ChemPubSoc Europe

DOI: 10.1002/slct.201801905

Chemistry SELECT Full Papers

Medicinal Chemistry & Drug Discovery

Synthesis and Antimalarial Evaluation of [1,2,3]-Triazole-Tethered Sulfonamide-Berberine Hybrids

Neha Batra,[a] Vinoth Rajendran,[b] Drishti Agarwal,[a] Ishan Wadi,[a] Prahlad C. Ghosh,[b] Rinkoo D. Gupta,[c] and Mahendra Nath*[a]

Malaria still remains a global health problem despite of the availability of effective control and treatment measures. In the present study, a novel series of [1,2,3]-triazole tethered sulfonamide-berberine hybrids were synthesized in good yields viaHuisgen [3+2] cycloaddition reaction of various primary, secondary and tertiary sulfonamide based azides with 9-O-(propyne)berberine chloride in *t*-BuOH:water (1:1) mixture containing a catalytic amount of sodium ascorbate and $CuSO_4.5H_2O$ at 90°C. After spectroscopic characterization, these novel hybrids were evaluated for their potency against asexual erythrocytic stages of *P. falciparum* (3D7) in vitro. Most of the synthesized compounds have shown significant antimalarial activity with IC_{50} values in the range of 0.1-20 μg/mL and were also found to be non-cytotoxic under tested conditions.

Introduction

Malaria is a tropical disease caused by parasites of genus *Plasmodium* and transmitted by female anopheles mosquitoes.[1] It is mainly endemic in tropical countries, with people living in countries of sub-Saharan Africa and South East Asia are at highest risk. Although, there are many species of malaria causing Plasmodia but only five are linked with human transmission. These five species are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*.[2] Out of these species, *P. falciparum* is the most lethal and contributes to majority of mortality and morbidity cases associated with malaria. According to recent figures provided by the World Health Organization, an estimated 216 million people contacted malaria in 2016 around the globe, with an estimated 445000 deaths, majority of which occurred in young children with less than five years of age.[3] Lack of effective vaccines coupled with rapid emergence and spread of drug resistance, especially in *P. falciparum* increases the severity quotient associated with the disease.[3–5]

Many successful antimalarial drugs such as chloroquine and hydroxychloroquine, function by targeting the hemoglobin degradation pathways.[6] Drugs such as chloroquine, sulfadoxine, pyrimethamine and artemisinin (**Figure 1**) are potent antimalarials with good pharmacokinetic profile with minimum side-effects. However, continuous emergence and spread of drug resistance in majority of these drugs has precluded their use to some extent and limited our options to fight this deadly disease. To tackle the threat of drug resistance, combination therapies were introduced as first line treatment for *P. falciparum* malaria worldwide. Combination therapy consists of multiple antimalarial drugs with variable chemotypes and diverse mechanism of action.[7] Artemisinin combination therapy, which is currently the preferred choice of treatment is under constant threat due to development of partial resistance in parasites to artemisinin component of the combination therapy.[8] Therefore, in order to combat drug resistance and increase the ammunition against the parasite, novel chemotherapeutic agents are needed which can effectively target asexual stages of the parasite and help in achieving our goal of malaria elimination.

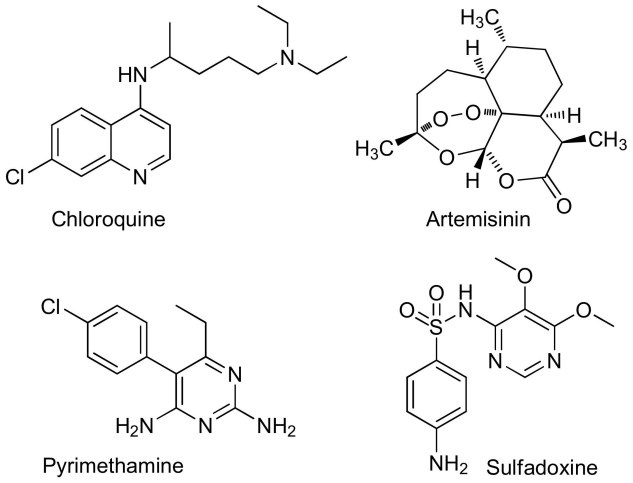
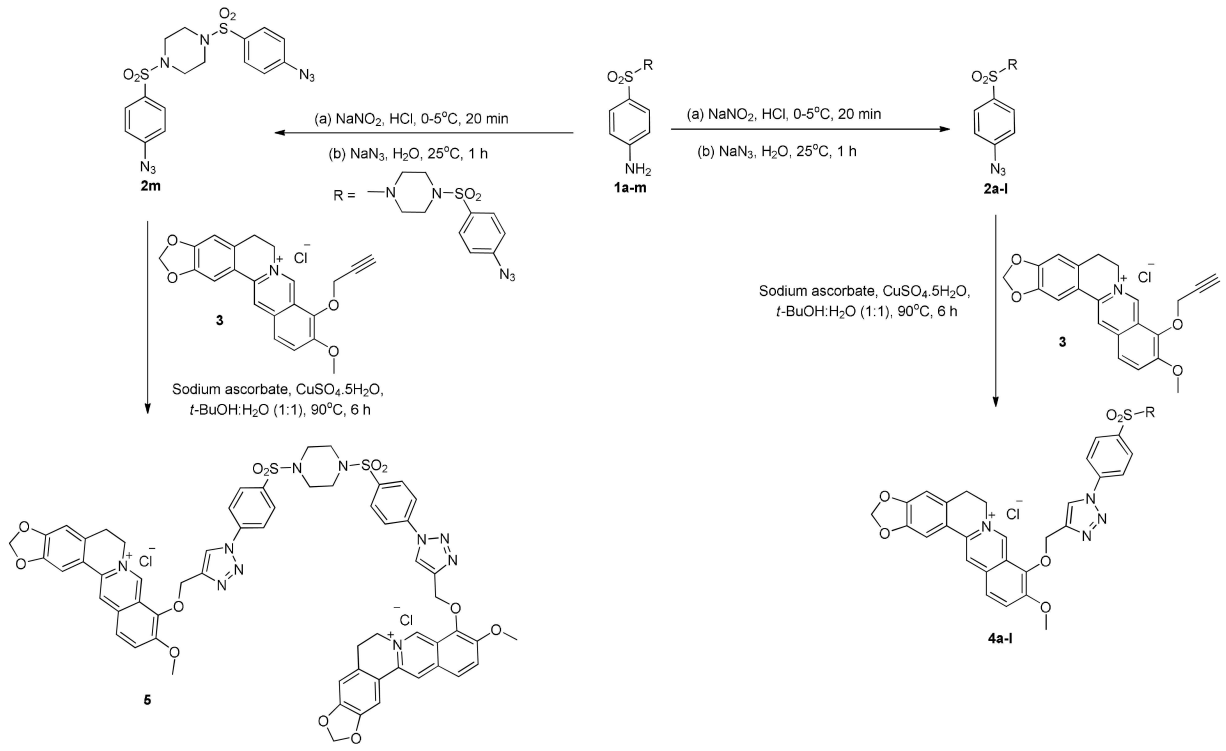


Figure 1. Structures of common antimalarial drugs.

[a] *Dr. N. Batra, D. Agarwal, I. Wadi, Prof. M. Nath*
Department of Chemistry, University of Delhi, Delhi 110 007, India
Tel.: +91 11 27667794 Extn. 186
E-mail: mnath@chemistry.du.ac.in
[b] *Dr. V. Rajendran, Prof. P. C. Ghosh*
Department of Biochemistry, University of Delhi South Campus, New Delhi 110 021, India
[c] *Dr. R. D. Gupta*
Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi 110 021, India

Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.201801905



Scheme 1. Synthesis of berberine-sulfonamide based [1,2,3]-triazoles.

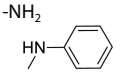
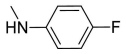
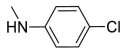
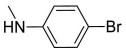
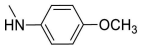
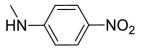
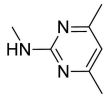
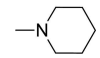
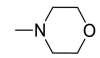
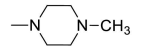
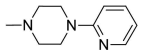
In context of novel antimalarial drugs, sulfonamide derivatives have emerged as recent subjects of great interest for their ease of synthesis and multiple pharmacological activities such as anti-microbial,[9] anti-cancer,[10] diuretic,[11] carbonic anhydrase inhibition[12] etc. With an attempt to achieve potent antimalarial compounds, in present study, a new series of hybrid molecules is created by linking sulfonamide moiety to another pharmacologically active natural isoquinoline alkaloid, berberine[13–26] through [1,2,3]-triazole linker to yield novel hybrids with potential antimalarial properties. Briefly, primary, secondary and tertiary sulfonamide based azides were reacted with 9-O-(propyne)berberine chloride under click reaction conditions to yield the desired products in good yields.

Results and Discussion

The synthesis of novel target molecules began with the preparation of secondary and tertiary sulfonamide based amines (**1b-g** and **1i-m**) according to the reported procedures.[27] In the next step, the primary, secondary and tertiary sulfonamide based amines (**1a-m**) were diazotized on the treatment with $NaNO_2$ in the presence of hydrochloric acid at 0°C for 20 minutes followed by the reaction with sodium azide to afford desired sulfonamide based azides (**2a-m**).[28] On the other hand, 9-O-(propyne)berberine chloride (**3**) was prepared in two steps under inert atmosphere. Firstly, the berberine chloride was demethylated on heating at 190°C in DMF and the resulting hydroxyl derivative reacted with propargyl bromide in presence of potassium carbonate in acetonitrile at 80°C to afford the desired berberine alkyne (**3**).[29] Finally, novel [1,2,3]-triazole tethered sulfonamide-berberine hybrids (**4a-l** and **5**) were constructed via Huisgen [3+2] cycloaddition reaction of azides (**2a-m**) and 9-O-(propyne)berberine chloride (**3**) in 50% aqueous *t*-BuOH containing a catalytic amount of sodium ascorbate and $CuSO_4.5H_2O$ at 90°C for 6 hours (**Scheme 1**). After purification, the structures of all target products were established on the basis of ^{1}H-NMR, IR and high resolution mass spectral analysis (**ESI**).

All the newly synthesized [1,2,3]-triazole tethered sulfonamide-berberine hybrids (**4a-l** and **5**) have been evaluated for their potential antimalarial activity against *P. falciparum*(3D7) by [^{3}H]-hypoxanthine incorporation assay.[30] In brief, *P. falciparum*infected erythrocytes at 4% hematocrit and 2% parasitaemia were incubated with varied concentrations of compounds for 24 hours at 37°C. For another 18 hours, 0.2 μCi/well [^{3}H]-hypoxanthine was added to each well and its uptake levels were used to determine the IC_{50} values which are summarized in **Table 1**. Chloroquine (CQ) was used as a positive control for the biological experiments. From the **Table 1**, it is evident that triazoles of all primary, secondary and tertiary sulfonamides demonstrate variable potency (0.1 μg/mL to 20 μg/mL) against *P. falciparum* in vitro. Primary sulfonamide-triazole-berberine **4a** demonstrated half maximal inhibitory concentration of 0.3 μg/mL. All secondary sulfonamide-triazole-berberine derivatives containing various electron attracting and electron releasing groups at *p*-position of an aryl ring attached to the sulfonamide scaffold showed comparable potency except the compound **4g**. Among the halogen substituted derivatives, the

Table 1. In vitro antimalarial activity of sulfonamide based berberine-triazole hybrids (**4a-l** and **5**) against *P. falciparum* (3D7) strain.

Entry	Compd.	R	Yield (%)	Antimalarial activity (after 42 h)	
				IC_{50} (μg/mL)	IC_{50} (μM)
1	**4a**	-NH_2	77	0.3	0.505
2	**4b**		78	0.2	0.298
3	**4c**		77	0.15	0.218
4	**4d**		96	0.1	0.142
5	**4e**		81	0.3	0.401
6	**4f**		80	0.5	0.715
7	**4g**		81	20	28.006
8	**4h**		81	4	5.737
9	**4i**		80	0.8	1.209
10	**4j**		80	0.5	0.753
11	**4k**		74	0.8	1.184
12	**4l**		83	1	1.352
13	**5**	-	78	0.5	0.399
14	CQ[a]	-	-	0.034	0.066

[a] CQ = Chloroquine

compound **4d**containing *p*-chlorophenylamino substituent is found to be the most active molecule with IC_{50} of 0.1 μg/mL. In comparison, the compound **4c** with *p*-fluorophenylamino group and **4e** with *p*-bromophenylamino group have displayed slightly higher IC_{50} values such as 0.15 μg/mL and 0.3 μg/mL, respectively.

Surprisingly, the presence of highly electron withdrawing nitro substituent at the *p*-position of aryl ring attached to the sulfonamide adversely affected the potency of compound **4g** (IC_{50} 20 μg/mL). However, other compounds in this series such as **4b** with phenylamino substituent and compound **4f** with *p*-methoxyphenylamino substituent have also shown better potency with the IC_{50} values 0.2 μg/mL and 0.5 μg/mL, respectively. In contrast, the tertiary sulfonamide-triazole-berberine analogues (**4i-l** and **5**) containing various heterocyclic substituents were also found to be significantly active against *P. falciparum and*demonstrated IC_{50} values in range of 0.5 μg/mL to 1.0 μg/mL. Collectively, our in vitro screening results suggest that the majority of synthesized sulfonamide based berberine-triazole hybrids are proved to be significantly active against *P. falciparum*. Furthermore, the synthesized hybrid compounds (**4a-l** and **5**) were found to be non-cytotoxic against the prostate cancer cells (PC-3) with CC_{50} values >200 μg/mL when evaluated by MTT assay.[31]

Conclusions

In summary, novel sulfonamide based berberine-[1,2,3]-triazole hybrids have been successfully synthesized in appreciable yields under click reaction conditions. On biological evaluation, most of the prepared compounds have shown significant potency against *P. falciparum* (3D7) strain in vitro and found to be non-cytotoxic against human prostate cancer cells. Our findings suggest that these compounds have potential to be considered as prototypes for the development of next generation antimalarials.

Supporting Information Summary

The experimental details for the syntheses and the biological evaluation of new compounds and their spectral data reported herein are provided in the Supporting Information.

Acknowledgements

Authors are thankful to University of Delhi for financial support under the scheme to strengthen R & D Doctoral Research Programme and USIC for providing ^{1}H-NMR, IR and high resolution mass data. NB and IW are thankful to UGC, India for providing the Senior Research Fellowship.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Antimalarial activity · berberine chloride · click chemistry · cytotoxicity · sulfonamides · synthesis · [1,2,3]-triazoles

[1] M. A. Phillips, J. N. Burrows, C. Manyando, R. H. Van Huijsduijnen, W. C. Van Voorhis, T. N. C. Wells, *Nat. Rev. Dis. Primers* **2017**, *3*, 17050.
[2] F. E. G. Cox, *Parasites Vectors* **2010**, *3*, 5.
[3] World Malaria Report **2016**. Geneva: World Health Organization; 2016. Licence: CC BY-NC-SA 3.0 IGO.
[4] N. J. White, *J. Clin. Invest.* **2004**, *113*, 1084–1092.
[5] N. J. White, *Lancet* **2010**, *376*, 2051–2052.
[6] a) L. M. Coronado, C. T. Nadovich, C. Spadafora, *Biochim. Biophys. Acta* **2014**, *1840*, 2032–2041;b) N. Klonis, D. J. Creek, L. Tilley, *Curr. Opin. Microbiol.* **2013**, *16*, 722–727.
[7] F. Nosten, P. Brasseur, *Drugs* **2002**, *62*, 1315–1329.
[8] M. Imwong, K. Suwannasin, C. Kunasol, K. Sutawong, M. Mayxay, H. Rekol, F. M. Smithuis, T. M. Hlaing, K. M. Tun, R. W. Van der Pluijm, R. Tripura, O. Miotto, D. Menard, M. Dhorda, N. P. J. Day, N. J. White, A. M. Dondorp, *Lancet Infect. Dis.* **2017**, *17*, 491–497.
[9] J. Drews, *Science* **2000**, *287*, 1960–1964.
[10] C. T. Supuran, *Expert Opin. Investig. Drugs* **2003**, *12*, 283–287.
[11] V. A. Zubkov, T. A. Tsapko, I. S. Gritsenko, A. L. Zagayko, L. V. Galuzinskaya, *Russ. Chem. Bull. Int. Ed.* **2012**, *61*, 1969–1974.
[12] a) C. T. Supuran, F. Briganti, S. Tilli, W. R. Chegwidden, A. Scozzafava, *Bioorg. Med. Chem.* **2001**, *9*, 703–714;b) F. Carta, A. Maresca, A. Scozzafava, D. Vullo, C. T. Supuran, *Bioorg. Med. Chem.* **2009**, *17*, 7093–7099.
[13] S. Samosorn, B. Tanwirat, N. Muhamad, G. Casadei, D. Tomkiewicz, K. Lewis, A. Suksamrarn. T. Prammanan, K. C. Gornall, J. L. Beck, J. B. Bremner, *Bioorg. Med. Chem.* **2009**, *17*, 3866–3872.
[14] H. S. Bodiwala, S. Sabde, D. Mitra, K. K. Bhutani, I. P. Singh, *Eur. J. Med. Chem.* **2011**, *46*, 1045–1049.

[15] S. Letasiova, S. Jantova, L. Cipak, M. Muckova, *Cancer Lett.* **2006**, *239*, 254–262.
[16] M. Bahar, Y. Deng, X. H. Zhu, S. S. He, T. Pandharkar, M. E. Drew, A. Navarro-Vazquez, C. Anklin, R. R. Gil, R. W. Doskotch, K. A. Werbovetz, A. D. Kinghorn, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2606–2610.
[17] J. L. Vennerstrom, J. K. Lovelace, V. B. Waits, W. L. Hanson, D. L. Klayman, *Antimicrob. Agents Chemother.* **1990**, *34*, 918–921.
[18] C. L. Kuo, C. W. Chi, T. Y. Liu, *Cancer Lett.* **2004**, *203*, 127–137.
[19] J. Wongbutdee, *Thai Pharm. Health Sci. J.* **2008**, *4*, 78–83.
[20] C. M. Tsang, Y. C. Cheung, V. W. Lui, Y. L. Yip, G. Zhang, V. W. Lin, K. C. Cheung, Y. Feng, S. W. Tsao, *BMC Cancer* **2013**, *13*, 619.
[21] C. Caliceti, P. Rizzo, A. F. Cicero, *Oxid. Med. Cell. Longev.* **2015**, DOI 10.1155/2015/723093
[22] Y. Zhou, H. Tao, Y. Li, M. Deng, B. He, S. Xia, C. Zhang, S. Liu, *Eur. J. Pharmacol.* **2016**, *789*, 109–118.
[23] M. Xiao, L. N. Men, M. G. Xu, G. B. Wang, H. T. Lv, C. Liu, *Eur. J. Pharmacol.* **2014**, *743*, 11–16.
[24] P. F. Hu, W. P. Chen, J. L. Tang, J. P. Bao, L. D. Wu, *Phytother. Res.* **2011**, *25*, 878–885.
[25] H. Zhao, T. Zhang, C. Xia, L. Shi, S. Wang, X. Zheng, T. Hu, B. Zhang, *J. Cell. Mol. Med.* **2014**, *18*, 283–292.
[26] a) W. J. Shan, L. Huang, Q. Zhou, F. C. Meng, X. S. Li, *Eur. J. Med. Chem.* **2011**, *46*, 5885–5893;b) Y. Ma, T. M. Ou, J. Q. Hou, Y. J. Lu, J. H. Tan, L. Q. Gu, Z. S. Huang, *Bioorg. Med. Chem.* **2008**, *16*, 7582–7591.
[27] a) P. Wang, C. Liu, T. Sanches, Y. Zhong, B. Liu, J. Xiong, N. Neamati, G. Zhao, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4574–4578;b) M. M. J. Al-Mudhafar, M. Q. Abdulkadir, A. A. Mohammed, F. A. Al-hilli, A. M. Hussian, *Pharm. Globale* **2012**, *6,* 1–10.
[28] N. Batra, B. Roy, S. Mazumder, M. Nath, *Ind. J. Chem.* **2015**, *54B*, 650–655.
[29] X. Jin, T. H. Yan, L. Yan, Q. Li, R. L. Wang, Z. L. Hu, Y. Y. Jiang, Q. Y. Sun, Y. B. Cao, *Drug Desg. Dev. Ther.* **2014**, *8*, 1047–1059.
[30] a) A. K. Singh, S. Rathore, Y. Tang, N. E. Goldfarb, B. M. Dunn, V. Rajendran, P. C. Ghosh, N. Singh, N. Latha, B. K. Singh, M. Rawat, B. Rathi, *PLoS One***2015**, *10*, DOI 10.1371/journal.pone.0139347;b) J. D. Chulay, J. D. Haynes, C. L. Diggs, *Exp. Parasitol.* **1983**, *55*, 138–146.
[31] J. Van Meerloo, G. J. Kaspers, J. Cloos, *Methods Mol. Bio.* **2011**, *731*, 237–245.

Submitted: June 22, 2018
Accepted: August 27, 2018