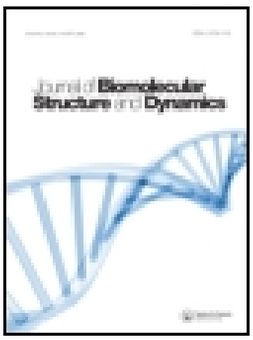

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Sulfonamide phenylalanine (SPA) series of analogues as an antibacterial, antifungal, anticancer agents along with p53 tumor suppressor-DNA complex inhibitor – Part 1

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Abstract

A series of N-[1-benzyl-2-oxo-2-substituted(ethyl)] benzene/*p*-toluene sulfonamide (K1–K12) are synthesized. Structure of the synthesized analogues has been confirmed by FT-IR, ^{1}H & ^{13}C NMR and ESI-MS spectroscopic techniques. All the synthesized analogues (K1-K12) have also been examined for their *in vitro* antibacterial and antifungal activities. Compounds showed good antibacterial and antifungal activity against standard drug. Anticancer study has been carried out on three cancer cell lines PC-3, MCF-7 and A549 on two different concentrations (mg/ml and μg/ml). The K4 sulfonamide analogue showed better anticancer activity amongst all analogues against PC-3 and A549 cell lines. K4 inhibit G0/G1 phase in cell cycle analysis experiment. All synthesized molecules (K1-K12) dock at junction p53-DNA and make hydrogen bonded with residues of p53 protein as per docking study. ADMET predictions of synthesized phenylalanine sulfonamide analogues (K1-K12) has been done using "Lipinski rule" and it has been observed that all synthesized analogues did not violate the rule. Electronic, chemical properties and mulliken atomic charges of analogues were calculated using density functional theory (DFT).

Keywords: phenylalanine sulfonamide; mitoxantrone; streptomycin sulfate; cell cycle; p53.

1. Introduction

Molecules having sulfonamide (SO_2NH_2) group in their structure have been used as medication towards number of diseases. Importance of sulfonamide was realized when sulfonyl amide, a key analogue of sulfonamide, revealed to be first antibacterial drug. Number of sulfonyl amide derivatives were synthesized and examined for antibacterial, anticancer, anti-inflammatory, antidiabetic, carbonic anhydrase inhibitor, diuretic, anti-thyroid, antiviral, dermatological, antiretroviral, anticonvulsants activities. Some sulfonyl hydrazine are said to have antitumor effects which prevent malignant cells from developing and lengthening [Shyam et al, 1990]. Chloroquinoxaline sulfonamide (CQS) was the first drug having -SO_2NH- feature considered to be an anticancer agent. Drug achieved *in-vitro* toxicity at higher concentration. It has been observed that drug arrest cell cycle in G0/G1 phase [Branda, Mccormack, and Perlmutter, 1988;

Tong et al, 1987]. Later on sulfabenzamide was proposed to effective anticancer agent. Unlike doxorubicin; sulfabenzamide induced antiproliferative effect due to autophagy. Minimum cell cycle arrest (G2/M phase) has been observed in T47D cells treated with sulfabenzamide. This is in contrast to doxorubicin wherein maximum accumulation of cells takes place in S-phase and to a lesser extent in G2/M phase [Mohammadpur, Safarian, Farahnak, Hasheminasl, and Sheibani, 2012]. Likewise another analogue, E7070, showed increase in G0/G1 phase and decrease in S-phase cells population on A549 lung cancer cell line. However, longer exposure would increase the G2/M fraction in A549 cells [Fukoka et al, 2001].

The structure of E7070 and E7010 (Fig. 1 (a & b)) analogues of sulfonamide is more or less same but their mechanism of action is different due to change in substituent group attached the sulfonamide functionality (-SO_2NH-). E7070 is proposed to show antitumor effect by targeting cell cycle at multiple points (includes both G1/S and G2/S transitions). Further inhibitory effect on p53 and p21 are considered to be plausible mode of action of E7070 [Yoshino et al, 1992; Yammato et al, 1998; Supuran 2003; Mohan et al, 2006; Hoyer-Hansen, Bastholm, Mathiasen, Elling, and Jaattela, 2005; Ozawa et al, 1996; Kesteren et al, 2002; Tsukahara et al, 2001]. Number of analogues of E7070 were synthesized and studied for anti-cancer activity [Mirian et al. 2011; Badawi, Ali and Ismail, 2008; Yokoi, Kuromitsu and Kawai, 2002]. From cell-cycle analysis, E7070 and sulfabenzamide proposed to inhibits G0/G1 phase of cell-cycle [Mohammadpur, Safarian, Farahnak, Hasheminasl, and Sheibani, 2012; Fukuoka et al, 2001]. (1R, 2R)-D-*threo*-2-(N-Myristoylamino)-1-(4'-nitrophenyl)-1,3-propanediol (B13) substituted with sulfonamide feature along with a $C_{13}H_{27}$ alkyl chain showed better cytotoxicity in comparison to B13. Sulfonamide group along with long alkyl chain increases antitumor activity and additionally the replacement of phenyl ring by naphthyl ring; significantly enhance the antitumor effects & hydrophobicity [Lee, Park and Im, 2011].

As per literature study G0/G1 phase have operational effects of p53, p21 and cyclin dependent kinase along with numerous DNA synthesizers. p53 accomplished to be the most commonly observed genetic factors in tumor growth. p53 inhibits tumor growth by way of blocking transformation of an activated oncogene, or stopping tumor formation. When p53 detects deformed DNA; it immediately signals the cell to start for cell cycle arrest and as the amino acid residues of p53 genes receive mutations; it lose its DNA binding function. Hence, DNA multiplication goes unchecked and it proliferates in uncontrolled manner which is proposed to be

the main cause of cancers. Approximately half of cancers show mutations of one p53 allele; and block the p53 tumor suppression properties. Over expression of p53 affects cell cycle arrest throughout G1 phase. It has been observed that in-presence of sulfabenzamide the anticancer activity of mutant p53 return to normal and induces autophagical cell death [Mohammadpur, Safarian, Farahnak, Hasheminasl, and Sheibani, 2012]. Hence the pathway, with the aid of which p53 initiates apoptosis, is a common target of sulfonamide drugs.

The indolenyl sulfonamides derivatives are proposed to be good antibacterial and antifungal agents. It is also suggested that these compounds can be good chelating agents. Sulfonamide with charged substituent group and O & N heteroatom can be effective antibacterial and antifungal agents. Molecules for multi-therapeutic materials with excessive selectivity have been prepared [Cohan, Youssoufi, Jarrahpour and Hadda, 2010]. The cytotoxic activity increases with substitution in para position of benzene ring. The activity towards Gram-positive and Gram-negative microorganism is reduced with the aid of larger alkyl substituents. Antibacterial activity increases when CH_3 group substituted on phenyl ring and decreases with $–OCH_3$ substitution [Shah, Rivera, and Ashfaq, 2013]. Further analogues of (1H-benzoimidazole) and (1H-benzoimidazole-1-carbonylphenylsulfamoyl) phenyl acetamide showed better activity against bacterial strains and no activity towards fungal strains [Kalidhar, Kumar, and Kaur, 2012]. 5-(bromo/nitropyridin-2-yl)benzamide molecules with benzamide and N-benzoyl-N-(5-bromopyridin-2-yl) trifluoromethane sulfonamide derivatives; having combination of benzamides and sulfonamides linkages and halogen substituents; had been proved to be highly potent antibacterial agents [Rai and Singh, 2011].

Therefore, we designed the synthesis of a series of N-[1-benzyl-2-oxo-2-substituted(ethyl)] benzene/*p*-toluene sulfonamide analogues (K1-K12) (Fig. 1 (d)). These K1-K12 analogues bear sulfonamide and amide functionalities with hydrophobic terminations. They are proposed to be anticancer, antibacterial and antifungal agent. Structure of new synthesized phenylalanine sulfonamide analogues (K1-K12) have been confirmed by FT-IR, ^{1}H & ^{13}C NMR and ESI-MS spectroscopic techniques. Petra Osiris Molinspiration (POM) analysis of synthesized phenylalanine sulfonamide analogues (K1-K12) has been performed to confirm their drug like behavior. The anticancer activities of synthesized sulfonamide phenylalanine analogues (K1-K12) has been studied against PC-3, MCF-7 and A549 cancer cell lines in comparison to Mitoxantrone (Fig. 1 (c)). Cell-cycle analysis of the best analogue (K4) has been carried out on

A549 cell line. The synthesized phenylalanine sulfonamide analogues (K1-K12) analogues showed good antimicrobial (antibacterial & antifungal) activities towards resistant/non-resistant strains. The docking study has been carried out with p53 tumor suppressor-DNA complex (1TUP) using Autodock software. The quantum chemical property of all synthesized phenylalanine sulfonamide analogues (K1-K12) has been calculated using density functional theory (DFT).

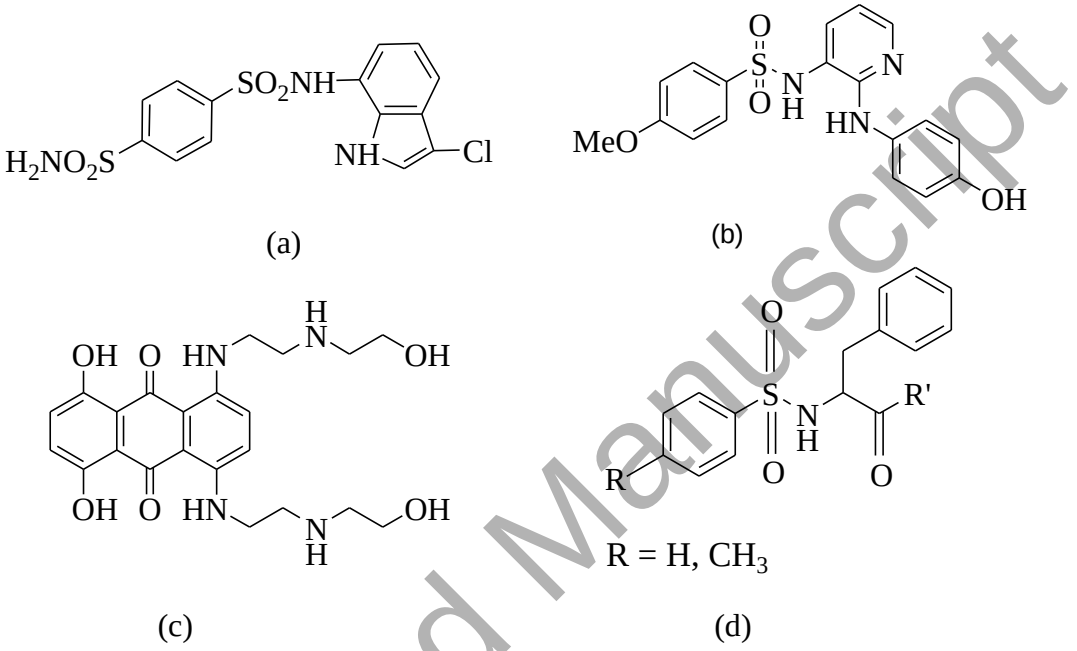


Fig. 1. (a) Structure of E7070 (b) structure of E7010 (C) structure of Mitoxantrone and (d) basic structure of K1-K12 synthesized series.

2. Materials and Methods

Solvents had been distilled and dried prior use. Reactions have been monitored on TLC (silica gel plates) with solvent system of benzene and methanol. Melting points were uncorrected and measured in open capillary tubes on Digital Melting/Boiling point apparatus. The electron spray positive (ES+) mass spectra were recorded on Waters Micromass Q TOF micro spectrometer**.** ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO on a BRUKER AVANCE II 400 NMR spectrometer. The records are described as chemical shift, multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, quin: quintet, m: multiplet, dd: doublet of doublets), coupling constant in Hz, and the number of protons. The FT-IR spectra were recorded on NICOLET 6700 of Thermo scientific USA using KBr pellets (500 - 4000cm^{-1}).

2.1 Procedure for synthesis of K1-K12 analogues

A mixture of benzene/p-toluene sulfonyl chloride (0.1 mole) and phenylalanine (0.1 mole) and NaOH solution (1N) stirred together at 65-70^{0}C for 4 hours. Reaction-mixture were cooled to 5^{0}C and concentrated HCl was added slowly to change the pH 6.5 [Mahajan, Patial and Sharma, 2002]. The precipitates of benzene/p-toluene sulfonyl phenylalanine has been collected and washed with distilled water and dried. 0.004 mole of benzene/p-toluene sulfonyl phenylalanine acids dissolved in dry benzene (10 ml). Distilled thionyl chloride (5ml) added to the same [Mahajan, Patial and Sharma, 2002]. The mixture was stirred at room temperature for 20 minutes and refluxed for 3 hours. To the acid chloride of benzene/p-toluene sulfonyl phenylalanine heterocyclic, aliphatic & aromatic anilines & substituted aromatic aniline (0.004 mole) in benzene are added drop wise. The temperature of the reaction mixture was raised to 48°C. Mixture is stirred at this temperature for 4-6 hours. The product was kept in ice overnight and extracted with chloroform and water. Product was crystallized [Reaction scheme in supplementary Fig. 1 and characterization in supplementary table 1 and 2].

2.2. In-silico bioactivity studies

The ADMET predictions of the synthesized analogues K1-K12 has been done by Molinspiration software (www.molinspiration.com). The physical descriptors like molecular weight, logP, hydrogen bond acceptors and hydrogen bond donor are calculated. The "Rule of 5" [Lipinski, Lombardo, Dominy, and Feeney, 2012] properties is a simple set of molecular descriptors used by Lipinski in formulating (logP<=5, molecular weight <=500, no. of hydrogen bond acceptors <=10 and no. of hydrogen bond donor <=5) calculated by use of molinspiration software. Total polar surface area is also calculated by a sum of fragment contributions of the molinspiration software [Lalitha and Sivakamasundari, 2010]. Molecular volume is acquired by fitting of some fragment contributions to real 3D volume of molecules [Ertl, Rohde, and Selzer, 2000; Clark, 1999]. Number of Rotatable Bonds – (nrotb is simple topological parameter defines the molecular flexibility) shown to be descriptor of oral bioavailability of drugs [Veber et al, 2002] are also calculated. GPCR ligand, Ion channel modulator, kinase inhibitor, nuclear receptor

ligand, protease inhibitors and enzyme inhibitors properties are calculated [Lagunin, Stepanchikova, Filimonov, and Poroikov, 2000; Pervez et al, 2010].

2.3. Antimicrobial studies

The *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus fumigatus* and *Fusarium moniliforme* strains has been used for antimicrobial studies. Mueller-Hinton agar (MHA) has been used as culture medium and DMSO was used as a solvent control. Streptomycin sulphate and Amphotericin B has been used as a standard for antibacterial and antifungal activities. All strains have been cultivated in for 15 h at 37^0C. The Mueller-Hinton agar (MHA) media diluted with water up to 1000 ml and sterilized at 120^0C for 45 min at 15-16 psi pressure in autoclave. Further media is cooled as much as to 40-45^0C and positioned on petri plates (6mm). The petri plates were covered and set aside for an hour and then incubated at 37^0C for 48 hrs. The zones of inhibition have been measured in mm [Barry, Coyle, Thornberry, Gerlach and Hawkinson, 1979] and results of synthesized analogues are compared with standard drug Streptomycin sulphate and Amphotericin B.

2.4. Anticancer activity

2.4.1. MTT assay [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay against PC-3 (Prostate cancer cell line)

The synthesized phenylalanine sulfonamide (K1-K12) analogues were examined for cytotoxic results against human prostate cancer (PC-3) cell line using MTT method and compared with control. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100units/ml) and streptomycin (100µg/ml) in a 5% CO_2– humidified atmosphere incubator. The cells were plated in a 96-well plate and treated with different concentrations of the compounds for 24 h in a CO_2 incubator (37^0C, 5% CO_2 and 90% relative humidity). The cytotoxicity was measured by adding 20µl of freshly prepared MTT solution (5 mg/mL in PBS, sterile filtered) to every well. Culture plates were gently stirred at150 rpm for 5 min and incubated for another 3 h in dark at 37^0C to allow metabolization of MTT. The purple formazan crystals were re-suspended by adding 5mg/ ml in methanol to every well [Mosmann, 1983]. The absorbance was examined at 570 nm on spectrophotometer. The cell

death was calculated: Cell death = 100 − [(test absorbance/control absorbance) × 100] as standard protocol.

2.4.2. SRB (sulforhodamine B) assay against MCF-7 (Human breast cancer cell line) and A549 (Human lung cancer cell line)

The cells have been inoculated into 96 well microtiter plates in 100 µL. After cell inoculation and before the addition of experimental drugs, plates were incubated at 37^0C, 5% CO_2, 95 % air and 100 % relative humidity for 24 hrs. Experimental drugs were dissolved in dimethyl sulfoxide at 100mg/ml and diluted upto 1mg/ml using water. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml and molar concentrations (10^{-4} mg/ml, 10^{-5} mg/ml, 10^{-6} mg/ml, 10^{-7} mg/ml) with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells having 90 µl of medium.

Further, plates have been incubated under standard conditions for 48 hours. Cells were fixed *in situ* by the mild addition of 50 µl of cold 30 % (w/v) TCA and incubated for 60 minutes at 4^0C. The supernatant discarded and plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid added to each of the wells followed by 20 minutes incubation at room temperature. Residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Absorbance was checked at a wavelength of 540 nm with 690 nm reference wavelength. Percentage growth inhibition was calculated as: [Ti/C] x 100 % [Vichai and Kirtikara, 2006; Skehn et al, 1990] as standard protocol.

2.4.3. Cell-cycle analysis

Standard protocol on flow cytometery has been followed. Cell culture was trypsinized and washed with phosphate buffered saline (PBS) and incubated at a density of 5×10^5 cells/well with 5 mL of complete medium in 6 well plates. Cells have been grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimitotic. Test samples dissolved in DMSO (Dimethyl sulfoxide) to make 10 mg/mL stock solution. Finally operating concentration made by way of further dilution of stock solution in entire

medium and several dilutions of the test compounds in 1 mL of whole medium was added. Alone cells (without treatment) are used as control. The plates were then incubated at 37^0C for 12 to 24 hours in 5% CO_2 incubator. After Incubation period, each adherent and floating cell have been harvested and centrifuged for 5 min. Pellet was washed with phosphate buffered saline (PBS) and again centrifuged for 5 min. Further, cell pellets were resuspended in 70% EtOH and fixed for 2h at 4^0C. Fixed cells were washed with PBS (14 mL) and centrifuged at 200g for 5 min. Cell pellets were resuspended in propidium iodide (PI) solution (50µg/mL in 0.1 % sodium citrate, 0.1 % Triton-X 100 and 20µg/ml RNAs A) for 30 min in dark. Samples were processed using an Amis flow cytometer. The distribution of cells in each phase of the cell cycle was calculated using IDEA software.

2.5. Docking studies

Docking studies of the synthesized analogues were carried out against p53 tumor suppressor-DNA complex to confirm the site of interaction in G0/G1 phase of cell cycle. Autodock4.2 docking tool (Scripps Research Instituite La Jolla, CA, USA) has been used for docking study. The 3D structures of ligands were constructed using the Pymol software (www.pymol.com). Gasteiger charges were assigned followed by merging of non-polar hydrogens. The PDB file of the structure of p53 protein (1TUP) [Eichhorn, Hiller, Hirschfelder, Frank and Efferth, 2013] was downloaded from protein data bank (www.rcsb.org). The Kollman charges were added to each atom and saved as protein.pdbqt. Default values of the parameters were used throughout the calculations. A grid parameter file (pro.gpf) of the protein was created using Auto Grid Tool. Calculated output was saved as pro.glg file. Grid points choosen to be 58 × 73 × 65, along the grid spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) protocol was applied for the protein fixed: ligand flexible model [Jenwitheesuk and Samudrala, 2003; Morris et al, 1998]. The LGA protocol includes a local minimization for the given fraction of the population. The LGA mixes a worldwide search for ligand conformation and orientation to perform energy minimization. The scoring function is calculated which is a sum of van der Waals, H-bonding and distance-dependent dielectric electrostatics as well as conformational torsional restriction entropy and empirical solvation energetics terms of the ligand-protein complex.

Docking calculations started with an initial population of 150 randomly positioned ligand conformations. Therefore, in total 100 search attempts (ga_run parameter) done for each ligand. The maximum number of energy evaluations and generations before the termination of the LGA run were 2.5×10^6 and 2.7×10^4, respectively. For the local search, pseudo-Solis and Wets-algorithm were applied. Other docking parameters have been set to default values. Final docking orientation lying within 2 Å of the root-mean square deviation (rmsd) tolerance of each was represented as the most favorable conformation with low free energy of binding (ΔG_b) [Jenwitheesuk and Samudrala, 2003; Morris et al, 1998]. The dock parameter file for the ligand was saved as lig.dpf. The ligands had been ranked in step with their binding free energy in kcal/mol and inhibition constant *Ki* in μMat 298.15 K. Overall free energy of binding (ΔG_{bind}) is consists of a sum of free energies measured for the ligand pose.

$\Delta G_{bind} =$

$\text{Intermolecualr Energy} + \text{Internal Energy} + \text{torsion Energy} - \text{Unbound system}'\text{senergy}$

(i)

$(\text{Intermolecular Energy} = a\Delta G_{vdw} + b\Delta G_{ele} + c\Delta G_{H-bond} + e\Delta G_{sol})$

2.6. Quantum chemical study

Density Functional Theory (DFT) method has been used to investigate electronic structural properties of designed ligand (K1-K12) in comparison to Mitoxantrone. The calculation has been done using DFT protocols. Geometry of the molecules are is optimized at B3LYP/6-311++(2d,p) level of theory using Gaussian 03W program package without any constraint on the geometry. Results were visualized by Gauss View 05. The electronic properties; HOMO-LUMO energies and mulliken charges were calculated. The dipole moment, chemical softness, electrophilicity index and chemical hardness were calculated and analyzed [Raj, Shoba, Ramalingam and Periandy, 2015].

3. Results and Discussion

3.1. In-silico Bioactivities studies

All synthesized phenylalanine sulfonamide analogues (K1-K12) were first screened for bioactivity study (Table 1). As per Lipinski rule, values for good membrane permeability must be logP ≤5, molecular weight ≤500; range of hydrogen bond acceptors ≤10 and wide array of hydrogen bond donor ≤5. Physical properties and bioactive properties of the synthesized phenylalanine sulfonamide analogues are calculated in comparison to E7070. All Sulfonamide analogues (K1-K12) showed near similar activity for four criteria i.e. GPCR (Guanine-protein coupled receptor) rate; Ion channel modulator; Kinase inhibitor and Nuclear Receptor Ligand. All synthesized molecules are arranged in ascending order for GPCR ligand and rated as K11>K12>K1>K3>K4>K8>K10>K2>K9>E7070, ion channel modulator was in ascending order and rated as K11>K12>K9>K6=K7>E7070>K2>K5>K10> K8>K4>K3>K1, kinase inhibitor was in ascending order and rated as K8>K1>K12>K11> K7>K6=K5=K10>K3=K9>K4>K2>E7070 along with nuclear receptor ligand rated as K12> K11>K6=K7>K5>K9>K2>K10>K3>K4>E7070>K8>K1. This has helped us to have an idea of probable bioactivity of the synthesized analogues (K1-K12) and kind of interaction with targeted molecule.

The synthesized phenylalanine sulfonamide analogue does not violate the "rule of 5". Therefore, we can say that synthesized phenylalanine sulfonamide analogues (K1-K12) are potential pharmacophore for antibacterial, antifungals, anticancer, antidiabetic activities and carbonic anhydrase inhibitor as well. Further, analogues showed good logP value (<5), which is a measure of the logarithm of octanol/water partition coefficient of organic molecules and measure the degree of hydrophilicity. High logP value indicates poor intestinal absorption. Synthesized sulfonamide phenylalanine (K1-K12) analogues having logP values less than five hence proposed to bear good membrane permeability and arranged in K12>K7>K11>K6>K10>K3>K4>K9> E7070>K2>K5>K8>K1 order. Total polar surface area (TPSA) is the measure of sum of surfaces of all polar atoms and polar bonds present in the molecule like oxygen, nitrogen and attached hydrogen. It predicts drug transport properties through intestine and blood brain barriers and rated as K1=K8>K2=K9>, K5=K6=K7=K11=K12 >K10=K3=K4>E7070.

Table 1

Bioactivity score of the synthesized phenylalanine sulfonamide analogues (K1-K12).

*Data	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	E7070
miLogP	0.59	1.99	3.06	2.55	1.90	3.60	4.28	1.03	2.44	3.50	4.05	4.73	2.18
TPSA	83.47	75.71	66.48	66.48	75.27	75.27	75.27	83.47	75.71	66.48	75.27	75.27	122.12
natoms	21	26	26	25	22	27	28	22	27	27	28	29	26
MW	305.36	374.46	372.49	358.46	318.40	380.47	414.91	319.3	388.49	386.52	394.50	428.94	385.85
nON	5	6	5	5	5	5	5	5	6	5	5	5	7
nOHNH	2	1	1	1	2	2	2	2	1	1	2	2	4
nviolations	0	0	0	0	0	0	0	0	0	0	0	0	0
Nrotb	6	6	6	6	6	7	7	6	6	6	7	7	4
Volume	259.92	330.03	337.85	321.05	280.86	335.71	349.25	276.4	346.59	354.41	352.27	365.81	284.52
GPCR L	0.23	0.13	0.22	0.21	0.16	0.00	0.00	0.19	0.09	0.17	-0.04	-0.04	0.04
ICM	-0.14	-0.26	-0.17	-0.19	-0.24	-0.29	-0.29	-0.21	-0.32	-0.23	-0.35	-0.34	-0.27
KI	-0.39	-0.21	-0.24	-0.23	-0.27	-0.27	-0.28	-0.40	-0.24	-0.27	-0.30	-0.31	0.30
NRL	-0.07	-0.23	-0.20	-0.18	-0.27	-0.38	-0.38	-0.08	-0.26	-0.22	-0.39	-0.40	-0.16
PI	0.48	0.47	0.51	0.58	0.55	0.31	0.26	0.43	0.40	0.44	0.25	0.20	0.48
EI	0.17	-0.01	0.04	0.02	0.03	-0.06	-0.09	0.09	-0.07	-0.02	-0.12	-0.14	0.30

*miLogP: logarithm of compound partition coefficient between n-octanol and water, TPSA: total polar surface area, nON: number of H-bond donor, nOHNH: number of H-bond acceptor, nVio: number of violations, Nrotb: number of rotational bonds; GPCR L = GPCR ligand, ICM = ion channel modulator, KI = kinase inhibitor, NRL = nuclear receptor ligand, PI = protease inhibitor, EI = enzyme inhibitor

3.2 Antimicrobial and antifungal studies

Molecules with -SO_2NH- and -NHCOOH- features are proposed to be essential for antimicrobial and antifungal activities. Numbers of drugs are currently in market as an antibacterial and antifungal agent bearing these features. The antibacterial study of synthesized phenylalanine sulfonamide analogues (K1-K12) was carried out on Gram-positive bacteria *Staphylococcus aureus,* and Gram-negative microorganism *Escherichia coli, Pseudomonas aeruginosa* and also on fungal strains like *Candida albicans, Aspergillus fumigatus* and *Fusarium moniliforme* (Table 2 & 3). K1-K12 series was also tested against multi-resistant strains *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli*. The synthesized phenylalanine analogues K2, K3, K4, K6, K10 and K12 showed better activity over *Staphylococcus aureus* and *Candida albicans* (resistant strains) but inactive for *E.coli* (Table 2 & 3). Out of all, K4 showed good activity against all strains. Structurally K4 differs from rest of analogues in series having pyrrolidine ring (heterocyclic) at on terminal. Due to flexibility in the ring structure; it might raises the hydrophobic character of molecule in comparison to rest and same may be responsible for good antibacterial action over Streptomycin sulphate. Likewise K10 having piperidine ring at one terminal showing better zone of inhibition in comparison to Streptomycin sulphate at 10mg/ml and 1mg/ml concentrations. The K4 analogue showed excellent activity in all examined fungal strains. K2 and K3 showed moderate activity against *Candida albicans* while K4 showed better activity. The K6 and K12 (with chloroaniline ring) showed good activity against *Fusarium moniliforme* in comparison to Amphotericin B at all concentrations. Molecules K2, K3 & K4 are structurally different from rest of the synthesized phenylalanine analogues (K1-K12). They are having heterocyclic ring at one terminal while rest of them is closing with aromatic/substituted aromatic ring. Sulfonamides carrying -SO_2NH_2- feature which lowers the polarity and alkyl group attached with it increases the hydrophobicity which crosses cell wall more easily [Awasthi, Vatsal and Sharma, 2019]. Therefore structure is not the only criteria for antibacterial and antifungal activities but the position of functional group and its orientation is also essential feature for biological activities.

Table 2

Antibacterial studies of synthesized phenylalanine sulfonamide analogues (K1-K12).

Species Name	Analogues	Zone of inhibition (mm)				Zone of inhibition Streptomycin sulphate (mm)
		10mg/ml	1mg/ml	0.1mg/ml	0.01mg/ml	
Pseudomonas aeruginosa	K4	16	13.5	13	12	20
	K10	12	12	-	-	20
Staphylococcus aureus	K4	-	11.5	-	-	24
Escherichia coli	-	-	-	-	-	

Table 3

Antifungal studies of synthesized phenylalanine sulfonamide analogues (K1-K12).

Species Name	Analogues	Zone of inhibition (mm)				Zone of inhibition Amphotericin B (mm)
		10mg/ml	1mg/ml	0.1mg/ml	0.01mg/ml	
Aspergillus Fumigatus	K4	20	15	15	10	30
Candida albicans	K2	Reduced growth	Reduced growth	Reduced growth	Reduced growth	20-24
	K3	Reduced growth	Reduced growth	Reduced growth	Reduced growth	20-24
	K4	20	10	10	10	20-24
Fusarium moniliforme	K4	20	10	10	10	20
	K6	10	-	-	-	20
	K12	10	24	24	20	20

3.3 Cytotoxic studies

The synthesized analogues were tested to check their anticancer potentials against different cancer cell lines.

3.3.1. MTT assay

The synthesized synthesized phenylalanine sulfonamide analogues (K1-K12) were screened for anticancer activity on Human Prostate cancer (PC-3) cell line in comparison with mitoxantrone at μg/ml concentrations. Cancer cell line was treated with different concentrations for fixed 48 hours. Mitoxantrone is showing cytotoxic effect at lower concentrations. Activity of all the analogues (K1-K12) on PC-3 cancer cell line is arranged as K4>K10>K2>K1>K5>K11>K6>K8>K9>K12>K7 at μg/ml concentration (Table 4 & Fig. 2). Interestingly analogue K4 showed good activity in K-series against PC-3 cell lines.

Table 4

IC_{50} value on PC-3 cell lines of synthesized phenylalanine sulfonamide analogues (K1-K12).

Analogues	IC_{50}(μM)
K1	2.92
K2	2.86
K3	3.20
K4	2.73
K5	3.12
K6	3.17
K7	3.32
K8	3.19
K9	3.20
K10	2.79
K11	3.16
K12	3.23
MTX	3.24

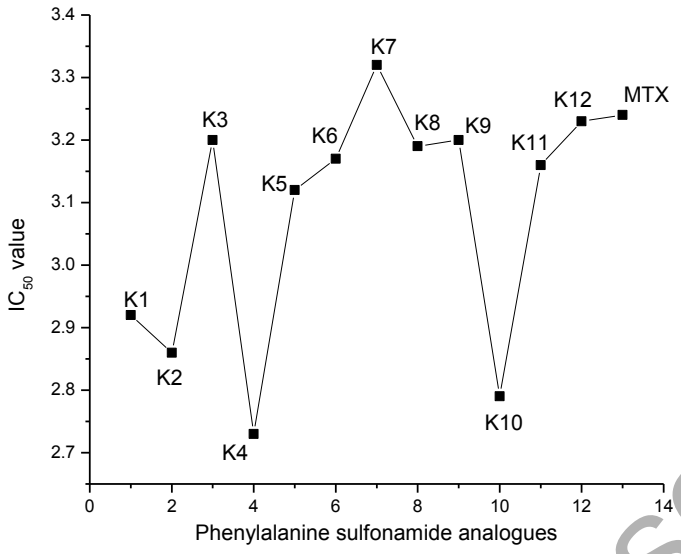


Fig. 2. Anticancer activity of synthesized phenylalanine sulfonamide analogues (K1-K12) on PC-3 cell line.

3.3.2. SRB assay

The synthesized series (K1-K12) have been screened for anticancer activity on Human breast cancer (MCF-7) and Human lung cancer (A549) cell lines at two concentrations i.e. µg/ml and molar concentrations along with standard drug Adriamycin and Mitoxantrone. Cancer cell lines were treated with different concentrations for fixed 48 hours. Mitoxantrone and Adriamycin are showing cytotoxic effect at lower concentrations.

All synthesized analogues K1-K12 are more or less showing similar activity at all concentrations (molar and µg/ml) on MCF-7 cell lines and A549 cell line. The activity of synthesized sulfonamide phenylalanine analogues (K1-K12) on A549 cancer cell line at µg/ml concentration is arranged as K10>K4>K12>K3>K8> K11>K9>K5>K2>K6>K7 (Fig. 3a) while at molar concentration the K4 analogue showed good activity amongst all synthesized analogues on A549 were arranged as K4>K10>K1>K3>K2> K9>K8>K11>K12>K6>K7 (Fig. 3b). Activity of all synthesized analogues against MCF-7 is arranged as K6>K3>K5>K1>K2>K12>K7>K9>K11> K8>K4 at molar concentration while K7> K2>K5>K6>K12>K9>K3>K1>K10>K4>K8>K11 at µg/ml concentration ((Table 5 and Fig. 4a, 4b). At both the concentrations molar as well as µg

molecules are showing more or less same pattern. However, LC_{50}, TGI and GI_{50} values are quite different from standard drugs mitoxantrone and adriamycin.

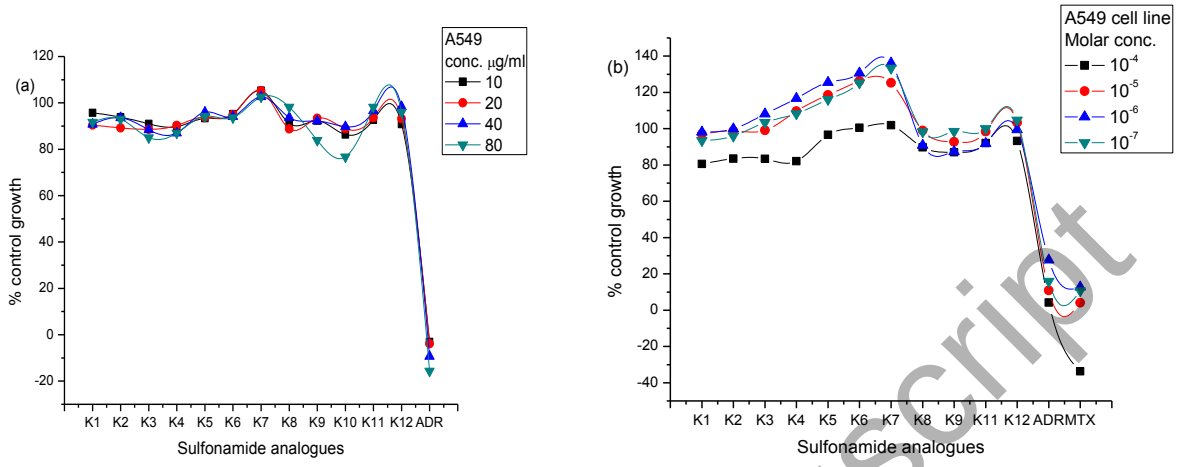


Fig. 3a. Cytotoxicity measure of synthesized phenylalanine sulfonamide analogues (K1-K12) on A549 cancer cell line µg/ml; (3b) at molar concentration.

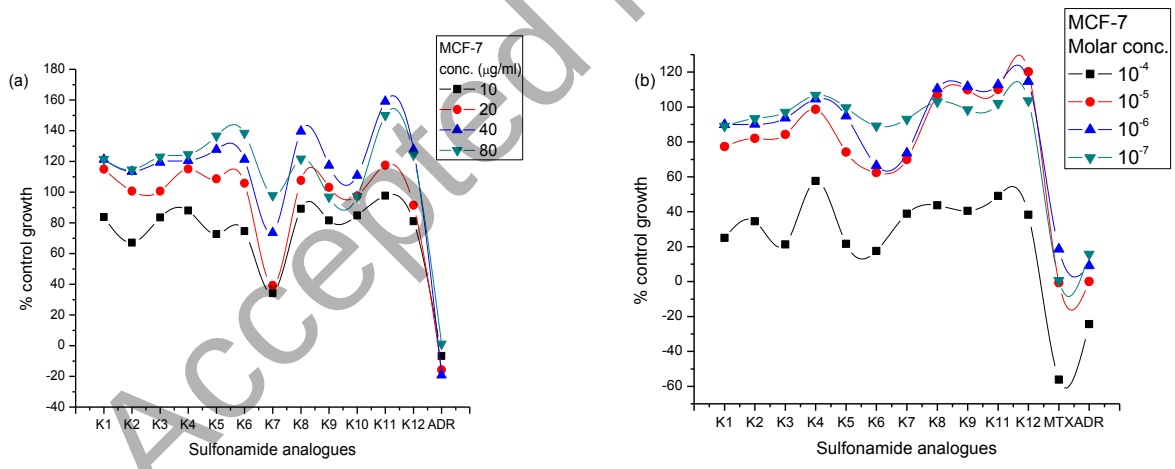


Fig. 4a. Cytotoxicity measure of synthesized phenylalanine sulfonamide analogues (K1-K12) on MCF-7 cancer cell line at µg/ml; **(4b)** at molar concentrations.

Table 5

LC_{50}, TGI and GI_{50} of synthesized phenylalanine sulfonamide analogues (K1-K12) on MCF-7 cancer cell line at molar concentrations.

Compounds	LC_{50}*	TGI*	GI_{50}*
K1	2.385	0.009	3E-05
K2	NE	0.036	7E-05
K3	0.559	0.004	3E-05
K4	56.50	3.106	0.002
K5	0.207	0.002	2E-05
K6	0.293	0.002	8E-06
K7	43.7	0.043	4E-05
K8	NE	0.325	6E-04
K9	NE	0.422	6E-04
K11	NE	1.888	0.002
K12	NE	0.281	7E-04
MTX	4E-04	1E-06	2E-09
ADR	0.023	3E-06	5E-10

*(LC_{50} = concentration of drug causing 50% cell kill; TGI = concentration of drug causing total inhibition of cell growth; GI_{50} = concentration of drug causing 50% inhibition of cell growth).

3.3.3. Cell-cycle analysis

In order to understand the mode of interaction of designed analogues at cellular level; detailed cell-cycle analysis of best screen analogue K4 has been carried out using flow cytometry. Cell-cycle analysis of K4 analogue of K series on A549 cell gives interesting results. Literature studies confirmed that Mitoxantrone arrests G1 and G2 phases of cellular cycle process and ultimately inhibit the cell growth [Khan, Lal, Kumar and Khan, 2010]. Further Mitoxantrone also promote the arrest of S-phase of cell cycle. G1 & G2 are the growth phases in cell-cycle analysis whereas S-phase is a synthetic phase in which DNA replication and DNA synthesis takes place. Cell undergoes apoptosis upon treatment with Mitoxantrone due to inhibition of cell growth phases (G1 & G2) in conjuction with inhibiting the DNA-replication/duplication process (S-phase). Direct assault on cell regulatory protein is suggested and play important role in controlling the regulation of G2/M and G1 phases. Morphological changes observed in analogues treated cells included cell shrinkage and extensive detachment of cell from cell culture substratum. These changes are characteristics of apoptotic cell death in phases of cell-cycle. The anticancer analogue E7070 having $–SO_2NH-$ feature is in clinical trials; reduce the S-phase along

with perturbation of cell-cycle in G1 phase; further E7070 block the G1 phase development in P388 murine leukemia cells [Lee, Park and Im, 2011]. The cell-cycle analysis of E7070 on A549 cell line showed an increase in G0/G1 phase and decrease in S phase fractions for 24 h while G2/M fraction increases after increased exposure for 48 & 72 h in A549 cells [Kesteren et al, 2002].

At molar concentration (10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}), K4 analogue showed better effect in comparison to Mitoxantrone & Adriamycin on A549 and PC-3 in cytotoxic study. Variations are observed in G0/G1 phases (initial phases) of cell cycle after analyzing data for K4 (Fig. 5). Hence enhancements of all chemical activities like deliver of proteins, doubling in number of cell organelles, and so forth has been observed. Biosynthetic activity is high throughout G1 phase which appear to be increased to 5.3% in comparison to control. However activities in G2 phase decrease by ~3.21% i.e. cell accelerating in G2 phase. 2.11% cell is arrested at S phase i.e. inhibiting the DNA replication mechanism. K4 analogue deregulates the cell cycle and arrest G0 and G1 phase by means of stressful biochemical system along with mild disturbance at S phase. It arrests 90% growth of cells in G0/G1 phase in comparison to control with 84.7% cent arrest. Deregulation of cell cycle is the first most important condition for the molecules/drugs to act as cytotoxic. Therefore, synthesized series (K1-K12) proposed to deregulates the cell cycle at G0 and G1 phase.

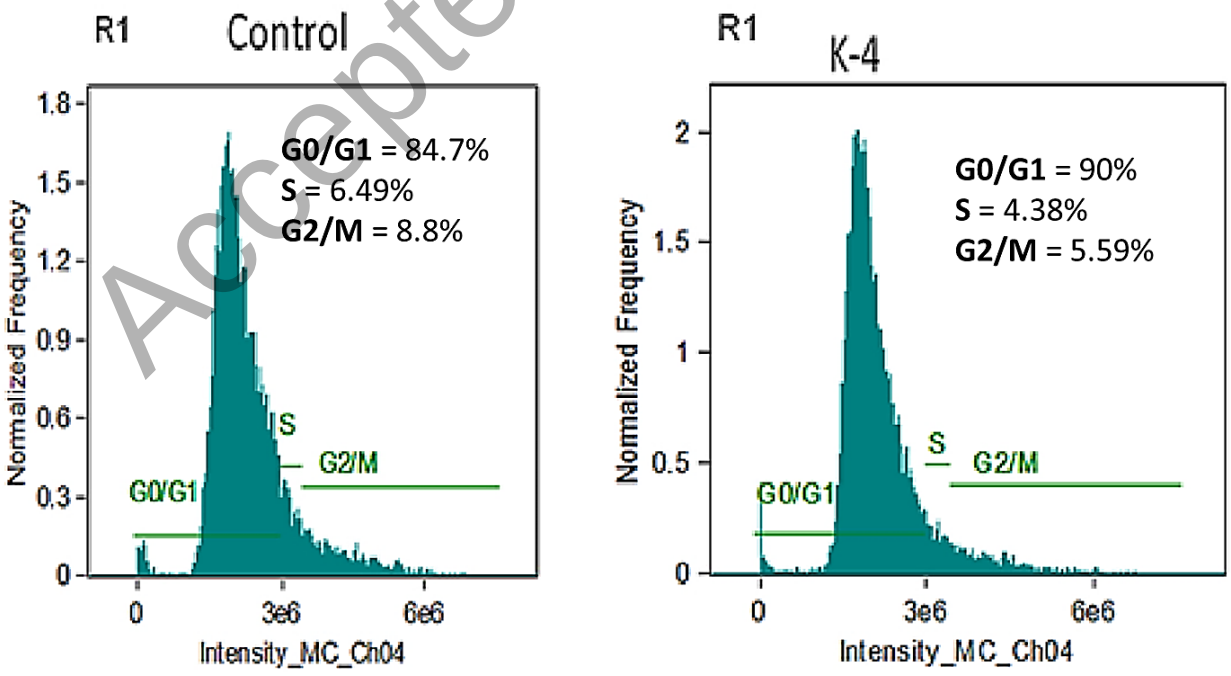


Fig. 5. Cell-cycle analysis of K4 on A549 cancer cell line.

3.4. Docking study

Binding energy (BE) and inhibition constant (Ki) of the synthesized phenylalanine sulfonamide analogues (K1-K12) are calculated against p53 protein (protein ID: 1TUP). From the cell-cycle analysis of K4; it is proposed that synthesized phenylalanine sulfonamide analogues (K1-K12) affect the growth of cancer cells at G0 and G1 phase. In G0 and G1 phase the p53, p21, cyclin dependent kinase activities are excessive, therefore, detailed binding study of synthesized analogues with p53 protein has been carried out. It has been observed that majority of mutations in p53 occurs between 102-292 residues and due to this p53 forgets DNA binding activity. This kind of p53 activity has been diagnosed in maximum cases of cancers. When residues of p53 undergo mutation; it lacks the ability to check DNA damage before permitting cell to go for cell division. Hence results in neoplastic growth [Cho, Gorina, Jaffrey and Pavletich, 1994]. The p53 tumor suppressor-DNA complex structural study (Fig. 6) confirmed that DNA binding is critical for biological activity of p53, and mutations at p53 inactivate the functioning.

The docking study identifies an appropriate pose of ligand inside the binding site of receptor p53, and also predicts the extent of affinity among ligand (synthesized analogues) and receptor p53. The search process begins from a random ligand location and orientation inside and outside the binding sites. By exploring the values of ligand translations, rotations and internal degree of freedom, it eventually reaches the bound conformation. The energy is estimated as binding free energy of all conformations of ligand with binding site of receptor p53.

Docking protocol is applied to study binding affinity of sulfonamide analogues (K1-K12) and mitoxantrone with p53 (1TUP). After creation of docking box, lowest energy conformer of every single of sulfonamide phenylalanine (K1-K12) analogues is allowed to dock inside the active sites. Comparative docking analysis of all synthesized sulfonamide analogues (K1-K12) and mitoxantrone with their common receptor site was carried out. The smaller the value of binding energy and inhibitory constant, the more effective the compound will be in terms of its bioactivity. The energy results were ranked according to binding energy, dock energy, torsional energy and inhibitory concentrations (*Ki)* (Table 6). The lower value of binding free energy indicates the greater stability of best docked conformations. Synthesized sulfonamide analogues (K1-K12) possess lower binding energies upon interaction with 1TUP in comparison to

mitoxantrone and K4 gives the best fit inside the binding site, followed by analogues K1, K8 and K12 analogues (Fig. 8).

Vander Waals and electrostatic interactions dominates binding energies between ligand and protein. Van der Waals terms are associated with shape complementarity among protein and ligand. Whereas electrostatic interaction are the Coulombic interaction between partial atomic charges. The atomic charges play a significant role in figuring out binding energy and binding structure. H-bonds are treated as an aggregate of electrostatic and van der Waals interactions. Total internal energy also plays important role in binding of complex. The greater is the internal energy; greater is the probability of intra as well as intermolecular interaction of ligand-for the protein alone, as well as for the ligand-receptor complex. The synthesized sulfonamide analogues (K1-K12) having internal energy (-1.86, -1.53, -1.92, -1.77, -1.66, -1.97, -1.94, -2.00, -1.51, -1.60, -2.03, -1.44) comparable with MTX (-1.15). There is large difference in the internal energy of K11 & K8 as compared with other analogues and considered to be least fit. Torsion energy related with the degree of freedom of ligands. Greater the degree of freedom, greater will be possibilities of change in the conformations. Therefore internal energy and torsional energy balanced each other but there is an overall increase in binding energy profile (Fig. 8 (a) and (b)). Synthesized phenylalanine analogues K1, K6, K7, K8, K11 and K12 showed an increase in torsion energy. An increase in torsion energy increases the overall binding energy of complex. Therefore K1 showed the least fit. All synthesized sulfonamides phenylalanine analogues (K1-K12) show hydrogen bonding interactions with amino acids on the active site of the protein p53 (Table 6). In addition to H-bonding, π-cation interactions have also been observed. K4 shows effective interactions with amino acids moieties ARG-248 in comparison to mitoxantrone (Fig. 7). The sulfonamide feature is common for all the synthesized sulfonamide analogues (K1-K12); whereas the substituent group differs at the *p*-position of ring A (R_1) and at the R_2 (Table 7). The change in substituent at R_1 and R_2 changes the conformation in free and bound state which leads to torsion angle variations (Fig. 9 (a) and (b)) and (Table 7). High flexibility in main chain angles i.e. Φ1, Φ2, Φ2", Φ3, Φ3", Φ4, Φ4" and Φ5 along with major changes in overall structure has been observed (Table 7).

Interestingly oxygen atom of SO_2 in K2 analogue makes H-bonds with ARG-248 and oxygen atom of heterocyclic ring (morpholine) makes H-bond with GLN-165 and other interactions with HIS 168, ASN 239, SER 241 residues of p53. However, mitoxantrone indicates interactions with

ARG-248, ASN 239 and LEU 137. Maximum inhibition and effective binding interactions is exhibited by K10 and K2 in comparison to mitoxantrone (Table 6) and binding interactions of analogues K1-12 with p53 tumor suppressor-DNA complex is confirmed (Fig. 7). K10 makes hydrogen bond with ARG 248 and MET 243 residues of p53.

Binding activity is based upon the existence of several functional groups present in the molecule. The polarity of functional group as well as configuration of ligand regulates the relative binding. Variation in the biological activity among the analogues depends on length and number of carbon atoms as well as the nature of attached functional groups. Incorporation of hydrophobicity in main chain and also in side group of main chain lowers the overall binding energy. The comparative results of anticancer and docking studies of all analogues support each other and are shown in (Fig. 10).

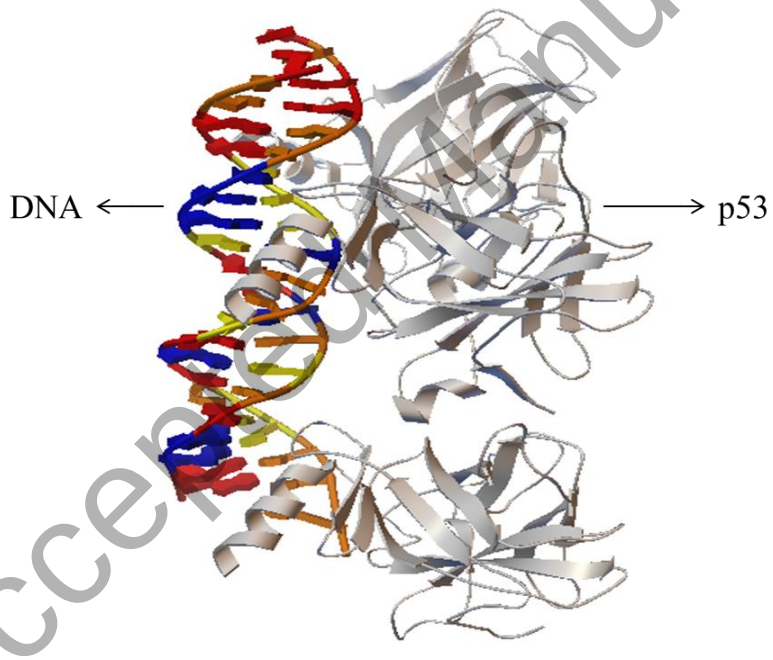


Fig. 6. Complete structure of p53 (1TUP).

Table 6

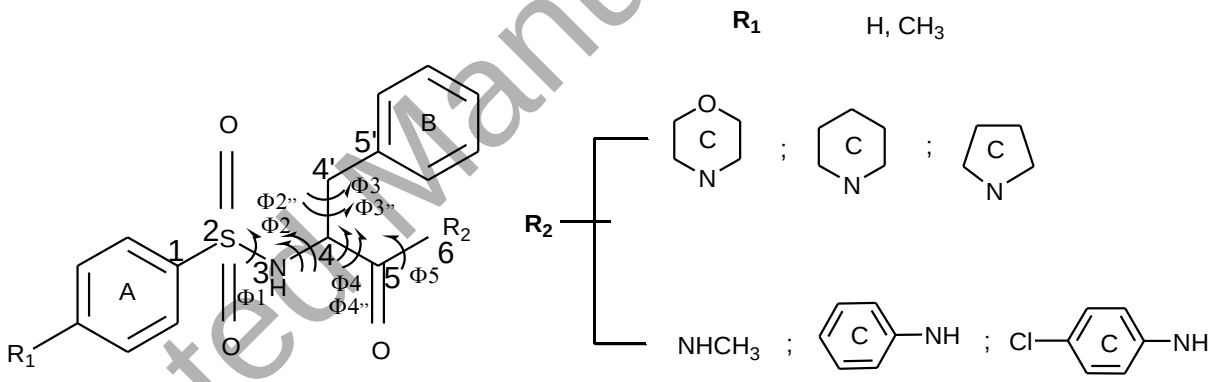
Binding energy (ΔG_b), docking energy, Inhibition constant and H-bond interactions of synthesized phenylalanine sulfonamide analogues (K1-K12) and reference compound.

S. No.	Analogues	Inhibition constant Ki (μM)	Binding energy (Kcal/mol)	Docking energy (Kcal/mol)	Torsional energy (Kcal/mol)	H-bond interactions	Distances (Å)	Number in cluster
1.	K-1	391.78	-4.65	-8.60	2.09	ARG 248 NH…O_2S MET 243 NH…O=C	2.031 1.713	2
2.	K-2	2.92	-7.55	-10.87	1.79	HIS 168 NH…O of morpholine ASN 239 NH…O=C SER 241 NH…O_2S	1.809 2.016 2.203	4
3.	K-3	5.00	-7.23	-10.94	1.79	ARG 248 NH…O_2S MET 243 NH…O=C	2.084 1.934	2
4.	K-4	3.81	-7.39	-10.95	1.79	ASN 239 NH…O=C MET 243 NH…O_2S	2.197 1.792	10
5.	K-5	15.26	-6.57	-10.02	1.79	ARG 273 NH…O_2S ARG 248 CO…$NHCH_3$	2.229 1.936	1
6.	K-6	16.10	-6.54	-10.60	2.09	ARG 248 NH…O_2S MET 243 NH…O=C	1.903 1.69	7
7.	K-7	6.85	-7.05	-11.07	2.09	ASN 239 NH…O_2S MET 243 NH…O=C	2.121 1.921	6
8.	K-8	179.67	-5.11	-9.20	2.09	ARG 248 NH…O=C ARG 248 NH…O-CH ASN 239 NH…O_2S	1.934 1.948 1.932	2

9.	K-9	14.85	-6.59	-9.89	1.79	ASN 239 NH…O_2S HIS 168 NH…O of morpholine	2.08 2.205	1
10	K-10	1.59	-7.91	-10.30	1.79	ARG 248 NH…O_2S MET 243 NH…O=C	2.106 1.851	3
11.	K-11	6.79	-7.05	-11.17	2.09	ARG 248 NH…O_2S MET 243 NH…O=C	2.057 1.724	2
12.	K-12	4.41	-7.31	-10.83	2.09	ASN 239 NH…O_2S MET 243 NH…O=C	2.192 1.735	1
13.	MTX	928.62nM	-8.23	-11.17	1.79	ASN 247 NH…OH LEU 137 NH…OH ASN 239 NH…NH ASN 239 NH…O oxygen Of MTX ring	1.712	4

Table 7

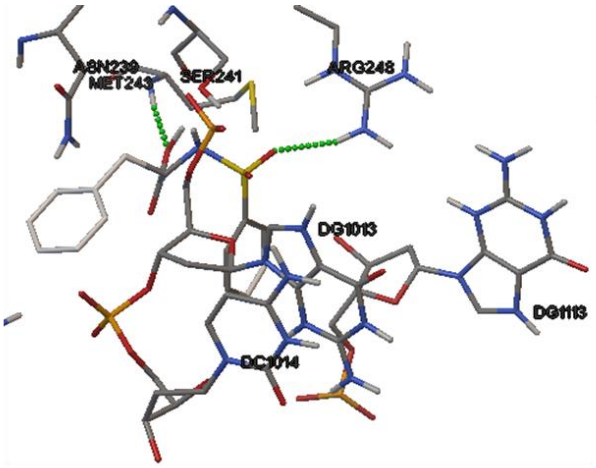
Torsional angles variation of synthesized phenylalanine sulfonamide analogues (K1-K12) before and after docking with p53 tumor suppressor.



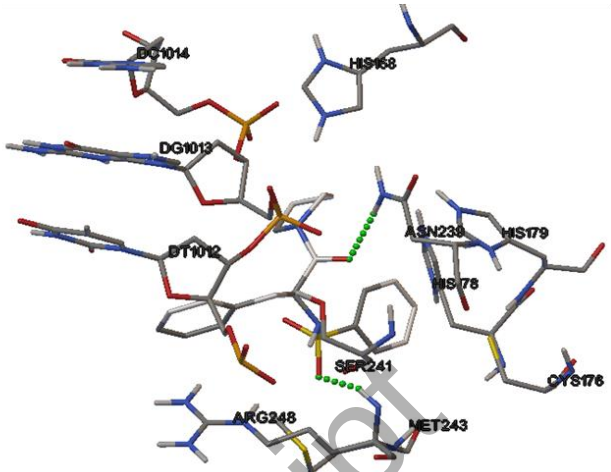
S. No.	Code	Ligands		$C_1S_2N_3C_4$ (Φ1)		$S_2N_3C_4C_5$ (Φ2'')		$S_2N_3C_4C_4'$(Φ2)		$C_5C_4C_4'C_5'$ (Φ3)		$N_3C_4C_4'C_5'$ (Φ3'')		$N_3C_4C_5R_6$ (Φ4)		$C_4'C_4C_5N_6$ (Φ4'')		$C_4C_5N_6C_7$ (Φ5)	
		R_1	R_2	Free	Docked	Free	Docked	Free	Docked	Free	Docked	Free	Docked	Free	Docked	Free	Docked	Free	Docked
1.	K1	H	OH	-110.16	19.311	-178.40	-90.75	61.63	149.35	66.56	80.00	-173.44	-154.07	39.49	-35.78	159.47	84.12	109.56	14.49
2.	K2	H		-110.16	122.98	-178.33	-106.11	61.63	133.90	66.58	133.81	-173.44	-106.25	36.69	125.20	156.69	-114.83	167.25	-62.41
3.	K3	H		-110.16	38.199	-178.33	-108.23	61.63	131.70	66.58	96.39	-173.44	-143.62	36.69	-161.23	156.69	-41.22	167.25	-62.32
4.	K4	H		-110.16	102.75	-178.33	-93.43	61.63	146.58	66.58	134.64	-173.44	-105.36	36.12	153.12	156.12	-86.89	-177.11	-177.11
5.	K5	H	NH_2CH_3	-110.16	80.72	-178.82	-130.55	61.63	109.88	66.31	-49.10	-173.44	71.15	39.59	-23.43	159.51	96.50	-179.98	-177.99

6.	K6	H		-110.16	41.44	-178.82	-151.15	61.63	89.33	66.31	95.95	-173.44	-143.85	39.59	-91.57	159.51	28.33	-179.98	-177.96
7.	K7	H		-110.16	165.31	-178.82	-139.17	61.63	101.28	66.31	135.59	-173.44	-104.13	39.59	2.63	159.51	122.55	-178.98	-179.98
8.	K8	CH_3	OH	-110.16	-88.74	-178.40	177.74	61.63	57.79	66.56	96.78	-173.44	-143.21	39.49	-16.11	159.47	103.88	109.56	-8.80
9.	K9	CH_3		-110.16	-24.30	-178.33	-112.48	61.63	127.49	66.58	-90.13	-173.44	29.82	36.69	117.61	156.69	-122.40	167.25	-62.41
10.	K10	CH_3		-110.16	143.96	-178.33	-115.70	61.63	124.29	66.58	126.69	-173.44	-113.33	36.69	91.402	156.69	-148.62	167.25	-62.38
11.	K11	CH_3		-110.16	51.73	-178.82	-105.11	61.63	135.30	66.31	90.96	-173.44	-148.76	39.59	178.47	159.51	-61.60	-179.98	179.99
12.	K12	CH_3		-110.16	-167.65	-178.82	90.69	61.63	-28.81	66.31	96.86	-173.44	-142.92	39.59	35.12	159.51	155.01	-179.98	180.00

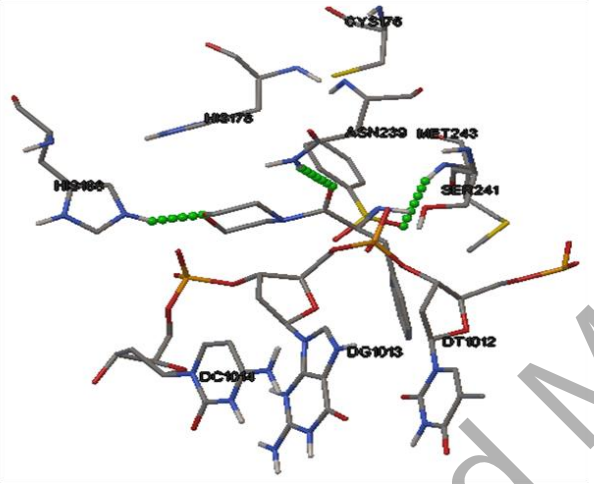
K1



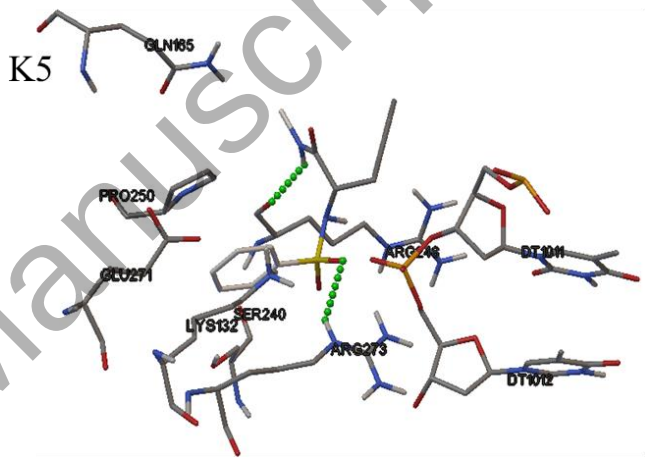
K4



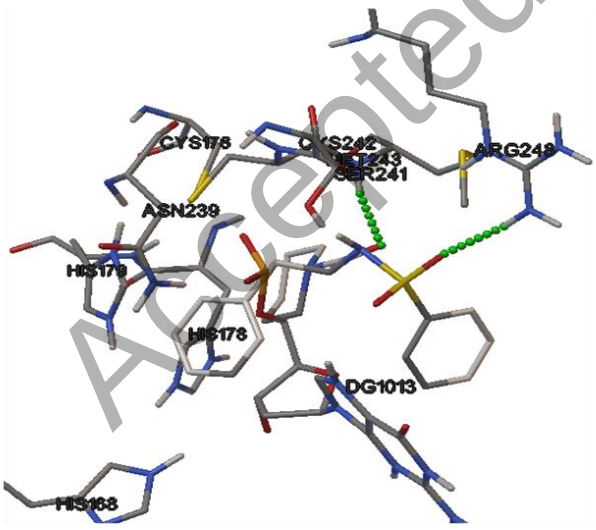
K2



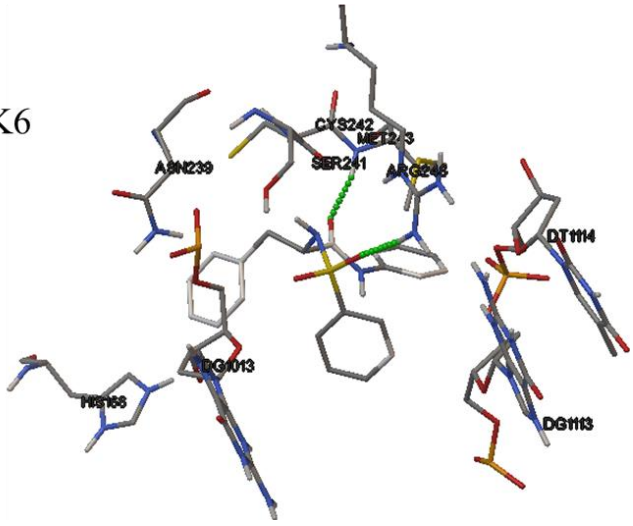
K5



K3



K6



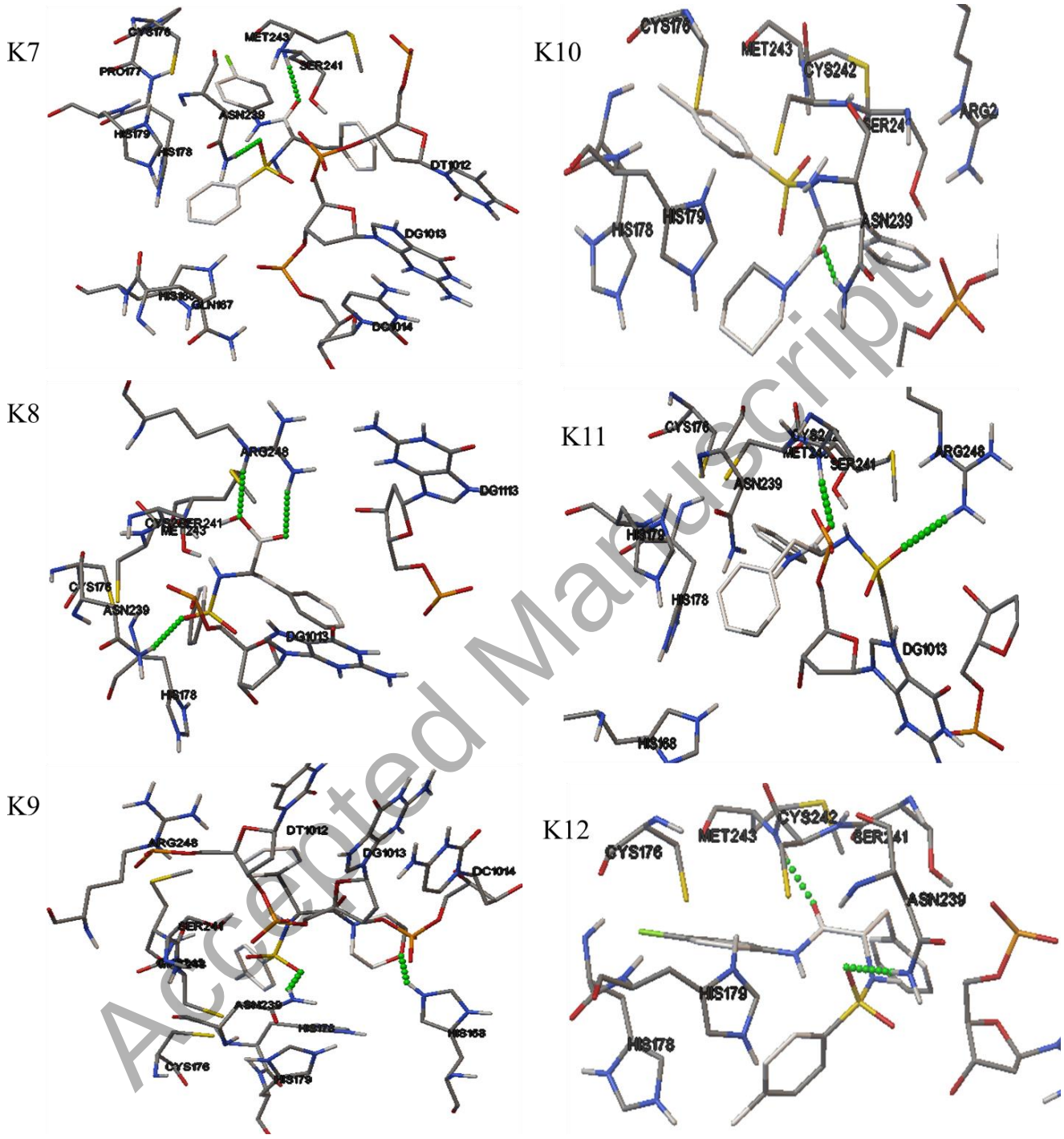


Fig. 7. H-bond interactions of synthesized phenylalanine sulfonamide analogues (K1-K12).

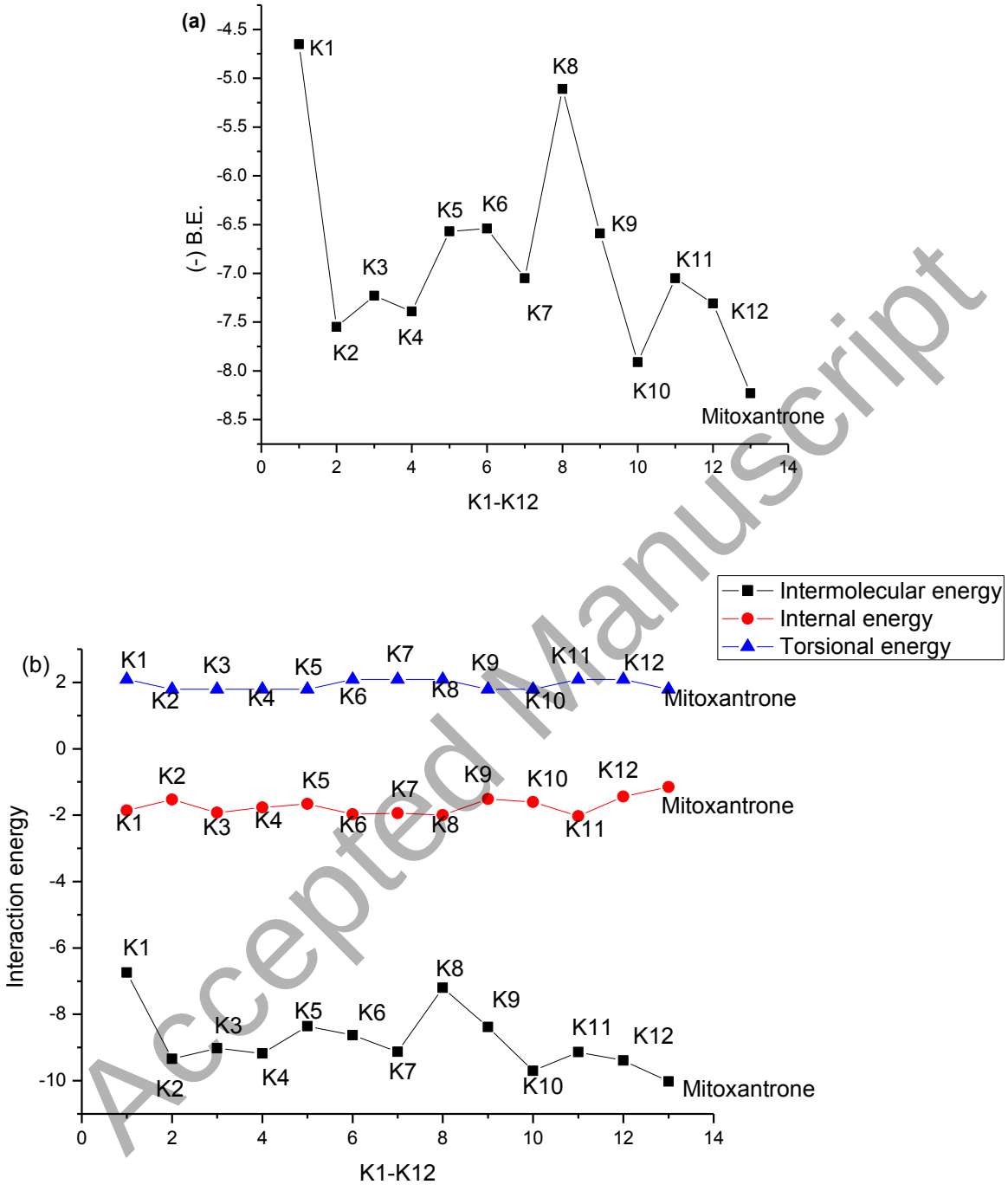


Fig. 8a. Binding energy profile of synthesized phenylalanine sulfonamide analogues (K1-K12); **(8b)** Intermolecular, internal, and torsional energy (Energy variations) of all (K1-K12) analogues with p53 tumor suppressor complex.

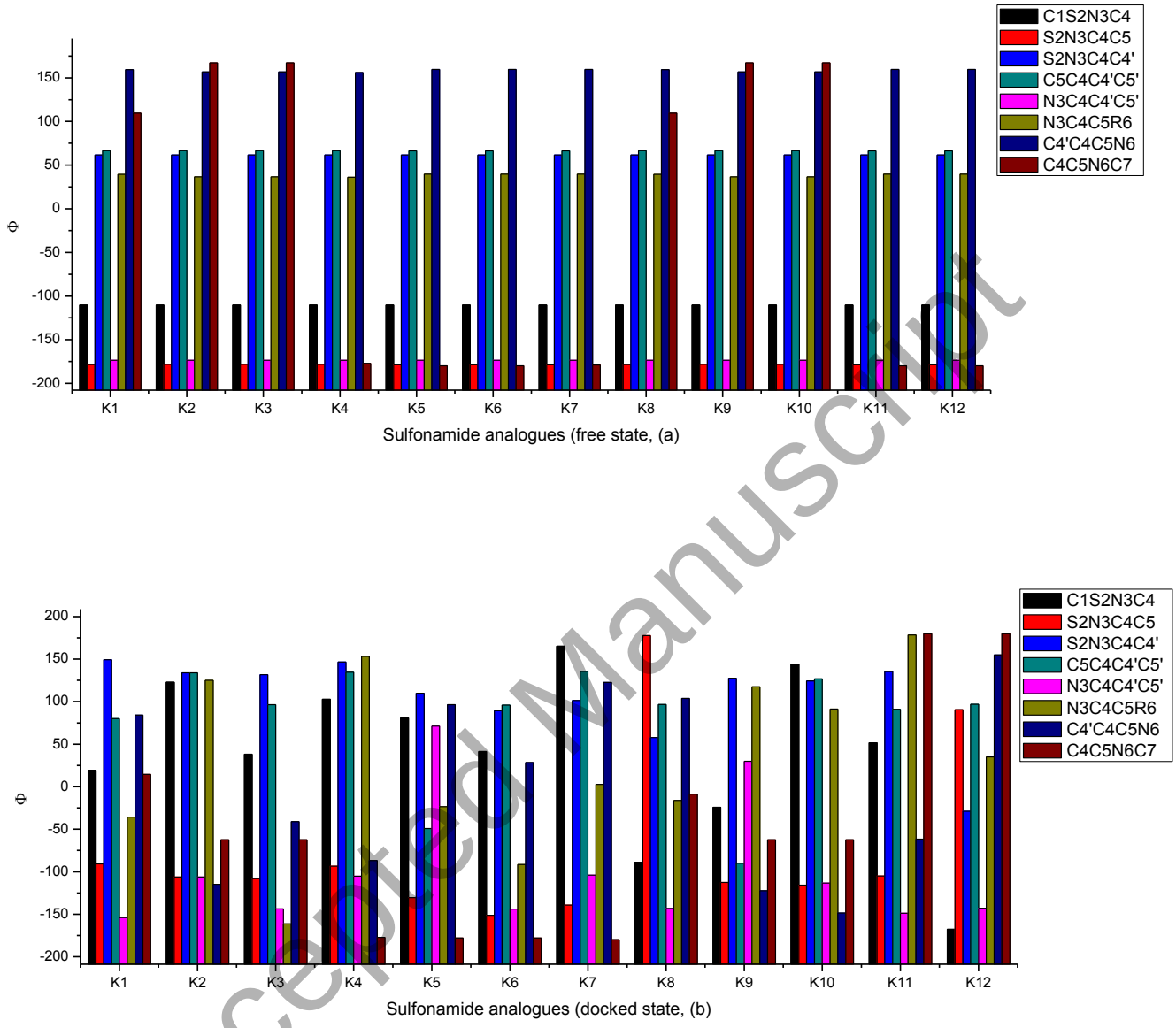


Fig. 9a. Torsional angles variation of synthesized phenylalanine sulfonamide analogues (K1-K12) in undocked (free) state; **(9b)** Torsional angles variation of synthesized phenylalanine sulfonamide analogues (K1-K12) in docked state.

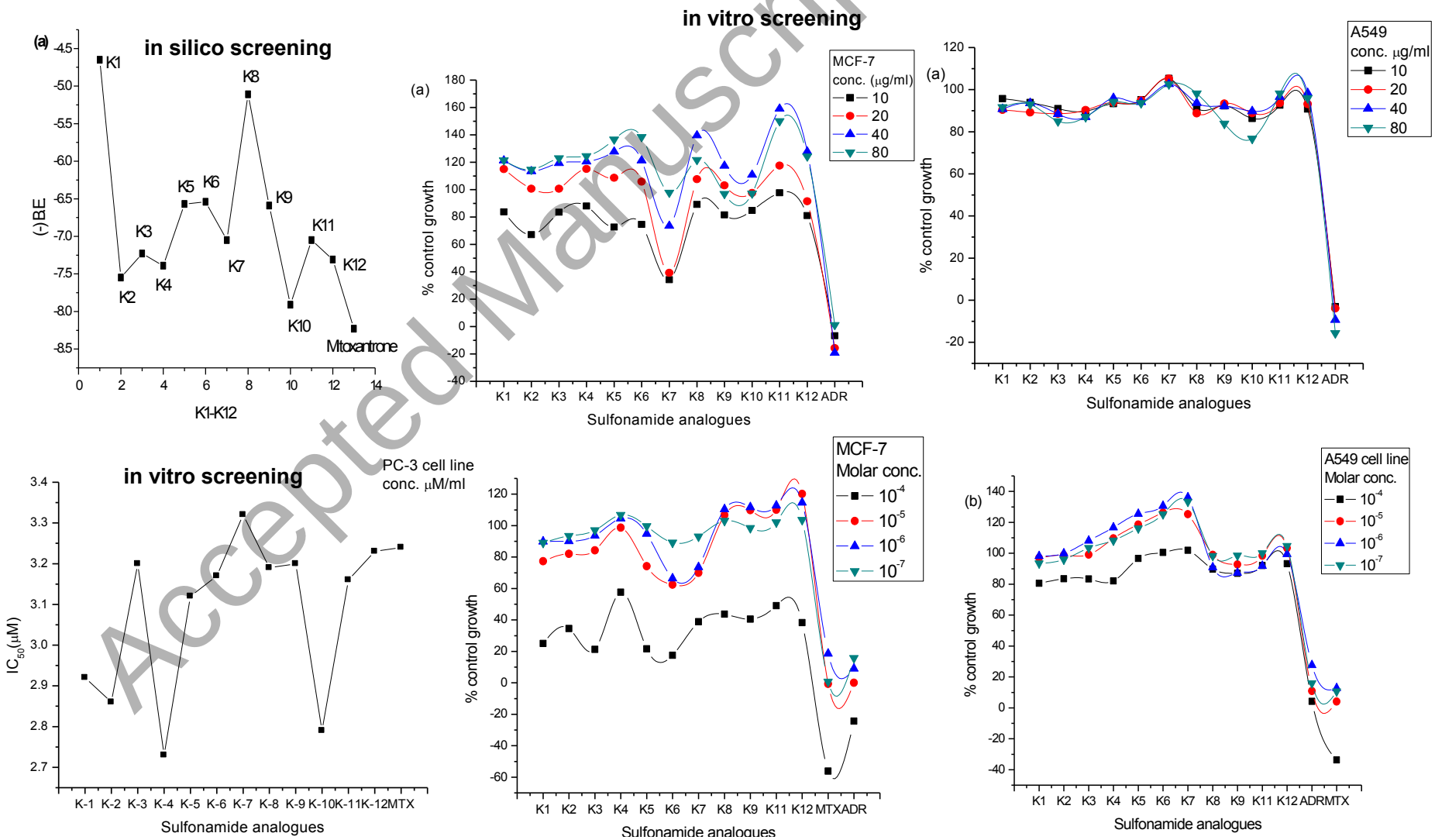


Fig. 10. *In-silico* study and *In-vitro* screening comparative of synthesized phenylalanine sulfonamide analogues (K1-K12) analogues K1-K12) (on PC-3, A549 and MCF-7 cancer cell line at molar and μg/ml concentration).

3.5 Quantum chemical studies

The frontier molecular orbitals theory is very extensively used for studying the electric and non-linear optical properties of organic compounds. The stabilization of the bonding molecular orbitals and destabilization of the antibonding can be observed by using overlapping of molecular orbitals. In molecular interaction, there are two critical orbitals that interact with each other; one is the highest occupied molecular orbital (HOMO) have the potential to donate the electron while another is lowest unoccupied molecular orbital (LUMO) has a potential to accept electron. The gap between HOMO and LUMO energy ascertains the kinetic stability, chemical reactivity and optical polarizability and chemical hardness-softness of molecules [Vatsal, Devi and Awasthi, 2018].

Table 8

Total energy, electronic states (HOMO and LUMO) and energy gaps for synthesized phenylalanine sulfonamide analogues (K1-K12) using DFT/B3LYP/6-311++G(d,p).

S. No.	E_{total}(Hartree)	E_{HOMO}(eV)	E_{LUMO} (eV)	$\Delta E_{HOMO-LUMO\ gap}$ (eV)
K1	-1334.141	-0.228	-0.117	0.111
K2	-1545.555	-0.209	-0.116	0.093
K3	-1509.661	-0.202	-0.112	0.090
K4	-1470.332	-0.198	-0.112	0.086
K5	-1353.605	-0.217	-0.110	0.107
K6	-1545.293	-0.214	-0.111	0.103
K7	-2004.915	-0.219	-0.114	0.105
K8	-1373.468	-0.226	-0.113	0.113
K9	-1584.882	-0.207	-0.112	0.095
K10	-1548.987	-0.201	-0.109	0.092
K11	-1584.619	-0.213	-0.108	0.105
K12	-2044.242	-0.218	-0.111	0.107
MTX	-1517.016	-0.181	-0.126	0.055

The hard molecules are less polarizable than the soft one because they need more energy for excitation. HOMO is localized on almost the entire molecule and LUMO is especially localized at the ring. Both the HOMO-LUMO is largely antibonding type orbitals and shape of HOMO and LUMO of sulfonamide analogues (K1-K12) are presented in Fig. 11. The HOMO-LUMO gap in these analogues (K1-K12) is in the range of 0.044-1.00 eV with an order of K8>K1>K5=K12>K11=K7>K6>K9>K2>K10>K3> K4>MTX (Table 8). Calculated HOMO-LUMO gap for K4 is 0.086 eV in comparison to MTX i.e. 0.055 eV. Large HOMO-LUMO gap

implies high kinetic stability and low chemical reactivity. It is energetically unfavorable to add electrons to high-lying LUMO and to extract electrons from low-lying HOMO in order to form activated complex of any potential reaction. Chemical properties are calculated to know the chemical hardness, chemical potential and electronegativity. Electrophilicity index (chemical applications) calculated and tabulated in Table 9. The chemical hardness is good indicator of chemical stability or thermodynamic stability. The better the associated electronegativity number, the more an element or atom or compound draw electron towards it. All these chemical properties of synthesized analogues (K1-K12) are comparable with standard drug mitoxantrone. Mulliken analysis is used to understand the charge distribution over chemical bonding. It facilitates the localization of positive and negative region in molecular space, at which protons and electrons accumulate. Thus chemically extensive regions, including bonds or lone pairs, can be identified. It also indicates the mechanism of electrophilic and nucleophilic substitutions at binding sites like carbonyl functionalities (-C=O, -C=O-N-) which are an important sites of the synthesized analogues (K1-K12). Due to bipolar nature of C=O bond, both nucleophilic and electrophilic attacks are viable at C and O sites and interaction with receptor is facilitated from this site. It is noted that the rate of nucleophilic addition on carbonyl analogue can be decreased by electron donating alkyl groups and enhanced by electron withdrawing groups inside the designed molecule and same has been very well taken care off in the present synthesize (K1-K12) series. This comprehensive study to certain extend confirms the variation in cytotoxicity and biological activities of sulfonamide series (K1-K12) from mitoxantrone.

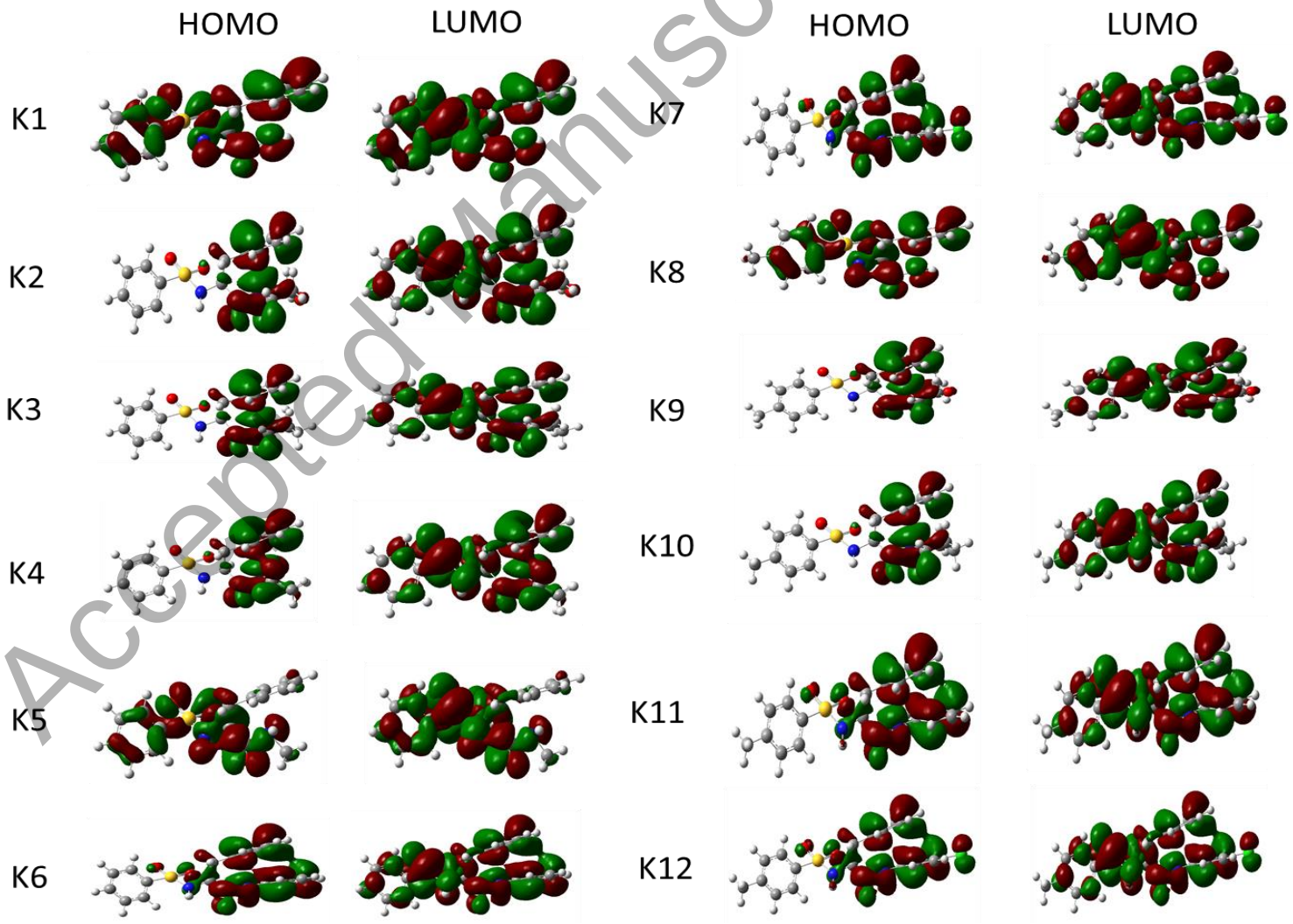


Fig. 11. Frontier molecular orbitals of synthesized phenylalanine sulfonamide analogues (K1-K12).

Table 9

Chemical hardness, electronegativity, chemical potential, electrophilicity index of synthesized phenylalanine sulfonamide analogues (K1-K12) and mitoxantrone.

S. No.	Ionization potential	Electron affinity	Dipole moment (Debye)	Electronegativity χ	Chemical Softness ξ	Chemical Hardness η	Electrophilicity index ω
K1	0.228	-0.117	3.462	-0.1725	9.009	0.0555	0.2680
K2	0.209	-0.116	4.835	-0.1625	10.752	0.0465	0.2839
K3	0.202	-0.112	3.103	-0.157	11.11	0.045	0.2738
K4	0.198	-0.112	3.362	-0.155	11.62	0.043	0.2793
K5	0.217	-0.110	3.319	-0.1635	9.345	0.0535	0.2498
K6	0.214	-0.111	2.385	-0.1625	9.708	0.0515	0.2563
K7	0.219	-0.114	4.375	-0.1665	9.522	0.0525	0.2638
K8	0.226	-0.113	4.109	-0.1695	8.849	0.0565	0.2539
K9	0.207	-0.112	5.551	-0.1595	10.526	0.0475	0.2673
K10	0.201	-0.109	3.767	-0.155	10.869	0.046	0.2608
K11	0.213	-0.108	3.017	-0.1605	9.523	0.0525	0.2453
K12	0.218	-0.111	5.119	-0.1645	9.345	0.0535	0.2523
MTX	0.181	-0.126	6.89	-0.1535	18.18	0.0275	0.4283

4. Conclusions

The proposed work is designing and synthesis of phenylalanine sulfonamide analogues (K1-K12) to be anticancer, antibacterial and antifungals agents. We have carried out detail computational bioactivity study of the synthesized sulfonamide phenylalanine (SPA) analogues. It has been observed that they do not violate the Lipinski Rule. In present research paper, twelve synthetic sulfonamide phenylalanine analogues (K1-K12) are reported. Compounds differ from each other; primarily based upon substituent/functional group attached at aliphatic termination

and aromatic ring A around $–SO_2$-NH group. Compounds (K1-K12) showed good antimicrobial (antibacterial & antifungal) activities over resistant and non-resistant strains. All the synthesized analogues are screened on PC-3, MCF-7 and A549 cancer cell lines. K4 screened out to be best and comparable to Mitoxantrone.The cell-cycle study of K4 confirms that K1-K12 series inhibit the G0/G1 phase. Docking study of (K1-K12) analogues with p53 tumor suppresser-DNA complex indicates effective p53-DNA-DRUG complex formation. The results of biological screening and docking studies support each other. K4 analogues showed comparable cytotoxic properties to Mitoxantrone.

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