Design, Synthesis of New Amino Acid Derivatives and Evaluated DNA Binding Activity, Anticancer and Antimicrobial Activity

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Abstract: Recently, sulfonamides have been reported to show significant antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity. The present work reports the synthesis some novel amino acid sulfadiazine derivatives using novel method, this may play a role in their anticancer activity. All the newly synthesized compounds were evaluated for DNA binding activity and antimicrobial activity, some synthesized compounds showed high DNA binding activity and antimicrobial activity. Some selected compounds were evaluated for anticancer activity against breast cancer cell line (MCF7) in vitro. All selected compounds showed interesting cytotoxic activities compared to a reference drug. [Journal of American Science. 2010;6(11):240-249]. (ISSN: 1545-1003).

Keywords: Peptide; Amino Acid; Anticancer; Antimicrobial; DNA Binding

1. Introduction

Sulfonamides posses many types of biological activities and representatives of this class of pharmacological agents are widely used in clinic as antibacterial [1], antithyroid [2], diuretic [3,4], hypoglycaemic [5] and anti-carbonic anhydrase [3,6]. From other studies, Sulfadiazine derivatives have been reported to show considerable antitumor activity [7,8]. Also, aryl/heteroaryl sulfonamides may act as antitumor agents through several mechanisms, such as disruption of microtubule assembly, angiogenesis inhibition, perturbation in the G1 phase, functional suppression of the transcriptional activator NF-Y, and most important suggested mechanism by inhibition of carbonic anhydrase isozymes(CA) [9-13]. After wildly evaluation, Sulfonamides were found act as carbonic anhydrase (CA) inhibitors [14].

On other hand, the structural changes of DNA Based on the interaction of small molecular weight ligands with DNA (deoxyribonucleic acid) have attracted attention in the medicinal design of anticancer and anti-AIDS drugs [15-22]. Moreover, peptide derivatives pose anti tumour effect [23-25].

In the light of these facts, the present study aim to syntheses of peptides series of sulfadiazinylacetlyl derivatives, and test the influence of these compounds toward the DNA binding affinity, anticancer activity and antimicrobial activity.

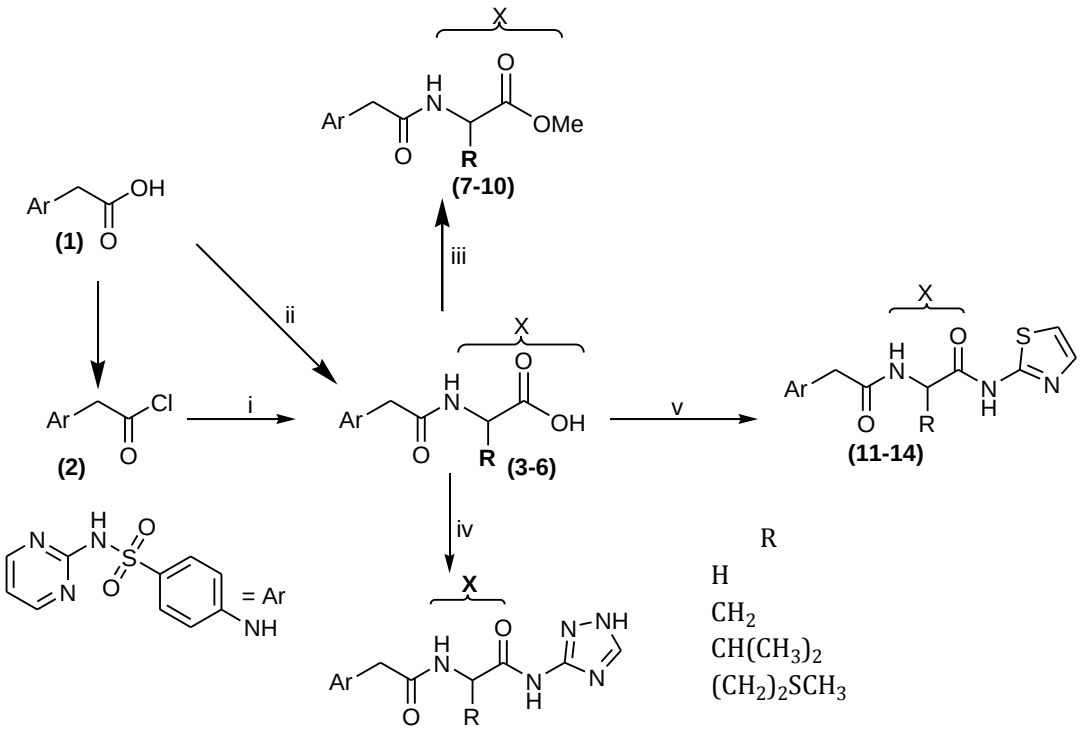
2. Results and discussion:

2.1. Chemistry:

In this paper, aimed to prepare bio molecules contain sulfonamide moiety using novel method. This method is convenient, high yield, high purity and completing chemical reactions in short time comparable with other methods which take several hours to days.

The synthetic route designed for the pseudo-peptide derivatives (**3-22**), was summarized in (Scheme 1,2). The reaction of acid chloride (**2**) with amino acids as nucleophiles, might seem hard to conduct. The reason might be due to characteristics of amino acids. Since, amino acids are amphoteric compounds, in solution a dipolar ion ($^{+}$NH3CH(R)-COO^{-}) is formed by a proton transformation from the carboxyl group to the nitrogen atom of amino group [26]. The amphoteric natures of amino acids decrease the electron density on nitrogen atom. Thus, the zwitterions amino acids possess lower nucleophility than amines and are difficult to react with acid chloride. In order to facilitate the reaction, adding an organic base such as triethyamine(TEA) to improve the reaction rate. The formations of sulfadiazinylacetyl amino acids (**3-6, 19**) were achieved by the reaction of (**2**) with suitable type of amino acids (Scheme 1,2) in tetrahydrofurane (THF/ TEA) media, or by heating Sulfadiazinyl acetic **(1)** with amino acids at 250 °C (Scheme 1,2).

The IR spectra of free amino acid derivatives (**3-6,19**) displayed a broad bands for (NH) and (OH) absorption between ν=3157–3382cm^{-1}. The (CO) group of carboxyl group occurred between ν=1672-1698 cm-1. Characteristic ^{1}HNMR of these amino acid derivatives (**3-6,19**) were displayed peaks between δ=11.82-12.65 ppm for the proton of the OH group, which disappeared immediately when treated with D_2O. Also, when compounds (**3-6,19**) were reacted with thionyl chloride (molar ratio) in methanol give the corresponding methyl esters (**7-10** and **20**; Scheme 1,2).



Reagent and conditions :i- amino acids/ THF/TEA/H_2O ii- heat 250°C iii-$SOCl_2$/MeOH
iv- 2-amino thiazole/ DCC/THF v- 2-amino triazole/ DCC/THF

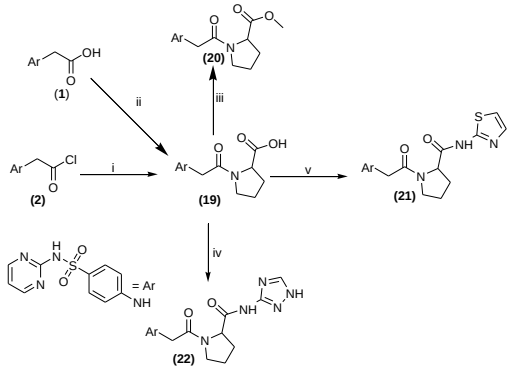
(SCHEME 1)

The IR spectra of amino acid methyl ester derivatives (**7-10** and **20**) disappeared a bands for (OH) of carboxyl group, which showed a characteristic peaks between δ=3.59-3.76 ppm for a proton (CH_3) of methoxy group. Thiazolo derivatives (**11-14 and 21**; Scheme 1,2) and Triazolo derivatives (**15-18** and **22**; Scheme 1,2) were prepared by the reaction of compounds (**3-7** and **18**) with 2-aminothiazole and/or 3-amino-1,2,4-triazol in presence of dicyclohexylcarbodiimide (DCC/ THF) media. ^{1}HNMR spectra showed that, The amide protons **(NH)** of thiazole derivatives (**11-14** and **21**), and triazole derivatives (**15-18** and **22**) were affected by the deshielding effect of CO group, and the chemical shift was δ= 9.91-13.33 ppm, which disappeared immediately when treated with D_2O. The results of chemical analyses of the synthesized compounds were summarized in Tables (1-3).

*Table (1): Showed physical data and elemental analysis of the synthesized compounds (**3-22**):*

*Table (2): Showed of ^{1}H-NMR in (CDCl3) results of synthesized compounds (**3-22**).*

*Table (3): Showed of IR and mass spectroscopy results of synthesized compounds (**3-22**).*



Reagent and conditions :i- amino acids/ THF/TEA/H_2O ii- heat 250°C iii-$SOCl_2$/MeOH
iv- 2-amino thiazole/ DCC/THF v- 2-amino triazole/ DCC/THF

(SCHEME 2)

2.2. Biological activity:

2.2.1. DNA Binding Assay:

The mechanism of several antitumor compounds and antitumor antibiotics depend on their interaction with DNA. In this work, the antitumor activities of the newly synthesized compounds were determined using DNA binding assay and methyl green DNA displacement assay [27,28]. In this method, a fixed amount of the ligand is spotted on the RP-18 TLC plates, followed by addition of known amount of DNA on the same spot. The plate was then developed and the position of unbound DNA was determined by

spraying the plates with anisaldehyde reagent. The free DNA was detected as a blue spot (R_f, MeOH–H2O, 8:2) on RP-18 TLC. It was demonstrated that, when DNA was mixed with compounds known to interact with it, e.g. ethidium bromide, the complex was retained at the origin. Compounds with high binding affinity to DNA remained on the base line or migrated for a very short distance, while compounds with poor binding affinity did not cause DNA to be retained at the origin [27].

2.2.2. Methyl Green-DNA Displacement Assay:

Methyl green reversibly binds polymerized DNA forming a stable complex at neutral pH [28]. The maximum absorption for the DNA-methyl green complex is 642-645 nm. This colorimetric assay was used to measure the displacement of methyl green with DNA by compounds, which having the ability to bind with DNA. The degree of displacement was determined spectrophotometrically by measuring the change in the initial absorbance of the DNA-methyl green solution in the presence of reference compound.

Table (4): Showed DNA binding activity of compounds (**3-22**) using methyl green DNA displacements assay as IC_{50} (concentration required for 50% decrease in the initial absorbance of the DNA/methyl green solution).

Results from (Table 4) indicate that, Compounds (**3, 6, 7, 8 and 10**) showed the highest affinity for DNA, which was demonstrated by retaining the complex at the origin or by migrating for a very short distances, and by measuring IC_{50}. Compounds (**5, 11, 18, 21 and 22**) showed moderate affinity, while compound (**12,14 and 15**) showed weak affinity.

From these results, the following points can be concluded. The combination of some amino acids with N4-actylSulfadiazinyl (**3-6 and 19**), showed high to moderate activity, except that combined with β-alanine and proline residue gave inactive against DNA. Also, the esterification of C-terminal amino acids of the synthesized compounds (**7-10, and 20**) gave high activity compounds, except that combined with valine(**9**) and proline (**20**) residue gave inactive compounds. Moreover, when elongation of the chain by introduced blocking moieties such as thiazoles (**11-14 and 21**) and\ or triazoles (**15-18 and 22**) showed moderate to week activity against DNA, except that contained β-alaninethiazole (**11**), valinetriazoles (**16**) and Methionintriazoles (**17**) residue gave inactive compounds.

2.2.2. In vitro anticancer screening :

In the present work, four newly synthesized compounds (**3,10,12 and 17**) were selected to evaluate their in vitro growth inhibitory activities against breast cancer cell line (MCF7). Doxorubicin was used as the reference drug in this study. The response parameter calculated was IC_{50} value (concentration required for 50% inhibition of cell viability).

The four selected compounds, were carefully selected to be representatives for all the twenty newly Synthesized compounds, which covering all structural variations in these work. These analogs, being of 2-(4-(N-pyrimidin-2-ylsulfamoyl) phenyl-amino) acetyl attached to (i) glycine moiety (**3**) which represented free amino acids series. (ii) L-methionine methyl ester moiety (**10**), which represented corresponding methyl ester series.(iii) -β-alaninyl-N2-thiazol (**12**) and L-valinyl-N3-4H-1,2,4-triazol" (**17**), which represented series attached with blocking moieties.

Table (5): showed the cytotoxic activity of the synthesized compounds in vitro as IC_{50} (concentration required for 50% inhibition of cell viability) compared to the reference drug Doxorubicin.

From Table (5), it was found that, all the tested compounds showed significant antitumor activities compared to reference compound. Also, variation in biological activity between the synthesized compounds was not very high. Moreover, free amino acid the most potent compound. Furthermore, amino acid methyl ester more potent than those has containing blocking moieties.

2.2.3.Anti-microbial activity:

All the synthesized compounds were screened for antimicrobial activities by paper disc diffusion technique (**29-33**). The tested micro-organism strains were: S. aureus (ATCC-9144), S. epidermidis (ATCC-155), E. coli (ATCC-25922), K. pneumoniae (ATCC-11298), A. niger (ATCC-9029), A. fumigatus (ATCC-46645).

Table (6): Showed the anti-microbial activity of the synthesized compounds and reference drug.

The result from (Table 6) showed that, most synthesized compounds were active against all tested micro-organisms with the range of MICs values for S. aureus(11.1-28.4 µg /ml), S. epidermidis (15.1-28.2 µ/ml), K. pneumoniae (14.6-33.8 µg /ml), E. coli (12.6-25.3 µg/ml), A. niger (11.6-32.2 µg /ml) and A. fumigates(13.4-32.3 µg/ml). The compounds (**3, 6, 7, 8, 9, 13, 15, 16, 20**) were exhibit in vitro anti-microbial activity against all micro-organisms at MICs of (11.1 -33.8 µg/ml). Compounds (10, 15 and 16) were exhibit in vitro anti-microbial activity against most micro-organisms at MICs of (18.7-32.2 µg/ml).

From the previous results, it was concluded that, sulfadiazinylacetyl combined with amino acid residues have antimicrobial activity against all tested microorganisms (**3-6and19**). Moreover, esterifii-cation of C-terminal of amino acids, and/or when elongation the chain by introducing blocking moieties gave biologically active compounds against all tested micro-organisms (**7-10 and20**), excepted that containing methionine methyl ester moiety (**10**), valinetriazol(**17**) and methionine tiazol(**18**) moiety.

In conclusion, the present work was aimed to design sulphonamide amino acid derivatives using novel method; it was clearly observed that the compounds containing free amino acid moieties and corsponding methyl ester moieties exhibit significant anticancer activity, DNA binding activity and antimicrobial activity. When these compounds attached with blocking group lead to decrease anticancer activity, DNA binding activity and antimicrobial activity. SO, the further modification of these compounds may be promising candidates for clinically useful drug agents.

3.EXPERMINTAL:

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (Rf) for analytical purposes was carried out on silica gel and developed with benzene-ethyl acetate (6:1) using iodine-KI (20%) solution as spraying agent. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The IR spectra of the compounds were recorded on a Perkin–Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm-1. The ^{1}HNMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. The mass spectra were recorded on a mass spectrometer HP model MS–QPL000EX (Shimadzu) at 70 eV. Elemental analyses (C,H,N) were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt.

3.1. Synthesise:

3.1.1.*Preparation of 2-(4-(N-pyrimidin-2-yl sulfamoyl)phenylamino)acetic acid (3-6 and19):*

General Procedures:

Mothed A:

A mixture of amino acids (1.5 equiv) was dissolved in water (25 ml), THF (15 ml) mixture and triethylamine (2 ml) was added, followed by portionwise addition of sulfadizinylacetyl chloride (**2**; 1 equiv) during 30 min. The temperature of the reaction mixture during the addition was kept at 10°C. Stirring continued for 2 hrs. at 20°C. (THF) was removed by concentration of the reaction mixture under reduced pressure; water (30 ml) was added and acidified with 2 M HCl to pH=5. The crude products were filtered and recrystallized from Ethanol. All the products (**3-6 and 19**) were chromatographically homogeneous by iodine and benzidine development.

Mothed B:

A mixture of amino acids (0.01 mol) and sulfanadiazine (**1,** 0.01 mol) was fused at 250 °C in an oil bath for 15 min. Fused mass was dissolved in ethanol and poured onto cold water, the solid obtained was recrystallized from ethanol.

3.1.2.Preparation 2-(4-(N-pyrimidin-2-ylsulf-amoyl)phenylamino)acetyl amino acid methyl esters (7-10 and 20):-

General Procedures:

A suspension of coupling reaction products (**3-7**; 1 equiv) in absolute methanol (150 ml) was cooled to -10°C and pure thionylchlorid (1 equiv) was added dropwise during one hour. The reaction mixture was stirred for an additional 34 hrs. at room temperature, and then kept overnight when the solvent was removed by vacuum distillation. The residual solid material was recrystallized from Ethanol. All the products (***7-10 and 20***) were chromatographically homogeneous by iodine and benzidine development.

3.1.3.Preparation of 2-(4-(N-pyrimidin-2-ylsulf-amoyl)phenylamino)acetylamino acid- N^{2}-thiazole (11-14and 21):

General Procedures:

To a solution of 2-aminothiazol (1.5 equiv) was dissolved in THF, the solution was stirred for 30 min. at 20°C, and cooled to 0 °C, free amino acids (**3-6and 19**;1equiv) in THF (50 ml) and DCC (1 equiv) were added to the above mixture. The reaction mixture was stirred for 6 h. at 0°C and for another 12 hrs. at room temperature. The crude material is diluted with EtOAc and washed with sat. aq. Na2CO3 (×2) and brine (×1), dried over sodium sulfate, evaporated, and purified by Ethanol to give desired products. The products (***11-14and 21***) were to be chromate-graphically homogeneous by iodine and benzidine development.

3.1.4. Preparation of 2-(4-(N-pyrimidin-2-ylsulf- amoyl)phenylamino)acetylamino acid - N^{3}-4H-1,2,4-triazol (15-18 and 22):

General Procedures

To a solution of 1H-1,2,4-triazol-3-amine (1.5 equiv) was dissolved in THF, the solution was stirred for 30 min. at 20°C, and cooled to O°C, free amino acids (**3-6 and 19**;1equiv) in THF (50 ml) and DCC (1 equiv) were added to the above mixture. The reaction mixture was stirred for 6 hrs. at 0°C and for another 12 hrs. at room temperature. The crude material is diluted with EtOAc and washed with

aqueous Na2CO3 (×2) and brine (×1), dried over sodium sulfate, evaporated, and purified by Ethanol to give desired products. The products (***15-18 and 22***) were to be chromatographically homogeneous by iodine and benzidine development.

3.2. Biological screening:

3.2.1. Evaluation of the degree of DNA binding:

3.2.1.1. DNA binding assay on TLC plates:

Analyses of the DNA binding affinity of the tested compounds were predeveloped first using methanol-water (8:2). The tested compounds were then applied (5 mg/ml in methanol) at the origin, followed by the spotting of DNA (1 mg/ml in methanol-water mixture (8:2) at the same positions at the origin. Ethidium bromide was used as a positive control. After complete spotting, the plates were developed with the same solvent system, and the positions of DNA were visualized by spraying the plates with anisaldehyde, which produces a blue colour with DNA. The intensity of the colour was proportional to the quantity of DNA added to the plate.

3.2.1.2. Colorimetric assay for the degree of DNA binding:

DNA\methyl green complex (20 mg) was suspended in 100 ml of 0.05M tris-HCl buffer (pH 7.5) containing 7.5 mM MgSO4 and stirred at 37 °C with a magnetic stirrer for 24 hrs. The calculated amounts of samples were placed in Eppendorf tubes, and (200 µl) of the DNA-methyl green solution was added to each tube. The samples were incubated in dark at room temperature, and after 24 hrs. the final absorbance of each sample was determined at (642-645) nm. The results were recorded in form of the IC50 for each compound, which is the sample concentration required to produce 50% decrease in the initial absorbance of the DNA-methyl green complex. Ethidium bromide was used as a positive control. The molar concentration required for 50% decrease in the initial absorbance of the DNA-methyl green complex was calculated and the results are given in Table 4.

3.3.2. Anticancer screening:

Antitumor screening was performed at the National Cancer Institute, Cancer Biology Department, Cairo, Egypt. Cytotoxic activity was measured in vitro for the selected synthesized compounds (3, 10, 15 and 18) using Skehan et al method(34). Cells were plated in 96-multiwellmicrotiter plate (104 cells/well) for 24 hrs. before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume.

Different concentrations of the compound under test (0, 1, 2.5, 5, and10 mg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 hrs. at 37 °C and in atmosphere of 5% CO2. After 48 hrs., cells were fixed, washed and stained with SRB (Sulfo-Rhodamine-B) stain. Excess stain was washed with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumour cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC50) was calculated and the results are given in Table 5.

3.3.3. Anti-microbial screening

Biological activities were carried out at the biogenetic engineering center, molecular biology unit, Al-Azhar University, Nasr city, Egypt. The anti-bacterial activity of the synthesized compounds was tested against two Gram- positive bacteria S. aureus (ATCC-9144) and S. epidermidis (ATCC-155), two Gram-negative bacteria E. coli (ATCC-25922), and K. pneumoniae (ATCC-11298) and two fungi namely A. niger (ATCC-9029)and A. fumigatus (ATCC-46645) using nutrient agar medium.

3.3.3.1. Paper disc diffusion technique:

The sterilized (autoclaved at 120 °C for 30 min) medium (40-50° C) was incubated (1ml/100ml. of medium) with the suspension (105 cfu ml-1) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (µg\ml-1 in methanol) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37 °C for 24 and 48 hrs. for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (100 µg/disc) were used as standard for antibacterial and anti-fungal activity respectively. The observed zone of inhibition is presented in Table 6.

4.3.3.3Minimum inhibitory concentration (MIC):

MIC of the compound was determined by agar streak dilution method (35). A stock solution of the synthesized compound (100 µg\ml-1) in methanol was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50 °C) containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately (105cfu ml-1) and applied to plates with serially

diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 and 48 hrs. for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of .the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in (Table6).

Table (1): Physical data for the compounds (3-22):

Cpd. No.	X	Yield %	M.P. °C	Color	$[\alpha]_D^{20}$	R_f	Molecular Formula (M.Wt.)	Elemental Analysis Calculated/Found		
								C	H	N
3	Gly	80	195-97	brown	__	0.55	$C_{14}H_{15}N_5O_5S$ 365	46.02 46.09	4.10 4.14	19.17 19.22
4	β-Ala	83	160-62	brown	__	0.60	$C_{15}H_{17}N_5O_5S$ 379	47.49 47.55	4.48 4.52	18.46 18.55
5	L-Val	83	182-84	brown	+116	0.68	$C_{17}H_{21}N_5O_5S$ 407	50.12 50.11	5.15 5.20	17.19 17.29
6	L-Met	75	191-93	brown	+160	0.64	$C_{17}H_{21}N_5O_5S_2$ 439	46.46 46.50	4.78 4.82	15.94 15.93
7	Gly-OMe	50	200-02	yellow	__	0.69	$C_{15}H_{17}N_5O_5S$ 379	47.49 47.85	4.48 4.52	18.46 18.48
8	β-Ala-OMe	57	176-78	yellow	__	0.80	$C_{16}H_{19}N_5O_5S$ 393	48.85 48.89	4.83 4.87	17.81 17.80
9	L-Val-OMe	45	210-13	yellow	+215	0.87	$C_{18}H_{23}N_5O_5S$ 421	51.30 51.29	5.46 5.50	16.62 16.66
10	L-Met-OMe	48	222-24	yellow	+179	0.86	$C_{18}H_{23}N_5O_5S_2$ 453	47.68 47.67	5.07 5.11	15.45 15.44
11	Gly-thiazol	80	247-49	brown	__	0.89	$C_{17}H_{17}N_7O_4S_2$ 447	45.63 45.65	3.80 3.83	21.91 21.94
12	β-Ala-thiazol	75	179-81	brown	__	0.65	$C_{18}H_{19}N_7O_4S_2$ 461	46.85 46.84	4.12 4.15	21.25 21.24
13	L-Val-thiazol	88	240-42	brown	+160	0.94	$C_{20}H_{23}N_7O_4S_2$ 489	49.07 49.12	4.70 4.74	20.04 20.03
14	L-Met-thiazol	77	167-78	brown	+184	0.79	$C_{20}H_{23}N_7O_4S_3$ 521	46.06 46.05	4.41 4.44	18.80 18.89
15	Gly-triazol	83	274-76	gray	__	0.84	$C_{16}H_{17}N_9O_4S$ 431	44.54 44.50	3.94 3.97	29.23 29.22
16	β-Ala-triazol	75	197-99	brown	__	0.90	$C_{17}H_{19}N_9O_4S$ 445	45.84 45.82	4.26 4.30	28.31 28.30
17	L-Val-triazol	67	140-42	brown	+102	0.81	$C_{19}H_{23}N_9O_4S$ 473	48.20 48.19	4.86 4.90	26.62 26.65
18	L-Met-triazol	82	154-56	brown	+193	0.87	$C_{19}H_{23}N_9O_4S_2$ 505	45.14 45.18	4.55 4.59	24.95 24.93
19	L-Pro	78	189-91	brown	+130	0.67	$C_{17}H_{19}N_5O_5S$ 405	50.37 50.36	4.69 4.72	17.28 17.27
20	L-Pro-OMe	30	208-10	yellow	+170	0.88	$C_{18}H_{21}N_5O_5S$ 419	51.55 51.54	5.01 5.05	16.70 16.73
21	L-Pro-thiazol	89	236-38	brown	+213	0.95	$C_{20}H_{21}N_7O_4S_2$ 487	49.28 49.27	4.31 4.34	20.12 20.11
22	L-Pro-triazol	92	248-50	brown	+180	0.92	$C_{19}H_{21}N_9O_4S$ 471	48.40 48.35	4.45 4.49	26.75 26.77

* Crystallization solvent: Ethanol (**3-22**).

Where Gly= Gycine, β-Ala = β-Alanine, Val= Valine, Met= Methionine, Pro= Proline.

Table2: Results of the chemical analyses (^{1}HNMR spectra).

Cpd.	^{1}H NMR spectra of compounds
3	*12.65(s, 1H, OH-COO**H**, exchangeable D_2O), 8.90(s,1H, NH- N**H**SO_2, exchangeable D_2O) 8.63 (s, 1H, NH-N**H**CH_2COOH-Gly, exchangeable D_2O) 8.49 (d, 2H,C**H**-primidine), 7.71-7.07 (m,5H,[4H, Ar-H+1H,CH-primidine]), 5.64 (s,1H, NH-N**H**CH_2CO exchangeable D_2O), 4.24 (s,2H,CH_2-Gly,), 4.06 (s,2H, NHCH_2CO).*
4	*11.82 (s, 1H, O**H**-carboxylic, exchangeable D_2O), 8.89(s, 1H, NH-N**H**SO_2, exchangeable D_2O), 8.49 (d, 2H,C**H**-primidine), 7.73-7.10 (m,6H,[4H, Ar-H + 1H,C**H**-primidine + 1H, CON**H**CH_2 CH_2,exchangeable D_2O]), 5.93(s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.09 (s,2H,CH_2- NHCH_2CO), 3.77-3.65(m,2H,CH_2-NHC$H_2$$CH_2$COOH), 2.56(t, 2H,$CH_2$-$CH_2$ CH_2COOH) .*
5	*11.92 (s, 1H, O**H**- carboxylic, exchangeable D_2O), 8.86(s, 1H, NH-N**H**SO_2, exchangeable D_2O), 8.47 (d, 2H,C**H**-primidine), 8.27(d, 1H, NH-N**H**CH-Val, exchangeable D_2O), 7.69-6.98(m,5H,[4H-Ar-H + 1H,C**H**-primidine]), 5.92 (s, 1H, NH-N**H**CH_2CO exchangeable D_2O), 4.16 (s, 2H, NHCH_2CO), 2.03-1.99 (m,2H,[1H,CH-C**H**$(CH_3)_2$COOH& 1H,CH-C**H**$CH(CH_3)_2$]), 098 (s, 6H,2CH_3-$(CH3)_2$) .*
6	*12.50 (s, 1H, O**H**-carboxylic, exchangeable D_2O), 8.90 (s, 1H, NH-N**H**SO_2 exchangeable D_2O), 8.53(d,2H,C**H**-primidine), 7.72-7.00(m,6H, [4H, Ar-H + 1H,C**H**-primidine + 1H, NH-N**H**CH-Met., exchangeable D_2O]), 5.09 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.59(t, 1H, CH-NHC**H**COOH), 4.13(s,2H,CH_2- NHCH_2CO), 2.67 (t, 2H, CH_2-CH_2SCH$_3$), 2.35(s,6H,2 **CH3**), 2.24-1.97 (m, 2H,CH_2-C**H2**CH_2SCH$_3$).*
7	*8.90 (s, 1H, NH-N**H**SO_2 exchangeable D_2O), 8.75(s, 1H, NH-N**H**CH_2COOCH$_3$, exchangeable D_2O), 8.49 (d,2H,C**H**-primidine), 7.72-7.07(m,5H, [4H, Ar-H + 1H,CH-primidine]), 5.64 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.31 (s, 2H,CH_2-CH_2COOCH$_3$), 4.05 (s, 2H, CH_2-NHCH_2CO), 3.75 (s, 3H,CH_3-COOCH_3).*
8	*8.39 (s, 1H, NH-N**H**SO_2), 8.00 (d, 2H,CH-primidine), 7.72-6.56(m,6H,[4H, Ar-H + 1H,CH-primidine + 1H, NH-N**H**CH_2CH_2exchangeable D_2O]), 4.00 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 3.95(s, 2H-CH_2CONH),3.67(s,3H,CH_3-COOCH$_3$), 3.60-3.58(m, 2H, CH_2- CH_2 CH_2COOCH$_3$),2.52(t,2H, CH_2- CH_2CH_2COOCH$_3$).*
9	*8.39 (s, 1H, NH-N**H**SO_2), 8.00(d,2H,C**H**-primidine), 7.72-6.56(m,6H,[4H, Ar-H + 1H,CH-primidine +1H,N**H**CHCOOCH$_3$, exchangeableD_2O]), 5.00 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.42(s,1H,CH-NHC**H**COOCH$_3$), 3.95(s, 2H-CH_2-NHCH_2CO), 3.67(s,3H, CH_3-COOCH_3), 3.14-3.04(m, 1H,CH-C**H**$(CH_3)_2$), 1.02 (d,6H,(CH_3)$_2$) .*
10	*8.90 (s, 1H, NH-N**H**SO_2,exchangeable D_2O), 8.49-8.48 (d, 2H,CH-primidine), 7.72-7.07(m,6H, [4H, Ar-H + 1H,C**H**-primidine + 1H, NH-N**H**CHCOOCH$_3$- Met. exchangeable D_2O]), 5.66(s, 1H, NH-N**H**CH_2exchangeable D_2O), 4.79(t,1H, CH-NHC**H**COOCH$_3$), 4.05(s, 2H, CH2-NHCH_2CO) 3.76(s,3H, CH_3-COOCH_3),2.67 (t,2H, CH_2-CH_2SCH$_3$, 2.34 (s, 3H, CH_3-SCH_3), 2.24-2.18 (m,2H, CH_2-C$H_2$$CH_2SCH_3$) .*
11	*13.26(s, 1H, NH, CON**H**-thiazole,exchangeable D_2O), 8.90(s, 1H, NH-N**H**SO_2), 8.64 (d,2H,CH-primidine),8.50(s, 1H, NH-CON**H**CH_2-Gly exchangeable D_2O), 7.52-6.95(m,7H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole]), 5.05 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.39 (s,2H, CH_2CONH-Gly), 4.00 (s, 2H, NHCH_2CO).*
12	*12.82(s, 1H, NH-CON**H**thiazole,exchangeable D_2O), 8.90(s, 1H, NH-N**H**SO_2), 8.55 (d, 2H,CH-primidine), 7.71-7.04(m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole+ 1H, NH-N**H**CH_2CH_2exchangeable D_2O]), 5.46 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.08 (s,2H, CH_2-NHCH_2CO), 3.79-3.69 (m, 2H,CH_2-NHC$H_2$$CH_2$CO), 2.79(t, 2H,$CH_2$- NHC$H_2CH_2$CO).*
13	*13.09(s, 1H, NH-CON**H**-thiazole, exchangeable D_2O), 8.90(s, 1H, NH-N**H**SO_2), 8.50-8.49(d,2H,CH-primidine), 7.67-7.09(m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole + 1H, NH-CON**H**CH, exchangeable D_2O]),5.03 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.83(s,1H, CH-C**H**CONH), 4.31 (s,2H, CH_2-NHCH_2CO),2.55-2.47(m, 1H,CH-C**H**$(CH_3)_2$), 1.08 (d,6H,2CH_3-(CH_3)$_2$).*
14	*13.32(s, 1H, NH- CON**H**-thiazole,exchangeable D_2O), 8.90(s, 1H, NH-N**H**SO_2), 8.54-8.52(d,2H,C**H**-primidine), 7.66-9.65 (m,8H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole+ 1H, NH-CON**H**CH,exchangeable D_2O]), 4.89 (s, 1H, NH- N**H**CH_2CO, exchangeable D_2O), 4.64(t,1H, CH-CONHC**H**CH_2CH_2), 4.26(s,2H, CH_2-NHCH_2CO), 2.77(t,2H,CH_2-CH_2SCH$_3$),2.34(s, 3H,CH_3-SCH_3), 2.23-2.17(m, 2H, CH_2-CH_2 CH_2 SCH$_3$).*
15	*9.92 (s, 1H, NH-CON**H**-triazole), 8.90(s, 1H, NH-N**H**SO_2), 8.70(s,1H, NH-NH-triazole), 8.57(s, 1H, NH- CON**H**CH, exchangeable D_2O), 8.50(d, 2H,CH-primidine), 7.69-7.05(m,6H,[4H,Ar-H + 1H,CH-primidine +1H, CH-triazole]), 5.62 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.31 (s,2H, CH_2-CONHCH_2-Gly), 4.12 (s,2H, CH_2-NHCH_2CO).*
16	*9.91(s, 1H, NH-CON**H**- triazole,exchangeable D_2O),8.90 (s, 1H, NH-N**H**SO_2), 8.68 (s, 1H, NH- triazole), 8.48 (d,2H,CH-primidine), 7.68-7.03(m,7H,[4H, Ar-H + 1H,CH-primidine+ 1H, CH-triazole+ 1H, NH-N**H**CH_2CH_2CONHexchangeable D_2O]), 5.60 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.09 (s,2H, CH_2-NHCH_2CO), 3.75-3.69 (m,2H, CH_2-C$H_2$$CH_2$CONH), 2.77 (t,2H, CH_2-CH_2CONH).*
17	*10.79(s, 1H, NH-CON**H**- triazole,exchangeable D_2O), 9.03 (s, 1H, NH-N**H**SO_2), 8.89 (s, 1H, NH-triazole), 8.58 (d,2H,CH-primidine), 7.03-7.73(m,7H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole+ 1H, NH-N**H**CHCONH exchangeable D_2O]),4.91 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.35(d, 1H,CH,CH-C**H**CONH-Val),4.17 (s, 2H, CH_2-NHCH_2CO), 1.91-1.83 (m,1H, CH-C**H**$(CH_3)_2$), 0.86 (d,6H,2 CH_3-(CH_3)$_2$).*
18	*11.03(s, 1H, NH-CONH- triazole,exchangeable D_2O), 9.95 (s,1H, NH- triazole), 8.89 (s, 1H, NH- NHSO$_2$), 8.56 (d, 2H,CH-primidine), 7.66-7.00 (m,7H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole+ 1H, NH-NHCHCONHexchangeable D_2O]), 5.19 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.49-4.42 (m, 1H, CH-C**H**CONH), 4.02 (s, 2H, CH_2-NHCH_2CO), 2.67 (t,2H, CH_2-CH_2S CH_3), 2.36 (s,3H, CH_3), 2.19-2.12 (m,2H, CH_2- C$H_2$$CH_2$S CH_3).*
19	*11.86(s, 1H, O**H**-carboxylic, exchangeable D_2O), 8.89 (s, 1H, NH- N**H**SO_2), 8.53(d, 2H,C**H**-primidine),7.69-7.03(m,5H,[4H, Ar-H + 1H,C**H**-primidine]), 5.16 (t, 1H,CH-C**H**-Pro), 5.09 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O), 4.50 (s, 2H, CH_2-NHCH_2CO), 3.81-2.37(m, 6H, 3CH_2-Pro).*
20	*8.89 (s, 1H, NH- N**H**SO_2), 8.55-8.54(d,2H,CH-primidine),7.72-7.06(m,5H,[4H, Ar-H + 1H,CH-primidine]), 5.21 (s, 1H, NH-N**H**CH_2CO, exchangeable D_2O),4.60(t,1H,CH-C**H**-Pro),4.01(s,2H,CH_2-NHCH_2CO), 3.59(s,3H,CH_3-COOCH_3), 2.44-2.23 (m,6H, 3CH_2-Pro).*
21	*13.33(s, 1H, NH-CON**H**-Thiazole,exchangeable D_2O), 8.90 (s, 1H, NH-N**H**SO_2), 8.50-8.49(d,2H,C**H**-primidine),7.67-7.13(m,7H, [4H, Ar-H + 1H,CH-primidine +2H, CH-thiazole]), 5.21 (s, 1H, NH-N**H**CH_2, exchangeable D_2O), 5.04(t,1H,CH-CH-Pro), 4.21(s, 2H, CH_2CO), 3.92-2.37 (m, 6H,3CH_2-Pro).*
22	*10.91(s, 1H, NH-CON**H**-triazole,exchangeable D_2O), 10.11 (s, 1H, N**H**-triazole), 8.90 (s, 1H, NH-N**H**SO_2), 8.53 (d, 2H,CH-primidine),7.72-7.04(m,6H, [4H, Ar-H + 1H,CH-primidine +1H, CH-triazole]), 5.01 (s, 1H, NH-N**H**CH_2, exchangeable D_2O), 4.40-4.34(t,1H,CH-C**H**-Pro),4.05 (s, 2H, CH_2-NHCH_2CO), 3.45- 2.39(m, 6H,3CH_2-Pro).*

Table 3: Results of the chemical analyses (IR and Mass spectra):

Compd.	IR (ν_{max} cm^{-1}) Selected bands	MS [M^+]
3	*(b)3350cm^{-1} (OH); 3235 (NH); 1672 (CO);1350 (SO_2).*	*(365,1.68)*
4	*(b)3364 (OH); 3157 (NH);3094 (CH-aromatic), 1690 (CO), 1355,1152 (SO_2NH).*	*(380,M+1,16.9)*
5	*(b)3370 (OH);3245 (NH);2888 (CH-ali), 1690 (CO), 1352,1152 (SO_2).*	*(407, 11.1)*
6		*(439,1.04)*
7	*3150 (NH), 1672 (CO), 1352,1152 (SO_2).*	
8	*3154 (NH), 1695 (CO), 1342,1155 (SO_2).*	*(393, 5.07)*
9	*3151 (NH); 1692 (CO).*	*(423,M+2,7.97)*
10	*3145 (NH); 1695 (CO).*	*(453, 11.25)*
11	*3265 (NH); 1720 (CO).*	*(448,M+1,24.3)*
12	*3260 (NH); 1722 (CO).*	*(462, M+1,1.02)*
13	*3198 (NH); 1723 (CO).*	*(489, 1.11)*
14	*3335 (NH);1718(CO).*	*(521,2.45)*
15	*3352 (NH); 1708 (CO).*	*(431,0.01)*
16	*3345(NH); 1702 (CO).*	*(445,0.89)*
17	*3358(NH); 1708(CO).*	
18	*3265 (NH),1656 (CO).*	*(505, 2.08)*
19	*3382 (OH); 3147 (NH) 1698 (CO).*	*(407,M+2,3.14)*
20	*3145 (NH) 1690(CO).*	*(420, M+1,1.86)*
21	*3358 (NH) 1690 (CO).*	(489,M+2, 26.3)
22	*3350 (NH) 1693 (CO).*	

Table 4. DNA binding activity of compounds (**3-20**) using methyl green DNA displacements assay.

NO.	IC_{50}(*μg/ml.*)*	IC_{50}(*μM.*)**	NO.	IC_{50}(*μg/ml.*)*	IC_{50}(*μM.*)**
EtBr	81 ±0.02	205.58	**3**	15 ±0.005	41.09
4	ND	ND	**5**	49 ±0.004	120.39
6	19 ±0.01	43.28	**7**	11 ±0.03	29.02
8	16 ±0.02	40.71	**9**	ND	ND
10	20 ±0.01	44.15	**11**	21 ±0.01	46.98
12	69 ±0.01	149.67	**13**	ND	ND
14	83 ±0.01	169.73	**15**	78 ±0.01	180.97
16	ND	ND	**17**	ND	ND
18	21 ±0.01	41.58	**19**	ND	ND
20	ND	ND	**21**	22 ±0.002	45.17
22	33 ±0.001	70.00			

ND) Not determined (Compounds having IC_{50} value > 100 μ/ml.).

*) IC_{50} Values: Represented IC50 obtained from three independent determinations (mean ± SD) required for 50% decrease in the initial absorbance of DNA-methyl green solution.

**) IC_{50} values: Concentration required for 50% decrease in the initial absorbance of DNA-methyl green solution.

Table 5: Growth inhibitory action (IC50) of the tested compounds against (MCF7) human cell lines.

NO.	IC_{50}(µg/ml.)*	IC_{50}(µM.)**
3	0.97	2.66
10	1.48	3.26
12	2.37	5.14
17	3.65	7.71
DOX	0.9	1.54

*) IC_{50} Values: represent IC_{50} obtained from three independent determinations.
**) IC_{50} values: concentration causing 50% inhibition of cell viability.

Table 6: Anti-microbial activity of the synthesized compounds.

	In vitro activity-zone of inhibition in mm (MIC in µg/ml)											
Comp No.	S.Aureus (ATCC-9144)		S.epidermidis (ATCC-155)		K.Pneum-oniae (ATCC-11298)		E.coli (ATCC-25922)		A.niger (ATCC-9029)		A.fumigatus(ATCC-46645)	
	A	M.I.C	A	M.I.C	A	M.I.C	A	M.I.C	A	M.I.C	A	M.I.C
3	16	28.4	18	15.1	13	33.8	13	15.6	17	26.2	35	13.4
6	14	27.9	19	15.2	19	18.9	22	25.3	15	18.8	13	32.3
7	18	22.6	19	30.1	19	11.6	25	21.8	18	19.4	14	29.8
8	22	11.1	27	20.1	20	14.6	17	14.8	21	18.8	27	11.8
9	26	17.3	16	17.2	26	19.1	18	22.6	19	20.2	24	21.8
10	15	23.8	7	—	7	—	9	—	11	32.2	14	30.2
13	26	22.4	12	27.4	16	22.9	16	17.3	19	20.2	19	22.2
15	18	18.9	13	18.8	15	32.9	14	21.8	19	30.2	22	18.2
16	22	21.6	17	15.8	16	11.2	22	12.6	13	11.6	15	31.2
17	17	23.8	19	22.8	20	17.2	21	15.6	22	31.1	7	—
18	25	18.9	16	18.8	17	19.1	7	—	25	21.8	22	18.2
20	19	14.9	19	28.1	20	15.2	21	12.6	17	11.9	15	31.2
Ciprofloxacin	29	0.2	31	0.39	30	0.1	29	0.3	—	—	—	—

Inhibition zone in millimeters.
Inhibition zone diameter: (7-13 mm) Weak active;
(14-20 mm) Moderate active;
(21-42 mm) High active.

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