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Vinyl sulfone-based inhibitors of trypanosomal cysteine protease rhodesain with improved antitrypanosomal activities

Huaisheng Zhang[a], Jasmine Collins[a], Rogers Nyamwihura[a], Olamide Crown[a,b], Oluwatomi Ajayi[a], Ifedayo Victor Ogungbe[a,*]

[a] Department of Chemistry, Physics, and Atmospheric Sciences, Jackson State University, Jackson, MS 39217, USA
[b] Department of Biochemistry, Federal University of Technology, Akure, Nigeria

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ABSTRACT

The number of reported cases of Human African Trypanosmiasis (HAT), caused by kinetoplastid protozoan parasite *Trypanosoma brucei*, is declining in sub-Saharan Africa. Historically, such declines are generally followed by periods of higher incidence, and one of the lingering public health challenges of HAT is that its drug development pipeline is historically sparse. As a continuation of our work on new antitrypanosomal agents, we found that partially saturated quinoline-based vinyl sulfone compounds selectively inhibit the growth of *T. brucei* but displayed relatively weak inhibitory activity towards *T. brucei's* cysteine protease rhodesain. While two nitroaromatic analogues of the quinoline-based vinyl sulfone compounds displayed potent inhibition of *T. brucei* and rhodesain. The quinoline derivatives and the nitroaromatic-based compounds discovered in this work can serve as leads for ADME-based optimization and pre-clinical investigations.

The Human African Trypanosomiasis (HAT), also known as sleeping sickness, is caused by two sub-species of *Trypanosoma brucei*, *T. b. gambiense* and *T. b. rhodesiense*. *T. b. gambiense* causes a chronic infection that is predominantly found in Central and West Africa while the infection caused by *T. b. rhodesiense* is acute in nature and localized to Eastern and Southern Africa.[1] Historically, lack of adequate and rapid diagnostic tools as well as lack of effective, safe, and accessible medicines to treat or prevent HAT resulted in the death of hundreds of thousands of people. In recent years, however, there has been a drastic reduction in the number of reported cases of HAT and infections caused by *T. b. gambiense* accounts for 97% of all HAT cases.[2] HAT caused by *T. b. gambiense* is targeted for elimination by the World Health Organization this year (2020). Nevertheless, there is a high probability for continued transmission of the parasite to humans from zoonotic reservoirs in both rural and urban areas in the coming future. In addition, limited access to primary healthcare services, especially in rural communities, makes it imperative to have safe, easily administrable, and effective curative or prophylactic agents for HAT. The approval of fexinidazole (**1**) as the first oral medicine to treat gambiense-HAT in late 2018, by the European Medicines Agency's Scientific Committee for Medicinal Products for Human Use (CHMP), represent a major step towards improving access to medicine and reducing hospitalization of HAT patients. Despite the effectiveness of fexinidazole in the treatment of gambiense-HAT, relapse has been observed in some patients. This suggests that the drug does not provide parasitological cure in all patients. In addition, fexinidazole induces nausea and vomiting, and its absorption is dependent on ingestion of food. These limitations demand both point-of-care observation and long-term monitoring for signs of recurrence by trained health staff. Because of these facts, a robust drug development pipeline for HAT is still essential (Fig 1).[3,4]

Our previous work on natural products-derived antitrypanosomal agents led to the discovery of a covalent inhibitor of rhodesain (**2**) that displayed moderate activity against *T. brucei*.[5] As a continuation of that work, a structure-activity relationship study was initiated to identify new antitrypanosomal compounds, with compound **2** serving as the starting point. The vinyl sulfone-based covalent warhead and the phenethyl side chain in compound **2** are prominent features of K777, an experimental anti-Chagas agent. The three major changes, as shown in Table 1 and outlined in Schemes 1 and 2, are i) replacing the phenethyl side chain, that occupies the P1 site in rhodesain's active site, with shorter/less bulky side chains (H, butyl, isobutyl, benzyl, and propyl groups), ii) replacing the quinoline ring with similar bicyclic motifs, and iii) replacing the quinoline ring with monocyclic heterocycles including nitroaromatic heterocycles.

The SAR revealed that replacing the phenethyl side chain with benzyl (**3**), isobutyl (**4**), propyl (**5**), butyl (**6**) or H (**7**) groups reduced

* Corresponding author.
E-mail address: ifedayo.v.ogungbe@jsums.edu (I.V. Ogungbe).

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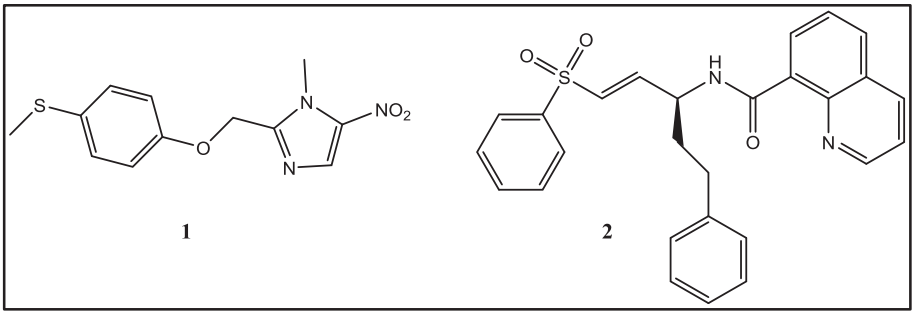


Fig. 1. Structures of fexinidazole (1) and previously reported covalent inhibitor of rhodesain (2).[5]

the antitrypanosomal activity of the analogues and they are generally less selective (*T. brucei*/Hep G2) than **2** (Table 1). Also, compounds **3** and **4** have much weaker inhibitory activity against rhodesain while compounds with propyl and butyl side chains, **5** and **6,** have comparable inhibitory activity against rhodesain as **2** (Table 1).

The results from rhodesain inhibition studies are consistent with previous work on analogues of K777, as inhibitors of cruzain and rhodesain, by Renslo and co-workers.[6] Renslo and co-workers previously reported that replacing a phenethyl group with a butyl group have very marginal impact on both enzyme inhibitory and antiparasitic activities. In this work, however, when the compounds' activities on trypanosomes and rhodesain are taken into consideration, the phenethyl side chain is the most selective (*T. brucei*/Hep G2) motif out of the six motifs investigated. Therefore, the phenethyl group was retained in our subsequent SAR studies.

The quinoline ring was then replaced with quinoxaline (**8**), as well as partially saturated quinoline derivatives such as methylated dihydroquinoline (**9**), methylated tetrahydroquinoline (**10**) and tetrahydroquinoline (**11**) rings. The compound bearing the quinoxaline motif, **8**, had similar inhibitory effects on *T. brucei* and rhodesain as **2** but it is less selective when compared to Hep G2 cells. However, compounds bearing the partially saturated and methylated dihydroquinoline and tetrahydroquinoline motifs (**9** and **10**) are more selective and have marginally higher activities against *T. brucei*. Paradoxically, compounds **9** and **10** have much weaker/slower inhibitory activity towards rhodesain (Table 1). Perhaps, the relative flexibility and bulkiness of the methylated and partially saturated rings in dihydroquinoline (**9**) and tetrahydroquinoline (**10**) provides selective binding to its target in *T. brucei*. In addition, it is possible that the partially saturated quinoline motifs in **9** and **10** are intrinsically less toxic than the bicyclic aromatic quinoline ring, as previously suggested for similar motifs.[7]

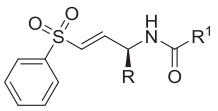
To further explore this series of vinyl sulfone-based compounds, the quinoline ring in **2** was replaced with monocyclic heterocycles (**12–17**). As shown in Table 1, the pyridine-based analog, **12**, was inactive against *T. brucei*, while the 1,4-dimethyl-1*H*-imidazole-based analog, **13**, had much weaker activity against *T. brucei*. The 1,3-thiazolidine-based compound, **14**, had similar potency against trypanosomes as **2**, but compounds **12–14** are generally less selective. Nevertheless, we found out that nitroaromatic-based compounds **16** and **17** are significantly more potent against *T. brucei* than **2,** and they both have an adequate degree of selectivity. It is well-established that protozoan parasites like trypanosomes and *Leishmania* are susceptible to nitroaromatics. In fact, the front-line therapies to treat HAT includes nitroaromatics such as nifurtimox, used in combination with eflornithine, and the recently approved fexinidazole (**1**).[8] Benznidazole, a nitroimidazole, is the front-line drug against Chagas disease. Another nitroimidazole derivative, DNDI-0690, is under clinical development for the treatment of visceral leishmaniasis by *DNDi*.[9] The susceptibility of pathogenic protozoans such as *T. brucei*, *T. cruzi* and *Leishmania* spp. to nitroaromatics is linked to selective bioactivation by type I nitroreductase.[10–13] It is worth pointing out, however, that despite the clinical use of nifurtimox, benznidazole and fexinidazole in the treatment of Chagas disease and HAT, concerns about the inherent mutagenic potential of nitro groups remain valid and require careful evaluation.[14]

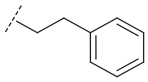
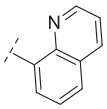
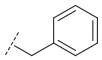
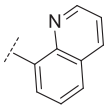
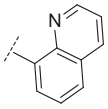
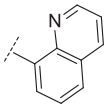
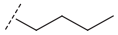
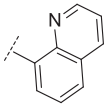
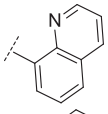
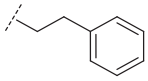
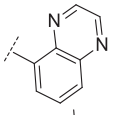
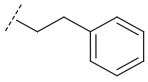
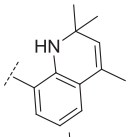
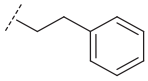
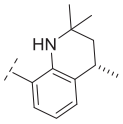
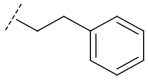
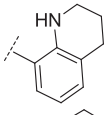
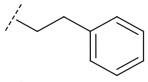
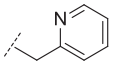
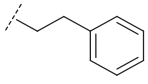
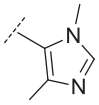
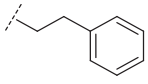
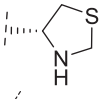
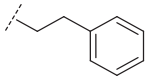
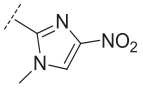
Also, development of resistance to nifurtimox and benznidazole by *T. cruzi*, and the discovery of cross-resistance to fexinidazole by nifurtimox-resistant *T. brucei* has led to suggestions that new antitrypanosomal nitroaromatics should only be used as one of the active ingredients in combination therapies.[8,15–19] Like combination therapies, dual-acting agents also present unique opportunities and challenges.[20,21] The potential *dual-acting* (inhibition of trypanosomal cathepsin L and trypanocidal action of the nitroaromatic moiety) nature of compounds **16** and **17** provide a unique opportunity to deliver two warheads in the same molecule. Dual-acting nitroaromatic compounds can potentially suppress or delay development of drug resistance to nitroaromatic-induced trypanocidal activity. It is important to point out that dual-acting compounds can potentially have a higher degree of off-target toxicity. The possibility that compounds **16** and **17** could act as dual-acting agents needs validation. Also, the compounds require multiparameter optimization of selectivity and pharmacokinetic properties. Boechat and co-workers have already shown that mutagenicity of nitro groups in trypanocidal nitroimidazoles depends on the location of the nitro group on the imidazole ring.[22] Therefore, the potential mutagenicity of nitroaromatic-based dual-acting compounds can conceivably be investigated and eliminated or minimized through systematic structure-activity relationship studies.

In order to understand the key structural features important for the inhibition of rhodesain by compounds that showed significant inhibition of the protease like **11** and **17** and those with much weaker inhibition like **9** and **10**, covalent docking was used to predict the top binding poses of each compound. As shown in Figs. 2 and 3 below, the phenethyl and phenyl sulfone moieties were predicted to occupy the P_1 site and P_1' site, respectively. This is consistent with the orientations of the two moieties in the rhodesain-K777 complex (**PDB 2P7U**) reported by Brinen and co-workers.[23] The dihydroquinoline moiety in **9** and the tetrahydroquinoline moiety in **10** and **11** were predicted to bridge the P_2 and P_3 sites.

The nitrofuran ring in **17** is predicted to occupy the P_2 site and it is solvent exposed. When compared with **9** and **10**, compound **11** is a better inhibitor of rhodesain. It is likely that the three methyl substituents on the partially saturated quinoline ring precludes efficient binding of **9** and **10** to rhodesain when compared to **11**. Also, the hydrophobicity of solvent-exposed methyl groups in **9** and **10** possibly negates the formation of solvent-stabilized rhodesain-inhibitor complex.

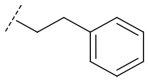
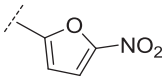
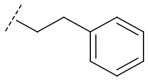
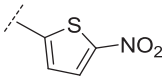
Table 1
Antitrypanosomal activity of compounds **2–19**. Compounds were assayed as described in the Supporting information. EC_{50} is the concentration that caused half-maximal trypanosomal viability. CC_{50} is the concentration that caused half-maximal cellular viability. SI is the selectivity index (CC_{50}/EC_{50}). [a]Percentage inhibition of rhodesain at 10 μM after 1 h.

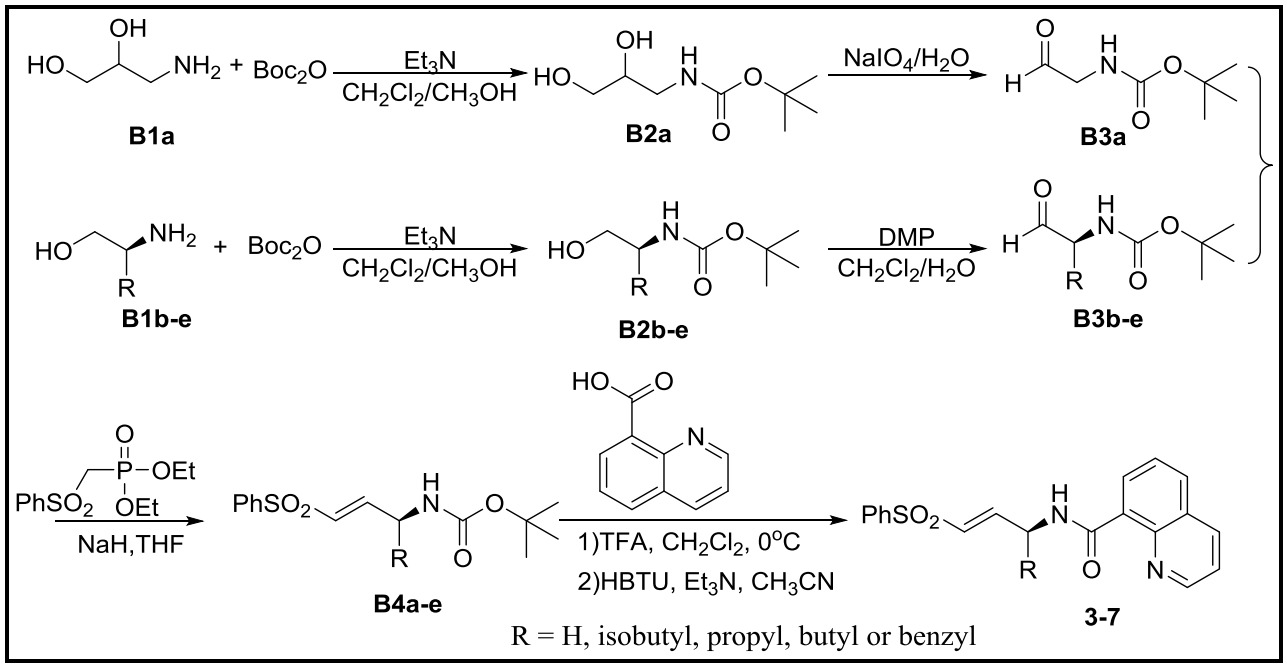


Entry	R	R^1	*T. brucei* EC_{50} (μM)	Hep G2 CC_{50} (μM)	SI	Rhodesain K_{inact}/K_i (M^{-1} s^{-1}) (% Inhibition at 10 μM)[a]
2			5.97 ± 0.12	> 80.00	13.40	99 (100 ± 1.13)[a]
3			11.66 ± 0.59	25.30 ± 1.14	2.16	−(15.27 ± 0.91)[a]
4			7.98 ± 1.11	15.11 ± 1.07	1.89	−(20.12 ± 7.48)[a]
5			9.23 ± 2.75	> 80.00	> 8.66	82.13 (93.53 ± 0.48)[a]
6			6.20 ± 0.88	36.50 ± 1.12	6.38	107.50 (96.23 ± 0.17)[a]
7	H		14.34 ± 2.29	55.47 ± 1.07	3.86	−(58.11 ± 4.39)[a]
8			5.90 ± 1.07	39.62 ± 1.04	6.71	292.6 (99.11 ± 0.06)[a]
9			1.88 ± 0.84	> 300.00	> 159.57	−(26.24 ± 5.79)[a]
10			3.02 ± 0.27	> 300.00	> 99.33	−(17.38 ± 8.03)[a]
11			6.30 ± 0.71	> 160.00	> 25.00	245.1 (100.5 ± 0.77)[a]
12			> 20	37.89 ± 5.45	–	−(69.92 ± 3.98)[a]
13			19.56 ± 5.83	34.43 ± 1.10	1.76	−(81.58 ± 0.85)[a]
14			6.94 ± 1.29	77.75 ± 1.08	11.20	161.3 (99.34 ± 0.05)[a]
15			7.04 ± 0.13	28.78 ± 1.08	4.08	59 (91.94 ± 1.04)[a]

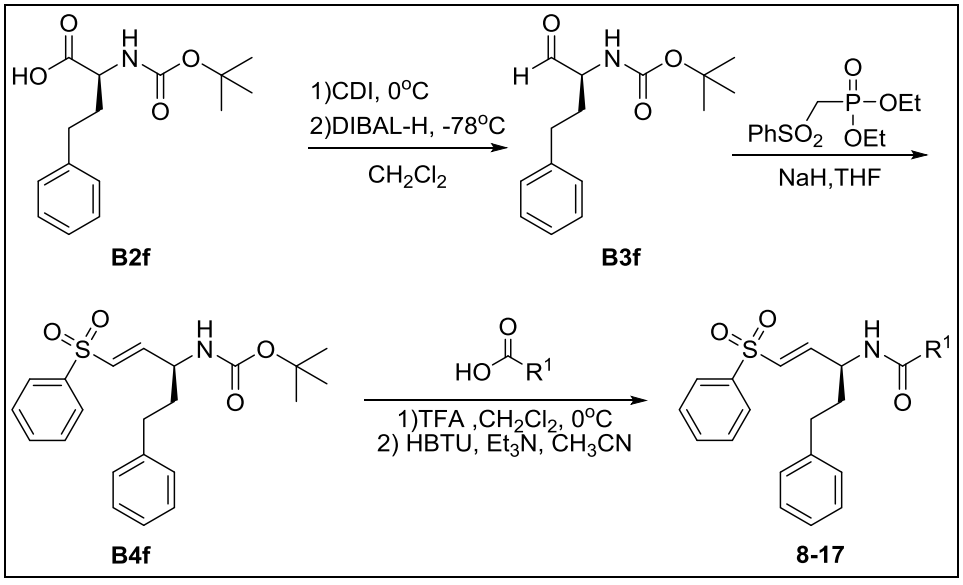
(*continued on next page*)

Table 1 (*continued*)

Entry	R	R^1	*T. brucei* EC_{50} (μM)	Hep G2 CC_{50} (μM)	SI	Rhodesain K_{inact}/K_i (M^{-1} s^{-1}) (% Inhibition at 10 μM)[a]
16			0.11 ± 0.01	5.17 ± 1.17	47.00	67 (78.08 ± 0.69)[a]
17			0.69 ± 0.05	12.43 ± 1.01	18.01	1297 (100.2 ± 0.04)[a]
Suramin	–	–	0.004 ± 0.00	–	–	–
Podophyllotoxin	–	–	–	< 0.5	–	–
E-64	–	–	–	–	–	(100.00 ± 0.01)[a]



Scheme 1. Synthesis of target compounds **3–7**. See Supporting information for description.



Scheme 2. Synthesis of target compounds **8–17**. See Supporting information for description.

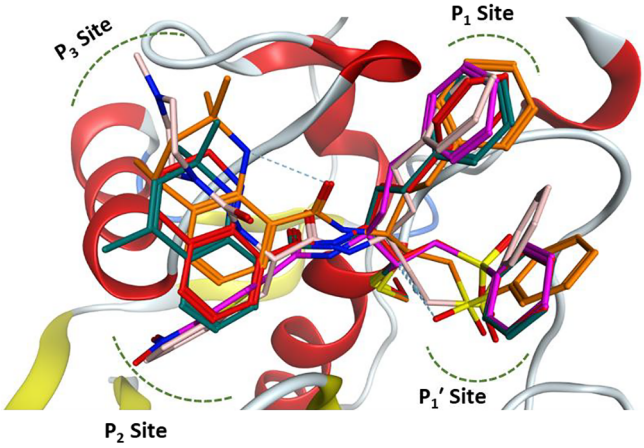


Fig. 2. Modelled complex of rhodesain and compounds 9 (green), 10 (orange), 11 (red), 17 (purple) and co-crystallized ligand K777 (pink). The blue dash depicts H-bond interaction.

In summary, a series of vinyl sulfone-based compounds were investigated in this work. In general, there is no clear correlation between enzyme inhibition and parasite growth inhibition. Although compounds with SI > 6 (T. brucei vs Hep G2) tend to have higher percentage inhibition of rhodesain at 10 μM. Compounds **9** and **10** were found to have the most selective antitrypanosomal activities but are relatively weak inhibitors of rhodesain while the nitrofuran and nitrothiophene-derivatives, **16** and **17**, have potent antitrypanosomal activity. Compounds **16** and **17** present an opportunity for the exploration of potential dual-acting agents against trypanosomes. Ongoing and future work will focus on using ADME experiments to determine any PK/PD shortcomings, synthesis of analogs (**9**, **10**, **16** and **17**) to address any ADME issues, followed by *in vivo* efficacy studies in mice models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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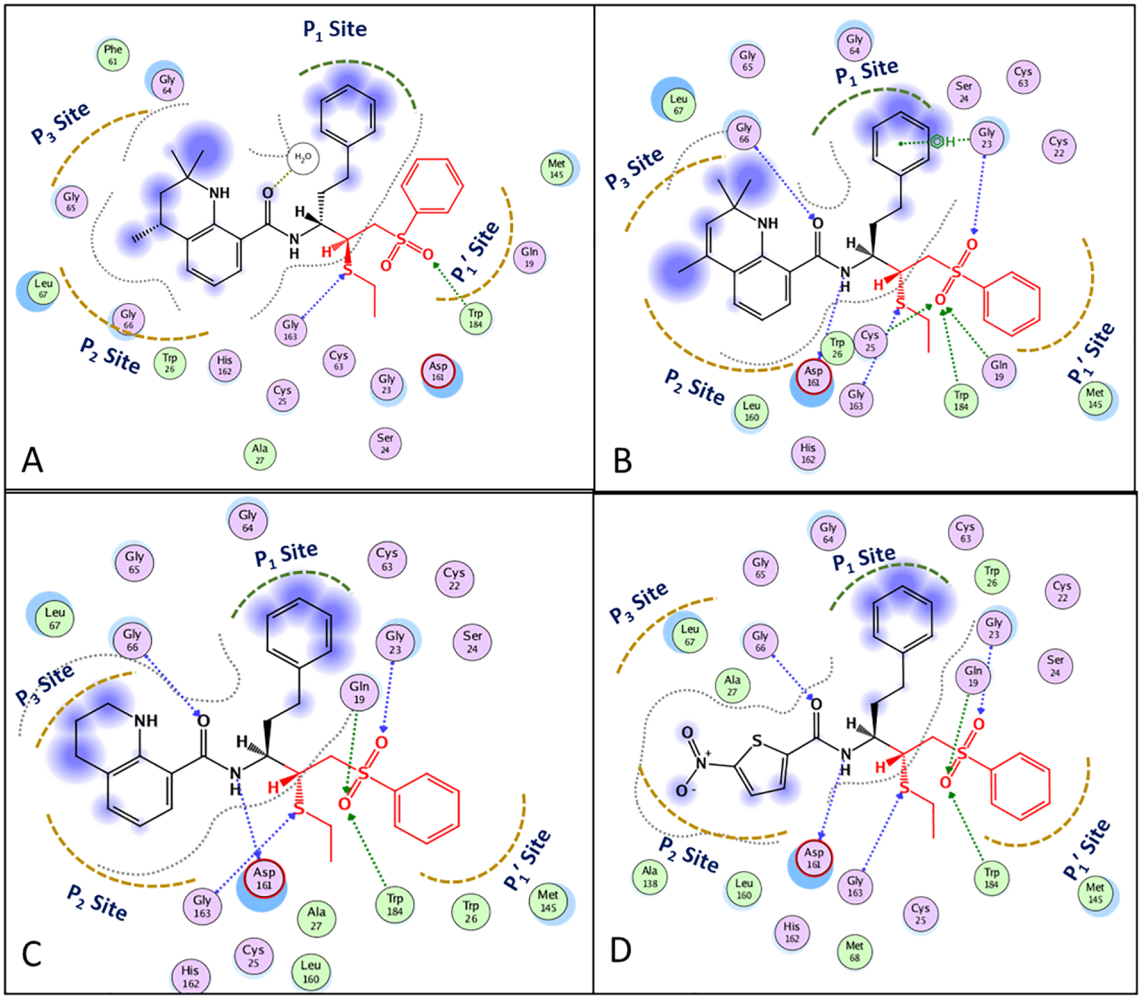

Fig. 3. Modelled complexes of rhodesain and compounds 9 (A), 10 (B), 11 (C) and 17 (D). H-bond interactions are depicted as the dash arrows. The 2D depictions of the proximity of active site residues to the compounds are shown as gray contours while the extent of exposure of the compound's atoms to solvent is shown as purple hues.

Appendix A. Supplementary data

Supplementary data (details of bioassays, synthesis and compound characterization) to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127217.

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