Original Article

Nostocine A Derivatives as Human DNA Topoisomerase II-alpha Inhibitor

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ABSTRACT

Introduction: Due to heavy morbidity and mortality from cancer, the designing of newer drugable molecules against breast cancer is the call of day. As, Schiff-base sulfonamides have been widely used in tumor treatments. **Methods:** Nostocine-sulfonamide (NS) Schiff-base molecules were designed with tools of bioinformatics against the target enzyme, human topoisomerase II-alpha (topo IIa) against breast cancer. The designed NS conjugates were assessed by RO5, ADMET and molecular docking. **Results:** Herein, these analogues, NS-20b (Nostocine A-sulfaphenazole), 12a (Nostocine A-sulfisoxazole) and 16b (Nostocine A-sulfamethazineare) are *N*-heteroaryl substituted sulfonamide moieties linked with pyrazolo[4,3-*e*][1,2,4]triazine of Nostocine A. **Conclusion:** These derivatives would act as potent inhibitors of topo IIa for breast cancer.
Key words: Pyrazolotriazine, Nostocine A, Cyanobacterium, *Nostoc spongiforium*, Breast cancer, Docking.

INTRODUCTION

Bioactive algal compounds have been lent for the development of pharmaceutical cascades in treating several human diseases, particularly cancer chemotherapy. Nowadays, phycocompounds from blue-green algae (cyanobacteria) Norharmane, Lyngbyabellin, Dolastatin and a few more have been placed in mainstream medicines for cancer treatment.[1] Indeed, isolated from *Nostoc spongiforium*, Nostocine A (7-Methyl-2,7-dihydro-3H-pyrazolo[4,3-*e*][1,2,4]triazin-3-one) is the naturally occurring phycocompound, which could be a future drug candidate against cancer in due modification with suitable chemical entities. Furthermore, literature indicates that the scaffold Pyrazolo[4,3-e][1,2, 4]triazine is considered as privileged molecules for various biological activities. Those scaffold linked with substituted sulfonamide moiety at C-5 position, which have shown greater inhibition with tyrosine kinase and urease.[2,3]

Schiff based compounds are versatile building blockers and synthetic precursors for various organic heterocyclic compounds, which have azomethine–CH=N- linked in their structures, with manifestation of a broad range of biological activities *viz*., antibacterial, antifungal, anticancer, antiviral properties and a few more.[4] Analogues of the phytocompound, isatin conjugated with sulfonamides have significant activities on tumor associated carbonic anhydrase.[5] Moreover, sulfonamide derivatives are essential pharmacophore entities with inhibit carbonic anhydrase, tyrosine kinase, topoisomerase.[6] Concomitantly, topoisomerase II-alpha (topo IIa) is an important class of enzyme used as marker for breast cancer, which has linked with cell proliferation with the HER2/neu gene.[7] Additionally, doxorubicin and etoposide are important classes of chemotherapeutic agents targeted to topo IIa that mediated DNA damage.

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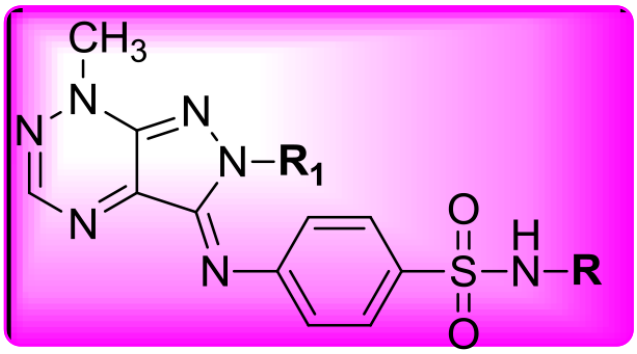


Figure 1: Designed Nostocine A analogues.

However, over and low expressions of cells lead to drug hypersensitive and resistance, respectively; in this regard, a drug-protein-DNA ternary complex would have enormous approaches for study of the drug-molecular interactions with topo IIa.[8] In the present study, forty NS (1a-20b) conjugates were virtually designed with Nostocine A (Figure 1); and those molecules were validated by Lipinski role (RO5), Adsorption, distributions, metabolism, excretion and toxicity (ADMET), Prediction of activity spectra for substances (PASS) prediction and molecular docking study with breast cancer associated protein, human DNA topoisomerase II-alpha ATPase/ADP (PDB:1ZXN). Indeed, conjugation of Nostocine A by linking with *N*-heteroaryl substituted sulfonamide entity through azomethine, which could develop as the future drug candidates against invasive breast cancer.

MATERIALS AND METHODS

Preparation of data set

Preliminarily, isolated Nostocine A molecule as a template was retrieved from online database PubChem. Concomitantly, a series of NS analogues were linked with the absolute sulfa drug congeners. Two-dimensional (2D) structures of the designed NS derivatives were drawn, allotted with proper 2D orientation with stereo optimization by ChemDraw Ultra-12. Furthermore, the designed congeners were optimized with ACD-Labs ChemSketch-2015 and energy minimization were executed by OpenBabel-2.4.

Drug-likeness and PASS prediction

The physicochemical properties of NS derivatives were predicted with the Lipinski's Rule of Five (RO5) by molinspiration software, Comprehensive Medicinal Chemistry (CMC) rule and World Drug Index (WDI). PASS predictions were evaluated with preloaded training data set and the obtained Probable Activity (Pa) and Probable Inactivity (Pi) value, individually.[9]

ADMET Validation

Pharmacokinetic and dynamic profiles of NS derivatives was examined with ADMET by PreADMET and cheminformatics web server with several statistical models – blood brain barrier (BBB), plasma protein binding, cell permeability, human intestinal absorption and Caco-2 cell permeability. Furthermore, toxicity assessment of each designed molecule was predicted with level and lethal doses$_{50}$ (LD_{50}) values by ProTox software. Thereafter, those compounds were passed above optimistic condition/refinery approaches subjected to molecular docking studies. Among all the designed NS derivatives, 40 compounds were used for the docking purpose.[10]

Molecular docking

The designed NS derivatives were used for virtual screening against the selected active site of the topo IIa and associated binding interactions. The crystal structure of topo IIa, 1ZXN (2.5 Å resolution) was retrieved from Protein Data Bank (www.rcsb.org/pdb) and deletion of non-protein molecules was done. Furthermore, the docking study was carried out by using AutoDock Tools 4.2. for ligand-receptor interaction with visualization by Discovery Studio R2 2017 and analyzed by PyMOL program.[11,12]

RESULTS AND DISCUSSION

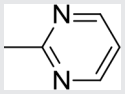
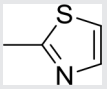
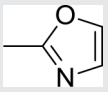
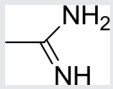
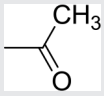
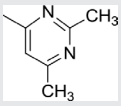
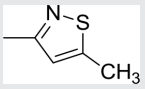
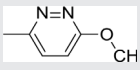
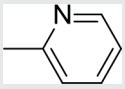
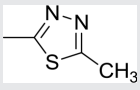
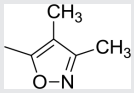
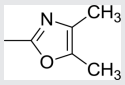
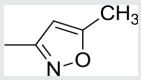
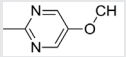
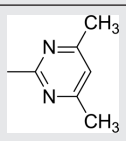
Drug likeness Properties

The prediction of drug like properties of all the desired compounds were carried out by RO5, these rules state that those compounds have been shown with physio-chemical properties, five different parameters viz. Molecular Weight (MW), Donor of H-bond number (HD), Acceptor of H-bond number (HA) and partition coefficient, the clog *P* value (octane/water). These NS congeners were assessed with RO5 for prediction of drug-likeness scores (Table 1). Indeed, all those molecules were significant for pharmacokinetics properties.

ADMET Profile

The NS analogues were examined with absorption permeability (blood-brain barrier, human intestinal absorption, P-glycoprotein substrate, P-glycoprotein inhibitorandrenalorganiccationtransporter);distribution (subcellular localization); metabolism (CYP450 substrates and inhibitors); toxicity (acute oral toxicity, AMES toxicity) and classes of toxicity parameters.

Table 1: Physiochemical parameters of NS analogues and docking scores.

Compound No.	Hybridization compounds	R	R1	Lipinski rule of five (RO5)					Docking score-PDBID (1zxn)
				MW (≤ 500 g/mol)	No. of H-ba (≤10)	No. of H-bd (≤5)	cLogP value (≤5)	tPSA (Å)	
NS1a	Nostocine A + Sulfadiazene		H	397	8	1	−0.22	108.37	−8.7
NS1b			CH_3	383	8	2	−0.6	117.56	−8.9
NS2a	Nostocine A + Sulfathiazole		H	402	8	1	0.52	98.28	−8.0
NS2b			CH_3	388	8	2	0.58	107.47	−8.4
NS3a	Nostocine A + Sulfaoxazole		H	386	8	1	−0.49	106.92	−8.1
NS3b			CH_3	372	8	2	−0.43	116.11	−8.6
NS4a	Nostocine A + Sulfoguanidine		H	361	7	4	−1.71	127.72	−8.7
NS4b			CH_3	347	7	5	−1.65	136.91	−8.7
NS5a	Nostocine A + Sulfacetamide		H	361	7	1	−0.94	102.15	−9.0
NS5b			CH_3	347	7	2	−0.88	111.34	−9.0
NS6a	Nostocine A + Sulfisomidine		H	425	8	1	0.25	106.36	−8.6
NS6b			CH_3	411	8	2	0.30	115.55	−8.9
NS7a	Nostocine A + Sulfasomizole		H	416	8	1	0.69	98.86	−9.1
NS7b			CH_3	402	8	2	0.75	108.05	−9.1
NS8a	Nostocine A + Sulfamethoxypyridazine		H	427	9	1	−0.26	117.55	−8.6
NS8b			CH_3	413	9	2	−0.20	126.74	−9.5
NS9a	Nostocine A + Sulfapyridine		H	396	7	1	0.34	97.41	−8.5
NS9b			CH_3	382	7	2	0.39	106.60	−8.7
NS10a	Nostocine A + Sulfanilamide	H	H	319	7	2	−0.78	97.75	−7.7
NS10b			CH_3	305	7	3	−0.73	106.94	−7.7
NS11a	Nostocine A + Sulamethizole		H	417	9	1	0.10	111.15	−8.0
NS11b			CH_3	403	9	2	0.16	120.34	−9.3
NS12a	Nostocine A + Sulfisoxazole		H	414	8	1	0.03	110.46	−9.9
NS12b			CH_3	400	8	2	0.09	119.65	−9.5
NS13a	Nostocine A + Sulfamoxole		H	414	8	1	0.320	106.82	−9.4
NS13b			CH_3	400	8	2	0.26	116.00	−9.4
NS14a	Nostocine A + Sulfamethoxazole		H	400	8	1	0.29	109.59	−8.9
NS14b			CH_3	386	8	2	0.35	118.78	−9.1
NS15a	Nostocine A + Sulfamethoxydiazine		H	427	9	1	−0.13	116.09	−8.7
NS15b			CH_3	413	9	2	−0.07	125.28	−9.5
NS16a	Nostocine A + Sulfamethazine		H	425	8	1	0.54	107.12	−9.5
NS16b			CH_3	411	8	2	0.60	116.31	−9.9

Continued...

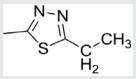
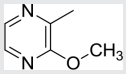
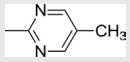
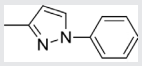
Table 1: Cont'd.									
Compound No.	Hybridization compounds	R	R1	Lipinski rule of five (RO5)					Docking score-PDBID (1zxn)
				MW (≤ 500 g/mol)	No. of H-ba (≤10)	No. of H-bd (≤5)	cLogP value (≤5)	tPSA (Å)	
NS17a	Nostocine A + Sulfaethidol		H	431	9	1	0.60	111.84	−9.6
NS17b			CH_3	417	9	2	0.66	121.03	−9.3
NS18a	Nostocine A + Sulfalene		H	427	9	1	−0.48	114.84	−8.5
NS18b			CH_3	413	9	2	−0.42	124.02	−9.0
NS19a	Nostocine A + Sulfaperine		H	411	8	1	0.18	108.55	−9.6
NS19b			CH_3	397	8	2	0.24	117.74	−9.2
NS20a	Nostocine A + Sulfaphenazole		H	461	7	1	1.14	102.61	−9.3
NS20b			CH_3	447	7	2	1.20	111.80	−10.2

Table S1: Absorption, distribution, metabolism and excretion predicted properties of NS analogues.				
ADMET		20b	12a	16b
Absorption				
Blood-Brain Barrier	BBB+	0.8282	0.7576	0.8021
Human Intestinal Absorption	HIA+	1	0.9876	0.9952
Caco-2 Permeability	Caco2-	0.535	0.5542	0.5454
P-glycoprotein Substrate	Non-substrate	0.795	0.8279	0.7825
P-glycoprotein Inhibitor	Non-inhibitor	0.7344	0.7311	0.6737
	Inhibitor	0.7594	0.5803	0.7118
Renal Organic Cation Transporter	Non-inhibitor	0.7557	0.8232	0.7375
Distribution				
Subcellular localization	Mitochondria	0.3607	0.3540	0.3439
Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.5823	0.5762	0.6514
CYP450 2D6 Substrate	Non-substrate	0.8433	0.8301	0.8441
CYP450 3A4 Substrate	Non-substrate	0.6169	0.5938	0.6043
CYP450 1A2 Inhibitor	Inhibitor	0.6462	0.8463	0.5185
CYP450 2C9 Inhibitor	Inhibitor	0.5308	0.5835	0.5516
CYP450 2D6 Inhibitor	Non-inhibitor	0.7412	0.8755	0.8769
CYP450 2C19 Inhibitor	Non-inhibitor	0.5371	0.8338	0.7078
CYP450 3A4 Inhibitor	Non-inhibitor	0.5389	0.8098	0.5268
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	0.5258	0.6265	0.7088
Excretion				
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9028	0.9583	0.8661
	Non-inhibitor	0.732	0.8465	0.7136
AMES Toxicity	Non AMES toxic	0.7193	0.7233	0.7146
Carcinogens	Non-carcinogens	0.7822	0.6868	0.7972
Fish Toxicity	High FHMT	0.9433	0.7953	0.7714
Tetrahymena Pyriformis Toxicity	High TPT	0.8786	0.795	0.8677
Honey Bee Toxicity	Low HBT	0.8295	0.8346	0.7918
Biodegradation	Not ready biodegradable	1	0.9901	1
Acute Oral Toxicity	III	0.6811	0.5949	0.6748
Carcinogenicity (Three-class)	Non-required	0.5879	0.6071	0.5353

Table S2: Molecular docking interaction of NS analogues with human DNA topoisomerase II-alpha.		
Compounds	**Conventional Hydrogen bonding interaction with amino acids**	**Hydrophobic interaction with active amino acids**
12a	SER:121,TYR:6	PHE:114,ARG:70, ILE:5, ILE:97,ALA:136, ILE:113, ASN:63
`16a	ARG:70,ASN:122,TYR:6	PHE:114, ALA 136, ILE:113, SER:121, ARG:7O, ILE:5, PRO:98
17a	GLY:135,ASN: 63, LYS:137, ILE:113	PHE:114,SER:120,ALA:136,TYR:134,ARG:70
19a	THR:184.	ILE:113, ASN:63,ILE:97, ALA:136, ARG:70, ASN:122,GLY:135,TYR:134 .
8b	SER:121,ASN:122,SER:120	ARG:70,THR:128,LYS:124
12b	TYR:134,GLY:135,TYR:06,ASN:67,SER:121	ILE:113,ILE:97,ASN:122,ARG:70,ALA:136.
15b	GLY:135,TYR:134,GLY:133, SER:120,ASN:122	ILE:113,ASN:63,ASN:132,LYS:126, ARG:70SER:121.
16b	ARG:70,ASN:63	THR:128,LYS:126,ARG:70,ILE:113,TYR:6,SER:121.
20b	SER:121,THR:184,ASN:92,ASP:66	ILE:113,PHE:114,ARG:70,

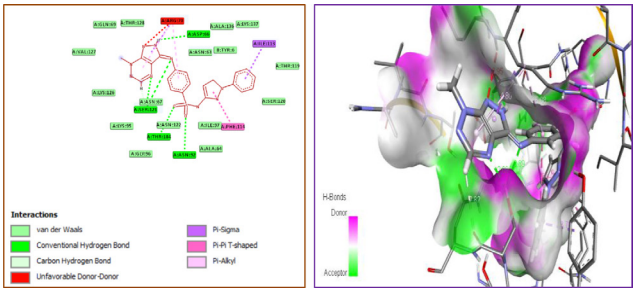


Figure 2: Molecular interaction of NS20b analogue with human topoisomerase II-alpha (PDBID:1ZXN).

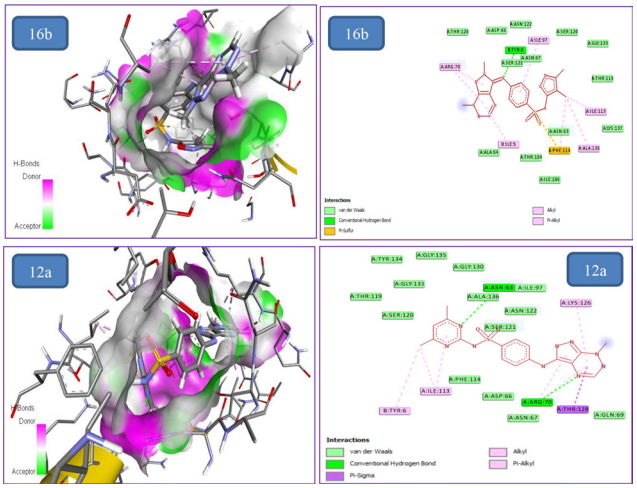


Figure S1: Molecular docking interaction of NS analogues 16b and 12a of with human DNA topoisomerase II-alpha.

These designed analogues were passed with due substructure pattern recognition (Table S1).

Molecular docking

In general, *in-silico* investigation was carried out to identify the potentiality and inhibitory action of the designed molecules against the breast-cancer associated enzyme human DNA topoisomerase II-alpha ATPase/ ADP. All these NS congeners were docked with cancer associated enzyme topo IIa (PDBID: 1ZXN). The compound NS20b had the lowest ΔG_{bind} energy -10.2 kcal/mol (Figure 2). Whereas the compounds NS10a and 10b have the highest energy values -7.7 kcal/mol (Table 1). The compound NS20b had interacted with SER:121, THR:184, ASN:92, ASP:66 by H-bonding of the active site of topo IIa; whereas ILE:113; and PHE:114 amino acids interacted with hydrophobic π-σ of phenyl and π-π pyrazole ring (Table S2; Figure S1).

CONCLUSION

In this present study, among all the Nostocine A analogues, compound NS20b, NS12a and NS 16b, were the effective-most bioactive congeners against topo IIa, which was assessed with drug likeness, ADMET and molecular docking interactions. Thus it concluded that, the structural modification of the designed natural compound analogues were potential inhibitors counter to breast cancer.

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CONFLICT OF INTEREST

No author declared any conflict of interest.

ABBREVIATIONS

NS: Nostocine-sulfonamide; **topo IIa:** topoisomerase II-alpha; **RO5:** Lipinski rule of five; **ADMET:** Adsorption, distributions, metabolism, excretion and toxicity; **PDB:** protein data bank; **LD_{50}:** lethal $doses_{50}$.

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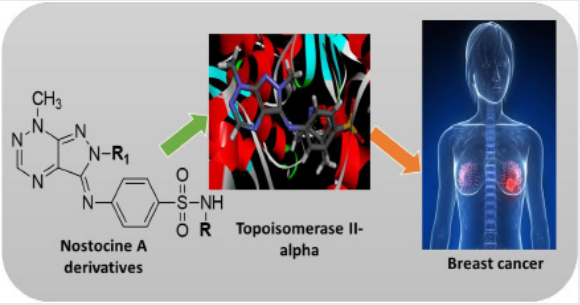
PICTORIAL ABSTRACT



SUMMARY

The designing of newer drug able molecules against breast cancer is described with a cyano-compound Nostocine A with Schiff-base condensing sulfonamides, which were designed with several advanced tools of bioinformatics against the target enzyme against breast cancer, the human topoisomerase II-alpha. Nostocine-sulfonamides (NS) were assessed by RO5, ADMET and molecular docking. The Nostocine A analogue compounds, NS-20b (Nostocine A-sulfaphenazole), 12a (Nostocine A-sulfisoxazole) and 16b (Nostocine A-sulfamethazineare) were the effective-most bioactive congeners against topo IIa. Thus, the structural modifications of the designed Nostocine A analogues were potential inhibitors against breast cancer.

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