Indian Journal of Biochemistry & Biophysics
Vol. 61, May 2024, pp. 305-313
DOI: 10.56042/ijbb.v61i5.6123

Interaction of a new triazole compound with Serum albumins and Proteolytic enzyme Bromelain by Steady state fluorescence and Molecular docking techniques

Sourav Misra[1], Sourav Pakrashy[1,2], Sandip Paul[3], Pawan Kumar Maurya[2], Pinki Saha Sardar[3], Adity Bose[1]* & Anjoy Majhi[1]*

[1]Department of Chemistry, Presidency University, Kolkata-700 073, West Bengal, India

[2]Indian Council of Medical Research-Centre for Ageing and Mental Health, Division of Non-Communicable Diseases, Kolkata-700 091, West Bengal, India

[3]The Department of Chemistry, The Bhawanipur Education Society College, Kolkata-700 020, West Bengal, India

Received 10 October 2023; *revised 01 March 2024*

Study of interaction of small molecules with serum albumins is very much essential in the perspective of pharmaceutical and food chemistry. Triazoles are nitrogen containing heterocyclic organic compounds and can show binding interaction with the amino acid residues of proteins via mainly H-bonding using the nitrogen atoms. Here we have synthesized a triazole based organic compound tert-butyl(6-oxo-6-(((1-(2-oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl) methyl)amino) hexyl)carbamate (SAM-1) using CuAAC reaction and investigated its interaction with serum albumins (BSA, HSA) and Bromelain (BMLN) using steady state fluorescence spectroscopy. The experimental results were further supplemented by Molecular docking. The theoretical ADMET (Absorption, Digestion, Metabolism, Excretion, Toxicity) predictions are also performed to check its drug-able nature. The experimental and theoretical studies indicate a good and spontaneous binding interaction (binding constant is of 10^5 order) of SAM-1 with both the serum albumins and Bromelain at 298K along with a good ADMET profile. As SAM-1 binds with Bromelain, it makes it suitable for oral absorption. In a nutshell SAM-1 can be considered as a potential drug candidate and can be further investigated for its medical effectiveness in future.

Keywords: ADMET, Bromelain, CuAAC, Fluorescence, Molecular docking, Serum albumins, Triazole

Cu(I)-catalyzed alkyne–azide 1,3-dipolar cycloaddition (CuAAC) reaction is commonly known as "click reaction". Click chemistry is highly efficient and reliable reactions, which has wide scope, gives high yield; stereospecific and most importantly proceeds under simple reaction conditions and involve straight forward procedures for product isolation[1]. The click reaction produces a 1,2,3-triazole ring, which is of interest in medicinal chemistry. Several 1,2,3-triazole-based coumarin compounds were previously synthesised *via* the CuAAC process and have a variety of applications, including medicines, and are already marketed as drugs[2]. 1,2,3-triazolecoumarin hybrids with high biological activity have recently been discovered[3].

Triazoles are basically heterocyclic rings involving three N-atoms. There are two isomeric form of it namely 1,2,3-triazole and 1,2,4-triazole. Triazoles are found to be present in different FDA -approved drugs like Radezolid, Rufinamide, Tazobactum etc. and are known for their ability to bind with different proteins and enzymes. The 1,4-disubstituted 1,2,3-triazoles are highly aromatic, planar, polar and stable towards hydrolysis. Hence 1,2,3 triazoles can show preference towards binding with amino acid residues (like tryptophan) present in a hydrophobic environment via H-bonding interaction involving the N-atoms of the ring. Therefore synthesis of a compound containing 1,2,3-triazole nucleus enhances the probability of the synthesized molecule to be a potential drug candidate[4,5]. Although triazoles are polar, they have less solubility in water. Hence a suitable drug carrier can be required to transport them to their site of action. Bromelein (BMLN), a proteolyticenzyme, derived from the stem of pineapple can be useful in this respect. We can consume about 12 g/day of BMLN without major side effects[6].

The most abundant plasma proteins are bovine serum albumin (BSA) and human serum albumin (HSA), which serve important roles in a variety of biological systems. The molecular weights of these

*Correspondence:
E-mail: anjoy.chem@presiuniv.ac.in; adity.chem@presiuniv.ac.in
Suppl. Data available on respective page of NOPR

two serum albumins (SA) are essentially identical (66 KDa and 66.5 KDa for BSA and HSA, respectively) and they have an 80 percent structural similarity[7]. The aromatic amino acids tryptophan, tyrosine, and phenylalanine are found in proteins and are capable of displaying intrinsic fluorescence. Because the fluorescence of tyrosine and phenylalanine is often challenging to notice, tryptophan is the most commonly employed amino acid of proteins for fluorescence investigation.[8]

The study of protein ligand binding is crucial in drug development and development systems. At the very early stages of research, a protein ligand binding study can tell if a ligand can operate as a medication or not. Photophysical investigations of protein-ligand interactions utilising various spectroscopic techniques improve our understanding of the binding process[8,9].

Bromelain (BMLN) is a proteolytic enzyme, most commonly derived from pineapple stems.[6] BMLN possesses a wide range of protease inhibitors, making it a powerful therapeutic agent[10]. Stem BMLN is commonly used to inhibit platelet aggregation, angina pectoris, bronchitis, sinusitis, surgical traumas, thrombophlebitis, and pyelonephritis, as well as to improve drug absorption, especially antibiotics, analgesics, anti-inflammatory, antitumoral, and antituberculosis activity[10,11]. BMLN's interactions with some bioactive compounds have been widely researched in recent years due to their promising medical applications[12]. However to the best of our knowledge till now no study of interaction has been carried out between BMLN and triazole-based molecule. Hence study of the interaction of a synthesized triazole with BMLN can be considered as an important area to investigate.

Pharmacokinetics is a branch of pharmacology which investigates what the body does to any drug molecule. Pharmacokinetic studies try to estimate the rate of absorption or distribution of any chemical inside the body, mechanistic pathways of metabolism and excretion of that chemical and the concentration of that chemical in plasma for a long period of time. To achieve these, four steps are defined in Pharmacokinetics, which are abbreviated as ADME (Absorption, Distribution, Metabolism, Excretion). Absorption deals to the movement of the chemical (drug) from its site of administration to the bloodstream. Distribution examines where the chemical travels to and the rate at which it arrives to the required sites. Metabolism can be defined as the biotransformation of a drug chemical by certain organs or tissues (*e.g*. liver, kidney, skin or digestive tract) so that the drug can be excreted. Finally excretion is the process through which the metabolized drug chemical get removed from the body[13,14].

In drug discovery and development, the researchers have to examine the activity of any drug chemical in the body to ensure safety and toxicity. ADME and toxicology studies, are a critical step in this process[15]. The data collected from ADME prediction tells researchers if the chemical can be considered as a drug molecule in a very early stage of drug design. In order to be an effective drug, any chemical compound must have a good ADME (Absorption, Distribution, Metabolism, Excretion) profile. In the field of drug design and research, before synthesis of any new chemical compound, it is essential to check its ADME profile[16-18].

Here in this paper, our work is mainly categorized into two parts namely, design and synthesis novel coumarin based triazole compound tert-butyl(6-oxo-6-(((1-(2-oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl) methyl)amino)hexyl)carbamate (SAM-1, Fig. 1) using CuAAC reaction (Scheme is shown in Fig. 2) and then the biophysical studies of the synthesized compound was carried out with both the serum albumins (BSA/HSA) and bromelain (BMLN). As a result, an attempt is made to evaluate the role of a novel compound in the development of biomedicines for the drug design process at a very early stage of the research by conducting a binding study of the newly synthesized compound with biomolecules.

Results and Discussion

Fluorescence quenching study

If addition of an external agent to the solution of fluorophore leads to decrease of the fluorescence intensity of fluorophore, then the external agent is called quencher and the phenomenon is fluorescence quenching. From the fluorescence quenching experiment,

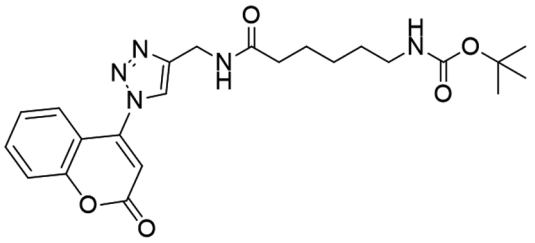


Fig. 1 — Structure of tert-butyl (6-oxo-6-(((1-(2-oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)hexyl)carbamate (SAM-1)

it was observed that SAM-1 can effectively quench the fluorescence intensity of the tryptophan emission of BSA, HSA, and BMLN (Fig. 3). The emission maxima was shifted towards shorter wavelength (blue shift) in the case of BSA (1.2 nm) and HSA (4 nm) but a reverse effect was observed for BMLN (2 nm red shift) with gradual addition of SAM-1. The blue shift indicates an increase in hydrophobicity in the microenvironment of the tryptophan residue while a red shift in emission spectrum indicates a decrease in hydrophobicity in the microenvironment of the tryptophan residue[7,19-21]. Therefore, we can conclude that the tryptophan moiety becomes more hydrophobic (i.e. gradually buried inside) with gradual addition of SAM-1 to BSA and HSA while for BMLN, the tryptophan moiety becomes much more exposed (less hydrophobic) on gradual addition of SAM-1 to it.

Stern-volmer plot

The quenching mechanisms can be broadly classified as static quenching and dynamic quenching. In case of static quenching, a non-fluorescent complex is formed between the fluorophore and quencher in the ground state of fluorophore. For dynamic quenching, fluorophore-quencher collision occurs in the excited state and this stops fluorescence emission of excited fluorophore. This Stern-Volmer plot is based on Stern-Volmer equation, which is as follows (Eq. 1):

$$F_0/F = 1 + K_{SV}[L] = 1 + k_q\tau_0[L] \quad \ldots (1)$$

where, F_0 and F represents fluorescence intensity of the in absence and in presence of quencher, [L] represents concentration of quencher in Molarity, τ_0 represents the average lifetime of the flourophore (which is nearly 5 ns for the tryptophan of BSA, HSA[3,4,7,19,20] and 10 ns for BMLN[12,22]). K_{SV} is called Stern-Volmer quenching constant and K_q represents bimolecular quenching constant.

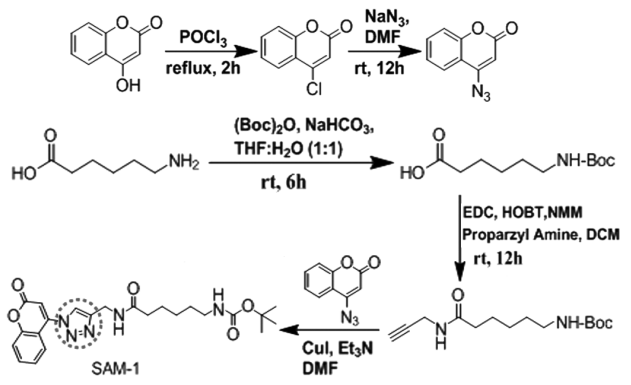


Fig. 2 — Synthesis of SAM-1

Higher the value of K_{SV} higher will be the extent of quenching. The order of K_{SV} value (Table 1) runs as: BSA<BMLN<HSA. This indicates that SAM-1 shows the most effective quenching with HSA and least effective quenching with BSA at room temperature (298K) (Fig. 4). The $K_q$value (Table 1) also runs as: BMLN<BSA<HSA. K_q represents the rate constant for quenching[8]. The trend in K_q value indicates that SAM-1 is able to quench the Trytophan emission of HSA at highest rate (fast) and quenching with BMLN occurs at lowest rate (slow) at room temperature.

Stern-Volmer plot is highly significant in order to predict the mechanism of quenching. If a linear Stern-Volmer plot is obtained, it indicates the presence of only one kind of quenching mechanism (either static or dynamic). From the slope of Stern-Volmer plot, we can determine k_q value. If the value of k_q is nearly equal or less than 2×10^{10} $M^{-1}\cdot s^{-1}$ then the quenching is considered to be dynamic and if k_q is greater than 2×10^{10} $M^{-1}\cdot s^{-1}$ then the quenching is considered to follow static quenching mechanism. In our study, k_q of SAM-1 is found to be much higher than the maximum possible value of scattered collision quenching constant (2×10^{10} $M^{-1}\cdot s^{-1}$) [20-24]. Hence, the quenching processes in both proteins follows static quenching mechanism (Fig. 4).

Binding constant and number of binding sites

The binding interaction can be evaluating by using modified Scatchard equation[25,26] shown as follows (Eq. 2)

$$\log[(F_0\text{-}F)/F] = \log K_b + n \log[Q] \quad \ldots (2)$$

where, where, F_0 and F represents the fluorescence intensities of serum albumins(BSA and HSA) and BMLN in the absence and presence of quencher molecule respectively, K_b is the binding constant of all the complexes formed (BSA-SAM-1, HSA-SAM-1, BMLN-SAM-1) and 'n' is the number of binding sites[27-31] (Table 2).

Table 1 — Stern-Volmer quenching constant (K_{SV}) and bimolecular quenching constant (k_q) of the complex formed due to the interaction of SAM-1 with BSA, HSA and BMLN in aqueous buffer (pH 7) at 298 K.

Systems	K_{sv} (M^{-1})	K_q ($M^{-1}s^{-1}$)	R^2
BSA-SAM-1	4.8×10^5	9.6×10^{13}	0.95
HSA-SAM-1	13.8×10^5	27.6×10^{13}	0.95
BMLN-SAM-1	8.2×10^5	8.2×10^{13}	0.97

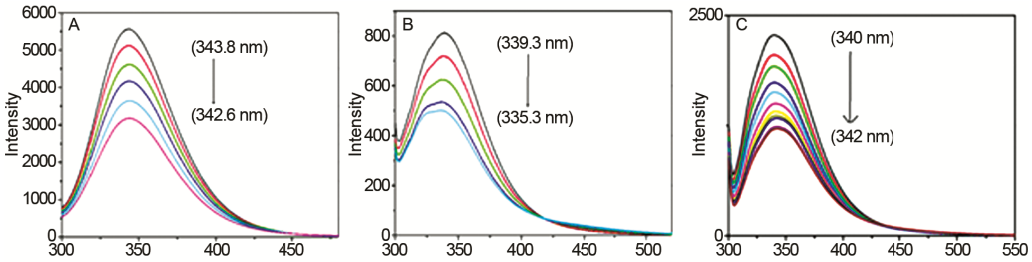


Fig. 3 — Room temperature (298K) fluorescence spectra of (A) Free BSA (5 µM) and its varying concentration of SAM-1 in aqueous phosphate buffer (pH 7). Excitation wavelength = 290 nm; excitation and emission band pass = 10 nm and 5 nm respectively; (B) Free HSA (5 µM) and its varying concentration of SAM-1 in aqueous phosphate buffer (pH 7). Excitation wavelength = 290 nm; excitation and emission band pass = 10 nm and 5 nm respectively; and (C) Free BMLN (5 µM) and its varying concentration of SAM-1 in aqueous phosphate buffer (pH 7). Excitation wavelength = 290 nm; excitation and emission band pass = 10 nm and 5 nm, respectively

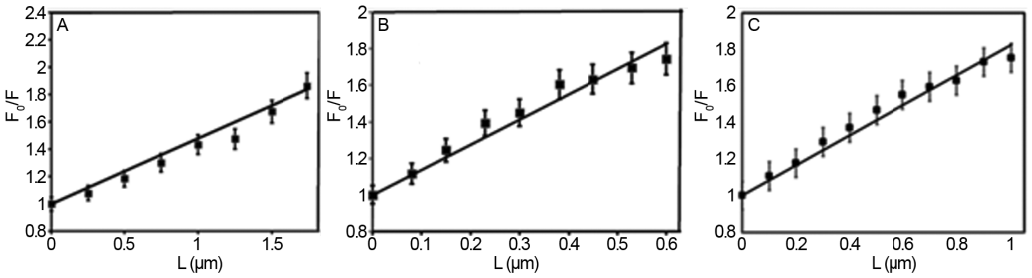


Fig. 4 — Stern-Volmer plots for fluorescence quenching at room temperature (298K) of (A) BSA-SAM-1 complex; λ_{exc}=290 nm, [BSA]=5 µM; Excitation band-pass = 10 nm and Emission band-pass = 5 nm; (B) HSA-SAM-1 complex; λ_{exc}=290 nm, [HSA]=5 µM; Excitation band-pass = 10 nm and Emission band-pass = 5 nm; and (C) BMLN-SAM-1 complex; λ_{exc}=290 nm, [BMLN]=5 µM; Excitation band-pass =10 nm and Emission band-pass = 5 nm

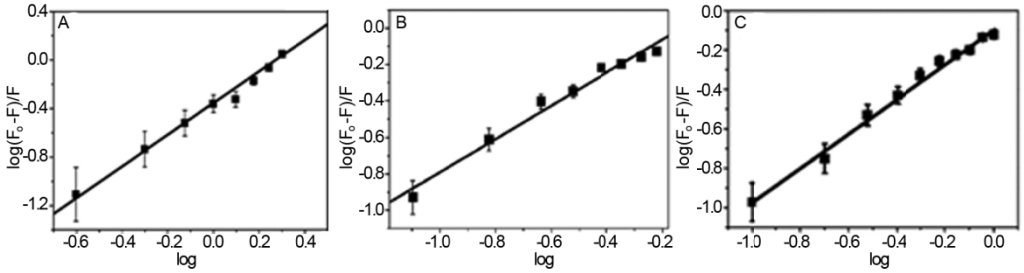


Fig. 5 — Double–logarithmic plots for binding constant determination at room temperature (298K) of (A) BSA-SAM-1 complex; λ_{exc}=290 nm, [BSA]=5 µM; Excitation band-pass = 10 nm and Emission band-pass = 5 nm; (B) HSA-SAM-1 complex; λ_{exc}=290 nm, [HSA]=5 µM; Excitation band-pass = 10 nm and Emission band-pass = 5 nm; and (C) BMLN-SAM-1 complex; λ_{exc}=290 nm, [BMLN]= 5 µM; Excitation band-pass = 10 nm and Emission band-pass = 5 nm

Table 2 — Binding constant (K_b), number of binding sites (n) and Change in Gibbs free energy (ΔG) of the complex formed due to the interaction of SAM-1 with BSA,HSA and BMLN in aqueous buffer (pH 7) at 298 K

Systems	$K_b(M^{-1})$	n	R^2	ΔG ($kJmol^{-1}$)
BSA-SAM-1	4.4×10^5	0.97	0.95	-3.89
HSA-SAM-1	13.2×10^5	0.97	0.96	-6.50
BMLN-SAM-1	8.06×10^5	0.99	0.97	-5.21

Higher the value of binding constant, higher will be the thermodynamic stability of the complex[31-35]. The order of binding constant of SAM-1 with the proteins and enzymes runs as- BSA<BMLN<HSA. Therefore it can be concluded that SAM-1 forms most stable complex with HSA and least stable complex with BSA under the experimental condition. The corresponding double-logarithmic plots are given in (Fig. 5).

The number of binding sites indicates number of available positions in the bio-macromolecule (here BSA, HSA, BMLN) where the ligand (here SAM-1) can bind[30-32]. The order of number binding sites also runs as: BSA=HSA<BMLN. This can be justified by the considering the presence of higher number of tryptophan residues (five) in case of BMLN compared to that of BSA and HSA[32-34]. However in all cases the value of n is nearly equal to 1 which suggests a 1:1 complex formation (Eq. 3) between SAM-1 with the bio-macromolecules (BSA, HSA, BMLN).

$$P + L \rightarrow PL \quad \ldots (3)$$

where P = BSA/HSA/BMLN, L= SAM-1

The change in Gibbs free energy (ΔG) indicates the spontaneity of the chemical reaction or process. Higher the negative value of ΔG, more spontaneous will be the process[31-35]. It is directly related to the equilibrium constant (here binding constant, K_b) as follows (Eq. 4):

$$\Delta G = -2.303\ RT \log K_b \quad \ldots (4)$$

The order of ΔG for the interaction of SAM-1 with the bio-macromolecules runs as BSA<BMLN<HSA. This indicates that SAM-1 binds most spontaneously with HSA and least spontaneously with BSA.

Molecular docking

The binding interaction of SAM-1 with BSA, HSA and BMLN were performed using the standard proceudre[36,37]. The grid box for 4OR0 (BSA) protein was taken as 73 × 30 × 92 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of – 9.6 Kcal/mol, grid box for 2BXG (HSA) protein was taken as 45 × 35 × 72 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of – 9.6 Kcal/mol and grid box for BMLN (1W0Q) was taken as - 2.6 × 4.2 × - 0.75 Å with size 51 × 36 × 36 Å along x- , y- and z- axes and ΔG value of – 9.5 Kcal/mol (Table 3). Considering the role of triazole in the protein-ligand complex (Fig. 6) it can be concluded that the nitrogen atoms of triazole ring

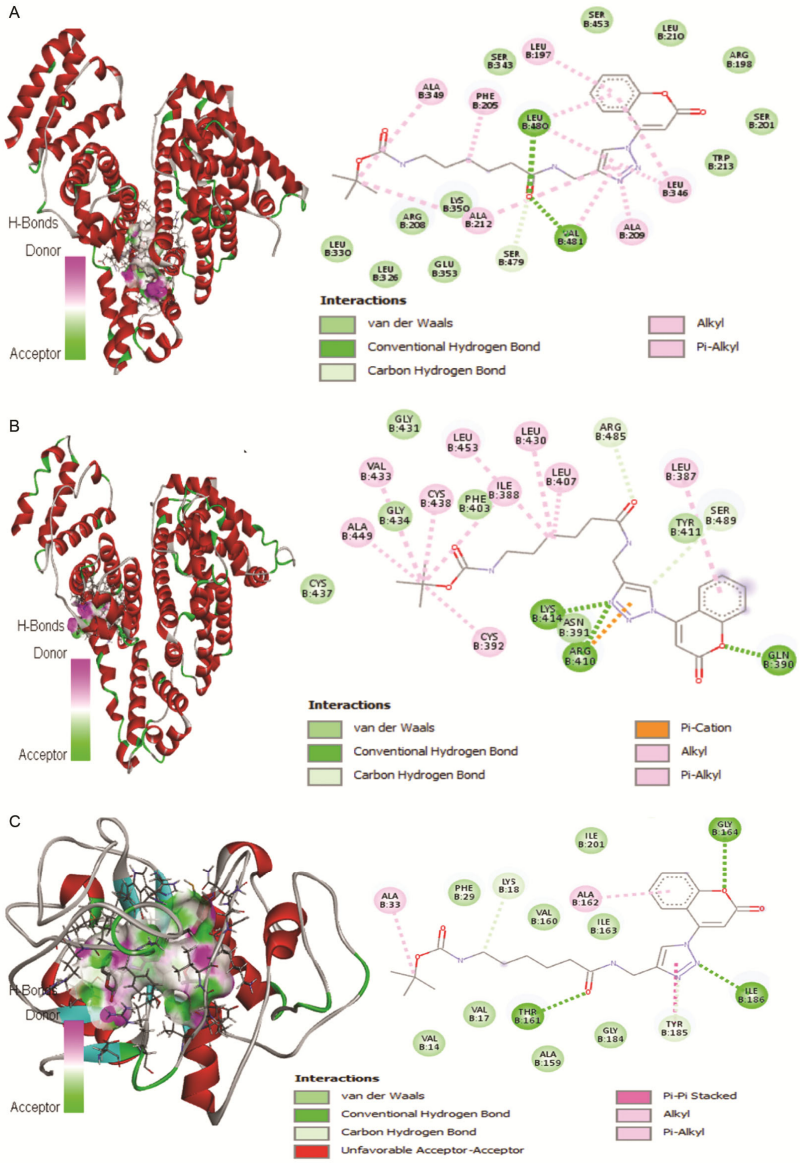
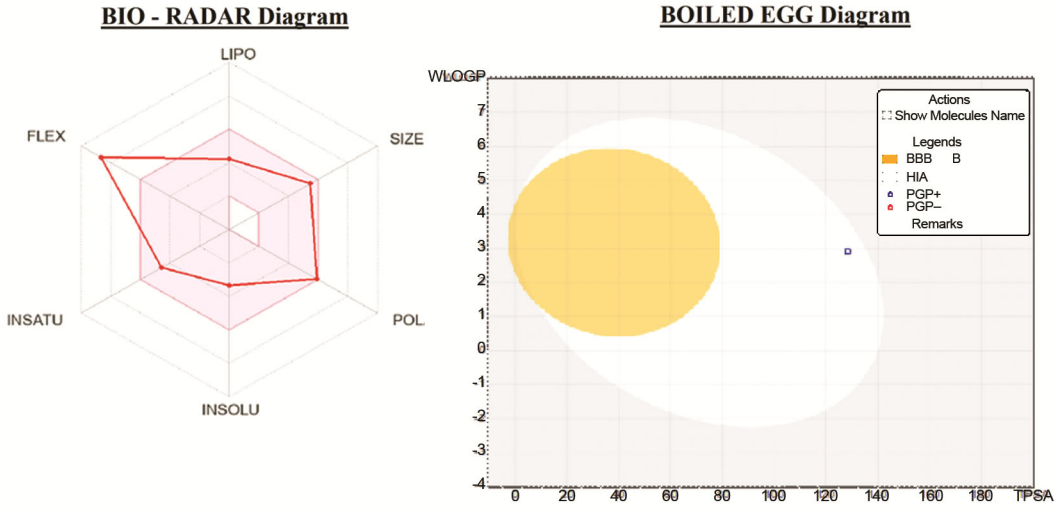


Fig. 6 — Molecular docked pose of (A) BSA-SAM-1 complex; (B) HSA-SAM-1 complex; and (C) BMLN-SAM-1 complex



Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-7.72 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	No; 1 violation: Rotors>10
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
TPSA	128.35 Å²

Fig. 7 — ADMET Analysis of SAM-1

Table 3 — Docking Scores for the interaction of SAM-1 with BSA, HSA and BMLN

Systems	PDB ID	Docking Score ΔG (kcal mol^{-1})
BSA-SAM-1	4OR0	-9.6
HSA-SAM-1	2BXG	-9.6
BMLN-SAM-1	1W0Q	-9.5

is involved in the hydrogen bonding interaction with the amino acid residues in case of HSA and BMLN; however such effect is found to be absent in case of BSA-SAM-1 complex.

ADME prediction

The result from SWISS ADME of SAM-1 came very satisfactory as it offers no violation from Lipinski's rule, Ghose rule, Egan rule and Muegge rule and it shows high gastrointestinal absorption as shown in (Suppl. Table S1) and it has a good bio-radar (Fig. 7) which makes it a fair candidate as orally admissible drug molecule[38]. In the boiled egg diagram (Fig. 7) if the small circle appears over the white zone then it HSA high gastrointestinal absorption, the blue colour tells that it would be effluxed out or metabolised by poly glycoproteins. The circle is far from the yellow zone which means it will not pass through the blood brain barrier[39-42].

Conclusion

SAM-1, a 1,2,3 triazole based compound is synthesized *via* CuAAC reaction and the interactions of the molecule with BSA, HSA and BMLN are investigated by using fluorescence spectroscopy. SAM-1 can effectively quench the tryptophan emission of both the serum albumins and BMLN. In the case of serum albumins, the interaction of SAM-1 with the tryptophan residue makes it to move towards inside (away from solvent, hydrophobicity increases) but reverse effect occurred for BMLN. The Stern-volmer plot and the K_q value indicates a static quenching mechanism. The molecule SAM-1 shows strong binding (of 10^5 order) with BSA, HSA and BMLN at room temperature under biological pH.

Binding with serum albumins makes it suitable to be considered as a potential drug candidate. As SAM-1 binds with BMLN, it makes it suitable for oral absorption, the theoretical ADMET prediction is also in consistent with it. Under biological condition the binding constant as well as the spontaneity of the process of interaction of SAM-1 runs as HSA > BMLN > BSA. So we can consider that SAM-1 can preferably bind with HSA in presence of BMLN and BSA. The binding interaction is also confirmed with molecular docking techniques which shows good binding potential. The ADMET prediction primarily gives conformation about its drug-able nature, subject to further studies.

Experimental

General information

All the reagents were of analytical grade, and chemicals and SA (BSA and HSA) were purchased from Sigma- Aldrich Chemicals Pvt. Ltd. All the solvents used were of spectral grade and further distilled by standard procedures. A phosphate buffer of pH 7 was prepared in triply distilled water and used for making experimental solutions. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica-gel plates (Merck, TLC Silica Gel 60 F_{254}, Cat. No. 1.05554.0001).

Synthesis of SAM-1

The compound 4-azido coumarin was synthesized followed by the literature and ^{1}H NMR value and melting point which is similar with the literature value.[25]

Preparation of BOC protected 6-Aminocaproic acid: Compound was synthesized by stirring an equivalent mixture of 6-aminocaproic acid and BOC anhydride with sodium bicarbonate in a solvent system of equal volume mixture of THF and water, at room temperature for 12 h.

Preparation of BOC protected 6-amino-N-(prop-2-yn-1-yl)hexanamide: BOC protected 6-Aminocaproic acid, HOBT and EDC were dissolved in DCM in a 100 mL round bottom flask. After 10 min of stirring, NMM was added. After 15 min of stirring propargyl amine (prop-2-yn-1-amine) was added drop wise. Then it was kept for 12 h of stirring. The reaction was monitored by TLC. The reaction mixture was poured into water and extracted with EtOAc. The layer was washed with saturated bicarbonate solution followed by brine solution. It was dried under anhydrous sodium sulphate and the solvent was evaporated to yield solid products. The product was then purified through column chromatography using 50% EtOAc in PET ether solution.

Preparation of tert-butyl (6-oxo-6-(((1-(2-oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl)methyl)amino) hexyl)carbamate (SAM-1): In a 50 mL two-neck round-bottom flask, 4-azido coumarin, BOC protected 6-amino-N-(prop-2-yn-1-yl) hexanamide and CuI (10 mol %) were dissolved in DMF under inert atmosphere at room temperature. Triethylamine (Et_3N) was added drop wise and then stirred for 5 h. The advancement of reaction was monitored by TLC. The mixture was filtered through a celite slurry of EtOAc. Cu was trapped in it. Then, eluted solution of EtOAc was washed with ammonium chloride (twice) carefully and then washed with water, saturated sodium bicarbonate, and brine solution. It was dried under anhydrous sodium sulphate, and the solvent was removed at reduced pressure. The crude product was purified by silica gel column chromatography (Fig. 2). The NMR and Mass spectrum of the compound are given in supporting information (Suppl. Fig. S1, S2, and S3).

Fluorescence quenching Study

The Fluorescence spectrum were recorded on a F-4700 Hitachi spectrofluorimeter equipped with a 1 cm path-length quartz cell and a circulating water bath, using an excitation wavelength of 290 nm. All the experiments were performed in micromolar range to avoid aggregation and inner filter effect. The solution of BSA (5 μM), HSA (5 μM) and BMLN (5 μM) were titrated separately with SAM-1 (0 to 2 μM) F_0 and F in all the cases being calculated considering the area under the emission curve of the fluorescence spectra.

Molecular docking study

Molecular docking of our designed moiety was further done with bovine serum albumin (BSA), human serum albumin (HSA) and Bromelain (BMLN). In order to do so we retrieved the proteins x-ray crystallographic structure in PDB format from RCSB PDB website (http://www.rcsb.org/pdb) bearing PDB ID's 4OR0 (BSA) and 2BXG (HSA) but x-ray crystallographic structure in PDB format is unavailable in the website so we took 1W0Q (BMLN) which is a formed structure through homology modelling, this modelled protein showed good result according to the positions occupied by its residues in Ramachandran Plot (Suppl. Fig. S4). Thus we used the PDB format of 1W0Q to dock with our designed

moiety[42]. All steps involving protein preparation, ligand preparation and other necessary ones for docking protocol done accordingly[36,37]. For visualising in many formats we used software's UCSF-Chimera and Discovery studio.

ADME prediction

To investigate the physicochemical properties of the secondary metabolites of all the plants. *In silico* ADME analysis was done, these are water solubility, lipophilicity and pharmacokinetics by using following website http://www.swissadme.ch, but the toxicity of these molecules cannot be investigated by using Swiss ADME, so help was taken from pkCSM - pharmacokinetics server to predict the Toxicity along with other ADME parameters of the molecules with their SMILE (Simplified Molecule Input Line Entry Specification) profile[15,36-38].

Acknowledgement

This research was supported by the Science and Engineering Research Board (SERB), India. We acknowledge SERB for research grant ref: EEQ/2019/000194. The authors are also thankful to the Presidency University, Kolkata for providing FRPDF research grant, laboratory and NMR facilities.

Conflict of interest

All authors declare no conflict of interest.

References

1 Kolb HC, Finn MG & Sharpless KB, Click chemistry: diverse chemical function from a few good reactions. *Angew Chem Int Ed*, 40 (2001) 2004.
2 Zhou CH & Wang Y, Recent researches in triazole compounds as medicinal drugs. *Curr Med Chem*, 19 (2012) 239.
3 Paul S, Roy P, Saha Sardar P & Majhi A, Design, Synthesis, and Biophysical Studies of Novel 1, 2, 3-Triazole-Based Quinoline and Coumarin Compounds. *ACS Omega*, 24 (2019) 7213.
4 Paul S, Ghanti R, Sardar PS & Majhi A, Synthesis of a novel coumarin derivative and its binding interaction with serum albumins. *Chem Heterocycl Compd*, 55 (2019) 607.
5 Hou J, Liu X, Shen J, Zhao G & Wang PG, The impact of click chemistry in medicinal chemistry. *Expert Opin Drug Discov*, 7 (2012) 489.
6 Pavan R, Jain S & Kumar A, Properties and therapeutic application of bromelain: a review. *Biotechnol Res Int*, 2012 (2012) 976203.
7 Sengupta P, Sardar PS, Roy P, Dasgupta S & Bose A, Investigation on the interaction of Rutin with serum albumins: Insights from spectroscopic and molecular docking techniques. *J Photochem Photobiol B Biol*, 183 (2018) 101.
8 Lakowicz JR, Principles of Fluorescence Spectroscopy; Springer: New York (2006) 3rd ed.
9 Paul S, Roy P, Das S, Ghosh S, Sardar PS & Majhi A. Addressing the Exigent Role of a Coumarin Fluorophore toward Finding the Suitable Microenvironment of Biomimicking and Biomolecular Systems: Steering to Project the Drug Designing and Drug Delivery Study. *ACS Omega,* 6 (2021) 11878.
10 Chakraborty AJ, Mitra S, Tallei TE, Tareq AM, Nainu F, Cicia D, Dhama K, Emran TB, Gandara JS & Capasso R, Bromelain a potential bioactive compound: a comprehensive overview from a pharmacological perspective. *Life,* 11 (2021) 317.
11 Heinicke RM & Gortner WA, Stem bromelain-a new protease preparation from pineapple plants. *Econ Bot*, 11 (1957) 225.
12 Li X, Yang Z & Bai Y, Fluorescence spectroscopic analysis of the interaction of papain and bromelain with l-ascorbic acid, α-tocopherol, β-carotene and astaxanthin. *Int J Biol Macromol*, 107(2018) 144.
13 Trilaksana H, Thanmayalaxmi D & Suvitha A, ADMET, Pharmacokinetic and Docking properties of the fungal drug 2- (2, 4-difluorophenyl)-1, 3-bis (1, 2, 4-triazol-1-yl) propan-2-ol by using Quantum computational methods . *Indian J Biochem Biophys*, 60 (2023) 58.
14 Roshan NS, Srikanth J, Swaminathan G, Rebekal J, Kannan R & Narasimha Rao G, Design, synthesis, characterization and *in vitro* evaluation of some novel thiol-substituted 1,3,4-oxadiazoles as GlmS inhibitors. *Indian J Biochem Biophys*, 60 (2023) 148.
15 Daina A, Michielin O & Zoete V, Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*, 7 (2017) 42717.
16 Chowdhury M, Sharma D, Das M & Dutta K, Molecular docking studies of natural and synthetic compounds against human secretory PLA2 in therapeutic intervention of inflammatory diseases and analysis of their pharmacokinetic properties. *Indian J Biochem Biophys*, 59 (2022) 33.
17 Yadav M & Eswari JS, Evolution of modern age drug discovery of lipopeptides and computer-aided drug discovery in India. *Indian J Biochem Biophys*, 59 (2022) 503.
18 Nambier MP, Jayadevan S, Babu BK & Biju AR, Computational studies on the structural variations of MAO-A and MAO-B inhibitors-An *in silico* docking approach. *Indian J Biochem Biophys*, 59 (2022) 276.
19 Sengupta P, Pal U, Roy P, Samanta T, Chattopadhyay N, Sen K & Bose A, Effect of a metal Ion in modulating the binding interaction of a dietary flavonoid with bovine serum albumin and DNA: a spectroscopic and theoretical approach. *Food Sci Technol*, 2 (2022) 114.
20 Zuo YQ, Zhong YS, Xu J, Rui YF, Wei YC, & Liu HY, Molecular simulation and spectroscopic studies on the interaction between perfluorohexadecanoic acid and human serum albumin. *Indian J Biochem Biophys*, 56 (2019) 185.
21 Gowda BG, Interaction of solifenacin succinate with bovine serum albumin by spectroscopic techniques and molecular modeling. *Indian J Biochem Biophys*, 58 (2021) 229.
22 Li X, Yang Z & Peng Y, The interaction of silver nanoparticles with papain and bromelain. *New J Chem*, 42 (2018) 4940.
23 Yu X, Lu S, Yang Y, Li X & Yi P, Study on the interaction between NCP-(4- hydroxycoumarins) and bovine serum albumin by spectroscopic techniques. *Spectrochim Acta Part A Mol Biomol Spectrosc*, 91 (2012) 113.

24 Fan Y, Yan J, Zhang S, Li J, Chen D & Duan P, Fluorescence spectroscopic analysis of the interaction of papain with ionic liquids. *Appl Biochem Biotechnol*, 168 (2012) 592.

25 Paul S, Sepay N, Sarkar S, Roy P, Dasgupta S, Sardar PS & Majhi, A, Interaction of serum albumins with fluorescent ligand 4-azido coumarin: spectroscopic analysis and molecular docking studies. *New J Chem*, 41 (2017) 15392.

26 Eftink MR & Ghiron CA, Fluorescence quenching studies with proteins. *Anal Biochem*, 114 (1981) 199.

27 Tian J, Liu J, Hu Z & Chen X, Interaction of wogonin with bovine serum albumin. *Bioorg Med Chem*, 13 (2005) 4124.

28 Ge YS, Jin C, Song Z, Zhang JQ, Jiang FL & Liu Y, Multi-spectroscopic analysis and molecular modelling on the interaction of curcumin and its derivatives with human serum albumin: A comparative study. *Spectrochim Acta Part A Mol Biomol Spectrosc*, 124 (2014) 265.

29 Tarannum A, Arif Z, Moinuddin, Alam K & Chandel TI, Biochemical and microscopy evidence on adverse effects of nitroxidized human serum albumin. *Indian J Biochem Biophys*, 59 (2022) 23.

30 Lissi E, Calderón C & Campos A, Evaluation of the number of binding sites in proteins from their intrinsic fluorescence: limitations and pitfalls. *Photochem Photobiol,* 89 (2013) 1413.

31 Wei XL, Xiao JB, Wang Y & Bai Y, Which model based on fluorescence quenching is suitable to study the interaction between trans-resveratrol and BSA?. *Spectrochim Acta - A: Mol Biomol Spectrosc,* 75 (2010) 299.

32 Bose A, Interaction of tea polyphenols with serum albumins: A fluorescence spectroscopic analysis. *J Lumin*, 169 (2016) 220.

33 Murachi T, Amino acid composition of stem bromelain. *Biochem*, 3 (1964) 932.

34 Ni Y, Zhu R & Kokot S, Competitive binding of small molecules with biopolymers: a fluorescence spectroscopy and chemometrics study of the interaction of aspirin and ibuprofen with BSA. *Analyst*, 136 (2011) 4794.

35 Rub MA, Khan JM, Asiri AM & Khan RH, Study on the interaction between amphiphilic drug and bovine serum albumin: A thermodynamic and spectroscopic description. *J Lumin*, 155 (2014) 39.

36 Pakrashy S, Mandal PK, Dey SK, Choudhury SM, Alasmary FA, Almalki AS, Islam MA & Dolay M, Design of a Structurally Novel Multipotent Drug Candidate by the Scaffold Architecture Technique for ACE-II, NSP15, and Mpro Protein Inhibition: Identification and Isolation of a Natural Product to Prevent the Severity of Future Variants of Covid 19 and a Colorectal Anticancer Drug. *ACS Omega*, 7 (2022) 33408.

37 Pakrashy S, Mandal PK., Nanda Goswami J, Dey SK, Choudhury SM, Bhattacharya B, Emmerling F, Alasmary FA & Dolay M, Bioinformatics and Network Pharmacology of the First Crystal Structured Clerodin: Anticancer and Antioxidant Potential against Human Breast Carcinoma Cell. *ACS Omega*, 7 (2022) 48572.

38 Sharma A, *In silico*-based studies on phytochemicals from native Indian plants as potential inhibitors of SARS-CoV-2. *Indian J Biochem Biophys*, 59 (2022) 653.

39 Dalal VK, Biswal AK, Patel D, Subramanyam R & Raghavendra AS, *In vitro* stability of various enzymes by proline from H2O2 mediated oxidative damage. *Indian J Biochem Biophys*, 59 (2022) 111.

40 Pires DEV, Blundell TL & Ascher DB, pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*, 58 (2015) 4066.

41 Ramaswamy S, Kongara D, Priyanka DL, Gade R, Krishna Raj R & Gayathri R, Synthesis, spectral characterization, antibacterial, cytotoxic evaluation and docking studies of new urea and thiourea derivatives. *Indian J Biochem Biophys*, 59 (2022) 767.

42 Swaroop AK, Patnaik SK, Vasanth P, Jeyaprakash MR, Praharsh Kumar MR, Jawahar N & Jubie S, Design and synthesis of novel quercetin metal complexes as IL-6 inhibitors for anti-inflammatory effect in SARS-CoV-2. *Indian J Biochem Biophys*, 59 (2022) 823.