

RESEARCH

Open Access



# One of the 5-aminosalicylates drug, mesalamine as a drug repurposing lead against breast cancer

Dayanand Swami<sup>†</sup>, Shafina Siddiqui<sup>†</sup>, Urwashi Kumar, Shine Devarajan<sup>\*</sup>  and Jyotirmoi Aich<sup>\*</sup> 

## Abstract

**Background:** Breast cancer is the world's second leading cause of death in women. The problem of chemoresistance in breast cancer is proving to be a challenge for researchers and several oncologists all around the world. Current treatment modalities are associated with severe toxicities and lower efficiency. Hence, there is an unmet need for the development of novel drugs that can be used as adjuvants in breast chemotherapy. One of the strategies used to overcome this problem and that has received scientific coverage over the years is 'Drug Repurposing'. For this purpose, a list of 5-aminosalicylates drugs were evaluated for their drug repurposing potential in breast cancer. Mesalamine, sulfasalazine, balsalazide, and olsalazine were docked with high expression signatures in cancer cells such as EGFR (epidermal growth factor receptor), ER $\alpha$  (Estrogen Receptor alpha), Aromatase, mTOR (mammalian target of rapamycin), ALOX5 (Arachidonate 5-lipoxygenase), and Topoisomerase II.

**Results:** Docking analysis revealed that the selected ligands (drug) exhibited good binding affinity for all receptors. Based on the specificity with receptors, mesalamine was further selected for in vitro functional validation in a breast cancer cell line. Cell-based cytotoxicity assay in MCF-7 (Michigan Cancer Foundation-7) cells demonstrated the anticancer potential of mesalamine in breast cancer with IC-50 (Inhibitory Concentration) of 6.358  $\mu$ M.

**Conclusions:** Significant morphological alterations were observed in breast cells treated with mesalamine. Further studies are warranted to explore the anticancer effect of mesalamine in breast cancer and its role in combination therapies to be used as an adjuvant in chemotherapy.

**Keywords:** Drug repurposing, Breast cancer, 5-Aminosalicylates, MCF-7, Chemoresistance, Mesalamine

## Background

Cancer has proved to be one of the major causes of death throughout the world, comprising around 7.9 million deaths, which accounts for 13% of total deaths (Siegel et al. 2021; Sung et al. 2021). Breast cancer constitutes around 10.4% of total cancer cases and is in the fifth rank for causing maximum mortalities (Bhattacharyya et al. 2020). A number of side effects like fatigue, nausea,

vomiting sensation, drastic hair loss, drying of the mouth, and gastric issues generally accompany chemotherapy. In certain cases, the cancer cells develop resistance against chemotherapeutic drugs and even inactivate them through metabolic pathways (Wang et al. 2019). The risk of recurrence is also seen in patients undergoing chemotherapy. The inability of drugs to suppress or inhibit the proteins involved in disease progression leads to higher rates of recurrence and ultimately mortality (Mahvi et al. 2018; Beckwitt et al. 2018).

Drug repurposing (also known as drug repositioning or drug reprofiling) is a new, interesting, simple-yet-effective strategy, which involves the identification of new therapeutic uses of a drug, beyond its prescribed indication

<sup>†</sup>Dayanand Swami and Shafina Siddiqui have contributed equally to this work

<sup>\*</sup>Correspondence: [shinedevraj@dypatil.edu](mailto:shinedevraj@dypatil.edu); [jyoti.aich@gmail.com](mailto:jyoti.aich@gmail.com)

School of Biotechnology and Bioinformatics, DY Patil Deemed to be University, CBD Belapur, Navi Mumbai, Maharashtra 400 614, India

by the food and drug administration (FDA) (Oliveira and Lang 2018). It has several impressive advantages, such as reusing drugs, therefore overcoming the obstacle of drug shortage and saving huge time, cost and effort; its already determined pharmacokinetic, pharmacodynamic, bio-availability, and toxicity profiles make it easier to enter further stages of clinical trials. In the last few years, this approach is known to have gained momentum, especially in the field of oncology (Zhang et al. 2020).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most well-known and widely used medicines, specifically for inflammation and pain relief. In addition to their antipyretic, antianalgesic, and anti-inflammatory properties, they have also been reported to show efficacy in treating deadly diseases like cancer and heart-related condition (Bindu et al. 2020; Wongrakpanich et al. 2018). One of the most common examples of drug repurposing of NSAIDs is the use of aspirin. It is reported to have shown anticancer and chemopreventive properties in colorectal cancer (CRC). Apart from this, several other anti-inflammatory drugs repurposed are Naproxen, Sulindac, Celecoxib, Licofelone, and Diclofenac for treating CRC (Pantziarka et al. 2016; Mohammed et al. 2018).

Aminosalicylates are one of the different classes of NSAIDs, most popularly prescribed for inflammatory bowel disease (IBD) and ulcerative colitis (UC). They are used to transport its active ingredient- 'mesalamine' (5-aminosalicylic acid) to the sites targeted (Veloso et al. 2021; Lim et al. 2016; Ye and Langenberg 2015). The structure of mesalamine "mimics" that of salicylic acid, but differing in containing an amino group at the 5th position on the ring. It is even considered a first-line therapy used for treating mild to moderate UC and IBD, along with controlling their remission (Perrotta et al. 2015). Recent experimental and epidemiological evidence has demonstrated the inhibitory potential of mesalamine in CRC. It hampers and inhibits the growth of CRC cells by inhibiting the wnt/ $\beta$ -catenin pathway and activating the epidermal growth factor receptor (EGFR), two of the most important targets for CRC cells (Dixon et al. 2021; Stolfi et al. 2013). This study aims to delineate the drug repurposing potential of mesalamine in breast cancer. It starts with in silico screening of the potent drug candidates in order to predict and assess the binding affinities of ligands within the binding site of target receptors and developing initial repositioning hypotheses. This is followed by functional validation of repurposed drug in an in vitro cancer model.

## Methods

### In silico studies

The binding modes and the impact of drugs on the targets were investigated by molecular docking technique.

AutoDock Vina (Trott and Olson 2010) and Molecular Graphics Lab (MGL) Tools were used for docking: Open Babel (O'Boyle et al. 2011) for 3D conformation analysis, PyMol (Seeliger and Groot 2010) and Ligplot<sup>+</sup> (Abdullah 2020) for 3D and 2D visualization of protein–ligand complexes, respectively, and BIOVIA Discovery Studio (DS) Visualizer (BIOVIA, Dassault Systèmes 2021) for visualizing proteins and their interactions with ligands.

### Library of compounds

A class of NSAIDs (aminosalicylates—mesalamine, sulfasalazine, balsalazide, and olsalazine) were retrieved from PubMed and docked with various general breast cancer targets to investigate and predict their binding energies. Prior to docking, they were prepared to show optimum results. Compounds belonging to the 'aminosalicylate' family of NSAIDs were retrieved from the PubChem database in Structure Data File (SDF) file format. Ligands were subjected to energy minimization by applying the 'MMFF94' force field. The structure files were converted to pdbqt format using open babel. The ligands were docked to the receptors using the multi docking approach. The compounds were selected considering the binding affinity (Kcal/mol), specificity, and molecular recognition during the procedure.

### Therapeutic targets

3D X-ray structures of all receptors were retrieved and downloaded in Protein Data Bank (PDB) format from Protein Data Bank. The proteins used for docking are EGFR (PDB ID: 3ERT), Aromatase (PDB ID: 3EQM), ER $\alpha$  (PDB ID: 1A52), ALOX 5 (PDB ID: 6N2W), mTOR (PDB ID: 4JSV), and Topoisomerase II (PDB ID: 1ZXM). The proteins were prepared by removing water molecules, docked ligand(s) and other heteroatoms (HETATM) (if any) using BIOVIA Discovery Studio (DS) visualizer and saving it as an individual compound. This was followed by its energy minimization through the Swiss-PDB Viewer (SPDBV). The compound was uploaded onto AutoDock software version 4.2. Polar hydrogens were added and merged with non-polar hydrogens, along with applying Histidine hydrogens. Kollman charges were applied to the protein to determine the electron density and charge distribution. Lastly, the grid box (or grid map) was set appropriately for each receptor and calculated by AutoGrid. It contains a 3D lattice of regularly spaced points, surrounding either completely or partially, and centered on a region of interest of the compound under study, with a default grid spacing of 0.375 Å.

### Property calculation of compounds

The compounds should possess a desirable biological property and toxicity. Hence, the selected compounds

were further evaluated based on toxicity parameters using the SWISSADME program (Daina et al. 2017). The canonical SMILES of the compounds were used for identifying the properties which could be influenced in toxicity studies. These properties are essentially needed for understanding the structural alerts which can alter the functions later in biological studies.

### Cell-based cytotoxicity assay

#### Reagents and drugs

Sterile DMEM (Dulbecco's Modified Eagle Medium) media (CELL Clone™), sterile FBS (Pan Biotech), Penicillin–Streptomycin solution 100U/L (CELL Clone™), sterile Trypsin–EDTA 0.05% solution (Gibco Life Technologies), and sterile 1X PBS were used. The tablet form of mesalamine (Mesacol 800 mg) was procured by Sun Pharma Laboratories Pvt Ltd respectively. MTT reagent (SRL), dimethyl sulfoxide (DMSO) and Trypan Blue 0.5% solution (HiMedia Pvt. Ltd) were used. The MCF-7 breast cancer cell line was procured from National Centre for Cell Science (NCCS), Pune.

#### Cell culture

The cytotoxic effect of mesalamine was further checked on MCF-7 (procured from NCCS), a breast cancer cell line. MCF-7 was maintained in DMEM supplemented with 10% FBS in 37 °C with 5% CO<sub>2</sub> in a humidified incubator supplemented with penicillin–streptomycin solution 100U/L.

#### Cell cytotoxicity assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was performed. Approximately, 5000 cells per 200µL of sterile complete medium were seeded into each well of the 96-well microtiter plate and incubated for 24 h. After incubation, different drug concentrations (5 µM, 20 µM, 50 µM and 100 µM) were added to each lane except the control and again incubated for 24 h. The stock concentration of mesalamine was prepared in dimethyl sulfoxide (DMSO). Subsequent dilution of mesalamine was prepared in complete media. At the end of 24 h of drug treatment, MTT solution (5 mg/ml) was freshly prepared, filtered, and added to each well and incubated for 4 h for the formation of formazan crystals. After incubation, the medium was discarded and 50 µl of DMSO was added to fully dissolve the crystals. The absorbance of each well was recorded using the Bio-Rad microplate reader at a wavelength of 570 nm. Relative cell viability was expressed as a percentage.

#### Phase-contrast microscopy

Approximately, 10,000 cells of MCF-7 were seeded in 500 µl of complete medium (DMEM along with 10%

FBS) into each well of 24-well plates. Cells were further incubated with different concentrations (5 µM, 20 µM, 50 µM and 100 µM) of mesalamine for 24 h. Untreated cells were labeled as control. Phase-contrast microscopy of control and treated MCF-7 cells was performed using Nikon ECLIPSE TS100.

### Survival analysis

Publicly available level 3 TCGA RNASeq data for breast cancer ( $n=1097$ ) and CRC ( $n=380$ ) along with clinical information of patients were downloaded using UCSC Xena Functional Genomics Explorer. Data were  $\log_2(x+1)$  transformed and RSEM (RNA-Seq by Expectation–Maximization) normalized. Known targets {arachidonate 5-lipoxygenase (ALOX5) and peroxisome proliferator-activated receptor gamma (PPARG)} of mesalamine were chosen from the DurgSurv database and probed into TCGA data. Patients were divided based on the expression median value for their respective gene and classified as High expression class and Low expression class. R software was used to do survival analysis using *survival* packages. *Survminer* R package was used to generate Kaplan Meier plot.

### Statistical analysis

The experimental data were shown as mean  $\pm$  SE. One-way ANOVA followed by the Turkey HSD post hoc test was used to calculate the significance between control and treated groups. IC-50 was calculated by GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com). Statistical significance was set at  $P \leq 0.05$ .

## Results

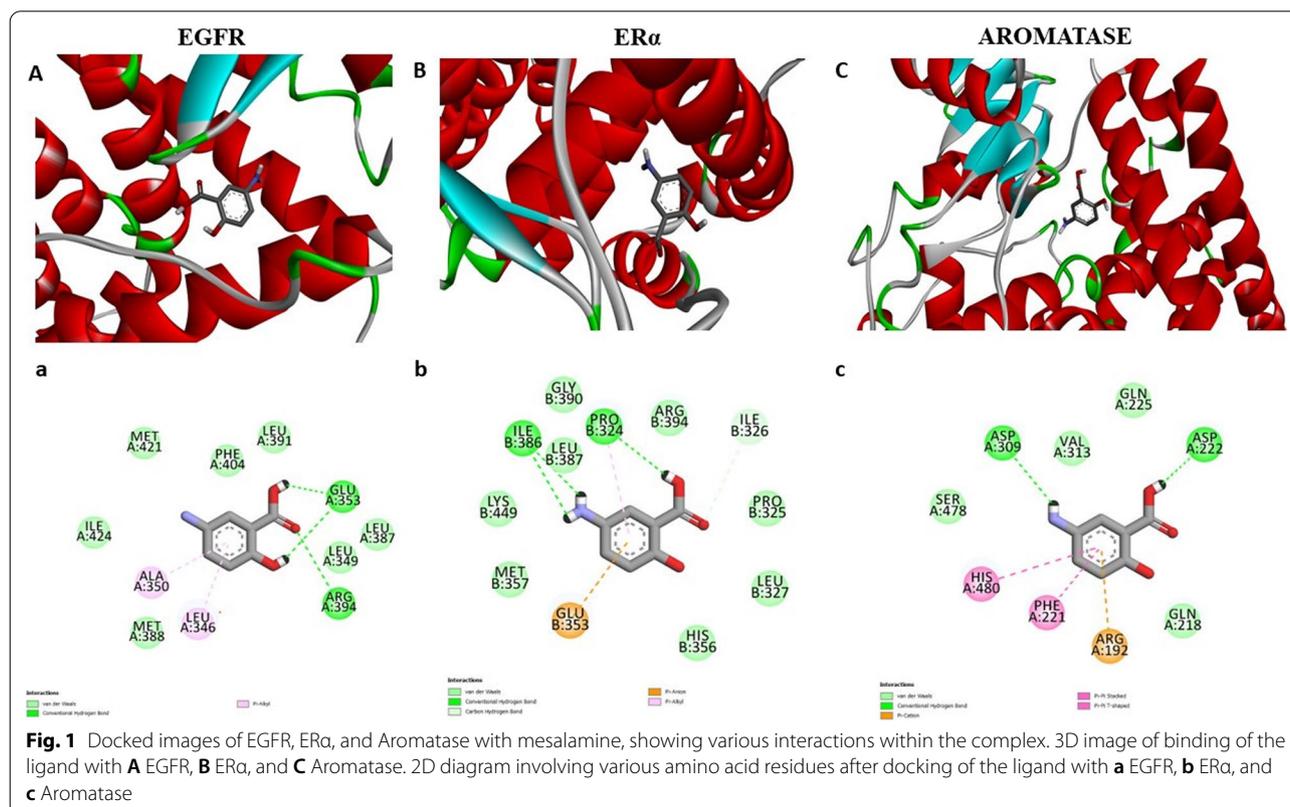
### Molecular Docking study

The docking analysis of aminosalicylates (mesalamine sulfasalazine olsalazine balsalazide) was done using AutoDock Vina software. The program generated 10 possible docking conformations of the ligand for each of the selected receptors. From the possible conformation, the best conformation was selected based on the binding energy and stability (number of hydrogen bonds, pi–pi, or van der Waals interactions). The grid box parameter used for docking was set as following Topoisomerase II:  $x=48$ ,  $y=34$ ,  $z=36$ , center:  $x=30.391$ ,  $y=-2.116$  and  $z=38.776$ ; Aromatase:  $x=44$ ,  $y=40$ ,  $z=42$ , center:  $x=86.146$ ,  $y=47.820$  and  $z=41.261$ . There are amino acids reported in the Topoisomerase II and Aromatase proteins that anchor the ligand in the binding site. In Topoisomerase II, GLU 87, ASN 91, ASN 120, SER 148 and LYS 168 are actively involved in the site. Whereas in Aromatase, ILE 305, ALA 306, ASP 309 (I helix), PHE 221 and TRP 224 (F helix), and ILE133 and PHE 134

from the BC loop also play a major role in the binding site. Moreover, VAL 370, LEU 372 and VAL 373 from the K helix  $\beta 3$  loop and LEU 477 and SER 478 in the  $\beta 8$ – $\beta 9$  loop also significantly affect the stability (Table 2) (BIOVIA, Dassault Systèmes 2021).

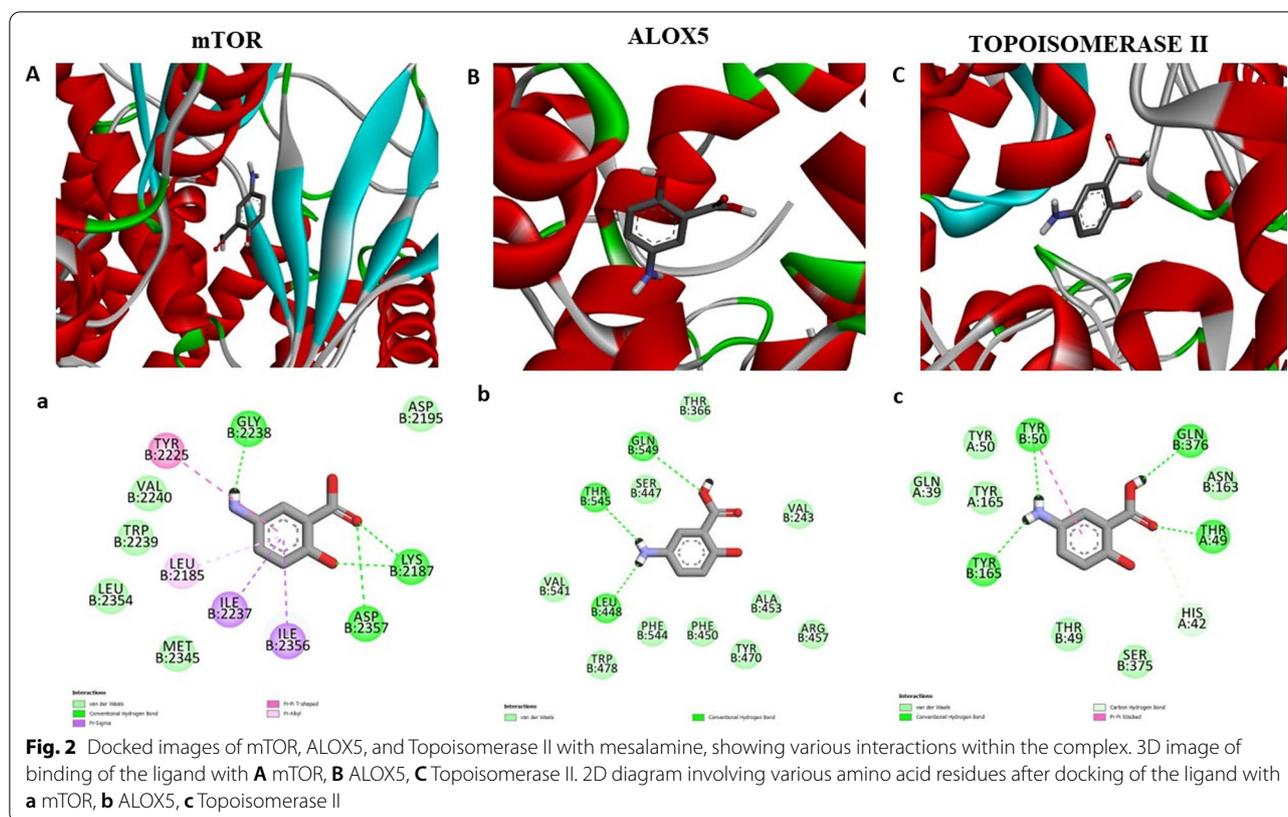
Molecular interaction studies (Fig. 1, Table 1) revealed binding affinities, different amino acids, and the interaction between mesalamine and different receptors [Fig. 1 (A,a-EGFR, B,b-ER $\alpha$ , C,c-Aromatase), Fig. 2 (A,a-mTOR, B,b-ALOX5, C,c-Topoisomerase II)]. Mesalamine showed moderate affinities for all. Olsalazine had the highest affinity for Topoisomerase II with binding energies of  $-9.4$  kcal/mol respectively (Table 1), whereas sulfasalazine and balsalazide had the highest affinities toward the receptor Aromatase with binding energies of  $-9.1$  and  $-8.1$  kcal/mol respectively (Table 1). Each docked ligand complex was stabilized with hydrogen bonds with pi–pi

interactions where non-covalent interactions involving pi systems dispersed electron densities. On further analysis, 2D Ligplot showed mesalamine in mesalamine -Topoisomerase II complex, formed four hydrogen bonds with Gln 376, Thr 49, Tyr 165, and Tyr 50, one carbon–hydrogen also showed with His 42 along with Pi–Pi stacked interaction of Tyr 50. The complex was stabilized with many six van der Waals forces as well (Fig. 1f). Olsalazine bound to Topoisomerase II with fourteen van der Waals interacting residues five conventional hydrogen bonds, Asn 120, Arg 98, Asn 150, Arg 162, Gly 164 and two pi–alkyl bonds with Ala 167, Ile 125 residues (Table 2). Docking analysis of sulfasalazine revealed that the ligand formed eight van der Waals forces, and five hydrogen bonds with Gly 439, Thr 310, Leu 372, Met 374, Arg 115. There were two pi–sigma interactions observed in Val 370, Leu 152. Interestingly, the Aromatase receptor



**Table 1** Binding affinity of aminosalicylic class of NSAIDs with each receptor

S. no.	Compounds	EGFR	ER $\alpha$	Aromatase	mTOR	ALOX5	Topoll	Progest
1	Mesalamine	$-5.7$	$-6.2$	$-6.0$	$-6.5$	$-6.0$	$-6.6$	$-5.9$
2	Sulfasalazine	$-8.0$	$-8.0$	$-9.1$	$-7.9$	$-8.2$	$-8.6$	$-8.0$
3	Balsalazide	$-7.3$	$-7.2$	$-8.1$	$-7.2$	$-7.4$	$-7.9$	$-7.5$
4	Olsalazine	$-6.9$	$-7.6$	$-7.8$	$-7.2$	$-7.9$	$-9.4$	$-8.4$



showed two pi–sulfur interactions with Cys 437 and Met 303. There were pi–alkyl bonds formed with Ala 306 and Ala 438 in the complex. At last, balsalazide formed fourteen van der Waals interacting residues, seven hydrogen bonds with Met 364, Pro 429, Cys 437, Thr 310, Val 369, Ser 314, and Val 370 (Table 2). However, Ala 306 and Phe 430 formed pi–sigma and pi–pi T-shaped interactions, respectively.

### Property calculation

The properties of the selected compounds were carried out using a server called ‘SWISS ADME’ (Daina et al. 2017). It is a well-known web server, mainly used to determine ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) and physicochemical features for studying the pharmacodynamics of drugs. Additional file 1: Tables S1 and S2 displays six important features that are usually determined prior to any pharmaceutical research. The partition coefficient between n-octanol and water ( $\log P_{o/w}$ ) is defined as lipophilicity and is also the affinity of a drug toward lipids (non-polar solvent) and denoted as ‘LogP’. Size is defined in terms of molecular weight in ‘g/mol’. Polarity is stated as topological polar surface area (TPSA) and takes all polar atoms of the molecule into account. Solubility of a molecule is

estimating the aqueous solubility of the compound and is defined as ‘Log S’. The ratio of  $sp^3$  hybridized carbons over the total carbon count of the molecule is taken and defined as ‘Fraction  $csp^3$ ’ for saturation. The flexibility estimates total number of rotatable bonds in the molecule that eventually determines affinity and pose upon binding to its receptor (Constantinescu et al. 2019; Sumalapao et al. 2020). It is clearly seen that the property of ‘Instauration’ does not fall in its ideal range for all four compounds, along with the property of ‘Polarity’ not being seen in majority of compounds.

### The cytotoxic effect of mesalamine on MCF-7 cells

To examine the effect of mesalamine on the proliferation of breast cancer cells, a cell-based cytotoxicity assay was performed using MTT. MCF-7 cells were seeded in 96-well plates (5000 cells/100  $\mu$ L). The next day, cells were treated with increasing concentrations of mesalamine for 24 h. The MTT assay relies on mitochondrial dehydrogenases converting MTT to a purple formazan product in the cell. The purple color produced by this method is proportional to the viability of the cells. In our experimental setup, the growth of breast cancer cells was suppressed in a dose-dependent manner upon treatment with mesalamine. Upon further analysis, the IC<sub>50</sub> value

**Table 2** Different amino acids and its interactions between ligands and receptors

Compounds	Parameters	EGFR	ERA	Aromatase	mTOR	ALOX5	Topoisomerase II	Progesterone
Mesalamine	Interacting Residues	Leu A:391, Leu A:387, Leu A:349, Met A:388, Ile A:424, Met A:421, Phe A:404, Glu A:353, Arg A:394, Ala A:350, Leu A:346	Arg B:394, Pro B:325, Leu B:327, His B:356, Met B:357, Lys B:449, Leu B:387, Gly B:390, Pro B:324, Ile B:386, Ile B:326, Glu B:353, Pro B:324	Ala A:306, Ala A:438, Gly A:436, Arg A:435, Trp A:141, Arg A:145, Arg A:115, Cys A:437, Ile A:132, Ile A:133	Asp B:2195, Met B:2345, Leu B:2354, Trp B:2239, Val B:2240, Lys B:2187, Asp B:2357, Gly B:2238, Tyr B:2225, Leu B:2185	Ile B:167, Val B:397, Tyr B:100, His B:624, Phe B:393, Ala B:388, Tyr B:383, Arg B:138, Tyr B:142, Asp B:166, Tyr B:383, Arg B:101	Asn B:163, Ser B:375, Thr B:49, Gln A:39, Tyr A:165, Tyr A:50, Gln B:376, Thr A:49, Tyr B:165, Tyr B:50, His A:42, Tyr B:50	Leu B:721, Gly B:722, Arg B:766, Val B:760, Met B:756, Leu B:887, Leu B:718, Met B:759, Met B:801, Phe B:778, Leu B:763, Met B:759
	Types of Interactions	Van der Waals, Conventional Hydrogen Bond, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen Bond, Pi-Sulfur, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Sulfur, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Pi T-shaped, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Pi T-shaped, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen, Pi-Pi stacked	Van der Waals, Conventional Hydrogen Bond, Pi-Pi T-shaped, Pi-Alkyl
	Total Number of H-Bonds	3	3	4	3	4	5	3
	Binding Affinity	- 5.7	- 6.2	- 6.0	- 6.5	- 6.0	- 6.6	- 5.9
Sulfasalazine	Interacting Residues	Phe A:404, Met A:388, Leu A:384, Trp A:383, Val A:533, Leu A:354, Leu A:536, Leu A:539, Thr A:347, Met A:421, Met A:343, Leu A:349, Glu A:353, Arg A:394, Asp A:351, Leu A:525, Ala A:350, Leu A:387, Leu A:346, Leu A:391	Leu B:403, Glu B:397, Pro B:325, His B:356, Leu B:387, Met B:357, Gly B:390, Glu B:323, Trp B:393, Arg B:394, Ile B:386, Lys B:449, Glu B:353, Ile B:326, Pro B:324, Leu A:346, Leu A:391	Phe A:148, Lys A:440, Met A:311, Ala A:443, Leu A:477, Phe A:134, Val A:373, Ala A:307, Gly A:439, Thr A:310, Leu A:372, Met A:374, Arg A:115, Val A:370, Leu A:152, Cys A:437, Met A:303, Ala A:306, Ala A:438	Leu B:2204, Val B:2227, Met B:2199, Gly B:2203, Gly B:1897, Gln B:1937, Asn B:1899, Leu B:1900, Arg B:2224, Gln B:2200, Pro B:1943, Asn B:1898	Asp A:156, Gly B:332, Pro B:331, Asn B:328, Leu A:153, Met A:145, Trp A:144, Asp B:290, Gln B:329, Ile B:330, Arg A:143, Glu A:146, Tyr A:515	Asn A:95, Asn A:120, Ala A:92, Thr A:215, Phe A:142, Ile A:141, Tyr B:34, Ser A:149, Thr A:159, Asp A:94, Lys A:157, Val A:158, Asn A:150, Asn A:91, Gln A:97, Arg A:98, Ile A:125	Asp B:697, Gly B:762, Met B:759, Leu B:758, Val B:729, Trp B:732, Lys B:822, Phe B:818, Pro B:696, His B:770, Arg B:766, Lys B:769, Trp B:765, Glu B:695, Gln B:775, Val B:698
	Types of Interactions	Van der Waals, Pi-Sulfur, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Sulfur, Pi-Sigma, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Sigma, Pi-Sulfur, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Alkyl, Carbon-Hydrogen Bond	Carbon-Hydrogen Bond, Conventional Hydrogen Bond, Pi-Sulfur, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen, Amide-Pi Stacked, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Unfavorable Donor, Pi-Alkyl
	Total Number of H-Bonds	0	4	5	5	11	4	3
	Binding Affinity	- 8.0	- 8.0	- 9.1	- 7.9	- 8.2	- 8.6	- 8.0

**Table 2** (continued)

Compounds	Parameters	EGFR	ERA	Aromatase	mTOR	ALOX5	Topoisomerase II	Progesterone
Balsalazide	Interacting Residues	Leu A:349, Leu A:387, Leu A:346, Thr A:347, Val A:533, Cys A:530, Tyr A:526, Lys A:529, Met A:528, Trp A:383, Leu A:384, Leu A:391, Phe A:424, Arg A:394, Glu A:353, Leu A:525, Ala A:350	Ile B:386, Leu B:387, His B:356, Met B:357, Lys B:449, Ile B:326, Leu B:320, Phe B:445, Leu B:323, Arg B:394, Glu B:353, Leu B:333, Trp B:393, Gly B:442, Gly B:390, Pro B:324	Asp A:371, Pro A:358, Ile A:398, His A:402, Arg A:365, Phe A:427, Ile A:132, Ala A:428, Phe A:368, Leu A:152, Met A:303, Met A:445, Met A:311, Ala A:443, Met A:364, Pro A:429, Cys A:437, Thr A:310, Val A:369, Ser A:314, Val A:370, Ala A:306, Phe A:430, Ala A:307	Gly B:1897, Gln B:1937, Gln B:2200, Pro B:1910, Pro B:2229, Ile B:2228, Ala B:2226, Tyr B:2225, Met B:2199, Arg B:2224, Gly B:2203, Leu B:2204, Asn B:1898, Thr B:2207, Glu B:2196, Val B:2227, Leu B:1900	Pro B:331, Gly B:332, Asp B:333, Arg A:143, Gln A:139, Asp A:507, Trp A:144, Met A:145, Asn B:335, Ile B:330, Glu B:334, Tyr A:515, Arg A:384, Gln A:385	Lys A:168, Gly A:164, Tyr B:34, Ile A:141, Lys A:156, Thr A:159, Asp A:94, Ser A:148, Thr A:147, Asn A:150, Ser A:149, Val A:158, Lys A:157, Gln A:97, Arg A:98	Asp B:697, Met B:692, Phe B:818, Gly B:762, Met B:759, Trp B:755, Gln B:725, Val B:729, Leu B:758, Trp B:732, Ser B:728, Lys B:822, Pro B:696, His B:770, Val B:698, Arg B:766, Lys B:769, Trp B:765
Olsalazine	Types of Interactions	Van der Waals, Conventional Hydrogen Bond, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen Bond, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Sigma, Pi-Pi T-Shaped, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond	Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen, Amide-Pi Stacked, Pi-Alkyl	Van der Waals, Conventional Hydrogen Bond
	Total Number of H-Bonds	3	6	7	3	5	5	4
	Binding Affinity	- 7.3	- 7.2	- 8.1	- 7.2	- 7.4	- 7.9	- 7.5
	Interacting Residues	Glu A:353, Leu A:349, Leu A:387, Thr A:347, Leu A:536, Leu A:354, Trp A:383, Leu A:384, Met A:343, Met A:421, Leu A:391, Phe A:404, Asp A:351, Leu A:346, Leu A:525, Ala A:350	Leu B:327, His B:356, Met B:357, Leu B:387, Ile B:386, Lys B:449, Gly B:390, Val B:446, Phe B:445, Pro B:325, Glu B:353, Arg B:394, Glu B:323, Trp B:393, Ile B:326, Pro B:324	Val A:369, Met A:364, Val A:370, Gly A:439, Phe A:148, Leu A:152, Ser A:199, Phe A:203, Met A:446, Met A:160, Met A:447, Pro A:429, Ala A:307, Met A:303, Thr A:310, Ser A:314, Ala A:306, Met A:311, Cys A:437, Phe A:430, Ala A:443	Thr B:2380, Gly B:2391, Val B:2389, Asn B:2385, Val B:2291, Phe B:2287, Leu B:2538, Thr B:2533, Leu B:2379, His B:2535, Thr B:2390, Glu B:2388, Met B:2387, Gln B:1405, Arg B:2381	Leu A:230, Arg A:221, Leu A:657, Gln A:656, Asn A:318, Tyr A:467, Leu A:237, Val A:321, Gly A:233, Ile A:320, His A:225, Glu A:228, Tyr A:234, Met A:231, Lys A:319	Thr A:215, Ala A:92, Phe A:142, Asn A:91, Ser A:148, Gly A:161, Asn A:163, Tyr A:165, Gly A:166, Lys A:168, Ile A:141, Lys A:123, Gly A:124, Asn A:95, Asn A:120, Arg A:98, Asn A:150, Arg A:162, Gly A:164, Ala A:167, Ile A:125	Gln B:725, Leu B:721, Gly B:722, Leu B:718, Met B:909, Leu B:715, Val B:903, Phe B:905, Tyr B:890, Leu B:797, Met B:756, Leu B:887, Met B:801, Val B:760, Met B:759, Asn B:719, Arg B:766, Cys B:891, Thr B:894, Phe B:778, Leu B:763

**Table 2** (continued)

Compounds	Parameters	EGFR	ERα	Aromatase	mTOR	ALOX5	Topoisomerase II	Progesterone
	Types of Interactions	Van der Waals, Conventional Hydro- gen Bond, Pi-Alkyl	Van der Waals, Conventional Hydro- gen Bond, Unfavorable Donor- Donor, Pi-Sigma Pi-Alkyl	Van der Waals Conventional Hydro- gen Bond, Pi-Sigma, Pi-Sulfur, Pi-Pi T-Shaped, Pi-Alkyl	Van der Waals Conventional Hydro- gen Bond, Unfavorable Donor- Donor, Pi-Alkyl	Van der Waals, Conventional Hydro- gen Bond, Pi-Pi T-Shaped, Pi-Alkyl	Van der Waals, Conventional Hydro- gen Bond Pi-Alkyl	Van der Waals, Conventional Hydro- gen Bond, Carbon-Hydrogen, Unfavorable Donor- Donor, Pi-Pi T-shaped, Pi-Alkyl
	Total Number of H-Bonds	1	4	5	6	3	5	4
	Binding Affinity	- 6.9	- 7.6	- 7.8	- 7.2	- 7.9	- 9.4	- 8.4

of mesalamine was determined to be 6.358  $\mu\text{M}$  (Fig. 3A). Figure 3B depicts the control for the rest of the MTT figures.

#### Phase-contrast microscopy

Control and treated cells were observed under an inverted light microscope, using phase-contrast microscopy to spot morphological changes and microphotographs were documented. Cells treated with increasing concentrations of mesalamine displayed significant morphological alterations at 5, 20, and 50  $\mu\text{M}$  (Fig. 3C, D, E). This change in morphology becomes more evident in cells treated with a higher concentration of mesalamine, i.e., > 100  $\mu\text{M}$  (Fig. 4F). A higher concentration of mesalamine (670  $\mu\text{M}$ ) resulted in cell apoptosis and degradation in a few cells, while the others were observed to have lost their morphology and become round in nature, with large vacuoles (Additional file 2: Figure S1). The cells seem to have been arrested in the G1 phase.

#### Survival analysis

To extend our findings, the gene expression levels of different targets of mesalamine in breast and colorectal cancer were undertaken. Mesalamine has already been reported to have protective effects against colorectal cancer in inflammatory bowel disease (Dixon et al. 2021). We did comparative studies based on the TCGA database (Atlas et al. 2019). We correlated the expression level of targets of mesalamine with the survival of breast and colorectal cancer patients. Known targets of mesalamine were retrieved from the DurgSurv database (Amelio et al. 2014). We examined the expression of ALOX5 (Arachidonate 5-lipoxygenase) and PPAR $\gamma$  (peroxisome proliferator-activated receptor gamma), in 1097 tumor samples of invasive breast and 380 samples of colorectal cancer from the cancer genome atlas (TCGA). The log-rank test was used to examine the difference in survival outcomes between low and high expression patient groups for each gene. Mesalamine has been reported to inhibit ALOX 5 (Nielsen et al. 1987; El-Nagar et al. 2018). In both cancer types, poor survival of cancer patients was associated with high expression ALOX5 with ( $P$  value = 0.10) (Fig. 4A, C). Interestingly, Kaplan–Meier survival plots displayed that high expression of PPAR $\gamma$  predicted shorter survival in breast cancer patients by TCGA database analysis but in the case of colorectal cancer high expression of PPAR $\gamma$  in the samples was associated with better survival probability ( $P$  value = 0.4) (Fig. 4B, D).

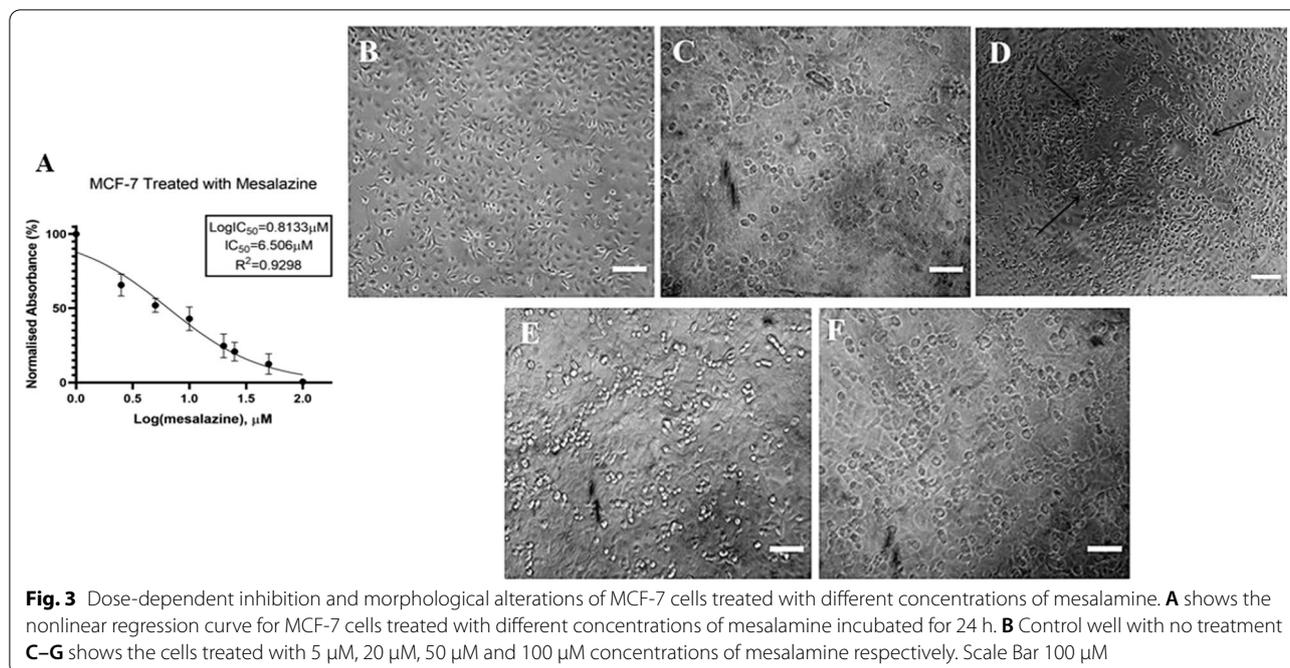
#### Discussion

In recent years, a plethora of studies have shed light on mechanisms that sustain cancer cell growth and survival and have pointed out the problem of cancer resistance

to chemotherapy and other treatment options currently available (Wang et al. 2019; Cree and Charlton 2017). Problems such as recurrence, metastasis, chemo, and radio-resistance have promoted drug repurposing as the go-to solution to find better candidates with fewer adverse side effects (Kirtonia et al. 2021). The repurposing or repositioning approach has since been repeatedly adopted and sought after as it drastically brings down the time and cost in comparison to de novo drug discovery methods. Traditional methods involve numerous trial and error experiments. Plethora of novel drugs show promising results in early stages but only few of them reach to phase 3 clinical trials and still it remains one of the biggest hurdles in cancer therapeutics (Parasrampuria et al. 2018). The current investigation deals with understanding the drug repurposing potential of 5-aminosalicylates in cancer via molecular screening followed by in vitro study.

Drugs that are potential candidates for repurposing should ideally have similar characteristic traits like market availability, acclaimed drugs and toxicity as that of conventionally used drugs. They need to exhibit a distinctive mode of action against the targeted condition and sufficient efficiency at the dosage administered (Pushpakom et al. 2019). Various drugs already being utilized for different conditions have shown immense potential in cancer therapeutics. Many off-patent pharmaceuticals have been proved to show properties against cancer by 16% of relevant clinical studies and 50% data from humans (Verbaanderd et al. 2020). Specifically, clinical trials have proven the efficacy of drugs like chloroquine, all-trans retinoic acid, verapamil, itraconazole and aspirin, against cancer (Correia et al. 2021). Along with these, mesalamine is another pharmaceutical showing activity against colorectal cancer and research is also being conducted to study its exact mechanism of action. These studies have indicated that strong doses of mesalamine hinder the development of colon cancer, and this obstruction is seen to be enhanced further when compounds derived from mesalamine are administered (Dixon et al. 2021).

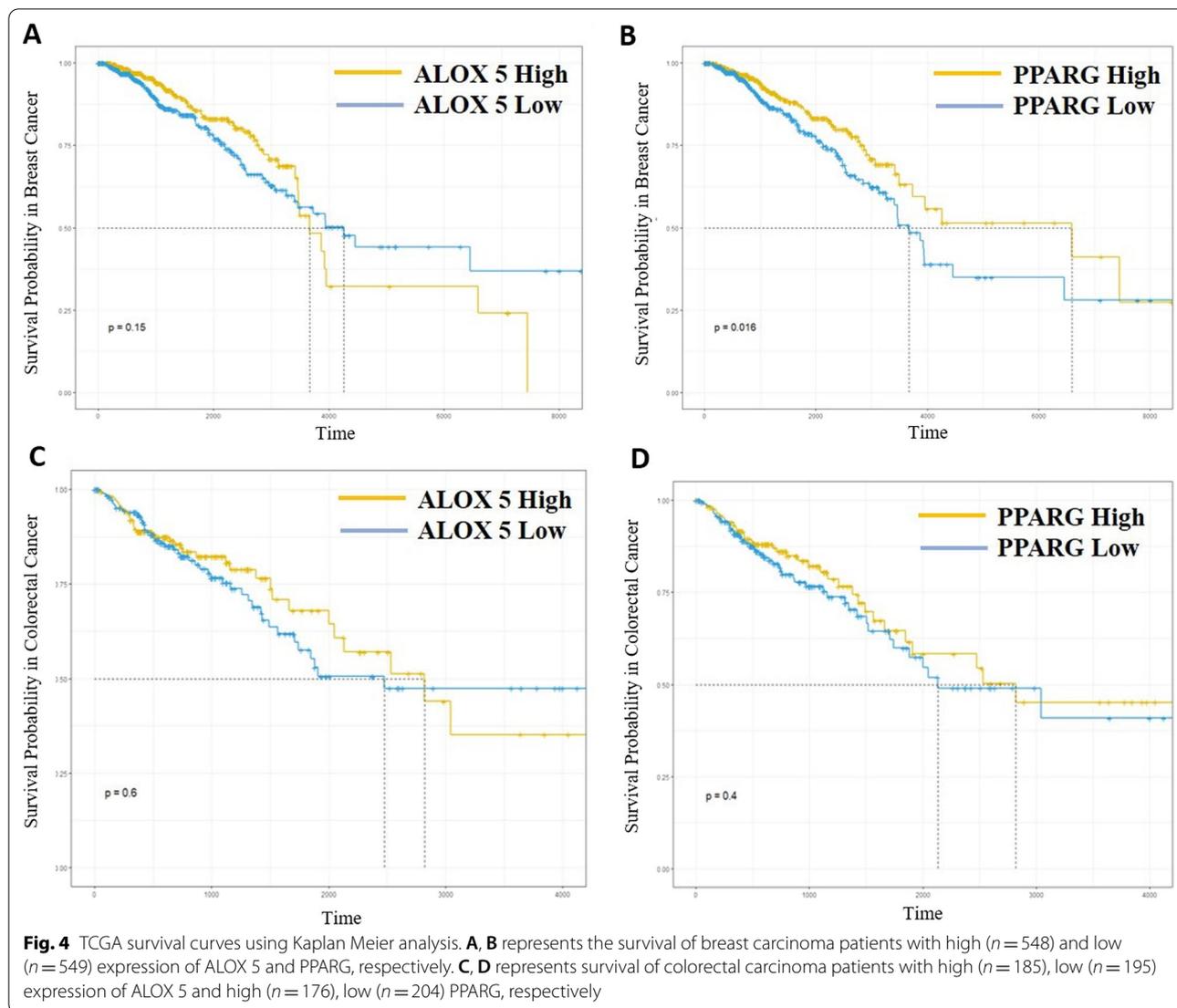
Mesalamine is the bioactive moiety of aminoacylates which are known to be dual inhibitors of COX2 and 5LOX receptors involved in the anti-inflammatory pathways and their role in cancer has been highlighted by many studies (Harris et al. 2020). Affinity toward other targets also points to the multi-target inhibition ability of mesalamine being a small molecule with a structure almost similar to that of aspirin. The target receptors selected for this study are well known to play a huge role in the development and progression of breast cancer. The proteins namely, EGFR, Aromatase, ALOX5, and mTOR are usually overexpressed in breast cancer



(Larionov 2018; Fu et al. 2020). The overexpression of EGFR results in the large size of the tumor, poor differentiation as well as clinical outcomes; Aromatase is responsible for regulating hyperplasia when circulating estrogen is not present; ALOX5 is known to play a critical role in inflammation tied to cancer such as breast and colorectal. mTOR gives a great advantage to the growth of tumors, activates protein synthesis, and suppresses autophagy (Harris et al. 2020; Sood et al. 2021; Hare and Harvey 2017; Sigismund et al. 2018). mTOR plays a central role on PI3K/Akt/mTOR pathway which is one of the critical pathway affecting cell survival and metabolism in normal and pathophysiological situations, especially in cancer (Mossmann et al. 2018). ER  $\alpha$  regulate the proliferation of breast cancer cells owing to their respective hormone ligands and by expressing oncogenic proteins (such as cyclin D1 and c-Myc) (Trabert et al. 2020; Xue et al. 2019). Topoisomerase II is responsible for DNA replication in the cells and has been a target for multiple chemotherapeutic agents (Arthi et al. 2021; You and Gao 2019). PPARs have already been reported to play a significant role in cancer cell proliferation and tumor growth (Gou et al. 2017). The role of EGFR is mostly in ER+ positive carcinoma and is a target for hormone and chemotherapy. In the present investigation, mesalamine along with sulfasalazine, balsalazide, and olsalazine was docked with different receptors and mesalamine showed moderate to strong affinity for all the receptors and was comparable for all the targets for which docking was performed as compared to other ligands.

Although sulfasalazine displayed a better binding affinity for all receptors, we still have chosen mesalamine for further functional validation in an in vitro model. Sulfasalazine, olsalazine, and balsalazide are the prodrug as compared to mesalamine (Sandborn and Hanauer 2003). Oral mesalamine has been shown to be safe and effective for both short and long-term use. Although sulfasalazine has been used for the therapeutics of ulcerative colitis, to avoid its side effects, direct application of mesalamine has been under investigation.

Cell-based cytotoxicity assay in MCF-7 demonstrated the anticancer potential of mesalamine. The IC<sub>50</sub> of mesalamine on MCF-7 cells was calculated to be 6.358 µM, with R-value of 0.9298, which implies a strong positive correlation between the increasing concentrations of mesalamine and its inhibitory effect on the MCF-7 cells. The same trend was observed while investigating morphological alterations and cytotoxic effects under phase-contrast microscopy. For a drug, the values of a potent inhibitor can lie anywhere between the range of nano-molar to milli-molar concentrations of the substance. For mesalamine, the anticancer and cytotoxicity effects could only be observed more evidently at higher concentrations, where cells were arrested in the initial stages of the cell cycle and/or were undergoing apoptosis. The plausible mechanism of action could be in accordance with that in colorectal cancer as the pathways contributing to the development and progression of cancer are alike in both disease conditions. Next, we performed an indirect correlation analysis between the expression



of target genes of mesalamine in breast and colorectal cancer with the survival of patients. Higher expression of ALOX5 was correlated with poor survival of both breast and colorectal cancer patients. Mesalamine is known to inhibit ALOX5 (Nielsen et al. 1987; El-Nagar et al. 2018). Therefore, if mesalamine is administered to breast cancer and colorectal cancer patients where expression of ALOX5 is elevated, it will lead to downregulation of ALOX5 and hence lead to better outcomes in breast and colorectal cancer, which fits, into our hypothesis of drug repurposing of mesalamine in breast cancer. A similar survival analysis was performed for PPARG in breast and colorectal cancer patients. There have been reports of the involvement of PPARG in mesalamine-mediated inhibition of colon cancer cells (Schwab et al. 2008). Survival analysis demonstrated high PPARG expression was correlated to shorter survival in breast cancer patients.

However, the opposite scenario existed for colorectal cancer patients. Mesalamine, being an agonist of PPARG will result in upregulation of its expression (Bertin et al. 2013). Therefore, when administered to colorectal cancer patients, it will elevate PPARG expression, which can lead to better survival outcomes. Breast cancer analysis resulted in contrasting observations. However, these all are indirect correlations and many more functional studies are warranted to comment on the efficacy of mesalamine in breast cancer.

### Conclusions

This explorative study aimed to address the anticancer potential of mesalamine, one of the 5-aminosalicylates drugs for breast cancer. Text mining and knowledge-based repurposing approach was employed, and we were able to identify a potential candidate mesalamine that could be

considered for drug repurposing in cancer. Based upon the results of the current investigation, mesalamine can further be considered for combination therapies with well-known anticancer drugs, the results of which will prove the potential of mesalamine to be repurposed for cancer chemotherapy as adjuvant therapy.

#### Abbreviations

FDA: Food and Drug Administration; NSAIDs: Non-steroidal anti-inflammatory drugs; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CRC: Colorectal cancer; EGFR: Epidermal growth factor receptor; MGL: Molecular Graphics Lab; SDF: Structure Data File; PDB: Protein Data Bank; HETATM: Heteroatoms; SPDBV: Swiss-PDB viewer; DMSO: Dimethyl sulfoxide; NCCS: National Centre for Cell Science; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RSEM: RNA-Seq by expectation-maximization; ALOX5: Arachidonate 5-lipoxygenase; PPARγ: Peroxisome proliferator-activated receptor gamma; ADMET: Adsorption, distribution, metabolism, excretion, and toxicity; TPSA: Topological polar surface area; TCGA: The Cancer Genome Atlas.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-022-00939-1>.

**Additional file 1.** Supplementary Tables.

**Additional file 2: Figure S1.** Morphological alterations of MCF-7 cells treated with 670 μM. Scale Bar: 100 μM.

#### Acknowledgements

The authors wish to thank the School of Biotechnology and Bioinformatics, D Y Patil Deemed to be University, for providing the necessary facilities.

#### Author contributions

DS and SS executed the experiments, collected data, and prepared original draft, along with reviewing and editing work. UK carried out the work of editing and reviewing. JA and SD supervised the entire study, conceptualized, managed resources, and edited and drafted original work. All authors have read and approved the manuscript.

#### Funding

Not applicable.

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

Not applicable.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare that they have no competing interests.

Received: 3 July 2022 Accepted: 21 September 2022

Published online: 26 September 2022

#### References

- Abdullah T (2020) Protein–ligand interaction analysis using LigPlot+. In: Bioinformatics review. <https://bioinformaticsreview.com/20200329/protein-ligand-interaction-analysis-using-ligplot/>. Accessed 23 Apr 2022
- Amelio I, Gostev M, Knight RA et al (2014) DRUGSURV: a resource for repositioning of approved and experimental drugs in oncology based on patient survival information. *Cell Death Dis* 5:e1051–e1051. <https://doi.org/10.1038/cddis.2014.9>
- Arthi M, Arun Kumar K, D'Cruze L, Rajendiran S, Joseph LD, Dev B (2021) The significance of topoisomerase II alpha in invasive breast carcinoma. *Cureus* 13(10):e18733. <https://doi.org/10.7759/cureus.18733>
- The Cancer Genome Atlas—computational tools—National Cancer Institute (2019). <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/tools>. Accessed 23 Apr 2022
- Beckwitt CH, Brufsky A, Oltvai ZN et al (2018) Statin drugs to reduce breast cancer recurrence and mortality. *Breast Cancer Res* 20:144. <https://doi.org/10.1186/s13058-018-1066-z>
- Bertin B, Dubuquoy L, Colombel J-F, Desreumaux P (2013) PPAR-γ in ulcerative colitis: a novel target for intervention. *Curr Drug Targets* 14:1501–1507. <https://doi.org/10.2174/13894501113149990162>
- Bhattacharyya GS, Doval DC, Desai CJ, Chaturvedi H, Sharma S, Somashekhar SP (2020) Overview of breast cancer and implications of overtreatment of early-stage breast cancer: an Indian perspective. *JCO Glob Oncol* 6:789–798. <https://doi.org/10.1200/GO.20.00033>
- Bindu S, Mazumder S, Bandyopadhyay U (2020) Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. *Biochem Pharmacol* 180:114147. <https://doi.org/10.1016/j.bcp.2020.114147>
- BIOVIA, Dassault Systèmes (2021) Discovery Studio Visualizer, Version 4.5. Dassault Systèmes, San Diego. <https://discover.3ds.com/discovery-studio-visualizer-download>
- Constantinescu T, Lungu CN, Lung I (2019) Lipophilicity as a central component of drug-like properties of chalcones and flavonoid derivatives. *Molecules* 24:1505. <https://doi.org/10.3390/molecules24081505>
- Correia AS, Gärtner F, Vale N (2021) Drug combination and repurposing for cancer therapy: the example of breast cancer. *Heliyon*. <https://doi.org/10.1016/j.heliyon.2021.e05948>
- Cree IA, Charlton P (2017) Molecular chess? Hallmarks of anti-cancer drug resistance. *BMC Cancer* 17(1):10. <https://doi.org/10.1186/s12885-016-2999-1>
- Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7:42717. <https://doi.org/10.1038/srep42717>
- Dixon SW, Collard TJ, Mortensson E, Legge DN, Chambers AC, Greenhough A, Creed TJ, Williams AC (2021) 5-Aminosalicylic acid inhibits stem cell function in human adenoma-derived cells: implications for chemoprophylaxis in colorectal tumorigenesis. *Br J Cancer* 124(12):1959–1969. <https://doi.org/10.1038/s41416-021-01354-5>
- El-Nagar M, Abdu-Allah H, Salem O, Kafafy AN, Farghaly H (2018) Novel N-substituted 5-aminosalicylamides as dual inhibitors of cyclooxygenase and 5-lipoxygenase enzymes: synthesis, biological evaluation and docking study. *Bioorg Chem* 78:80–93. <https://doi.org/10.1016/j.bioorg.2018.02.023>
- Fu Y, Zou T, Shen X, Nelson PJ, Li J, Wu C, Yang J, Zheng Y, Bruns C, Zhao Y, Qin L, Dong Q (2020) Lipid metabolism in cancer progression and therapeutic strategies. *MedComm* 2(1):27–59. <https://doi.org/10.1002/mco.2.27>
- Gou Q, Gong X, Jin J et al (2017) Peroxisome proliferator-activated receptors (PPARs) are potential drug targets for cancer therapy. *Oncotarget* 8:60704–60709. <https://doi.org/10.18632/oncotarget.19610>
- Hare S, Harvey A (2017) mTOR function and therapeutic targeting in breast cancer. *Am J Cancer Res* 7:383
- Harris RE, Beebe J, Schwartzbaum J (2020) Chemoprevention of breast cancer by cyclooxygenase and lipoxygenase inhibitors. *World Acad Sci J* 2:14–18. <https://doi.org/10.3892/wasj.2020.34>
- Kirtonia A, Gala K, Fernandes SG et al (2021) Repurposing of drugs: an attractive pharmacological strategy for cancer therapeutics. *Semin Cancer Biol* 68:258–278. <https://doi.org/10.1016/j.semcancer.2020.04.006>
- Larionov AA (2018) Current therapies for human epidermal growth factor receptor 2-positive metastatic breast cancer patients. *Front Oncol* 8:89. <https://doi.org/10.3389/fonc.2018.00089>

- Lim W-C, Wang Y, MacDonald JK, Hanauer S (2016) Aminosaliculates for induction of remission or response in Crohn's disease. *Cochrane Database Syst Rev*. <https://doi.org/10.1002/14651858.CD008870.pub2>
- Mahvi DA, Liu R, Grinstaff MW et al (2018) Local cancer recurrence: the realities, challenges, and opportunities for new therapies. *CA Cancer J Clin* 68:488–505. <https://doi.org/10.3322/caac.21498>
- Mohammed A, Yarla NS, Madka V, Rao CV (2018) Clinically relevant anti-inflammatory agents for chemoprevention of colorectal cancer: new perspectives. *Int J Mol Sci* 19:2332. <https://doi.org/10.3390/ijms19082332>
- Mossmann D, Park S, Hall MN (2018) mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer* 18(12):744–757. <https://doi.org/10.1038/s41568-018-0074-8>
- Nielsen OH, Bukhave K, Elmgreen J, Ahnfeldt-Rønne I (1987) Inhibition of 5-lipoxygenase pathway of arachidonic acid metabolism in human neutrophils by sulfasalazine and 5-aminosalicylic acid. *Dig Dis Sci* 32:577–582. <https://doi.org/10.1007/BF01296156>
- O'Boyle NM, Banck M, James CA et al (2011) Open Babel: an open chemical toolbox. *J Cheminform* 3:33. <https://doi.org/10.1186/1758-2946-3-33>
- Oliveira E, Lang K (2018) Drug repositioning: concept, classification, methodology, and importance in rare/orphans and neglected diseases. *J Appl Pharm Sci*. <https://doi.org/10.7324/JAPS.2018.8822>
- Pantziarka P, Sukhatme V, Bouche G, et al (2016) Repurposing DRUGS IN ONCOLOGY (ReDO)—diclofenac as an anti-cancer agent. <http://ecancer.org/en/journal/article/610-repurposing-drugs-in-oncology-redo-diclofenac-as-an-anti-cancer-agent>. Accessed 6 Apr 2022
- Parasrampur DA, Benet LZ, Sharma A (2018) Why drugs fail in late stages of development: case study analyses from the last decade and recommendations. *AAPS J* 20(3):46. <https://doi.org/10.1208/s12248-018-0204-y>
- Perrotta C, Pellegrino P, Moroni E et al (2015) Five-aminosalicylic acid: an update for the reappraisal of an old drug. *Gastroenterol Res Pract* 2015:e456895. <https://doi.org/10.1155/2015/456895>
- Pushpakom S, Iorio F, Eyers PA et al (2019) Drug repurposing: progress, challenges, and recommendations. *Nat Rev Drug Discov* 18:41–58. <https://doi.org/10.1038/nrd.2018.168>
- Sandborn WJ, Hanauer SB (2003) Systematic review: the pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. *Aliment Pharmacol Ther* 17(1):29–42. <https://doi.org/10.1046/j.1365-2036.2003.01408.x>
- Schwab M, Reynders V, Loitsch S et al (2008) PPARgamma is involved in mesalazine-mediated induction of apoptosis and inhibition of cell growth in colon cancer cells. *Carcinogenesis* 29:1407–1414. <https://doi.org/10.1093/carcin/bgn118>
- Seeliger D, de Groot BL (2010) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des* 24:417–422. <https://doi.org/10.1007/s10822-010-9352-6>
- Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer statistics, 2021. *CA Cancer J Clin* 71(1):7–33. <https://doi.org/10.3322/caac.21654>
- Sigismund S, Avanzato D, Lanzetti L (2018) Emerging functions of the EGFR in cancer. *Mol Oncol* 12(1):3–20. <https://doi.org/10.1002/1878-0261.12155>
- Sood A, Lang DK, Kaur R, Saini B, Arora S (2021) Relevance of aromatase inhibitors in breast cancer treatment. *Curr Top Med Chem* 21(15):1319–1336. <https://doi.org/10.2174/1568026621666210701143445>
- Stolfi C, De Simone V, Pallone F, Monteleone G (2013) Mechanisms of action of non-steroidal anti-inflammatory drugs (NSAIDs) and mesalazine in the chemoprevention of colorectal cancer. *Int J Mol Sci* 14:17972–17985. <https://doi.org/10.3390/ijms140917972>
- Sumalapa DEP, Villarante NR, Agapito JD, Asaad AS, Gloriani NG (2020) Topological polar surface area, molecular weight, and rotatable bond count account for the variations in the inhibitory potency of antimycotics against *Microsporium canis*. *J Pure Appl Microbiol* 14(1):247–254. <https://doi.org/10.22207/JPAM.14.1.25>
- Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71:209–249. <https://doi.org/10.3322/caac.21660>
- Trabert B, Sherman ME, Kannan N, Stanczyk FZ (2020) Progesterone and breast cancer. *Endocr Rev* 41(2):320–344. <https://doi.org/10.1210/endo/rev/bnz001>
- Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455–461. <https://doi.org/10.1002/jcc.21334>
- Veloso PM, Machado R, Nobre C (2021) Mesalazine and inflammatory bowel disease—from well-established therapies to progress beyond the state of the art. *Eur J Pharm Biopharm* 167:89–103. <https://doi.org/10.1016/j.ejpb.2021.07.014>
- Verbaanderd C, Rooman I, Meheus L, Huys I (2020) On-Label or off-label? Overcoming regulatory and financial barriers to bring repurposed medicines to cancer patients. *Front Pharmacol* 10:1664. <https://doi.org/10.3389/fphar.2019.01664>
- Wang X, Zhang H, Chen X (2019) Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist* 2(2):141–160. <https://doi.org/10.20517/cdr.2019.10>
- Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J (2018) A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging Dis* 9:143. <https://doi.org/10.14336/AD.2017.0306>
- Xue M, Zhang K, Mu K et al (2019) Regulation of estrogen signaling and breast cancer proliferation by an ubiquitin ligase TRIM56. *Oncogenesis* 8:1–14. <https://doi.org/10.1038/s41389-019-0139-x>
- Ye B, van Langenberg DR (2015) Mesalazine preparations for the treatment of ulcerative colitis: Are all created equal? *World J Gastrointest Pharmacol Ther* 6:137–144. <https://doi.org/10.4292/wjgpt.v6.i4.137>
- You F, Gao C (2019) Topoisomerase inhibitors and targeted delivery in cancer therapy. *Curr Top Med Chem* 19(9):713–729. <https://doi.org/10.2174/1568026619666190401112948>
- Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. *Signal Transduct Target Ther* 5(1):113. <https://doi.org/10.1038/s41392-020-00213-8>

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)