-4), 3.41 (3H; s, OCH_3), 3.39 (1H; dd, H6-eq), 2.79–2.68 (4H; m, $2CH_2S$), 2.15–2.05 (18H; s, 6 CH_3), ^{13}C NMR: δ, 174.40 ,174.32 (2C, 2C=O), 107.80 (1C, C-1), 86.10 (1C, C-4), 81.62, 81.60 (2C, $2OC(CH_3)_3$) 80.82 (1C, C-2), 79.60(1C, C-3), 75.15(1C, C-5), 73.22, 73.20, 72.25, 70.52, 70.38 (5C, $5CH_2O$), 71.00 (1C, C-6), 69.60 (1C, CH_2O), 67.60, 67.58 (2C, $2CH_2COOR$), 54.45 (1C, OCH_3), 33.10, 33.08 (2C, CH_2S), 27.90, 27.89, 27.86, 27.85, 27.80, 27.78 (6C, $6CH_3$).

Synthesis of compound 10

Compound 8 (1g, 2.7mmol), bromoacetonitrile (0.25g, 2.1mmol), and tetra butyl ammonium bromide (0.32g, 1mmol) were cooled down to (-5ºC) before being subjected to the general phase transfer catalyst procedure (2.3.7). After chromatography, the resulting product was the macrocyclic compound 10, obtained as a yellow syrup. (0.4g, 36.3%). m. p. = (185-187) ºC.

Compound (10): Chemical Formula: $C_{19}H_{30}N_2O_8S$, Molecular Weight: 446.52, FT-IR peaks compound 10 (3066, 2953, 1379, 2236, 1213, 764, 648) cm^{-1}, ^1H NMR: δ (ppm), 5.80 (*J*=3.5 Hz, 1H; d, H-1), 4.79–4.73 (4H; m, 2 CH_2CN), 4.10 (*J*=10.1, 9.1 Hz, 1H; dd, H-5),4.04–4.00 (4H; m, 2CH_2O), 3.82 (*J*=10.9, 4.9 Hz, 1H; dd, H6-ax), 3.72– 3.61 (10H; m, H-2, H-3, 4CH_2O), 3.5 (*J*=9.3 Hz, 1H; dd, H-4), 3.42 (1H; m, H6-eq), 3.36 (3H; s, OCH_3), 2.54–2.48 (4H; m, 2CH_2S), ^{13}C NMR: δ, 128.39, 126.13 (2C, C≡N), 102.10 (1C, C-1), 81.65(1C, C-4), 81.54(1C, C-2), 80.97 (1C, C-3), 78.72 (1C, C-5), 75.26, 72.35, 72.12, 72.04, 71.98 (5C, CH_2O), 70.47(1C, C-6), 68.81(1C, CH_2O), 66.11, 66.14(2C, 2CH_2CN),56.50(1C, OCH_3), 30.95(2C, 2CH_2S).

Synthesis of Compound 11

Compound 8 (1g, 2.7 mmol) dissolved in pyridine (20 mL) reflux to (65°C) before adding the 2-bromoacetic acid (0.5 g, 4.9 mmol) and stirring overnight. The organic solvent was evaporated and the residue was dissolved in chloroform (100 mL) and washed several times with (5%) HCl solution and then with water twice, dried over anhydrous magnesium sulfate and the organic solvent was evaporated to give after chromatography the macrocycle 11 as a dark yellow syrup (0.7 g, 53.3%). m. p. = (168-170) ºC.

Compound (11): Chemical Formula: $C_{19}H_{32}O_{12}S$, Molecular Weight: 484.51, FT-IR bands compound 11 (2978, 2932, 1764, 1393, 1253, 664) cm^{-1}, ^1H NMR: δ (ppm), 5.86 (*J*=3.5 Hz, 1H; d, H-1), 4.68–4.58 (4H; m, 2CH_2COOH), 4.10 (*J*=10.1, 9.1 Hz, 1H; dd, H-5), 3.94–3.90 (4H; m, 2CH_2O), 3.82 (*J*=10.9, 4.9 Hz,1H; dd, H6-ax), 3.77-(*J*=9.7, 3.6 Hz, 1H; dd, H-2), 3.75 (*J*=10.1, 9.1 Hz, 1H; dd, H-3), 3.69 – 3.60 (8H; m, 4CH_2O), 3.50 (*J*=9.3 Hz; 1H; t, H-4), 3.4 (3H; s, OCH_3), 3.42 (*J*=10.9, 4.9 Hz,1H; dd, H6-eq), 2.52– 2.46 (4H; m, 2CH_2S), ^{13}C NMR: δ 174.1 (2C, 2C=O), 107.88 (1C, C-1), 84.72 (1C, C-4) 79.77 (1C, C-2), 78.98(1C, C-3), 75.2 (1C, C-5), 73.12, 73.10,72.07, 70.70, 70.56 (5C, 5CH_2O), 70.40, (1C, C-6), 68.92 (1C, CH_2O), 68.13 (2C, 2CH_2COOH), 54.82 (1C, OCH_3), 32.46, 32.44 (2C, 2CH_2S).

Cell line with breast cancer cell (MCF-7)

Culturing of cancer cell lines

Cells of all types were cultured at 37 ºC with 5-8% CO2. The subculturing of MCF-7 breast cancer cells involved trypsin treatment. DMEM media was selected based on the specific growth requirements of the cells. To put it briefly, 5 mL of HBSS was used to rinse the cells after the medium was aspirated. Subsequently, 3 mL of 1× Trypsin/EDTA (Lonza) was added, and the cells were incubated for 10-20 minutes. To each cell, 7.5 mL of McCoy's 5A and DMEM media were added for resuspension, and this process was repeated twice. All cell pellets underwent centrifugation at 200× g for 5 minutes. After resuspending the pellet with 6 mL of each media, complete media was used. Finally, a hemocytometer was used to count the cells in order to determine the proper quantity of cells to be seeded in the next studies.

Cytotoxicity assays – MTT

A 96-well plate with a flat bottom was seeded with $1x10^5$ cells/mL of complete media per well (DMEM + 10% FBS for MCF-7 cells, grown in McCoy's 5A medium + 10% FBS). Each well contained 100 µl of the media. After that, the plate was incubated for 24 hours at 37°C with 5-8% CO_2. Media removal and subsequent washing of cells with 100 µl HBSS were performed using a multichannel pipette. The novel compounds were prepared at varying concentrations (50, 100, and 200 µmolar) in DMEM + 1% FBS. Four replicate wells were treated by adding 100 µl of each concentration. Included in the controls were media devoid of any cells, chemicals not treated, and positive cell death (treated with 1% Triton X-100). The compounds were removed after three hours of incubation at 37°C with 5% CO2, and the cells were twice rinsed with HBSS. Following a 3-hour incubation at 37°C with 5-8% CO2 in the dark, 100 µl of MTT (Sigma M5655) at 0.5 mg/ml in free medium was added to each well. The medium was then gently removed, and the cells were once again rinsed with HBSS. Ultimately, a multichannel pipette was used to inject 100 µl of DMSO to each well. After allowing the plates to solubilize in the dark for one hour at room temperature, the absorbance at 540 nm was measured using an LT-4000 microplate read.

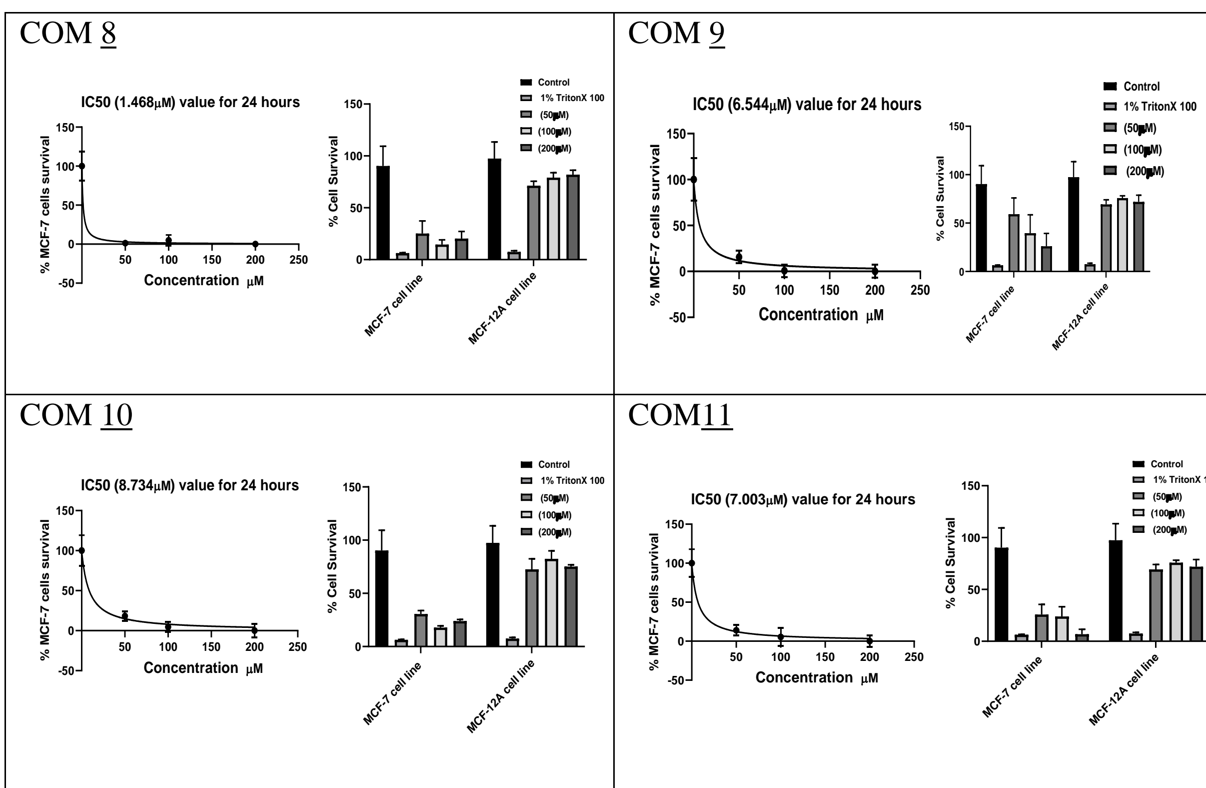


Figure 2. Cytotoxic activity with IC_{50} of compounds against MCF-7 breast cancer and normal cell lines.

MCF-12A cell lines: Breast normal cells, MCF-7: Breast cancer cells. After incubation overnight, the cells were treated with a new model compound for 24hrs, and then the cells were washed and treated with MTT reagent 5% for 3hrs as explained in section 3.1.2 The results are the mean of 3 independent experiments with triplicates.

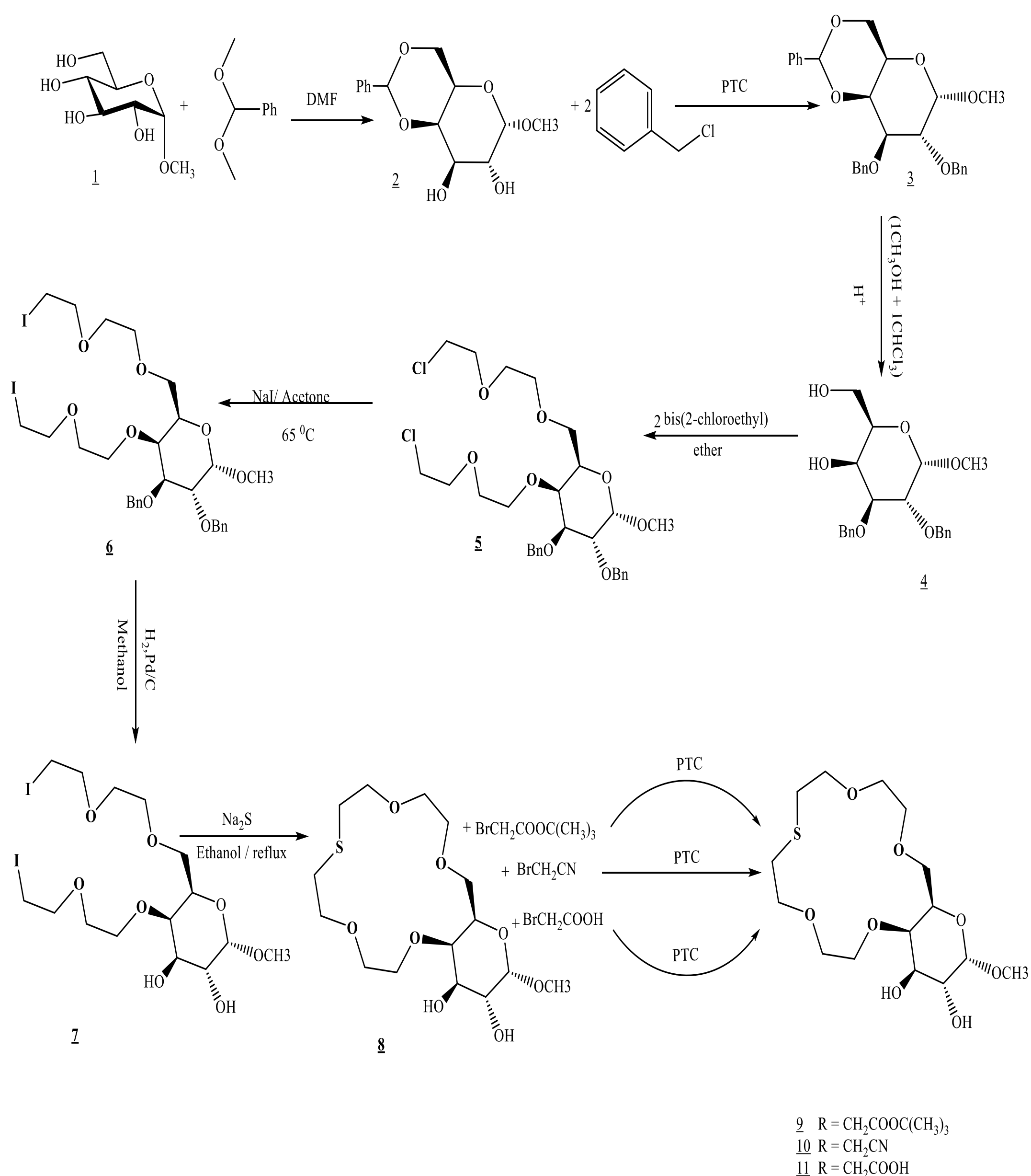
In silico antiviral assay

The computational calculations were conducted by configuring parameters for the grid box using the docking program VINA WIZARD integrated into PyRx software version 0.8 [39]. The docking utilized the crystallographic structure (PDB ID: 3ELJ) obtained from (PDB) the Protein Data Bank with a resolution of 1.8 Å, sourced from the Research Collaboratory for Structural Bioinformatics (http://www.RCSB.org). The ChemSketch was used for drawing the 3D (mol. format) Chemical structure of artificial ligand (http://www.cambridgesoft.com/software/overview .aspx). The PDB File (.pdb format) target structure has been prepared. The co-crystallized ligands were identified and deleted before processing. Water molecules were detached; both polar and nonpolar hydrogens were added. Gasteiger charges were computed using Molegro Virtual Docker software *version* 0.8 (Thomsen & Christensen, 2006) and Chimera software *version* 1.8.1

(http://www.rbvi.ucsf.edu/chimera) before submission to PyRx software. Type amino acids sequence and hydrogen bonds and its length (dotted lines) showing interactions with ligand structures were predicted using $LigPlot^+$ software (Laskowski & Swindells, 2011).

Results and discussion

The Thia-crown ether compound was prepared from an Methyl α-D-glucopyronside by a multi-step synthesis approach as illustrated in Scheme **1**.(Xie & Bogliotti, 2014) The synthetic scheme is significantly influenced by the distinct reactivity of the hydroxyl groups in the sugar, particularly in the case of methyl glucopyranoside. The synthesis plan is started with the protection of 4,6-position in the starting material with the common benzylidene protecting group (Therisod & Klibanov, 1987), which is selective and easy to introduce by simple treatment of glycoside **1** with benzaldehyde dimethyl acetal catalyzed by $TsOH.H_2O$ in DMF. To build up macrocycles on 4,6-positions, compound **2** was further protected by another more stable group, i.e. benzyl group, which is highly appropriated. The benzyl is stable and easy to introduce by phase transfer catalyst conditions. In addition, the diversity of protecting groups allows the selective removal of one of them on command (Hu, 2022). Under acidic conditions, such as using glacial acetic acid or $TsOH.H_2O$ in a chloroform: methanol mixture (50:50), the benzylidene group is eliminated, yielding products at the 4- and 6-positions(Badrinarayanan, 2016). These conditions can only remove the benzylidene group left the benzyl groups on 2- and 3-positions intact. The removal of benzylidene acetal leave the free hydroxyls on the 4- and 6- positions ready to be a good precursor(Wang & Demchenko, 2019). for construction of the macrocycle with specific chiral centers on the glucose moieties so compound 4 was subsequently treated with a large amount of bis(2-chloroethyl) ether is added to act as solvent as well as reactant under basic condition (NaOH) in the presence of phase transfer catalyst, (PTC) in room temperature to produce the compound 5 (Zhou et al., 2020).



Scheme 1. Synthesis of Thia-crown ether glucopyranoside derivatives

The transformation of chlorine to iodine in compound 5, which contains two chlorine atoms, was carried out by reacting it with sodium iodide (NaI) in acetone at its boiling point. This resulted in the formation of compound 6, which is now bis-iodo substituted. This modification was aimed at enhancing the electrophilic nature of the carbon atoms directly bonded to the halide moieties (Mishra et al., 2016). The compound 7 was synthesis to avoid the removal of benzyl protecting groups after converting it to the corresponding thia-crown ethers via hydrogenation(Fuji et al., 1979). The sulfur in the structure may be poison the palladium catalyst which is used for the removal of protecting groups, and hence, synthesis of other derivatives(Blaser et al., 2001).

Compound 6 was subjected to the hydrogenation reaction in presence of palladium catalyst to furnish compound 7 quantitively. This compound was then cyclized with slightly excess sodium sulfide nonahydrate in ethanol to produce macrocycle 8. The excess sodium sulfide of 1.5 equivalent could improve the yield significantly by reducing the side product. The high yield of ring closure can be interpreted in terms of the sodium templating effect. The sodium cation liberated after the first attack of Na2S was played as a template to pre-organize the ring (Götz et al., 2015). while the prepared Thia-crown ether with free hydroxyls in 2,3-positions were used to produce new derivatives 9,10 via phase transfer catalyst, (PTC) under basic condition (NaOH) with tert-butyl bromoacetate respectively and acetonitrile. Moreover, the acetylation of hydroxyl groups can also produce an ester(acetate groups) functionalized compound 11 macrocycle(Khalaf & Hasan, 2012). The newly synthesized derivatives underwent assessment for their potential as anticancer agents against both the MCF-7 breast cancer cell line and the non-cancerous MCF-12A cell line. In general, the synthesized compounds exhibited concentration-dependent effects on all cell lines, displaying a spectrum of anticancer activities ranging from potent to moderate effects. As the concentration increased, the cytotoxic effects intensified, leading to a corresponding decrease in cell viability. Notably, compound 8 demonstrated high activity against cancer cells, while compounds 9 and 11 exhibited greater activity against MCF-7 compared to compound 10. Furthermore, all compounds showing anticancer effects were more potent, as indicated by lower IC50 values, than capecitabine, an anticancer reference drug. The selection of capecitabine allowed for a comparative analysis of its docking binding affinity with activated protein kinase 8 (PDB 3ELJ) and a comparison with the novel conformers presented in Table 1. The target protein, 3ELJ, is the product of the MAPK8 gene in Homo sapiens (Human) and plays a crucial role in gene expression regulation by phosphorylating transcription factors (Hayes et al., 2009).

Table 1. Calculated scores for the molecular docking of novel compounds against mitogen-activated protein kinase 8 (PDB code 3ELJ) of breast cancer.

Ligands	**Binding energy (-Kcal mol^{-1})**	**RMSD ub^{-1*}**	**RMSD lb^{-1**}**
Com. 8	-9.4	0.227	0.227
Com. 9	-8.3	0.378	0.378
Com. 10	-7.8	7.114	3.371
Com. 11	-7.4	7.229	3.17
Xeloda (Capecitabine)- PubChem CID; 60953	-6.8	7.012	3.286

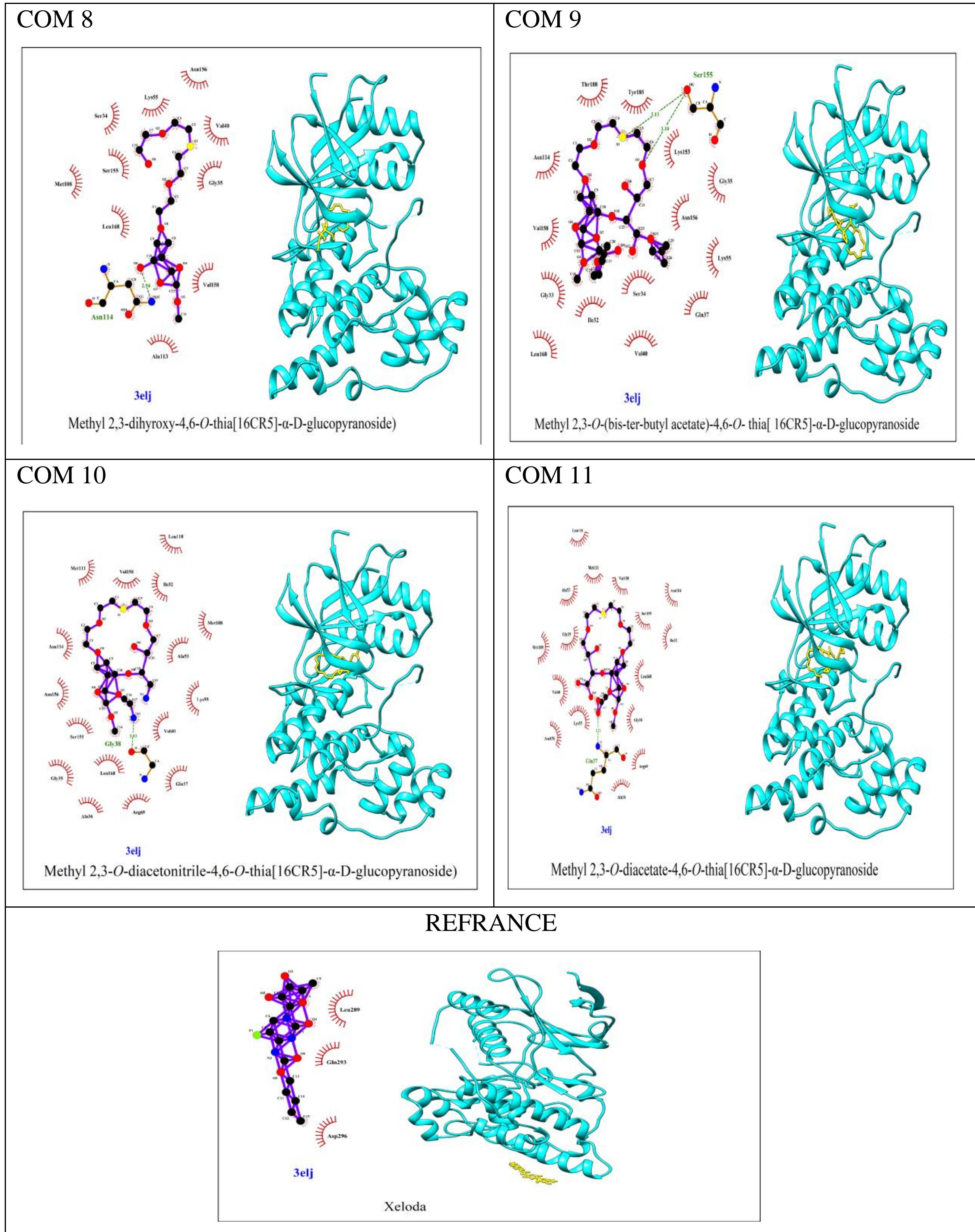


Figure 3. Binding affinity with 3ELJ with respect to capecitabine.

Based on docking results, 3ELJ-capecitabine demonstrations: Hydrophobic contacts involve corresponding atoms, non-ligand Asp296; Gln293; and Leu 289. capecitabine presented negative binding affinity with protein of breast cancer, mitogen-activated protein kinase 8 at -6.8 (kcal/mol) and docked via hydrophobic interactions (Figure 3; REFERENCE). The discovery of the new anti-tumor targets is a remarkable feature of our paper, which opens up a new prospect for multi-drug anti-tumor research. Wherever, 3ELJ-Methyl 2,3-dihyroxy-4,6-O-thia[16CR5]-α-D-glucopyranoside) demonstrations: non-ligand bond. Corresponding atoms and non-ligand Val158, Gly35, Val140, Asn156, Lys55, Ser34, Ser155, Met108, Leu168 and Ala113 involved in hydrophobic interactions. Hydrogen bonds between Methyl 2,3-dihyroxy-4,6-O-thia[16CR5]-α-D-glucopyranoside) and Asn114. 3ELJ- Methyl 2,3-O-(bis-ter-butyl acetate)-4,6-O- thia[16CR5]-α-D-glucopyranoside, demonstrations: non-ligand bond. Corresponding atoms and non-ligand Gln37, Lys55, Asn156, Gly35, Lys153, Tyr185, Thr188, Asn114, Val158, Gly33, Leu168, Ile32, Val140 and Ser34 involved in hydrophobic interactions. One H-bond was identified between Methyl 2,3-dihyroxy-4,6-O-thia[16CR5]-α-D-glucopyranoside) and Asn114 of 3ELJ in a molecular docking study. The O and S groups of Methyl 2,3-O-(bis-ter-butyl acetate)-4,6-O- thia[16CR5]-α-D-glucopyranoside took part in hydrogen bonds with Ser155 of 3ELJ were detected among these compounds. In addition, they formed several hydrophobic interactions with mitogen-activated protein kinase 8 (Figures, COM 8, COM9).3ELJ-Methyl-2,3-O-diacetonitrile-4,6-O-thia[16CR5]-α-D-glucopyranoside), demonstrations: non-ligand bond. Corresponding atoms and non-ligand Arg69, Gln37, Val140, Lys55, Ala53, Met108, Ile32, Leu110, Val158, Met11, Asn114, Asn156, Ser155, Gly35, Leu168 and Ala36 involved in hydrophobic interactions. Hydrogen bonds between Methyl 2,3-O-diacetonitrile-4,6-O-thia[16CR5]-α-D-glucopyranoside), and Gly38.3ELJ-Methyl-2,3-O-diacetate-4,6-O-thia[16CR5]-α-D-glucopyranoside, demonstrations: non-ligand bond. Corresponding atoms and non-ligand Ala36, Arg69, Gly38, Leu168, Ile32, Ser155, Asn114, Val158, Met111, Leu110, Ala53, Gly35, Met108, Val140, Lys55 and Asn156 involved in hydrophobic interactions. Hydrogen bonds between Methyl 2,3-O-diacetate-4,6-O-thia[16CR5]-α-D-glucopyranoside and Gln37. Methyl 2,3-O-diacetonitrile-4,6-O-thia[16CR5]-α-D-glucopyranoside), similar to Methyl 2,3-O-diacetate-4,6-O-thia[16CR5]-α-D-glucopyranoside, suitably docked with 3ELJ through hydrogen bond and hydrophobic interactions 8 (Figures, COM 10, COM 11).

Conclusion

A straightforward, useful, and effective synthesis procedure was created to create a variety of novel macrocyclic chiral derivatives with easy access to the sugar-linked crown moiety from readily available, affordable D-glucose in good yields. The structures of macrocyclic chiral derivatives are verified by mass spectroscopy, ^{1}HNMR, FT-IR, and ^{13}CNMR. In vitro anticancer properties of the recently synthesized macrocyclic derivatives were assessed on human breast cancer cell lines (MCF-7) and non-cancer cells (MCF-12A cell line). The results of *in silico* analysis showed that among the novel binders, Methyl 2,3-dihyroxy-4,6-*O*-thia[16CR5]-α-D-glucopyranoside) was higher negative BA to 3ELJ then capecitabine and would be regarded as a lead change to candidate to design anticancer drugs.

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