Egypt. J. Chem. **Vol. 64,** No. 7 pp. 3465 - 3474 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antiproliferative Evaluation and Molecular Docking studies of some Sulfonyl-α-L-amino acid Derivatives coupled with Anisamide Scaffold

Alaaeldin M. F. Galal,[a]* Khaled Mahmoud[b]

[a]*Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, 33 El Bohouth St., Dokki, Giza, 12622 Egypt.*

[b]*Pharmacognosy Department, National Research Centre, El-Behooth St., 12622 Dokki, Giza, Egypt.*

Abstract

A series of sulfonyl-α-L-amino acid derivatives coupled with anisamide scaffold, previously synthesized, were evaluated *in vitro* for their antiproliferative activity against human cell lines namely, Caucasian breast adenocarcinoma (MCF7), hepatocellular carcinoma (HEPG2), Colon cell line (HCT116), and pancreatic cell line (PaCa2) and comparing their results with skin fibroblast cell line (BJ1) as a normal cell line, using MTT cell proliferation assay. The results showed that 2-(4-{[(5-Chloro-2-methoxy-benzoyl)amino]methyl}phenyl)sulfonyl-L-cysteine (**5**), 2-(4-{[(5-Chloro-2-methoxy-benzoyl)amino]methyl}phenyl)sulfonyl-L-glutamine (**14**), and 2-(4-{[(5-Chloro-2-methoxy-benzoyl)amino]methyl}phenyl)sulfonyl-L-tryptophan (**18**) were the most active molecules against HEPG2, MCF7, and PaCa2 cell lines with IC_{50} 51.9, 54.2, and 59.7 µg/ml respectively. On contrary, 2-(4-{[(5-Chloro-2-methoxy-benzoyl)amino]methyl}phenyl)sulfonyl-L-valine (**3**) has a high selectivity index for (MCF7) and (PaCa2) cell lines at, IC_{50} 90.9 and 69.5 µg/ml, respectively. Similarly, 2-(4-{[(5-Chloro-2-methoxy-benzoyl)-amino]methyl}phenyl)sulfonyl-L-glycine (**1**) and 2-(4-{[(5-Chloro-2-methoxy-benzoyl)amino]methyl}phenyl)sulfonyl-L-lysine (**10**) have cytotoxic selectivity towards (HEPG2) cell line with a high selectivity index with IC_{50} 85.1 and 87.0 µg/ml, respectively. Moreover, a docking study was performed to predict the correct binding geometry for each binder and compare it with its activity.

Keywords: Antiproliferative; Sulfonyl-α-L-amino acids; cell lines; Molecular docking

1. Introduction

Cancer is the second most common cause of death worldwide, and it is a global health issue facing humanity [1]. Based on GLOBOCAN's estimates, around the world, there were approximately 19.3 million new cancer cases and 10.0 million deaths in 2020 worldwide. Over the years, the burden has shifted to developing countries, which currently account for around 57% of cases and 65% of cancer deaths worldwide [2]. Cancer is a class of diseases in which a group of cells exhibits rapid division of abnormal cells, which leads to uncontrolled growth beyond the usual boundaries of the normal state, invasion, and sometimes metastasis [3,4]. Cancer cells can spread to other parts of the body through the bloodstream or lymphatic system [5,6]. Thus, thousands of synthetic and natural compounds could be explored for use as anti-cancer agents through the application of cell culture tools *in vitro* [7]. The National Cancer Institute was the first institute to establish a program for this purpose. It has introduced several factors that are now part of standard cancer care after lengthy automated studies to determine their actual pathways that influence the growth of cancer cells [8]. As such, inhibiting cancer cell proliferation is an important approach to disease therapy. Early on, cytotoxic compounds were used to treat cancer, but there was a high risk due to the possibility of causing damage to normal and healthy cells. However, the development of new classes of anticancer agents with both effective and selective toxicity towards cancer

*Corresponding author e-mail: alaa17767@yahoo.com.; (Alaaeldin M.F. Galal).
Receive Date: 22 February 2021, Revise Date: 04 March 2021, Accept Date: 07 March 2021
DOI: 10.21608/EJCHEM.2021.64272.3381

cells is attracting great interest due to the unwanted side effects of many chemotherapy drugs.

Since the discovery of the antibacterial drug (Prontosil), as the first sulfonamide drug, sulfa drugs have had a prominent place in therapies [9]. Soon after, sulfonamide-containing antidiabetics [10] and carbonic anhydrase inhibitors [11,12] arrived on the market and continue until the day now. In recent decades, research into these categories has continued to be active although it has also moved to premium applications [13-16]. Sulfonamide-bearing compounds have been reported to display anti-inflammatory (COX-2 inhibitors) [17], anti-proliferative [18-21], anti-HIV [22,23], anti-Aβ fibrillogenesis [24], and anti-parasitic [25,26], among other properties, and display the diversity of their biological potentials.

Anisamide derivatives are the compounds containing amide group, in the trans-planner conformation [27], as a functional group that has been β found to acquire donor properties and show a wide range of biological activities. A broad spectrum of biological activity is described to be associated with many heterocyclic compounds. Anisamide nucleus is a gainful targeted case of liposome in a potent carrier for targeted doxorubicin to human prostate cancer cells. An anisamide derivative holds a high affinity for sigma receptors and is used for the treatment of human malignancies including prostate cancer [28]. Also, human lung cancer cells often overexpress the sigma receptor and thus, can be targeted with anisamide derivatives [29].

Compounds containing sulfonamide moiety and anisamide scaffold in the same structure have received great attention in a part of our studies due to the diverse chemotherapy potentials. Some compounds were showed strong antifungal activity through the inhibition of dihydropterate synthase (DHPS) enzyme [30] and, others have anti-*Helicobacter pylori* efficiency and Inosine 5′-monophosphate dehydrogenase (IMPDH) inhibitors [31]. Also, other compounds were showed potent antiproliferative activity against human cancer cell lines through cell cycle arrest at the G2/M phase and induced apoptosis [32].

Continuing the investigation of sulfonamide derivatives as potent anticancer agents [19,31,32,33], a new series of sulfonyl-α-L-amino acid derivatives coupled with anisamide scaffold was synthesized based on the strategy of drug design and was reported before [31]. In this study, the synthesized compounds were evaluated *in vitro* against five types of human cell lines. Four were carcinoma cell lines namely, Caucasian breast adenocarcinoma (MCF7), Hepatocellular carcinoma (HEPG2), Colon cell line (HCT116), and Pancreatic cell line (PaCa2) and comparing their results with Skin fibroblast cell line (BJ1) as a normal cell line, using MTT cell proliferation assay. Moreover, a docking study was performed to predict the binding geometry for each tested compound at the colchicine-binding site of tubulin, binding affinity, and compare it with its activity.

Experimental

Chemistry

Sulfonyl-α-L-amino acid derivatives (**1**-**20**) (Fig. 1) under investigation were synthesized according to the method described elsewhere [31]. The structure of the synthesized compounds was confirmed by mp, HR-MS, and NMR tools.

Biological evaluation

Antiproliferative evaluation

Cell lines:

The antiproliferative evaluation was carried out on five types of human cell lines *in vitro*. Four were carcinoma cell lines: Caucasian breast adenocarcinoma (MCF7), Hepatocellular carcinoma (HEPG2), Colon cell line (HCT116), and Pancreatic cell line (PaCa2), and a normal cell line: Skin fibroblast cell line (BJ1), following the previously published procedure [34].

Cell culturing:

The cells were cultured as monolayers and suspended in Dulbecco's modified Eagle medium (DMEM-F12) for all cell lines under test, supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.

Proliferation assay on human cell lines

Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

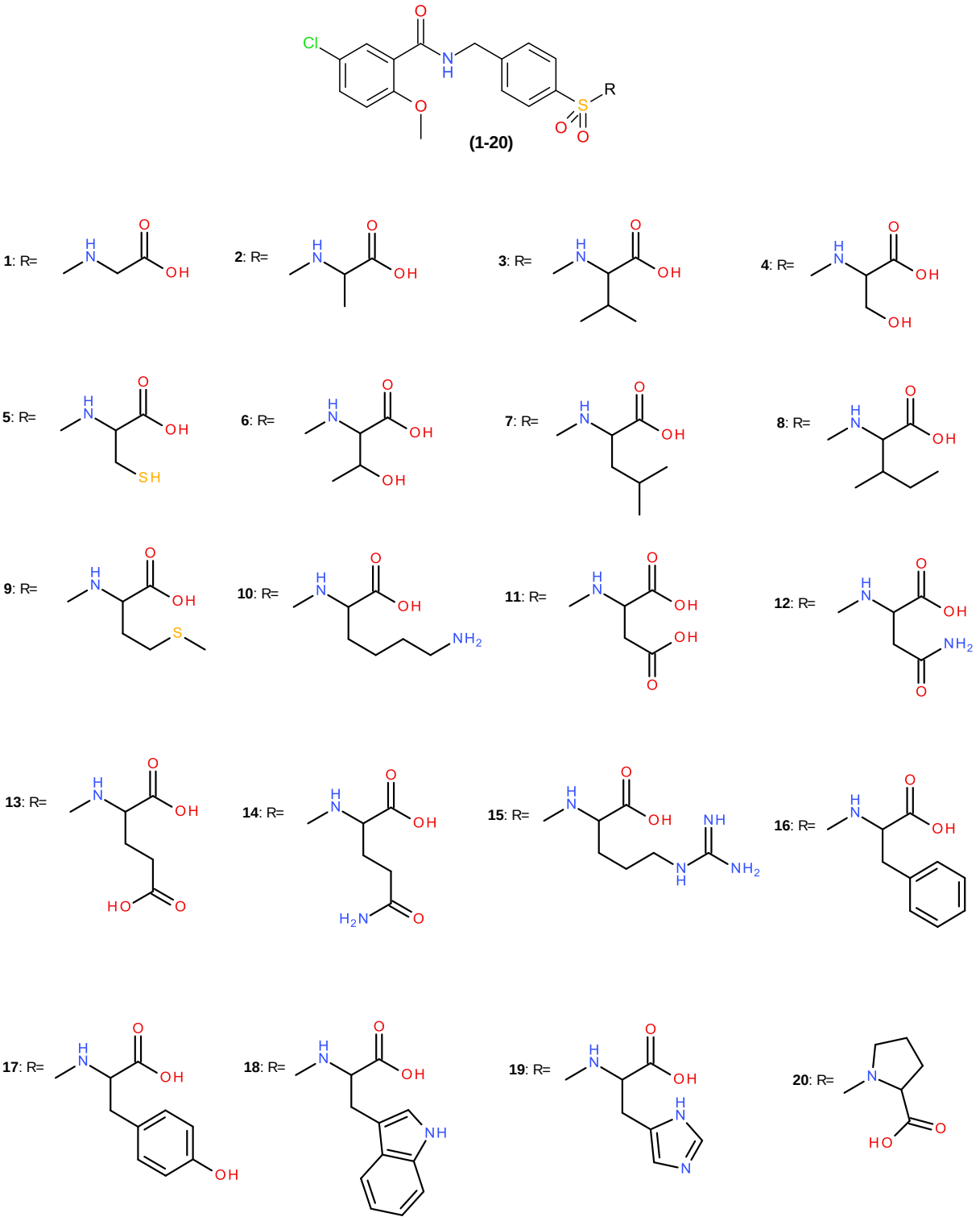

Fig. 1 A series of sulfonyl-α-L-amino acids coupled with anisamide scaffold derivatives (**1**-**20**)

bromide) to purple formazan [34]. All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM-F12 medium for all cell lines under test, 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2. Cells were batch cultured for 10 days, then seeded at a

concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 hr under 5% CO_2 using a water-jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, a fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with tested compounds. Compounds were tested at a concentration of 100 µg/ml to determine the most promising compounds. Then the most promising compounds were screened at different concentrations to give a final concentration of (100-50-25-12.5-6.25-3.125-1.56 and 0.78 µg/ml). After 48 hr of incubation, the medium was aspirated, 40 µl MTT salt (2.5 µg/ml) was added to each well and incubated for a further 4 hr at 37 ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37 ºC. A positive control composed of 100 µg/ml was used as a known cytotoxic natural agent that gives 100% lethality under the same conditions [35,36].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with the vehicle) using an independent t-test by SPSS 11 program. DMSO is the vehicle used for the dissolution of compounds and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

(1-(Reading of compound / Reading of negative control)) x 100

Probit analysis was carried for IC_{50} determination using SPSS 11 program.

In the present study, the degree of selectivity (SI) of the synthetic compounds is expressed as SI=IC_{50} of a pure compound in a normal cell line/IC_{50} of the same pure compound in a cancer cell line, where IC_{50} is the concentration required to kill 50% of the cell population.

Docking study:

The file corresponding to X-ray diffraction of tubulin-colchicine complex (PDB ID: 4O2B) was downloaded from protein data bank (http://www.rcsb.org/pdb/welcome.do) with resolution 2.3 A°. Its energy was minimized using the YASARA Energy Minimization Server (http://www.rcsb.org/pdb/welcome.do), all bound water, ligands, and cofactors were removed. The 2D structure of the compounds under study was created using BIOVIA Draw 2019 program (Dassault Systems). The PDB file corresponding to the 3D structures was created and energy minimized using VEGA ZZ 3.2.1.33. The pdbqt file format of the protein and the synthesized compounds were created using MGL Tools 1.5.7 (www.mgltools.Scripps. edu). Docking calculation was performed using Auto Dock Vena and PyRx 0.8 (www.mgltools.scripps.edu). The results were analyzed based on the binding of the ligand at the inhibitory pocket using PyMol 1 (www.pymol.org).

Results and Discussion

Chemistry

Twenty sulfonyl-α-L-amino acid derivatives (**1-20**) (Fig. 1) were synthesized according to the method described elsewhere [31] and their structures were confirmed by mp, high-resolution mass spectrometry and, ^1H and ^{13}C nuclear magnetic resonance measurements.

Biological evaluation

In vitro antiproliferative activity and cytotoxicity effect.

In the current study, a series of sulfonyl-α-L-amino acids coupled with anisamide scaffold derivatives (**1-20**) were evaluated for their antiproliferative activity against five types of human cell lines *in vitro*. Four were carcinoma cell lines namely, Caucasian breast adenocarcinoma (MCF7), Hepatocellular carcinoma (HEPG2), Colon cell line (HCT116), and Pancreatic cell line (PaCa2) and comparing their results with Skin fibroblast cell line (BJ1) as a normal cell line; the well-known anticancer agent Doxorubicin was used as a positive control. The results were expressed in terms of IC_{50} values. It is well known that stopping proliferation is a characteristic feature of normal growing cells after they reach a confluent monolayer in a culture dish. Conversely, cancer cells continue their multiplication and they do not have the contact inhibition phenomenon (the process by which cells stop proliferating when they reach confluence, despite the

availability of extracellular nutrients and growth factors) [37,38]. Consequently, the carcinoma cell lines were incubated overnight at 37 °C to get about 70–75% confluence while normal cell lines must be fully confluent at (time zero). Finally, the percentage of dead cells was calculated after treatment.

Primary screening

At first, the sulfonyl-α-L-amino acid derivatives (**1**-**20**) were screened against the four human cancer cell lines and the BJ1 at 100 µg/ml to estimate the derivatives that have activity more than 50%. Table 1 showed that nine compounds gave activity more than 50% on the HEPG2 cell line, nine compounds on the PaCa2 cell line, and ten compounds on the MCF7 cell line. Also, compounds **5**, **17**, **2**, **11**, and **4** have potent cytotoxic effect on the HEPG2 cell line with 86.5%, 75.3%, 73.5%, 68.8%, and 67.2% respectively. Similarly, the compounds **18**, **2**, **8**, **17**, **7**, and **3** showed potent cytotoxic activity on the Paca2 cell line within the range of 84.4% to 73.3%. The compounds **14**, **16**, **7**, **18**, **17**, **15**, and **8** have a strong to moderate cytotoxic potency on the MCF7 cell line with 84.4%, 83.4%, 64.7%, 63.5%, 60.4%, 56.2%, and 54.3% respectively. The compounds **16**, **11**, and **18** have a moderate to weak cytotoxic effect on the HCT116 cell line with 42.6%, 26.4%, and 25.3% respectively. Regarding the normal cell line BJ1, the compounds **9**, **3**, **19**, **1**, **6**, **10**, **12**, and **13** were considered highly safe with less than 6.5%, and the cytotoxic activity of compounds **20**, **4**, **11**, and **5** against BJ1 did not exceed 19.5%. So, the promising compounds were subjected to secondary screening to calculate their IC_{50} and their selectivity index.

Table 1: Percentage of the cytotoxic effect of sulfonyl-α-L-amino acid derivatives against the tested human cancer cell lines and their cytotoxic effect on the BJ1 normal cell line at 100 µg/ml

Compound	Amino acid moiety	HCT116	HEPG2	MCF7	PaCa2	BJ1
1	Gly	5.3%	59.4%	24.9%	24.7%	2.5%
2	Ala	2.3%	73.5%	42.2%	79.3%	84.2%
3	Val	5.9%	41.6%	49.8%	73.3%	1.3%
4	Ser	23.2%	67.2%	51.8%	26.3%	12.3%
5	Cys	3.8%	86.5%	51.2%	44.4%	19.5%
6	Thr	13.4%	5.9%	6.8%	36.6%	3.6%
7	Leu	1.5%	28.1%	64.7%	74.2%	74.6%
8	Ile	4.8%	54.2%	54.3%	77.7%	76.3%
9	Met	2.4%	3.8%	5.6%	36.7%	1.2%
10	Lys	20.6%	55.8%	34.8%	50.9%	3.7%
11	Asp	26.4%	68.8%	52.9%	30.6%	12.8%
12	Asn	1.8%	12.5%	42.3%	33.7%	5.8%
13	Glu	2.5%	2.9%	10.5%	25.8%	6.5%
14	Gln	4.9%	15.4%	84.4%	31.3%	65.8%
15	Arg	2.1%	28.2%	56.2%	39.5%	53.8%
16	Phe	42.6%	31.5%	83.4%	51.2%	61.2%
17	Tyr	20.5%	75.3%	60.4%	75.2%	47.2%
18	Trp	25.3%	60.1%	63.5%	84.4%	55.2%
19	His	1.2%	6.3%	1.3%	20.8%	2.4%
20	Pro	9.2%	13.2%	38.3%	58.9%	11.5%
DMSO		1%	1%	3%	1%	1%
Doxorubicin		100%	100%	100%	100%	100%

Secondary Screening

The fifteen promising sulfonyl-α-L-amino acid derivatives were evaluated their activity against three human cancer cell lines (HEPG2, MCF7, and PaCa2 cell lines) and the normal skin fibroblast cell line to calculate their IC_{50} and selectivity index (Table 2). The results showed that compounds **5**, **14**, and **18** were found to be the most active derivatives against HEPG2, MCF7, and PaCa2 cell lines with IC_{50} 51.9, 54.2, and 59.7 µg/ml respectively. Despite their activities were found to be half of the doxorubicin, but they have a poor selectivity index (1.77, 1.5, and 0.98). On contrary, compound **3** has a high selectivity

index >9 for breast (MCF7) and pancreatic (PaCa2) cancer cell lines >11, due to its low cytotoxicity on the normal cell line but its IC_{50} represents one-third of the drug (90.9 and 69.5 µg/ml). Similarly, compounds **1** and **10** have a high cytotoxic selectivity index (>9) to liver carcinoma (HEPG2) cell line but its IC_{50} represents one-fourth of the drug (85.1 and 87.0 µg/ml).

Table 2: IC_{50} (µg/ml) for promising compounds and their selectivity index.

compound	IC_{50} (µg/ml) HEPG2	Selectivity Index	IC_{50} (µg/ml) MCF7	Selectivity Index	IC_{50} (µg/ml) PaCa2	Selectivity Index	IC_{50} (µg/ml) BJ1
1	85.1	>9	ND	ND	ND	ND	>800
2	73.1	0.60	ND	ND	64.6	0.67	43.9
3	ND	ND	90.9	>9	69.5	>11	>800
4	80.6	>4	92.6	>4	ND	ND	>300
5	51.9	1.77	93.3	0.9	ND	ND	92.1
7	ND	ND	82.9	0.69	68.2	0.84	57.7
8	90.1	0.77	88.7	0.78	65	1.06	69.5
10	87.0	>9	ND	ND	ND	ND	>800
11	77.1	>4	91.0	>4	ND	ND	>400
14	ND	ND	54.2	1.5	ND	ND	81.4
15	ND	ND	87.5	>3	ND	ND	>300
16	ND	ND	63.2	1.36	ND	ND	86.2
17	64.8	1.46	83.2	1.14	69.4	1.3	95.1
18	84.4	0.69	74.5	0.78	59.7	0.98	58.7
20	ND	ND	ND	ND	85.7	0.16	13.5
negative control	0 %		0 %				
Doxorubicin	21.6		26.1		28.3		

ND not determined due to having less than 50% cell death

Docking study:

Microtubules are the key component of the cytoskeleton of eukaryotic cells and play a critical role in cell division. So, microtubule dynamics was found to be an important target for developing new anti-cancer drugs. Early, it was reported that the sulfonamide derivatives play a great role as antitumor drugs by the pound to the colchicine-binding site of tubulin, which further prevents the polymerization of microtubule [**Error! Bookmark not defined.**]. Consequently, it led to mitotic cell division arrest at the G2/M phase [39,40].

In the present study, docking calculation was carried out for the compounds under investigation using the Auto dock Vina software. The docking calculations aim to predict the binding geometry for each binder and compare it with its activity. The binding affinity (kcal mol^{-1}) and hydrogen bonds formed with the surrounding amino acids of the inhibitory pocket of the receptor tubulin were used to predict the test compound's binding modes (Table 3). Colchicine was used as a reference drug. The docking results were analyzed in the inhibitory pocket of tubulin using PyMol 1 (www.pymol.org). Their binding affinities ranged from −10.8 to −9.0 kcal mol^{-1}, while colchicine has an affinity of −10.3 kcal mol^{-1} and formed a hydrogen bond with VAL 181:A (Table 3). Also, the sulfonamide and carboxylic groups of the investigated compounds play a great role in their activity by forming strong hydrogen bonds with Gln11, Asn101 in chain A and Asn249, Lys254 in chain B. The only exception is compounds **3** and **20**, which formed hydrogen bonds with Ser178, Arg221 in chain A and Thr353 in chain B. On the other hand, the anisamide moiety extended to the lipophilic region, which is surrounded by Ser178 of chain A and Leu248, Ala250, Leu255, Lys352 of chain B. Figs. 2, 3, and 4 represent the binding modes of compounds **5**, **14** and **18**. The 3D structures were created by PyMol 1 (www.pymol.org), while the 2D pose views were created using the Hamburg University, Centre of Bioinformatics server (http://proteinsplus.zbh.uni-hamburg.de/ #poseview).

The structure of tubulin was deleted from the view to clarify the docked conformers.

The present study supported the results published by Galal, Abdelaziz, Toner, and Liu [19,32,41,42]. It was reported that the synthesized compounds mediated their anticancer action through the binding to the colchicine-binding site of tubulin and exert part of their action through arresting cell division at the G2/M phase.

Table 3: The binding affinity (kcal mol^{-1}) and hydrogen bonds formed with the surrounding amino acids of the tubulin-binding pocket.

compound	Amino acid moiety	Docking affinity kcal mol^{-1}	Hydrogen bonds	
			Chain A	Chain B
1	Gly	-9.9	Asn101, Tyr224	Gln247, Asn249, Lys254
2	Ala	-10.0	Gln11, Asn101	Asn249, Lys254
3	Val	-9.4	Ser178, Arg221	Thr353
4	Ser	-9.8	Gln11, Asn101, Tyr224	Asn249, Lys254
5	Cys	-9.1	Gln11, Asn101	Asn249, Lys254
6	Thr	-9.4	Gln11, Asn101, Tyr224	Gln247, Asn249, Lys254
7	Leu	-9.4	Gln11, Asn101	Asn249, Lys254
8	Ile	-9.4	Gln11, Asn101	Asn249, Lys254
9	Met	-9.0	Gln11, Asn101	Asn249, Lys254
10	Lys	-9.2	Gln11, Asn101	Asn249, Lys254, Thr353
11	Asp	-9.2	Gln11, Asn101, Ser178, Arg221, Tyr224	Asn249, Lys254
12	Asn	-9.1	Asn101, Ser178, Arg221	Gln247, Asn249, Lys254
13	Glu	-9.3	Gln11, Asn101	Gln247, Asn249, Lys254
14	Gln	-9.6	Gln11, Asn101	Gln247, Asn249, Lys254
15	Arg	-9.8	Gln11, Asn101, Ser178	Asn249, Lys254
16	Phe	-10.2	Gln11, Asn101, Tyr224	Asn249
17	Tyr	-10.0	Asn101, Tyr224	Asn249, Lys254, Thr353
18	Trp	-10.8	Gln11, Asn101, Thr 179, Arg221, Thr224	Asn249, Thr353
19	His	-9.9	Gln11, Ser178, Tyr224	Asn249
20	Pro	-9.1	Ser178, Arg221	Thr353
Colchicine		-10.3	Val181	-

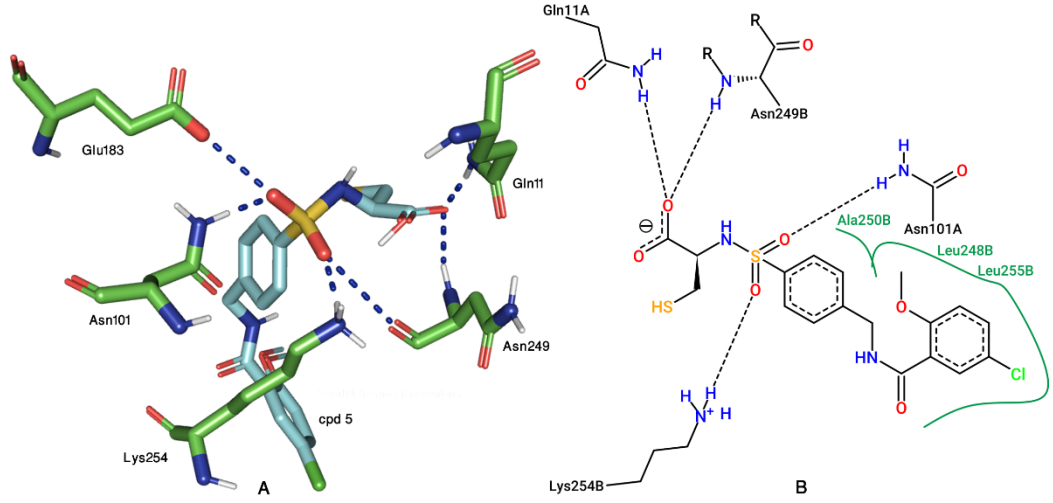

Fig. 2: (A) The 3D binding mode of compound **5** (blue color) showing the polar contact (blue dashed lines) with residues in the active site of tubulin (green color), which include intermolecular hydrogen bonds with Gln11, Asn101 of chain A and Asn249, Lys254 of chain B in addition to π-sulfur interaction with Glu183 and Asn249. (B) The pose view of residues in the active site of tubulin interacting with compound **5**. The black dashed lines indicate hydrogen bonds; the green solid lines show hydrophobic interactions.

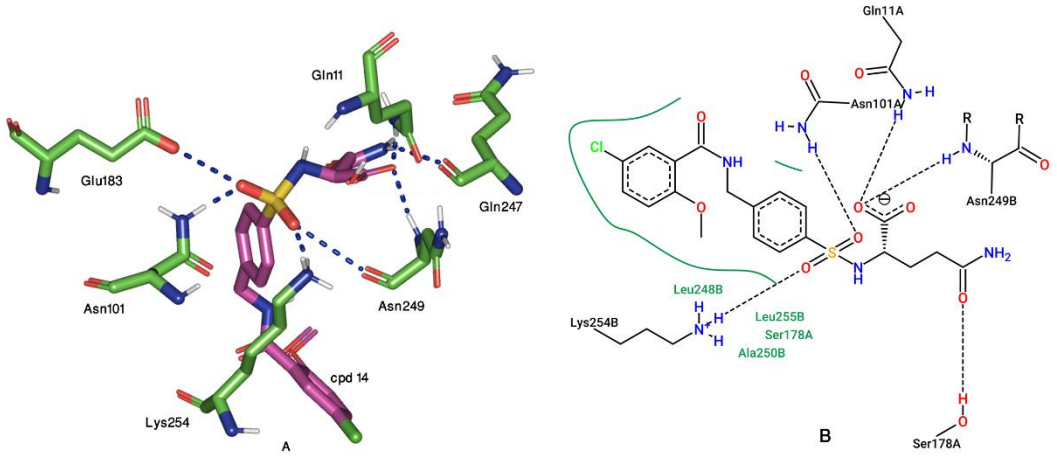

Fig. 3: (A) The 3D binding mode of compound **14** (cyan color) showing the polar contact (blue dashed lines) with residues in the active site of tubulin (green color), which include intermolecular hydrogen bonds with Gln11, Asn101 of chain A and Gln247, Asn249, Lys254 of chain B in addition to π-sulfur interaction with Glu183 and Asn249. (B) The pose view of residues in the active site of tubulin interacting with compound **14**. The black dashed lines indicate hydrogen bonds; the green solid lines show hydrophobic interactions.

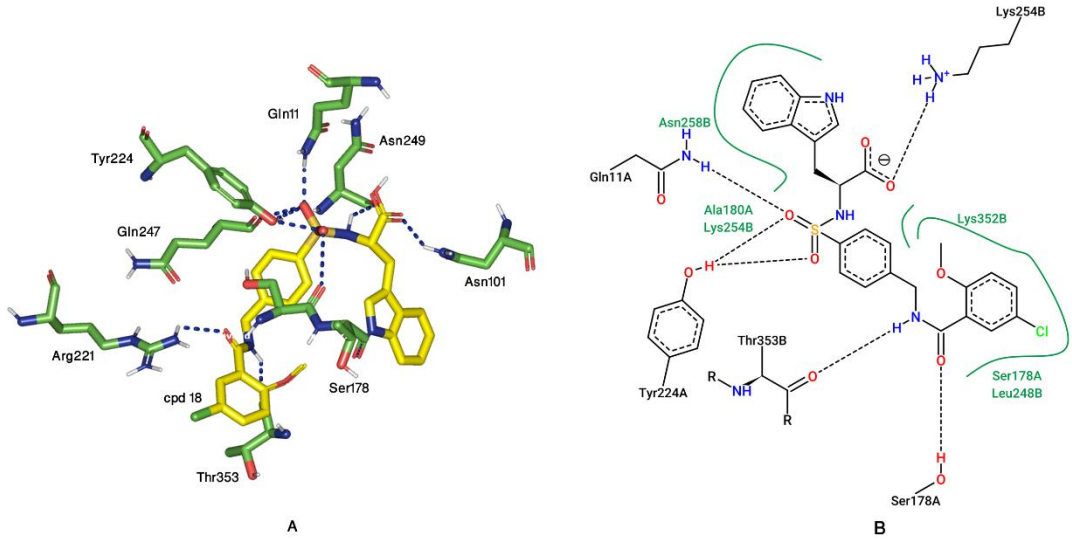

Fig. 4: (A) The 3D binding mode of compound **18** (yellow color) showing the polar contact (blue dashed lines) with residues in the active site of tubulin (green color), which include intermolecular hydrogen bonds with Gln11, Asn101, Thr 179, Arg221, Thr224 of chain A and ASN 258, Thr353 of chain B in addition to π-sulfur interaction with Ser178 and Tyr224. (B) The pose view of residues in the active site of tubulin interacting with compound **18**. The black dashed lines indicate hydrogen bonds; the green solid lines show hydrophobic interactions.

Conclusion

Series of sulfonyl-α-L-amino acid derivatives were evaluated for their antiproliferative activities against four types of human cancer cell lines *in vitro* namely, Caucasian breast adenocarcinoma (MCF7), Hepatocellular carcinoma (HEPG2), Colon cell line (HCT116), and Pancreatic cell line (PaCa2) and comparing their results with Skin fibroblast cell line (BJ1) as a normal cell line, using Doxorubicin as a positive control. The results showed that compounds **5**, **14**, and **18** were the most active molecules against HEPG2, MCF7, and PaCa2 cell lines with IC_{50} 51.9, 54.2, and 59.7 µg/ml respectively. Docking studies reveal that sulfonyl-α-L-amino acid derivatives **5**, **14**, and **18** mediated their anticancer action through

binding to the colchicine-binding site of tubulin by arresting cell division at the G2/M phase.

Conflicts of interests

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors would like to thank Dr. Atef G. Hanna for valuable advice and guidance, and the National Research Centre, Egypt, for the laboratory facilities and financial support.

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