DE-NOVO DRUG DESIGN OF NOVEL 1,2,3–TRIAZOLE-NAPHTHAMIDE AS AN INHIBITOR OF SARS-COV-2 MAIN PROTEASE: SYNTHESIS, BIOINFORMATICS AND BIOPHYSICAL STUDIES

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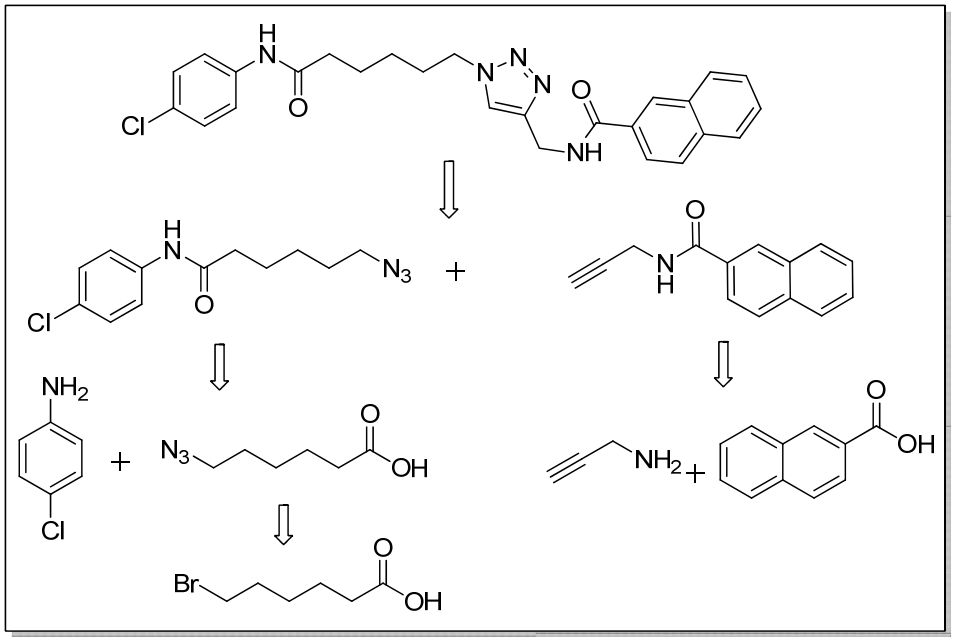
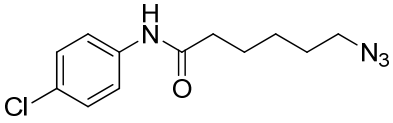


Figure S1: Retro Synthetic Pathway of SSAM-1

Synthetic methodology and NMR data:

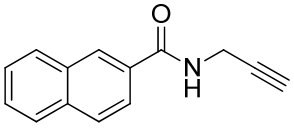
Synthesis of 6-azido-N-(4-chlorophenyl)hexanamide(3):



Compound **3** was synthesized following the literature.[5,25]

[1]**H NMR (400 MHz, ($CDCl_3$),** δ: 1.445 (m, 2H), 1.629 (m, 2H), 1.744 (m, 2H), 2.361 (t, 2H), 3.281 (t, 2H), 7.46 (d, 2H), 7.296 (d, 2H).

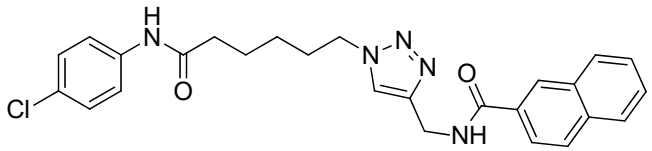
Synthesis of N-(prop-2-yn-1-yl)-2-naphthamide (5):



Compound **5** was synthesized following the literature.[5,25]

[1]H NMR (400 MH_Z, ($CDCl_3$), δ: 2.220 (s, 1H), 4.240 (s, 2H), 7.329 (d, 1H), 7.477 (d, 2H), 7.510 (d, 1H), 7.774 (d, 2H), 7.843 (t, 1H,), 8.227 (s,1H).

Synthesis of oftriazole-naphthamide(6):



Compound **SSAM-1** was synthesized following the literature.[5,25]

^{1}H NMR (400 MHz, $(CD_3)_2SO$)), δ: 1.26 (m, 2H), 1.57 (m, 2H), 1.85 (m, 2H), 2.283 (t, 2H), 4.365 (t, 2H), 4.570 (s, 2H), 7.316 (d, 2H), 7.321 (d, 2H), 7.533 (s, 1H), 7.552 (s, 1H), 7.632 (d, 1H), 7.958 (d, 1H), 8.021 (d, 1H), 8.066 (d, 1H), 8.192 (s, 1H), 8.217 (d, 2H); **^{13}C NMR (100 MHz, $(CD_3)_2SO$),δ**: 171.73, 169.02, 138.73, 130.39, 129.07, 128.70, 127.20, 126.72, 125.90, 125.81, 125.46, 123.26, 121.02,36.65, 35.34, 31.22, 30.14, 26.02,24.93.

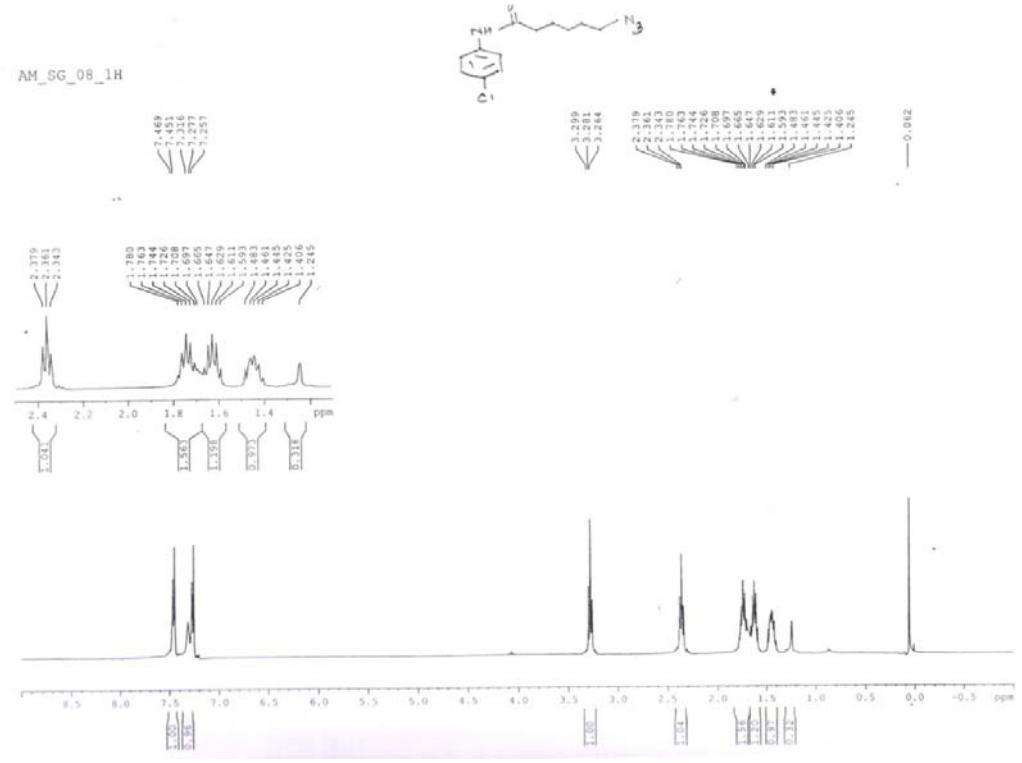


Figure S2. ^{1}H NMR spectra of compound **3**

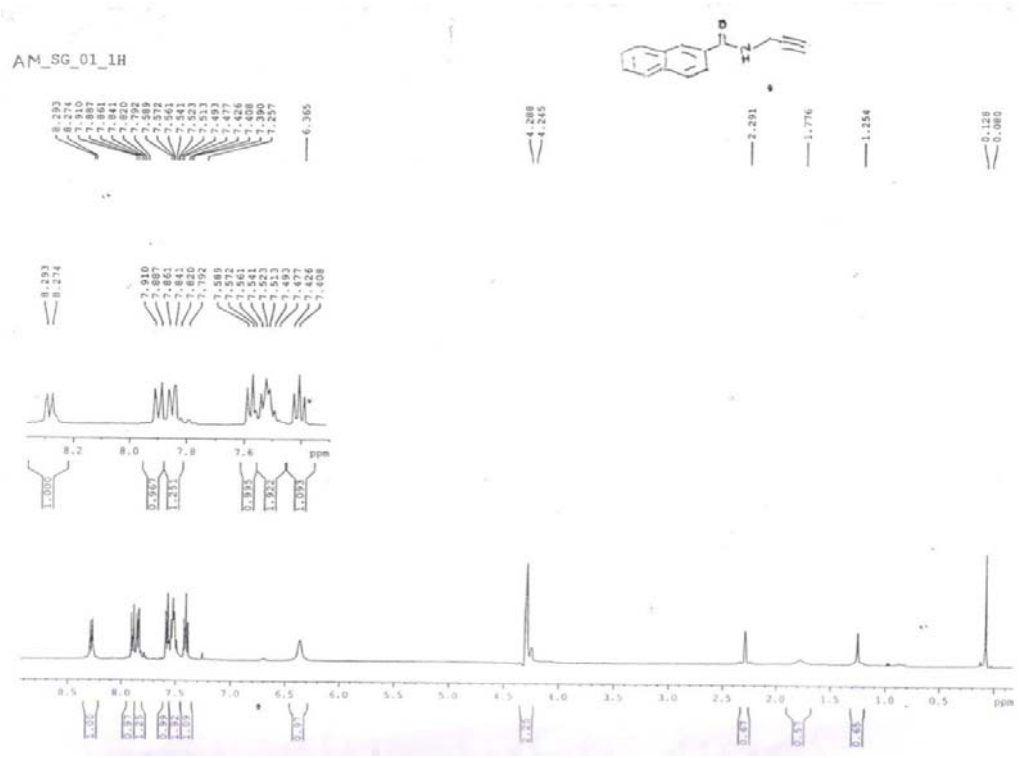


Figure S3. ^{1}H NMR spectra of compound **5**

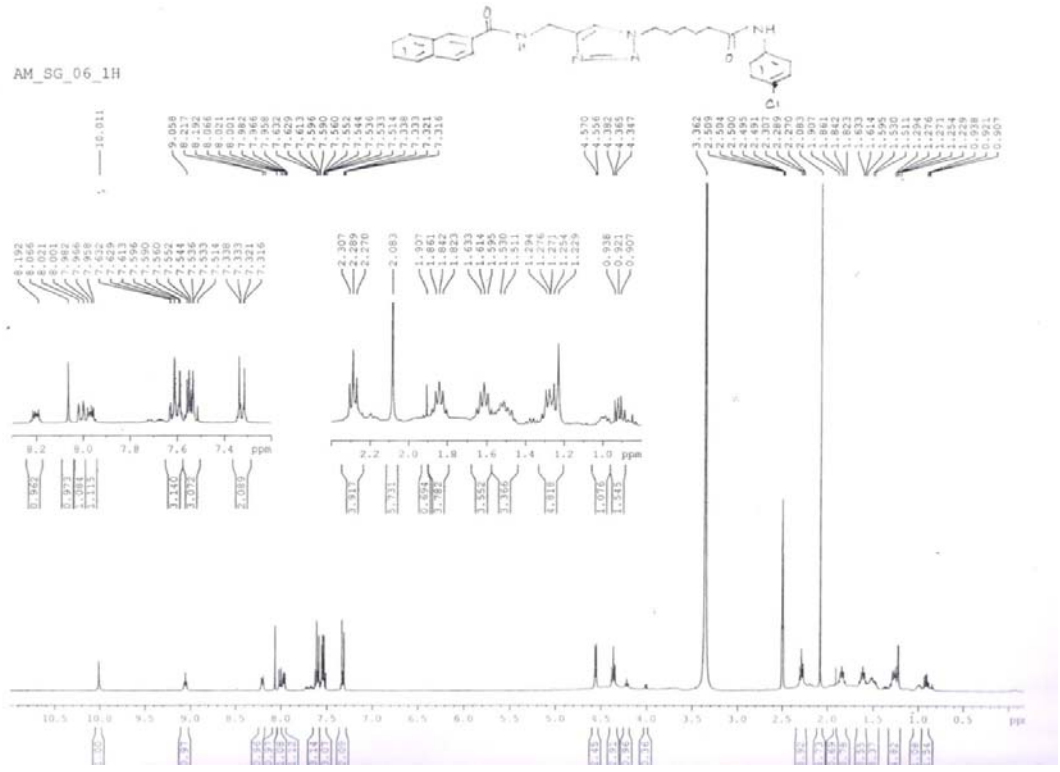


Figure S4. ^{1}H NMR spectra of compound **SSAM-1**

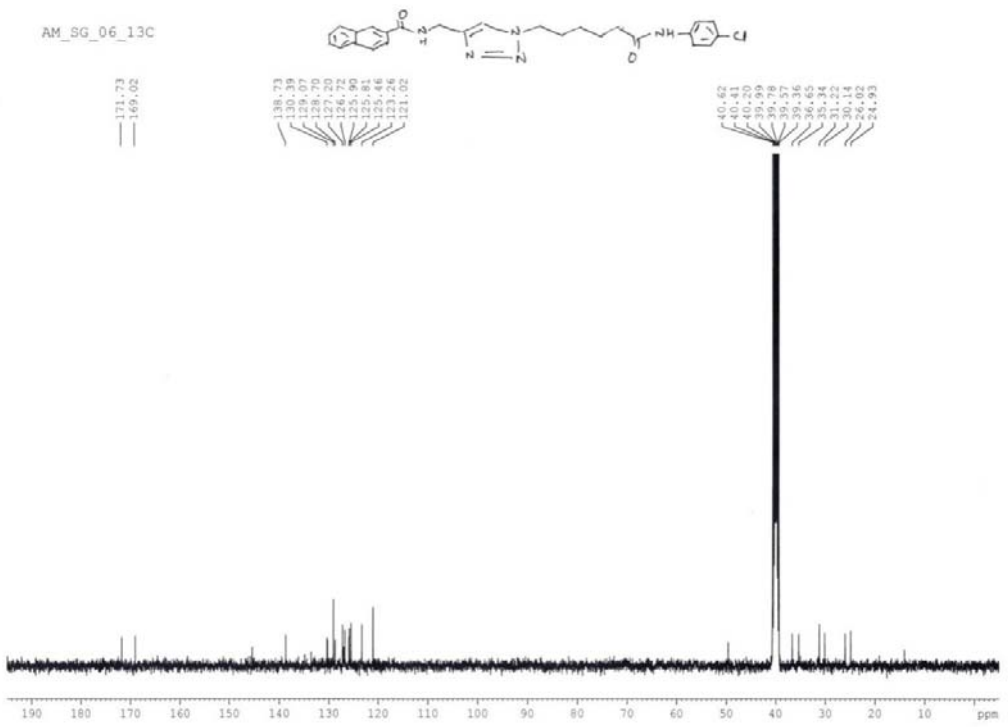
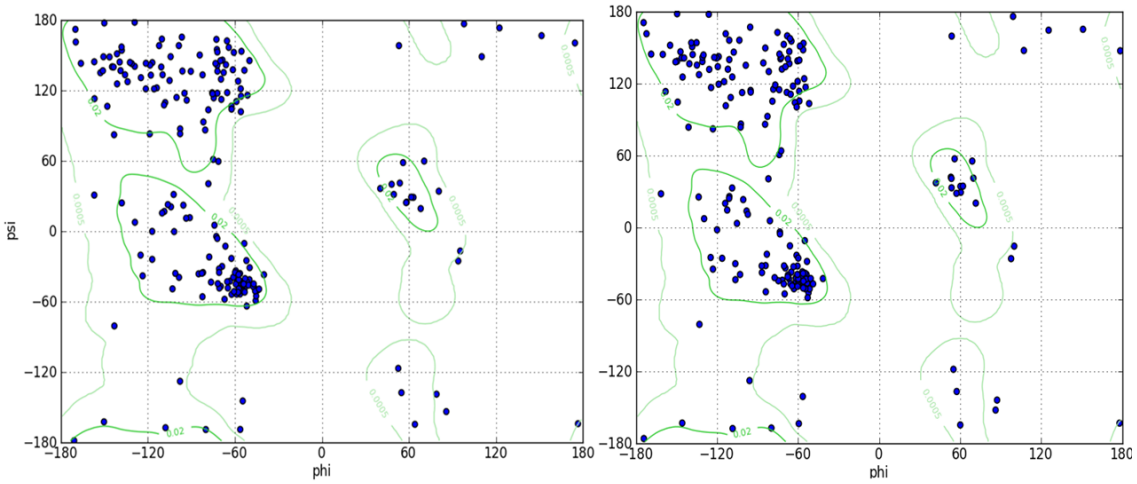


Figure S5. ^{13}C NMR spectra of compound **SSAM-1**



(a) 1W0Q - BMLN (b) 1W0Q – Minimized - BMLN

Figure S6. Ramachnadran Plot of Bromelain protein

Table S1: ADMET Prediction from pKCSM

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-4.88**	Numeric (log mol/L)
Absorption	Caco2 permeability	**0.47**	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	**89.921**	Numeric (% Absorbed)
Absorption	Skin Permeability	**-2.751**	Numeric (log Kp)
Absorption	P-glycoprotein substrate	**Yes**	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	**Yes**	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	**Yes**	Categorical (Yes/No)
Distribution	VDss (human)	**-0.154**	Numeric (log L/kg)
Distribution	Fraction unbound (human)	**0.019**	Numeric (Fu)
Distribution	BBB permeability	**-0.445**	Numeric (log BB)
Distribution	CNS permeability	**-2.227**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	**Yes**	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	**Yes**	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	**Yes**	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	**Yes**	Categorical (Yes/No)
Excretion	Total Clearance	**-0.23**	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.323**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**Yes**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.449**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**1.884**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**Yes**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	**0.351**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**-0.449**	Numeric (log mM)

SSAM-1 + BSA,HSA,BMLN interaction : UV absorption Spectroscopy

SSAM-1+ BSA

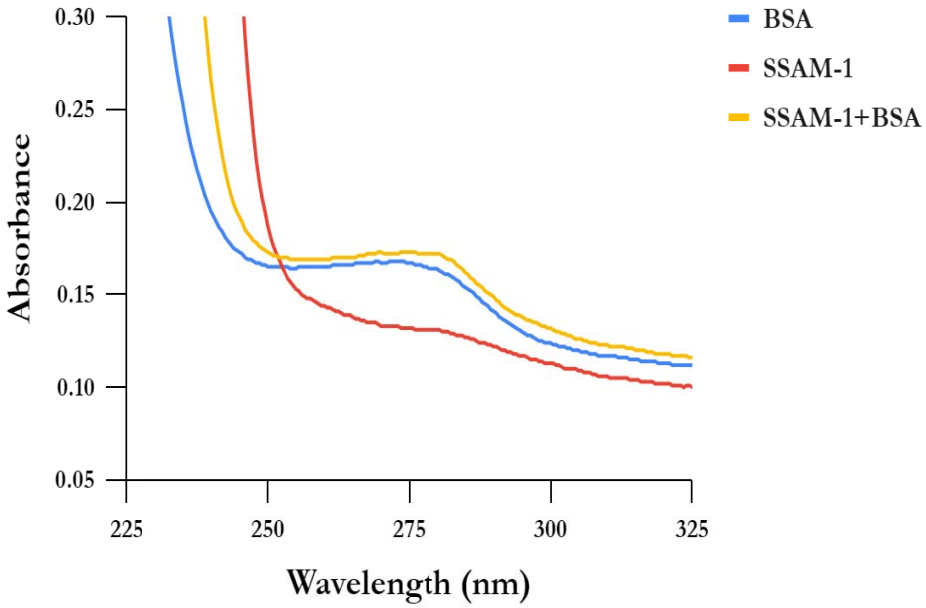


Figure S7. UV-Vis spectrum of SSAM-1-BSA system

SSAM-1+ HSA

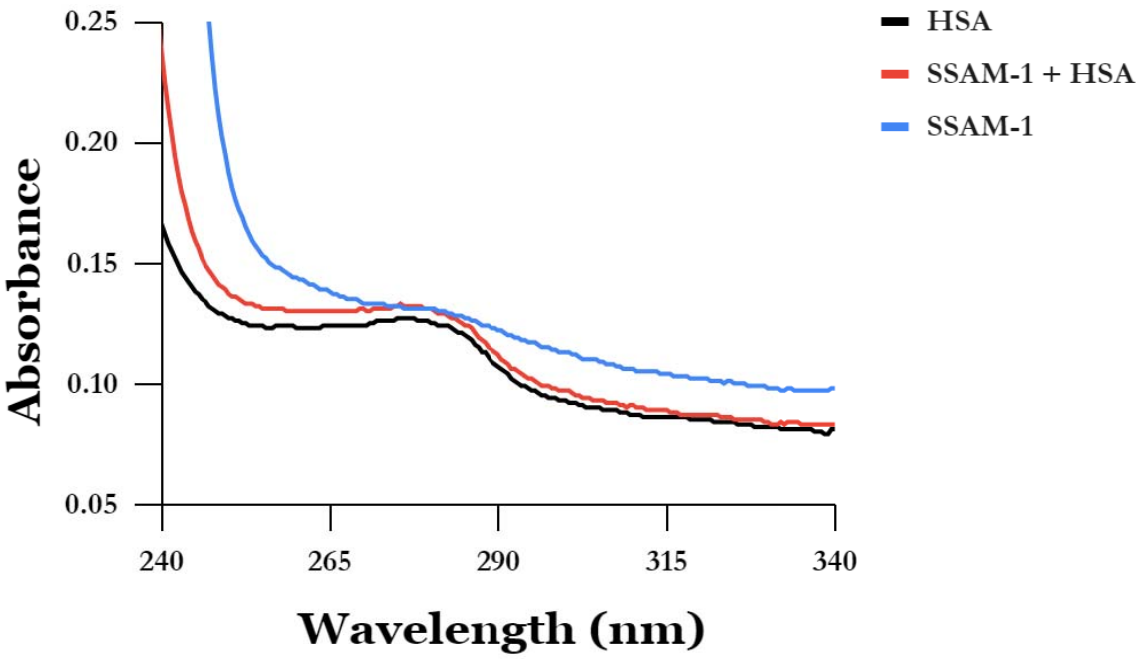


Figure S8. UV-Vis spectrum of SSAM-1-HSA system

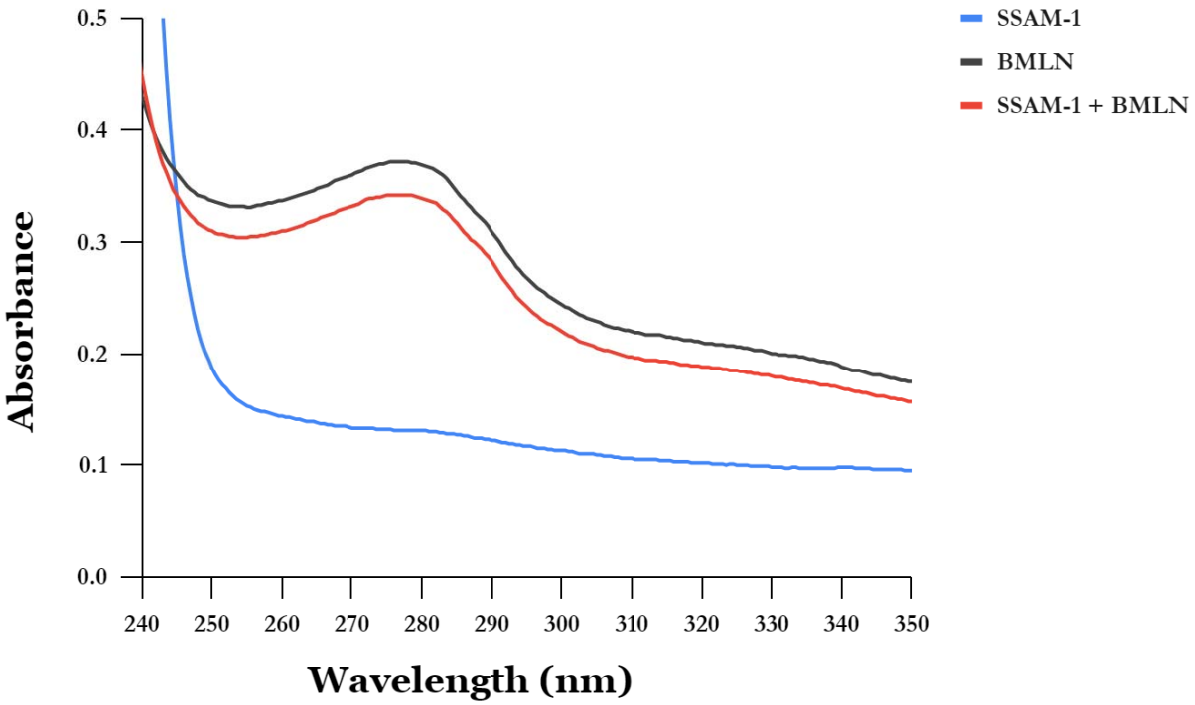


Figure S9. UV-Vis spectrum of SSAM-1-BMLN system

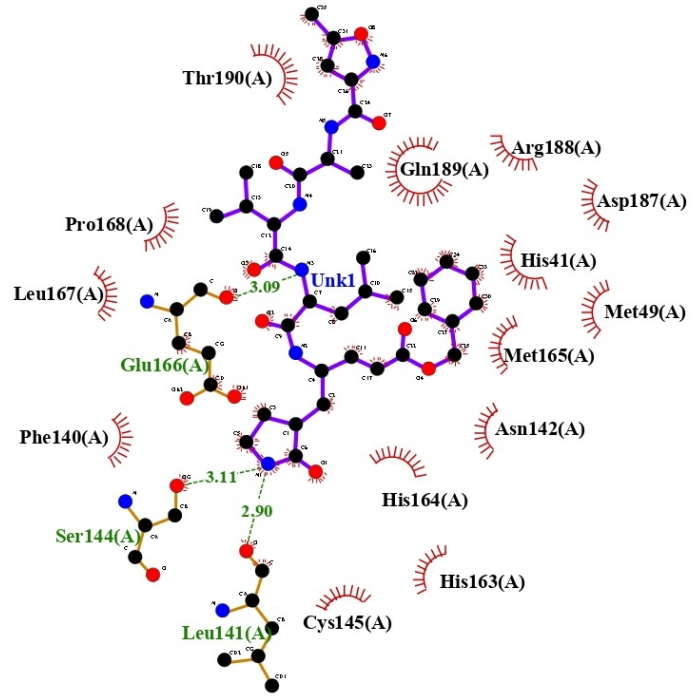


Figure S10. Docked structure of 6LU7 with N3 ligand

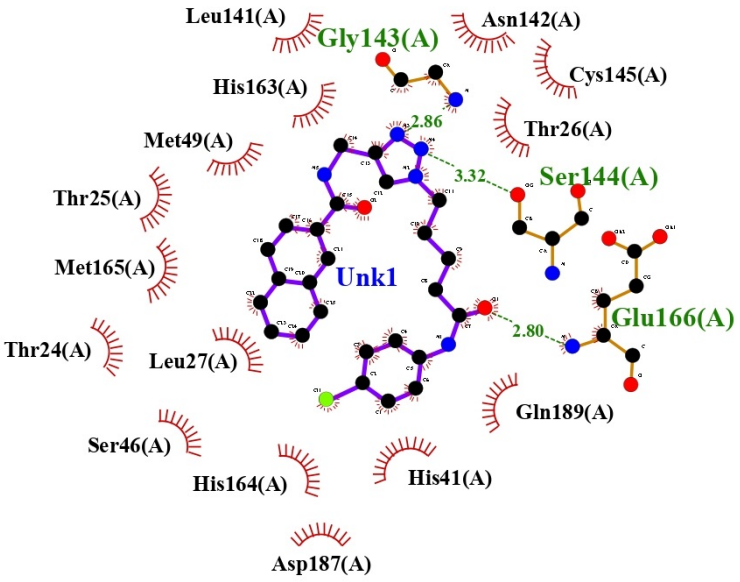


Figure S11. Binding region of SSAM-1 in 6LU7

Competitive binding study using Molecular docking (in BSA and HSA)

BSA has three binding sites which are reflected in three PDB IDs (4OR0, 6QS9 and 4JK4) and HSA has two binding sites which are reflected in two PDB IDs (2BXG and 2BXD). To understand the most probable binding sites of SSAM-1 in BSA and HSA we have conducted following in-silico analysis. The binding interaction of SSAM-1 with BSA in comparison with its bound ligands *viz*. Naproxen, Ketoprofen, 3,5-diiodosalicylic acid are given in Table S2, Table S3 and Table S4 at the respective ligand binding site. The binding interaction of SSAM-1 with HSA in comparison with its bound ligands such as Ibuprofen and Warfarin are given in Table S5 and Table S6 at the respective ligand binding site. Here we considered the chain B of the proteins BSA and HSA. The grid box for 4OR0 protein was taken as 73 × 30 × 92 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of - 11.6 Kcal/mol, grid box for 6QS9 protein was taken as -79 × 5 × 37 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of - 10.8 Kcal/mol, grid box for 4JK4 was taken as 37 × 23 × 44.98 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of - 10.4 Kcal/mol, grid box for 2BXG protein was taken as 45 × 35 × 72 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of - 10.4 Kcal/mol, grid box for 2BXD was taken as 42 × 36 × 52 Å with size 30 × 30 × 30 Å along x- , y- and z- axes and ΔG value of - 10.5 Kcal/mol.

Table S2: Results of the Docking of BSA with Naproxen and SSAM-1.

Compound Name	Protein Name	Docking Score (Kcal/mol)
		PDB-ID - 4OR0
Naproxen	BSA	- 8.7
SSAM-1	BSA	- 11.6

The docking poses shows the interaction of triazole region as it gets primarily involved in the formation of strong hydrogen bond and amide pi-stacking or pi-sigma bond respectively with protein 4OR0 (BSA) (Figure S12).

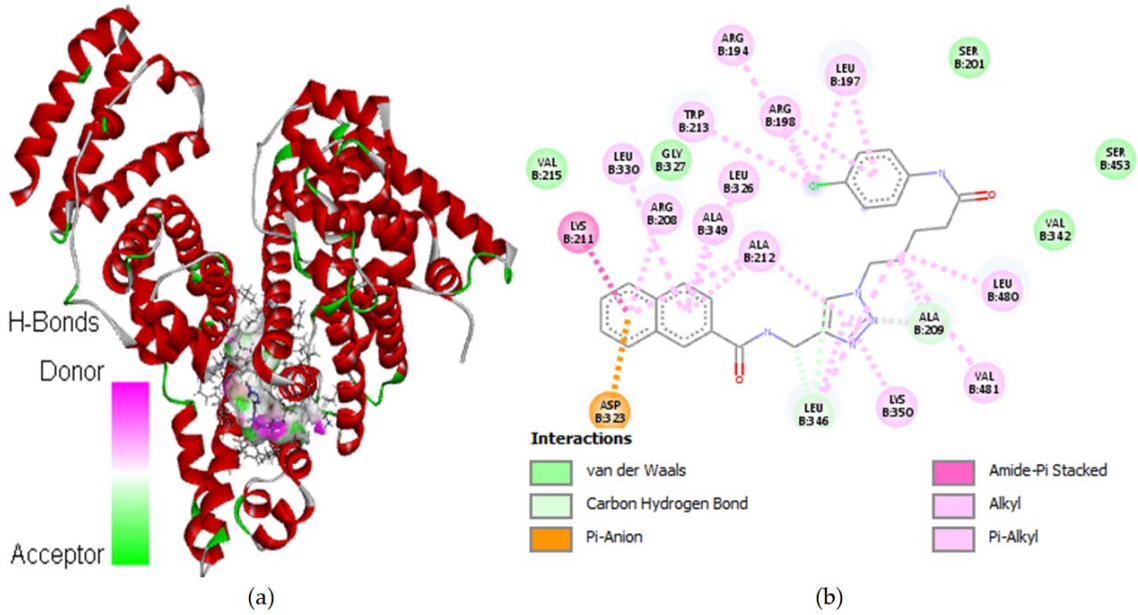
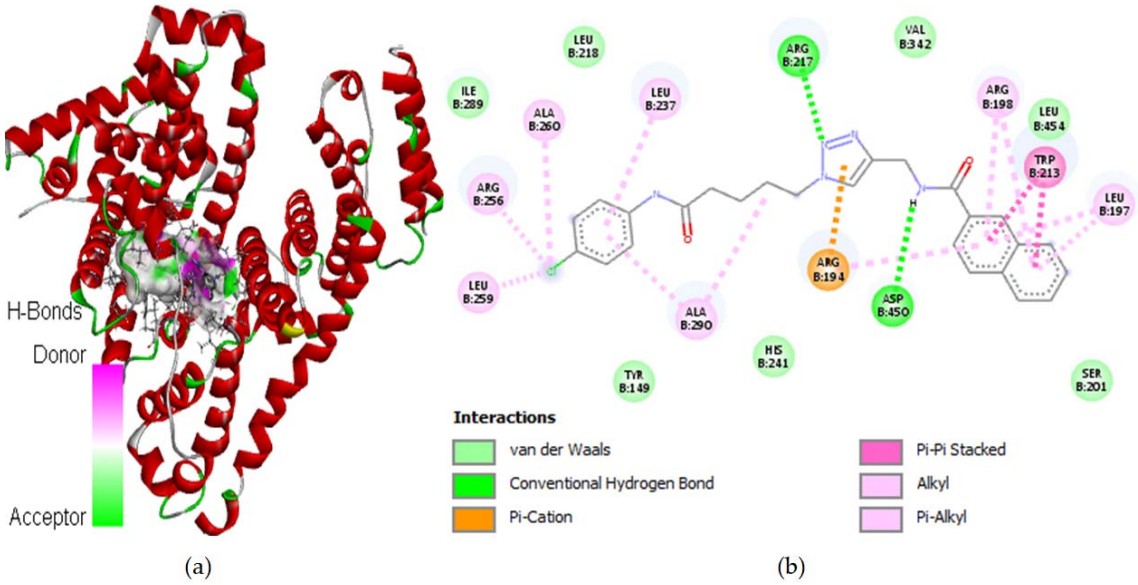


Figure S12: Docking Poses of SSAM-1 with 4OR0 (BSA).

Table S3: Results of the Docking of BSA with Ketoprofen and SSAM-1.

Compound Name	Protein Name	Docking Score (Kcal/mol)
		PDB-ID – 6QS9
Ketoprofen	BSA	- 8.4
SSAM-1	BSA	- 10.8

The docking poses upholds the importance of triazole region as it gets primarily involved in the formation of strong hydrogen bond and pi-cation bond respectively with protein 6QS9 (BSA) (Figure S13).



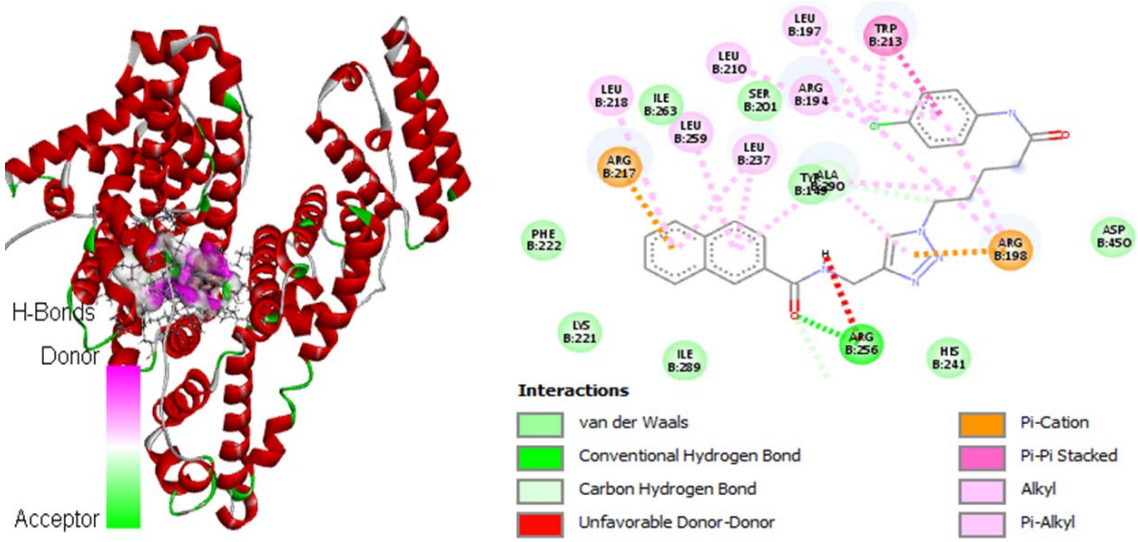
(a) (b)

Figure S13: Docking Poses of SSAM-1 with 6QS9(BSA).

Table S4: Results of the Docking of BSA with 3,5-diiodosalicylic acid and SSAM-1.

Compound Name	Protein Name	Docking Score (Kcal/mol)
		PDB-ID – 4JK4
3,5-diiodosalicylic acid	BSA	- 6.2
SSAM-1	BSA	- 10.4

The docking poses upholds the importance of triazole region as it gets primarily involved in the formation of vanderwaals bond and pi-cation bond respectively with protein 4JK4 (BSA) (Figure S14).



(a) (b)

Figure S14: Docking Poses of SSAM-1 with 4JK4(BSA).

Table S5: Results of the Docking of HSA with Ibuprofen and SSAM-1.

Compound Name	Protein Name	Docking Score (Kcal/mol)
		PDB-ID – 2BXG
Ibuprofen	HSA	- 7.1
SSAM-1	HSA	- 10.4

The docking poses upholds the importance of triazole region as it gets primarily involved in the formation of pi-pi stacking and pi-alkyl bond formation respectively with protein 2BXG (HSA) (Figure S15).

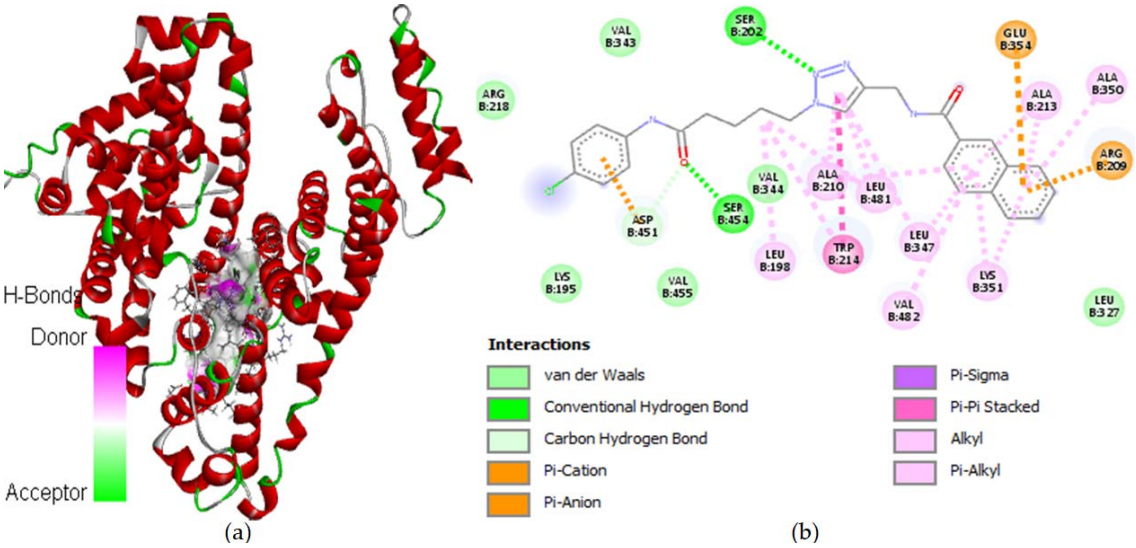


Figure S15: Docking Poses of SSAM-1 with 2BXG(HSA).

Table S6: Results of the Docking of HSA with Warfarin and SSAM-1.

Compound Name	Protein Name	Docking Score (Kcal/mol)
		PDB-ID – 2BXD
Warfarin	HSA	- 9.5
SSAM-1	HSA	- 10.5

The docking poses upholds the importance of triazole region as it gets primarily involved in the formation of either strong hydrogen bond or amide pi-stacking or pi-anion bond formation respectively with protein 2BXD (HSA) (Figure S16).

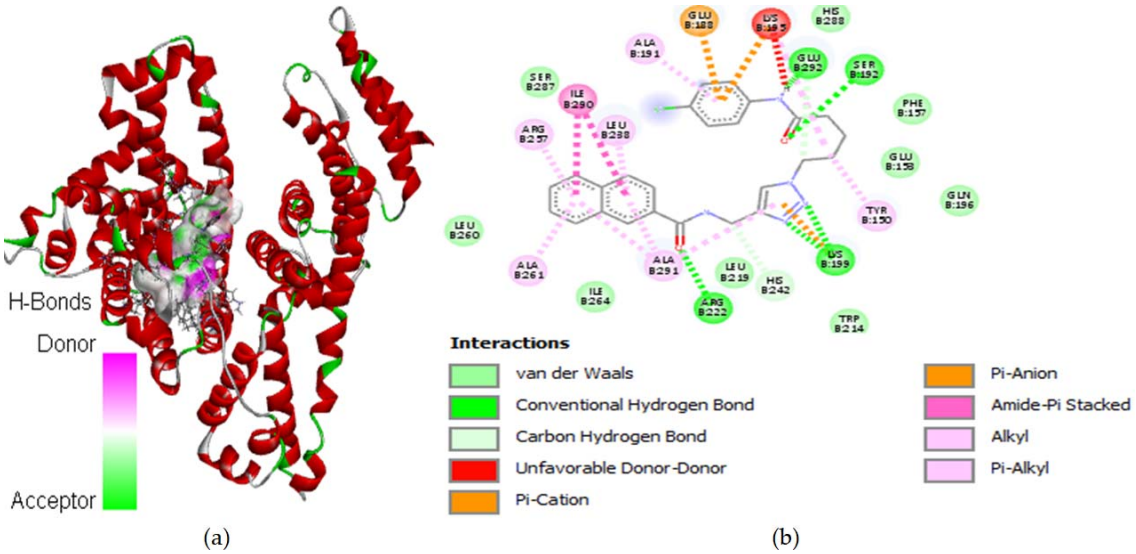


Figure S16: Docking Poses of SSAM-1 with 2BXD (HSA).

In all the docking analysis, it was found that the binding score of SSAM-1 is greater than all the bound ligands such as Naproxen (Table S2), Ketoprofen (Table S3), 3,5-diiodosalicylic acid (Table S4) and Ibuprofen (Table S5), Warfarin (Table S6).

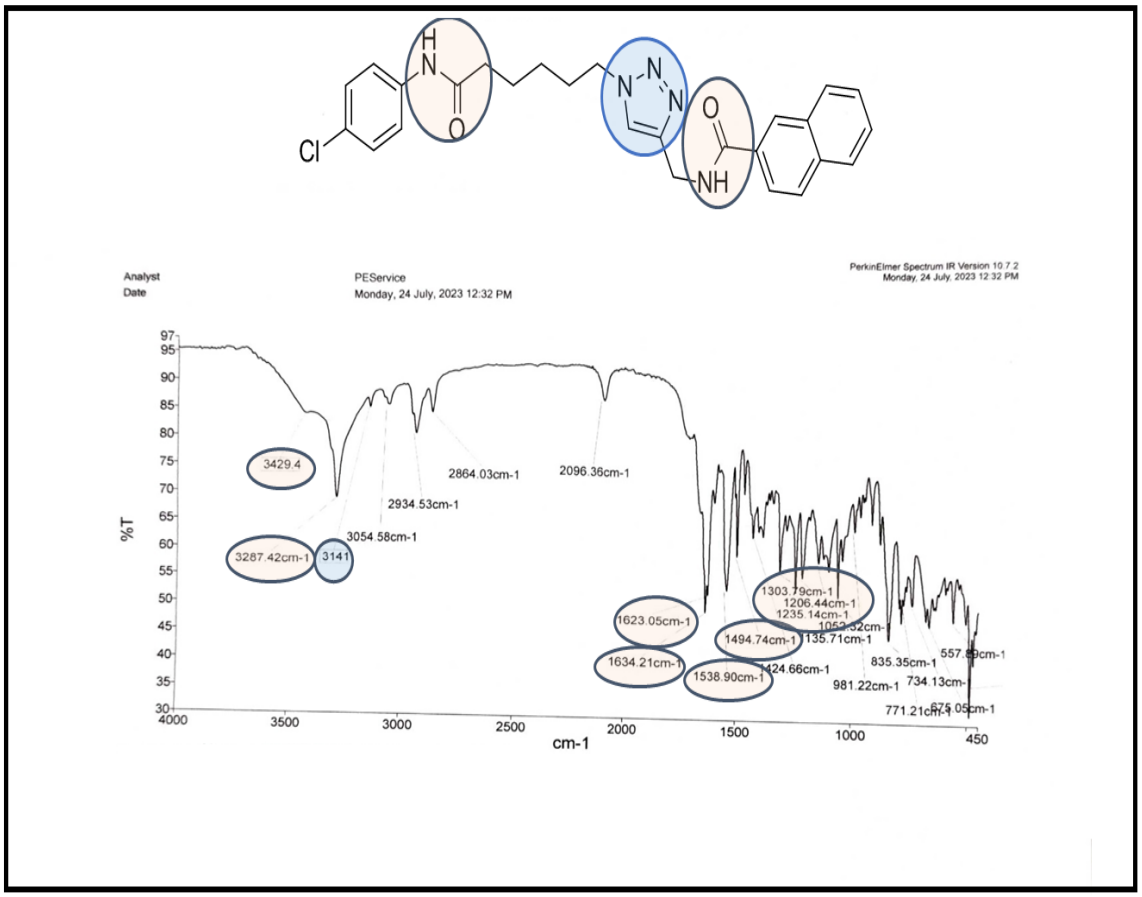


Figure S17: FT-IR spectrum of SSAM-1

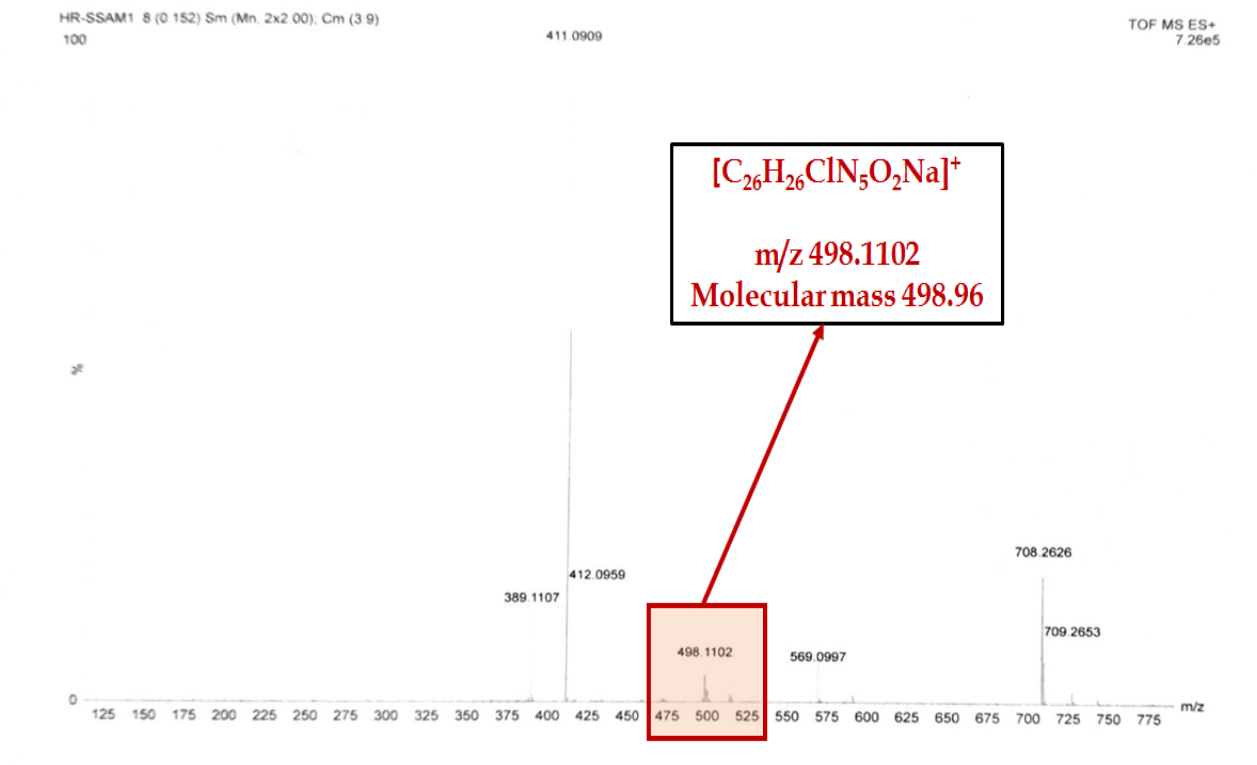


Figure S18: EI-MS spectrum of SSAM-1