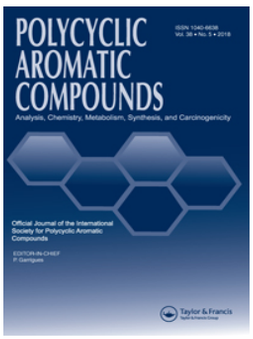

Polycyclic Aromatic Compounds

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Low Molecular Weight Non-Peptidyl Antimalarial Leads: Lichen Metabolite, Usnic Acid and Its Analogues

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ABSTRACT

The search for newer antimicrobial agents is a developing and continuing process. With an aim to discover new antimalarial leads from natural sources, a unique lichen-derived metabolite namely, usnic acid (USA) and their analogues were screened against a chloroquine-sensitive *Plasmodium falciparum* 3D7 line using an *in vitro* parasite growth inhibition assay. A bio guided fractionation of USA, the molecule of interest, derived from the ether extract of the lichen, *Usnea undulata*, reported to exhibit significant protease enzyme inhibitory activity, was used as a natural product scaffold and was chemically modified to yield three hydrazine, viz usnic acid anhydro phenyl hydrazone (S1), usnic acid anhydro 4-nitro phenyl hydrazone (S2), usnic acid anhydro 2,4-dinitro phenyl hydrazone (S3) and two amino compounds namely usnic acid anhydro o-phenylene diamine derivative (S5) and usnic acid anhydro piperazine derivative (S6) respectively. These low molecular weight non-peptidyl newer chemical entities (USA, S1, S2, S3, S5 and S6) were tested *in vitro* against the malaria parasite, *Plasmodium falciparum* and among all, the hydrazine moiety, usnic acid anhydro 4-nitro phenyl hydrazone (S2) was shown to the most active compound with an IC_{50} of $1.4{\cdot}10^{-5}$ M when compared to the parent pharmacophore, USA with an IC_{50} of $6.5{\cdot}10^{-5}$ M. The results reveal that lichen metabolite; USA offers a large scaffold for combinatorial outputs in developing lead candidates with potent antimalarial activity.

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Antimalarial; parasite growth inhibition assay; semisynthetic derivatives; usnic acid

1. Introduction

Malaria, an endemic disease killing 1–2 million people annually[1] has attained more interest worldwide due to the alarming resistance of *Plasmodium* to existing therapies. Of the four different types of malaria, *P. falciparum* is the most dreadful of all which is responsible for 95% of malaria related deaths. Among key potential drug target proteolytic systems in the malaria parasite *P. falciparum* are plasmepsins particularly plasmepsin II, a family of hemoglobin-degrading aspartyl proteases, which has fundamental importance in cellular protein turnover. Inhibition of plasmepsin II blocks parasite growth, primarily due to inhibition of hemoglobin degradation that

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serves as a source of amino acids for parasite growth. Recent studies have provided a lot of emphasis on these plasmepsin II aspartyl proteases of *P. falciparum*[2] as potential chemotherapeutic targets in the treatment of malaria. The compounds that have been investigated so far are large molecular weight peptidyl compounds which not only possess poor pharmacokinetics but also undergo degradation *in vivo*.

In our continuous efforts in searching for small molecular weight non-peptidyl novel antimalarial leads, the acylphloroglucinols were found to be effective, reported to possess putative antiplasmodial activity.[3,4] In this regard, we have taken up usnic acid (USA), a dibenzo-furandione acylphloroglucinol reported to exhibit weak antimalarial activity.[5] The unique feature of this molecule is that it is both enantiomeric forms have equipotent biological potency. The molecule is a biosynthetic product characteristic of several epiphytic lichens such as *Usnea, Evernia, Cladonia.*[6] Its antibacterial[7] and antimycotic properties as well as its antimitotic properties, activity against human neoplastic cell lines[8–10] are well known and recently USA has also been reported to exhibit antiviral activity.[11]

Relatively, small inhibitors that display non-peptide functionality about an appropriate isostere have been the focus of efforts to improve cell permeability and pharmacokinetic properties.[12] Recent studies have clearly thrown a large volume of evidence that targeting a specific enzyme of pathogenic bacteria and parasites is a rationale and highly effective parameter in drug design.[13,14] In this regard, initial experiments with cysteine protease non-peptidyl inhibitors have been performed with promising results.[15] Based on the above results, the molecule of interest, USA exhibiting significant trypsin inhibitory activity was further subjected to molecular docking simulations wherein, we attempted combinatorial chemistry and structure-based designed methods to develop novel scaffolds in the process of newer chemical entities (NCE) proposed to exhibit profound antimalarial activity.

Modification of chemical structure of natural product is vital, often preferred route in the design of new drugs. Several workers have attempted preparation of some semisynthetic derivatives of USA,[16,17] enabling to understand the stability and type of substitution possible with USA. However, there were very few reports on the biological and pharmacological evaluation of USA as antimalarial candidates.

Thus, the present focus of our work is to isolate the molecule of interest, USA from lichens and screen for enzyme inhibitory activity, and then, to prepare a few semisynthetic derivatives of USA (through molecular docking simulations), proposed to be useful in the malaria treatment. USA and the designed derivatives were then subjected to a preliminary *in vitro* parasite growth inhibition assay for testing the antimalarial activity.[18–20]

2. Experimental

2.1. Materials and methods

Lichen specimens were collected from Mahendragiri hills, Kanyakumari. The sample was dried at room temperature for 48 h. Dr. D. K. Upreti, Lichen Laboratory, National Botanical Research Institute (CSIR), Lucknow, UP, authenticated them as *Usnea undulata* Stirton (Authentication No: NBRI/LICH/DH-2004-75).

2.2. General experimental procedures

2.2.1. Spectroscopic parameters

IR spectra were recorded on a Thermo Nicolet Nexus 670 FTIR spectrometer using DTGS KBr.1H NMR spectra was obtained on Bruker AM- 300 instruments; MS analysis was taken on a JEOL JMS-HX300 mass spectrometer. The measurement of melting points was performed with

Yanaco MP-S3 micro melting point apparatus (Yanagimoto, Kyoto, Japan). Ultraviolet absorption spectra of the purified active fractions were recorded on a Lambda 25 spectrophotometer (Perkin Elmer) in methanol. Percentage purity was analyzed using HPLC (Shimadzu, Kyoto, Japan) system consisting of two LC-20AD pumps, SPDM20A diode array detector, SIL-20AC auto sampler, DGU-20A3 degasser and CBM-20A system controller. HPLC was performed using a reverse phase Inertsil ODS-3V (250 × 4.6 mm, 5 µm) column in a mobile phase (freshly prepared, filtered through a Millipore filter of pore size 0.45 µm and sonicated for 15 min) consisting of a mixture of water and acetonitrile in 1:1 ratio under isocratic mode of elution with sample injection volume of 20 µL at a flow rate of 1 mL/min for 30 min at 30 °C and detected at 254 nm. Thin-layer chromatography was performed on silica gel 60 F 254 TLC plates (Merck), using hexane: ethyl acetate (1:1 ratio) as mobile phase with compounds visualized by spraying with 10% (v/v) H_2SO_4 in an ethanol solution. Silica gel (100–200 mesh) was used for column chromatography. All solvents used for chromatographic isolation were of analytical grade and purchased from Merck.

2.3. Extraction, isolation and identification of the bioactive compound, USA

Lichen specimen Samples (∼1000 g) by wet weight were collected, washed and air dried and stored at 4 °C until used. A day before extraction, they were brought to complete dryness, by heating at 80 °C for 24 h and manually grinded to a fine powder. The dried material in small quantities was dissolved in a smaller volume (5–10 mL) of solvent evaporated and to the same little more quantity was dissolved and re-evaporated; the resulting crude material recovered as a powder and weighed to calculate the mass yield (mg g^{-1}).

Extraction was carried out as per the standard protocols by successive solvent extraction techniques by gradient elution method and three extracts viz. ether (SU-I), dichloromethane (SU-II) and acetone extracts (SU-III) were collected, checked with T.L.C. and accordingly pooled, concentrated and processed further.

2.4. Search for low molecular weight non-peptidyl enzyme inhibitors by trypsin assay

The three lichen extracts (SU-I, SU-II and SU-III) were screened for preliminary trypsin inhibitory activity.[15] Based on the results obtained from the above study, the most promising ether extract showing high inhibitory activity was processed further to isolate the bioactive constituent, USA[21,22] by column chromatography, purified and identified by physiochemical[23,24] and spectral properties.[25]

2.5. Molecular docking studies

USA and the designed analogues were subjected to molecular docking simulations using glide module v5.5 (Schrodinger suite 2009) against aspartic proteinase plasmepsin II - PL II (*P. falciparum*) and dihydro folate reductase-DHFR (*P. falciparum, P. vivax)*, in order to identify the binding mode, binding energy and hydrogen bond interactions of ligands with respect to the PL II and DHFR proteins. The docking results of the lichen metabolite, USA and its analogues were reported in our previous study.[26]

2.6. Preparation, purification and characterization of the semisynthetic derivatives of USA

The parent compound USA was chemically modified based on the rational drug design and structural design, to yield three hydrazone[27] namely, usnic acid anhydro phenyl hydrazone (S1), usnic acid anhydro 4-nitro phenyl hydrazine (S2) and usnic acid anhydro 2, 4- dinitro phenyl

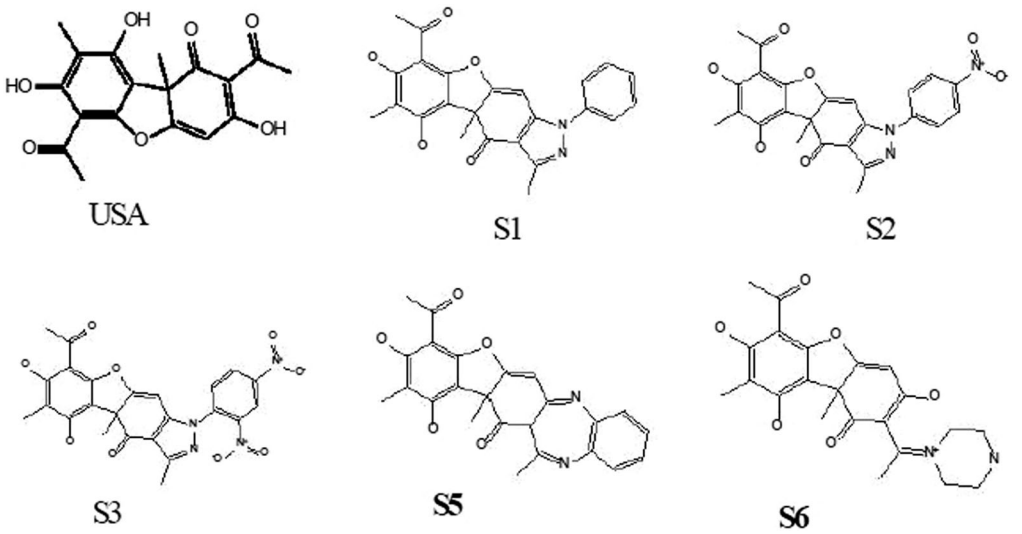

Figure 1. Chemical structure of usnic acid and its derivatives.

hydrazone (S3) and two amino[28,29] viz. usnic acid anhydro o-phenylene derivative (S5) and usnic acid anhydro piperazine derivative (S6). The chemical structure of the compounds is shown in Figure 1. The compounds SUA, S1, S2, S3, S5 and S6 were checked for purity by HPLC[30] and characterized by physical, chemical and spectral properties.

2.7. Antimalarial screening of USA and its derivatives

2.7.1. Materials

The *P. falciparum* 3D7 strain was obtained from the Malaria Research and Reference Reagent Resource Center (MR4). All other biochemical reagents were from Sigma or Serva. Plasmid isolation kits were from Qiagen or MACHEREY-NAGEL; cell culture reagents were from Lonza and Invitrogen; restriction and DNA modifying enzymes were from New England Biolabs; and SYBR Green 1 was from Invitrogen.

2.7.2. Parasite culture

In vitro parasite culture was done according to the protocols approved by the Institutional Biosafety Committees of CCMB and UCSF. *P. falciparum* was cultured in human erythrocyte sat 2% hematocrit in the presence of a gas mixture (5% CO_2, 5% O_2 and 90% N_2) in RPMI 1640 medium supplemented with 41.1 mg/L hypoxanthine, 300 mg/L glutamine, 2.5% human serum and 0.5% Albumax II.[31] Synchrony was maintained by serial treatment with 5% D-sorbitol.[32] For parasite isolation, the culture was centrifuged at 894 g for 5 min, the supernatant was aspirated off, the pellet was treated with ice-cold 0.05% saponin (in PBS) for 5 min to lyse erythrocytes and the sample was centrifuged at 12,096 g for 5 min at 4 °C. The supernatant was discarded, the pellet was washed twice with ice-cold PBS to remove residual hemoglobin and parasites were recovered by centrifugation at 12,096 g for 5 min at 4 °C. Genomic DNA (gDNA) was isolated from late trophozoite/schizont stage parasites using the Puregene Blood Core Kit B (Qiagen).

2.7.3. Parasite growth inhibition assays

USA, the molecule of interest and its semisynthetic derivatives were assessed for inhibition of *P. falciparum* erythrocytic stage development by parasite growth inhibition assays according to the

method adopted by Smilkstein et al.[33] The fluorescence of chloroquine treated culture was subtracted from inhibitor-treated and DMSO-containing cultures to account for background fluorescence. Fluorescence values of inhibitor-treated cultures were normalized as percentage of DMSO-treated cultures plotted against inhibitor concentrations and analyzed using nonlinear regression analysis to determine IC_{50} concentrations for the inhibitors.[34]

3. Results and discussion

The present investigation relates to dibenzofuran derivatives useful as antimalarial agents against the dreadful parasite of interest, *P. falciparum*. A large number of antimalarial compounds of natural, chemical or biotechnological origin currently on the market fail during the treatment due to the onset of resistance and/or toxicity. Consequently, there is an urgent need to identify new chemical entities (NCE) possessing antimalarial properties with desirable biological activity and toxicity profiles for enhanced treatment.[35,36]

The use of USA in combinatorial synthesis has been described.[37] The lipophilia (due to the intramolecular hydrogen bonds) of USA, together with its systemic toxicity, is a characteristic which has limited its use. Hence, structural modifications were made on the dibenzofuran ring without disturbing the pharmacopore architecture of the parent nucleus, to produce new, more active and less toxic compounds for the treatment of malaria. Three hydrazine and two amino derivatives were attempted in this regard, namely usnic acid anhydro phenyl hydrazone (S1), usnic acid anhydro 4-nitro phenyl hydrazone (S2), usnic acid anhydro 2,4-dinitro phenyl hydrazone (S3), usnic acid anhydro o-phenylene derivative (S5) and usnic acid anhydro piperazine derivative (S6).

USA: A pale yellow powder, yield 2.1 g (96.367% pure by HPLC analysis), soluble in DMSO, N,N-DMF, DCM, Toluene, Benzene with a melting point ranging from 203 °C and R_f value of 0.74 in Hexane: ether: formic acid (5:4:1), identified using 1% ferric chloride and appearance of one blue fluorescent spot at 350 and 254 nm under UV light, UV (MeOH) λ_{max}(nm): 328, 285, 231, IR υ_{max}(film)/cm-^{1}: 2829.82 (C-H Str),1692.49 (ester, C = O), 1632.54 (C = O str), 1542.78 (aromatic), 1190.09/1220.13 (Aromatic ester), 846.86 (aliphatic alcoholic ester);^{1}HNMR: 13.32δ (7-OH), 11.04δ (5-OH), 5.98δ (HC-1), 2.67δ ($COCH_3$), 2.11δ (Aro CH_3), 1.77δ (C-CH_3) and Mass (ESI) of 367 (m + Na).

Usnic acid anhydro phenyl hydrazone (S1): A pale yellow solid, yield 343 mg (99.830% pure by HPLC analysis), soluble in DMSO, N,N-DMF and DCM with a melting point of 156 °C and R_f value of 0.69 in hexane:ethyl acetate (9:1), identified using 1% ferric chloride and appearance of one blue fluorescent spot at 350 nm under UV light, UV (MeOH) λ_{max} (nm): 221, 253, 287 and 370 mμ, IR υ_{max}(film)/cm-^{1}: 2925.14 (Aliphatic C-H Str), 1630.05 (C = N Str), 1504.42 (C = C Str), 1438.31(C-H Bending), 1371.04 (C-N Str); ^{1}HNMR: 13.29δ (7-OH), 11.09δ (5-OH), 7.57δ (Aromatic proton), 6.24δ (HC-1), 2.55δ ($COCH_3$), 2.08δ (Aromatic CH_3), 1.79δ (C-CH_3) and Mass (HRMS) of 417.14431 (m + H).

Usnic acid anhydro 4-nitro phenyl hydrazone (S2): A dark yellow solid, yield 426 mg (99.757% pure by HPLC anaylsis), soluble in DMSO, N,N-DMF and DCM with a melting point of 150 °C and R_f value of 0.55 in Hexane:ethyl acetate (9.5:0.5), identified using 1% ferric chloride and by appearance of one blue fluorescent spot at 350 nm under UV light, IR υ_{max} (film)/cm-^{1}: 2923.38 (Aliphatic C-H str), 1674.78 (C = O Str), 1632.30 (C = N Str), 1510.22 (Aromatic C-NO_2Str), 1437.91 (CH_3 Bending), 1284.10/1340.18 (Aromatic str); ^{1}HNMR: 13.31δ (7-OH), 10.89δ (5-OH), 7.26δ (Aromatic proton), 6.32δ (HC-1), 2.65δ ($COCH_3$), 2.59δ (CH_3-C = N-), 2.11δ (Aromatic CH_3), 1.83δ (C-CH_3) and Mass (HRMS) of 462.12958 (m + H).

Usnic acid anhydro 2,4-dinitro phenyl hydrazone (S3): A orange solid powder, yield 412 mg (94.319% pure by HPLC analysis) soluble in DMSO, N,N-DMF, DCM and methanol with a melting point of 182 °C and R_f value of 0.61 in Hexane:ethyl acetate (5:5), identified using 1% ferric

Table 1. Trypsin activity in extracts of *Usnea undulata*.

S.No	Inhibitor/Extract	Inhibition (%)
1	Standard inhibitor	100
2	SU-I	100
3	SU-II	70
4	SU-III	NIL

SU- II: Dichloromethane extract.
SU- I: Ether extract.
SU- III: Acetone extract.

Table 2. Trypsin Activity of usnic acid and its analogues.

S.No	Inhibitor/Extract	Inhibition (%)
1	Standard Inhibitor	100
2	S-1	60
3	S-2	80
4	S-3	88
5	S-4	48
6	S-5	77
7	S-6	50

USA: Usnic acid.
S1: Usnic acid anhydro phenyl hydrazone.
S2: Usnic acid anhydro 4-nitro phenyl hydrazone.
S3: Usnic acid anhydro 2,4-dinitro phenyl hydrazone.
S5: Usnic acid anhydro o-phenylenediamine derivative.
S6: Usnic acid anhydropiperazine derivative.

chloride and appearance of one blue fluorescent spot at 350 nm under UV light, IR υ_{max}(film)/cm-^{1}: 3291.64 (N-H Str), 2927.23 (Aliphatic C-H str), 1619.09 (C = N Str), 1517.04 (Aromatic C-NO2 Str), 1448.91 (CH_3 Bending), 1330.45/1283.05 (Aromatic CN str); ^{1}HNMR: 13.34δ (7-OH), 11.20δ (5-OH), 7.26δ (Aromatic proton), 5.94δ (HC-1), 2.69δ ($COCH_3$), 2.31δ (CH_3-C = N-), 2.05δ (Aro CH_3), 1.84δ (C-CH_3) and Mass (ESI) of 529 (m + Na).

Usnic acid anhydro o-phenylamine derivative (S5):A pale brown solid, yield 313 mg (96.525% pure by HPLC analysis), soluble in DMSO, N,N-DMF, DCM and ethyl acetate with a melting point of 172 °C and R_f value of 0.64 in Hexane:ethyl acetate (8:2), identified using 1% ferric chloride and appearance of one dark blue fluorescent spot at 350 nm under UV light, IR υ_{max}(film)/cm-^{1}:3472.69 (N-H Str), 2923.03 (Aliphatic C-H str), 1694.38 (C = O Str), 1627.12 (C = N Str), 1531.61 (C = C Str), 1365.75 (C-N Bending), 1265.41 (CH Str), 1194.81/1061.06 (C-N bending); ^{1}HNMR: 13.36 (7-OH), 11.81 (5-OH), 7.01 (Aromatic proton), 5.86δ (HC-1), 2.69δ ($COCH_3$), 2.55δ (CH_3-C = N-), 2.10δ (Aromatic CH_3), 1.75δ (C-CH_3) and Mass (HRMS) of 435.15506 (m + Na).

Usnic acid anhydro piperazine derivative (S6): A pale brown solid, yield 313 mg (96.554% pure by HPLC analysis), soluble in DMSO, N,N-DMF, DCM and ethyl acetate with a melting point of 143 °C and R_f value of 0.64 in Hexane:ethyl acetate (7:3), identified using 1% ferric chloride and appearance of one dark blue fluorescent spot at 350 nm under UV light, IR υ_{max} (film)/cm^{-1}:3460.06 (O-H Str), 3249.60 (N-H Str), 3006.23 (Aro C-H Str), 2929.84 (Aliphatic C-H Str), 1700.00 (C = O Str), 1633.83, 1572.84 (C = N Str), 1505.24 (C = C Str), 1446.40 (C-H Bending), 1369.72, 1291.25 (C-N Str); ^{1}HNMR: 13.31 (7-OH), 11.03 (5-OH), 7.27 (Aromatic proton), 5.98δ (HC-1), 2.68δ ($COCH_3$), 2.10δ (Aromatic CH_3), 1.63δ (C-CH_3) and Mass (HRMS) of 413.17071 (M).

In an attempt to identify potent inhibitors of malarial parasites, initial preliminary biochemical screening assays on the *Plasmodium* protease enzyme, trypsin was performed for the crude extracts, isolated USA and its analogues as tabulated in Tables 1 and 2. The results of the crude extracts showing high inhibitory activity were found to be in the order of SU-I (100%) >SU-V (70%) inhibition respectively, arbitrarily fixing the inhibition caused by the standard inhibitor,

Table 3. Inhibitory Concentration values of usnic acid and its derivatives.

S.No	Compound	Inhibitory concentration 50 [IC50a (M)]
1	USA	6.5·10-5
2	S1	1.98·10-5
3	S2	1.4·10-5
4	S3	8.13·10-5
5	S5	2.13·10-5
6	S6	7.28·10-5

USA: Usnic acid.
S1: Usnic acid anhydro phenyl hydrazone.
S2: Usnic acid anhydro 4- nitrophenyl hydrazone.
S3: S2: Usnic acid anhydro 2, 4- nitrophenyl hydrazone.
S5: Usnic acid anhydro o-phenylene diamine derivative.
S6: Usnic acid anhydro piperazine derivative.

mercuric chloride as 100% inhibition. The decrease in the enzyme activity was a measure of the inhibitory activity. Similarly, the inhibitory results of USA and its derivatives were found to be in the order of S2 (88%) > S1 (80%) > S5 (77%) > USA (65%)> S6 (50%) > S3 (48%).

Therein, USA and its derivatives were screened for their antimalarial property by parasite growth inhibition assay and the IC_{50}values were determined by nonlinear regression analysis.The results (Table 3) indicated that the semisynthetic derivatives of USA possessed significant antimalarial activity in the order S2 ($1.4{\cdot}10^{-5}$M) > S1 ($1.98{\cdot}10^{-5}$M) > S5 ($2.13{\cdot}10^{-5}$M) when compared to the parent compound USA ($6.5{\cdot}10^{-5}$M). This reveals that the lichen metabolite, USA might offer effective scaffolds in further design and synthesis of potent antimalarial leads in the chemotherapy of malaria.

4. Conclusion

Current malaria drug discovery and design focuses on the well-established stages of malaria by both phenotype and target-based approaches. Recent studies have paid more attention to the drug targeting malaria parasite, *P. falciparum* for treatment and cure of malaria with low risk of resistance. Accordingly, plasmepsin II aspartyl proteases of *P. falciparum* were used for studying the control mechanism of malaria through a truly high through put screening system and novel targets were developed. In this regard, the present study has identified usnic acid anhydro 4-nitro phenyl hydrazone as the most active and most selective aspartyl *P. falciparum* inhibitor, first based on a preliminary biochemical enzyme inhibitory screening report for the crude extracts and synthesized molecules, second, by CADD studies and finally, by subjecting to an *in vitro* growth inhibition assay, wherein the growth inhibition at parasite level was assessed and reported. Among all the molecules under study, the hydrazine moiety, usnic acid anhydro 4-nitro phenyl hydrazone (S2) shows significant *in vitro* antimalarial activity (IC_{50}: $1.4{\cdot}10^{-5}$) against the test organism followed by S1, S5, USA, S6 & S3 compounds with IC_{50} values of $1.98{\cdot}10^{-5}$M, $2.13{\cdot}10^{-5}$M, $6.5{\cdot}10^{-5}$M, $7.28{\cdot}10^{-5}$M and $8.13{\cdot}10^{-5}$M, respectively. The results conclude that the lichen metabolite, USA and its analogues have a good scope of therapeutic importance in showing antimalarial activity. However, there arised a question on the comparison of the biological and pharmacological activities of USA enantiomers. Most of the available data refer to (+)-USA, while the left-handed isomer has been less often significantly studied. Nevertheless, more studies, especially on (−)-USA, are needed to give a final explanation for the similarities and differences between both USA enantiomers. These should be especially directed to steric structure–activity relationship of the enantiomers, tested under the same experimental conditions, which may help to explain the possible mechanisms of their actions.[38] In addition, the biological targets underlying antimalarial, antimycobacterial and antibacterial activities of lichen metabolites warrant further studies. This study also highlights the need to explore the *in vivo* potential of the above

lichen metabolites for enumerating the exact mechanism of action contributing to the antimalarial activity so as to produce novel bioactive molecules as potential chemotherapeutic agents in antimalarial drug design and discovery.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1. D. Wyler, "Malaria: Overview and Update," *Clinical Infectious Diseases* 16, no. 4 (1993): 449–58.
2. S. E. Francis, D. J. Sullivan Jr., and D. Goldberg, "Hemoglobin Metabolism in the Malaria Parasite *Plasmodium falciparum*," *Annual Review of Microbiology* 51, no. 1 (1997): 97–123.
3. L. Verotta, "Are Acylphloroglucinols Lead Structures for the Treatment of Degenerative Diseases?" *Phytochemistry Reviews* 1, no. 3 (2002): 389–407.
4. I. P. Singh, J. Sidana, P. Bansal, and W. J. Foley, "Phloroglucinol Compounds of Therapeutic Interest: Global Patent and Technology Status," *Expert Opinion on Therapeutic Patents* 19, no. 6 (2009): 847–66.
5. L. Verotta, G. Appendino, E. Bombardelli, and R. Brun, "In Vitro Antimalarial Activity of Hyperforin, a Prenylated Acylphloroglucinol. A Structure–Activity Study," *Bioorganic and Medicinal Chemistry Letters* 17, no. 6 (2007): 1544–8.
6. C. F. Culberson, *Chemical and Botanical Guide to Lichen Products* (Chapel Hill: University of North Caroline Press, 1969), 171.
7. J. G. Rowe and M. D. Saenz, "Additional contribution to the study of the antimicrobial activity and identification of lichenic substances in some lichens from southern Spain," *Annales Pharmaceutiques Francaise* 49 (1991): 278–85.
8. S. M. Kupchan and H. L. Kopperman, "l-Usnic Acid: Tumor Inhibitor Isolated from Lichens,"*Experientia* 31, no. 6 (1975): 625–752.
9. M. Cardarelli, G. Serino, L. Campanella, P. Ercole, F. De Cicco Nardone, O. Alesiani, and F. Rossiello, "Antimitotic Effects of Usnic Acid on Different Biological Systems," *Cellular and Molecular Life Sciences* 53, no. 8 (1997): 667–72.
10. E. R. Corrache, M. Carrasco, M. E. Escudero, L. Velaquez, A. M. S. deGuzman, and F. Giannini, "Study of the cytotoxic and antimicrobial activities of usnic acid and derivatives," *Fitoterpia* 69 (1998): 493–501.
11. N. B. Perry, M. H. Benn, N. J. Brennan, E. J. Burgess, G. Ellis, D. J. Galloway, S. D. Lorimer, and R. S. Tangney, "Antimicrobial, Antiviral and Cytotoxic Activity of New Zealand Lichens," *The Lichenologist* 31, no. 6 (1999): 627–36.
12. D. E. Goldberg, J. Mulzer, and R. Bohlmann, eds., *Ernst Schering Workshop Foundation*, vol. 32 (Berlin: Springer-Verlag, 2000), 183–205.
13. J. Greer, "Comparative Modeling Methods: Application to the Family of the Mammalian Serine Proteases," *Proteins: Structure, Function, and Genetics* 7, no. 4 (1990): 317–34.
14. T. S. Haque, A. G. Skillman, C. E. Lee, H. Habashita, I. Y. Gluzman, T. J. A. Ewing, D. E. Goldberg, I. D. Kuntz, and J. A. Ellman, "Potent, Low-Molecular-Weight Non-Peptide Inhibitors of Malarial Aspartyl Protease Plasmepsin II," *Journal of Medicinal Chemistry* 42, no. 8 (1999): 1428–40.
15. M. Kunitz, "Crystalline Soybean Trypsin Inhibitor," *Journal of General Physiology* 30, no. 4 (1947): 291–310.
16. O. Vertinan, H. Vertinan, and A. E. Kortekangas, "Antibiotic activity of some amino compound derivatives of l-usnic acid. I Sulfonamides, isonicotinoyl hydrazide," *Sounmen Kemi* 27B, no. 3 (1954): 18–20. Issue **1995,** B28, 125.
17. J. P. Kutney, I. H. Sanchez, and T. Yee, "Studies in the Usnic Acid Series. III. The Base Catalyzed Usnic Acid–Isousnic Acid Rearrangement. The Synthesis of (+)-Isousnic Acid," *Canadian Journal of Chemistry* 54, no. 23 (1976): 3721–30.
18. B. Marc-Antoine, A. C. Le Lamer, J. G. Delcros, I. Rouaud, P. Uriac, J. Boustie, J. C. Corbel, and S. Tomasi, "Synthesis and cytotoxic activities of usnic acid derivatives," *Bioorganic & Medicinal Chemistry* 16, no. 14 (2008): 6860–6.

19. M. S. Amine, M. H. M. Ahmed, K. A. Hebash, and A. A. El-Sheikh, "Utilization of fatty acids in heterocyclic synthesis part 9: dual activity of novel triazoles as antimicrobial and surface active agents," Olaj, Szappan, Kozmetika, 59 (2010): 49–55.
20. M. Natic, Z. Tesic, K. Andelkovic, I. Brceski, S. Radulovic, S. Manic, and D. Sladic, "Synthesis and Biological Activity of Pd(II) and Cu(II) Complexes with Acylhydrazones of Usnic Acid," *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry* 34, no. 1 (2004): 101–13.
21. M. Koegh, "2′-*O*-demethylpsoromic acid from usnea sp.," *Phytochemistry* 15 (1976): 1801.
22. G. P. Briner, G. E. Gream, and N. V. Riggs, "Chemistry of Australian Lichens. I. Some Constituents of *Parmelia conspersa* (Ehrh.) Ach., *Ramalina fraxinea* (L.) Ach., *Usnea barbata* (L.) Wigg., and *U. ceratina* Ach., from the New England Region," *Australian Journal of Chemistry* 13, no. 2 (1960): 277.
23. C. F. Culberson and H. D. Kristinsson, "A Standardized Method for the Identification of Lichen Products," *Journal of Chromatography A* 46 (1970): 85–93.
24. C. F. Culberson, "Conditions for the Use of Merck Silica Gel 60 F254 Plates in the Standardized Thin-Layer Chromatographic Technique for Lichen Products," *Journal of Chromatography A* 97, no. 1 (1974): 107–8.
25. M. A. Rashid, M. A. Majid, and M. A. Quader, "Complete NMR Assignments of (+)-Usnic Acid," *Fitoterapia* 70, no. 1 (1999): 113–5.
26. E. Susithra, S. Meena, S. Chamundeeswari, D. Rajasekhar, and Chekkara Ethiraj Varalakshmi, "Molecular docking studies of lichen metabolites as malarial protein inhibitors: Plasmepsin II protease and dihydro folate reductase," *Journal of Chemical and Pharmaceutical Research* 6, no. 6 (2014): 86–97.
27. D. H. R. Barton and T. Bruun, "Chapter 122. Some Observations on the Constitution of Usnic Acid," *Journal of the Chemical Society (Resumed)* (1953): 603–9.
28. J. P. Kutney and I. H. Sanchez, "Studies in the usnic acid series. I. The condensation of (+)-usnic acid with aliphatic and aromatic amines," *Canadian Journal of Chemistry* 54 (1976): 2795–803.
29. S. K. Manaktala, S. Neelakantan, and T. R. Seshadri, "Condensation products of usnic acid with amino compounds," *Indian Journal of Chemistry* 5 (1967): 29–33.
30. G. B. Feige, H. T. Lumbsch, S. Huneck, and J. A. Elix, "Identification of Lichen Substances by a Standardized High-Performance Liquid Chromatographic Method," *Journal of Chromatography A* 646, no. 2 (1993): 417–27.
31. W. Trager and J. B. Jensen, "Human Malaria Parasites in Continuous Culture," *Science* 193, no. 4254 (1976): 673–5.
32. C. Lambros and J. P. Vanderberg, "Synchronization of *Plasmodium falciparum* Erythrocytic Stages in Culture," *The Journal of Parasitology* 65, no. 3 (1979): 418–20.
33. M. Smilkstein, N. Sriwilaijaroen, J. X. Kelly, P. Wilairat, and M. Riscoe, "Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening," *Antimicrobial Agents and Chemotherapy* 48 (2004): 1803–6.
34. P. S. Sijwali and P. J. Rosenthal, "Gene Disruption Confirms a Critical Role for the Cysteine Protease Falcipain-2 in Hemoglobin Hydrolysis by *Plasmodium falciparum*," *Proceedings of the National Academy of Sciences of the United States of America* 101, no. 13 (2004): 4384–9.
35. D. Rathore, *Expert Opinion on Investigational Drugs*, vol. 14, no. 7 (London, GB: Ashley Publications Ltd., 2005), 871–83.
36. N. C. Waters, In *The Patent Literature, 2000-2003, Expert Opinion On Therapeutic Patents, Informa Healthcare*, vol. 14, no. 8 (GB, 2004), 1125–38.
37. S. Tomasi, S. Picard, C. Lainé, V. Babonneau, A. Goujeon, J. Boustie, and P. Uriac, "Solid-Phase Synthesis of Polyfunctionalized Natural Products: Application to Usnic Acid, a Bioactive Lichen Compound," *Journal of Combinatorial Chemistry* 8, no. 1 (2006): 11–4.
38. A. Galanty, P. Paśko, and I. Podolak, "Enantioselective Activity of Usnic Acid: A Comprehensive Review and Future Perspectives," *Phytochemistry Reviews* 18, no. 2 (2019): 527–48.