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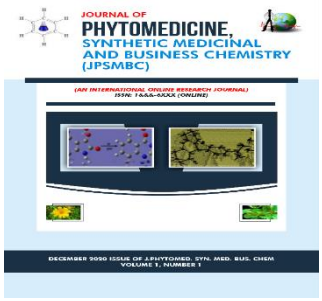

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Synthesis of a Spiro-quinoxaline Derivative from Quinoxalinone and Oxalyl Chloride and In Silico Approach to Antimalarial Properties of its Acylhydrazone Analogs

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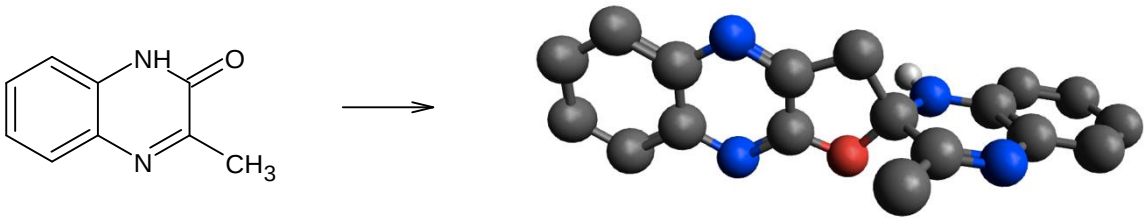
Graphical Abstract



Abstract

Background- Quinoxaline derivatives have occupied a prominent place among various classes of heterocyclic compounds, with many found to display a broad spectrum of biological activities including anthelmintic, antibacterial, anticancer, anti-inflammatory, antimalarial, antiviral and as kinase inhibitors. Malaria disease is one caused by a plasmodium parasite and known to occur mostly in poor, tropical and subtropical areas of the world. Some countries lack the resources to treat and control the disease with early diagnosis.

Methods- A spiro-furoquinoxaline derivative, 3'-methyl-1'*H*,3*H*-spiro[furo[2,3-*b*]quinoxaline-2,2'-quinoxaline] **1**, was synthesized via thermal reaction of a methylquinoxalinone with oxalyl chloride and fully characterized based on FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

The *Plasmodium falciparum* transketolase (*Pf*Tk) is a suitable/novel target in antimalarial chemotherapy. With a view to exploit the spiro-furoquinoxaline framework as a lead in antimalarial chemotherapy development, the binding of modelled 3'-carbonylhydrazone derivatives of **1** with the *Plasmodium falciparum* transketolase (PfTk) was studied, using computational techniques to conduct a virtual screening of a local library of the modelled compounds and filter out the compounds that are potential high inhibitors of the *P. falciparum* transketolase and with optimum pharmacokinetic properties to facilitate oral bioavailability.

Results-- Acylhydrazones are an important class of heterocyclic compounds with varied biological activities and suitable for new drug development. A spiro-furoquinoxaline derivative has been synthesized in 54 % yield. A series of its modelled 3'-acylhydrazono derivatives were subjected to virtual screening by molecular docking with *P. falciparum*

transketolase and ADME analysis. Three compounds were found to have high binding affinity for the target protein (-8.5 to -10.3 kcal/mol) and with the best pharmacokinetic profile and may be considered as lead compounds for further development as potential antimalarial agents.

Conclusion- This study highlights the synthesis of 3'-methyl-1'*H*,3*H*-spiro[furo[2,3-*b*]quinoxaline-2,2'-quinoxaline]. The identified three modelled compounds, (E)-N'-(naphthalen-1-ylmethylene)-1'H,3H-spiro[furo[2,3-b]quinoxaline-2,2'-quinoxaline]-3'-carbohydrazide (5), (E)-N'-(2-(allyloxy)benzylidene)-1'H,3H-spiro[furo[2,3-b]quinoxaline-2,2'-quinoxaline]-3'-carbohydrazide (82) and (E)-N'-(4-butylbenzylidene)-1'H,3H-spiro[furo[2,3-b]quinoxaline-2,2'-quinoxaline]-3'-carbohydrazide (105) may be a starting point in the rational design of inhibitors of *Plasmodium falciparum* transketolase (*Pf*Tk) and possible antimalarial agents.

Keywords: synthesis; spiro-furo[2,3-*b*]quinoxaline; molecular docking; malaria; transketolase.

Introduction

Malaria disease is one caused by a plasmodium parasite, typically transmitted to humans via the bites of infected mosquitoes, when released into the bloodstream. It can also be transmitted from mother to baby at birth or through the use of shared needles, organ transplant or a transfusion. *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale and Plasmodium knowlesi* are the five kinds of malaria parasites that can infect humans [1]. However, *Plasmodium falciparum* is the predominant parasite species that is most likely to cause severe malaria and death.

Malaria is known to occur mostly in poor, tropical and subtropical areas of the world. According to the World Health Organization (WHO) [2], there were about 241 million cases of malaria worldwide, with an estimated number of malaria deaths of 627 000 in 2020 and Africa as home to about 95 % of malaria cases and deaths. Nigeria had the highest number of global malaria cases (27 % of global malaria cases) in 2019 and accounted for the highest number of deaths (23 % of global malaria deaths). However, it is to be noted that the problem of malaria is not restricted to tropical countries solely, but of world-wide concern, as a result of the volume of international travel [3].

Many of the compounds (for example, quinine, artemisinin) for the treatment of malaria are derived from natural sources (including plants, animals and microbes) and many of them are N-containing compounds (alkaloids). For example, between 2010-2017, out of about 450 isolated natural products showing antiplasmodial property, about 32 % are alkaloids [4]. Quinine and artemisinin are the two most successful antimalarial drugs isolated from medicinal plants (*Cinchona officinalis* and *Artemisia annua*, respectively). Some other examples of natural, semi-synthetic and synthetic antimalarial drugs include artesunate, artemether, chloroquine, amodiaquine, hydroxychloroquine, piperaquine, primaquine, tafenoquine, mefloquine, febrifugine and atovaquone (in combination with proguanil).

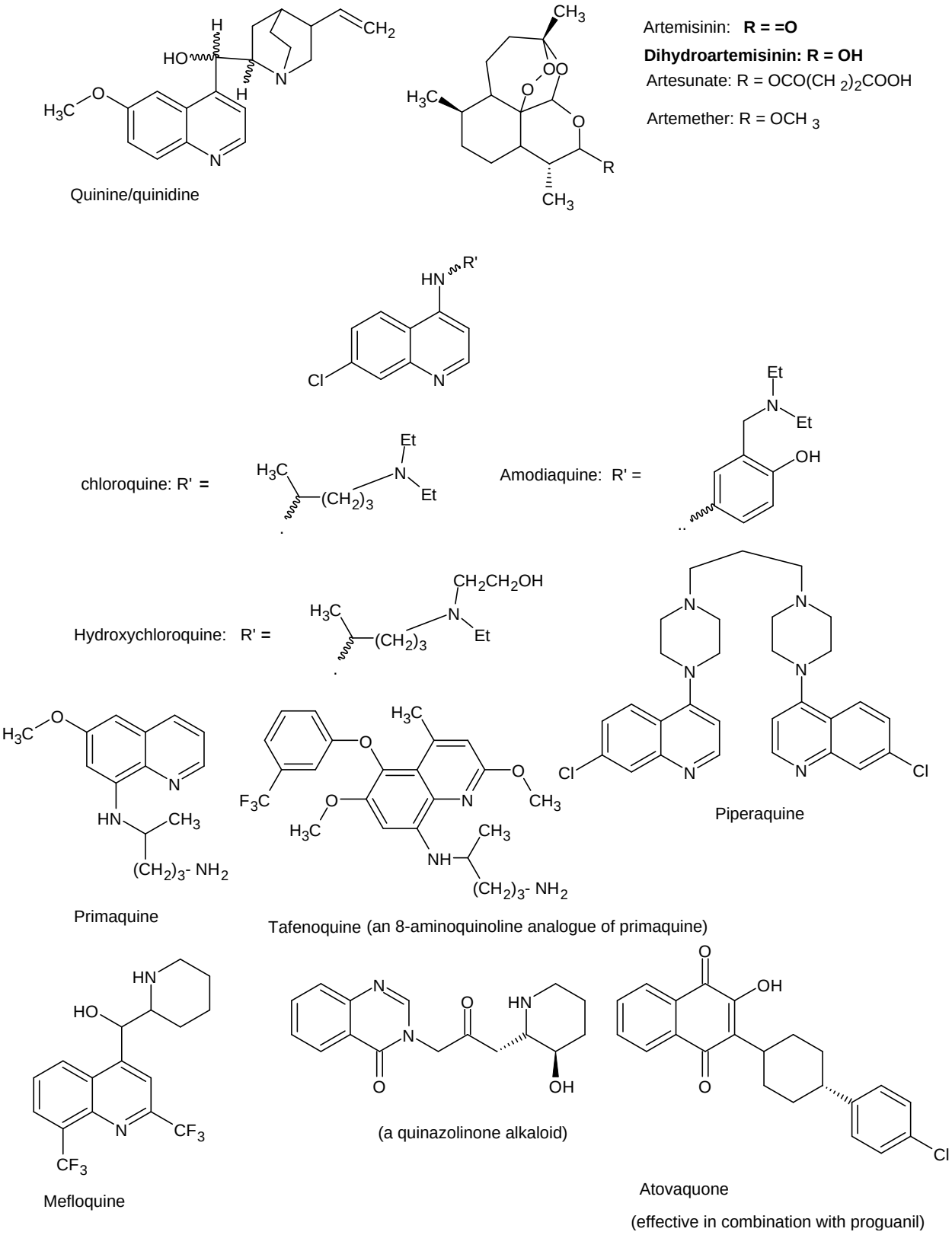


Fig. 1. Structures of some examples of natural, semi-synthetic and synthetic antimalarial drugs.

Nitrogen-based heterocyclic compounds form an integral part of many pharmacologically active molecules, thus their prominence in the fields of organic-medicinal chemistry and pharmaceutical industry [5]. A class of N-heterocyclic compounds is the quinoxalines having prominent pharmacological effects like antifungal, antibacterial, antiviral, and antimicrobial properties. Some quinoxaline and quinoxaline 1,4-di-N-oxide analogs of chalcones have been reported to be active against *Plasmodium falciparum* strains [6]. Also, fused quinoxalines, such as azolo-and pyrrolo-quinoxaline, are known to exhibit antibacterial, anticancer, anti-obesity, antiparasitic, antiviral, cardiomodulator, immunomodulator and neuromodulator activities and PARP-1 inhibitors [7, 8, 9]. In addition, Shekhar and co-workers have reported the antimalarial activity, of a series of novel N-alkyl- pyrido-quinoxaline derivatives, in vitro against chloroquine sensitive (3D7) and drug resistant (Dd2) strains of *Plasmodium falciparum*, with some of them shown to be more potent than their structural standard analog, ciprofloxacin [10].

On the other hand, fused furo-N-heterocycles, for example, furo-quinoxalines, have not been explored extensively for their potential biological activities, perhaps as a result of their limited or cumbersome accessibility. Some furo[2,3-b]quinoxaline derivatives have been reported to show encouraging pharmacological properties in vitro/in vivo, including possession of strong anti-inflammatory activity in chronic inflammatory models and moderate analgesic activity compared to the reference drug [11].

Efforts to control human malaria via drugs have been thwarted by the emergence of *Plasmodium falciparum* (malaria) parasite resistance to the commonly used antimalarial drugs (i.e. reduced clinical effectiveness). This growing resistance to current antimalarials poses a grave threat to human health (malaria control). Hence, there is a great need to discover and design new antimalarial compounds -showing a greater safety and efficacy profile. It is also important that the new drugs being developed target other biochemical pathways in the *plasmodium* apart from those that the current antimalarial drugs target because of the mutations that have already occurred that confer drug resistance to the *plasmodium*. In this study therefore, novel fused heterocyclic nitrogen compounds would be modelled and investigated as potential inhibitors of the *Plasmodium falciparum* transketolase (*Pf*Tk). The transketolase is a pivotal enzyme of the non-oxidative branch of the pentose phosphate pathway (PPP) and performs different functions in the malaria parasite including

pentose sugar supply for nucleotide synthesis, helps in replication and survival of the parasite [12, 13]. Moreover, the biochemical analysis of *Pf*Tk shows least homology with its human host [14]. All these make it a potential target for an antimalarial drug discovery effort. Potential transketolase inhibitors are yet to be developed and the major hindrance is the inability to get a crystalline form of the protein in order to get coordinates that may be used as target for an *in-silico* study as well as the difficulty associated with getting pure forms of the protein in reasonable quantities for a follow-up *in-vitro* study. Furthermore, a common problem that is associated with synthetic compounds is their inherent toxicity and low bioavailability. This study therefore seeks to identify some new potential *P. falciparum* transketolase inhibitors using computational techniques to model the transketolase protein, conduct a virtual screening of a local library of modelled compounds and filter out the compounds that are potential high inhibitors of the *P. falciparum* transketolase and with optimum pharmacokinetic properties to facilitate oral bioavailability.

Although efforts have been made to develop an effective malaria vaccine, stage- and species-specific short-lived immunity crippled these efforts [15]. The malaria parasite indeed has a remarkable ability to evade the immune system [16]. Therefore, antimalarial chemotherapy will be a mainstay for the treatment of malaria infection in the wake of the unavailability of a long-lasting vaccine protection [15].

2. Materials and methods

All solvents and chemicals were commercially available from BDH Chemicals Ltd and Sigma-Aldrich and used without further purification. The melting point was determined on a Gallenkamp (variable heater) melting point apparatus, uncorrected. The progress of the reactions was followed up by the TLC technique (silica gel 60 F_{254} tlc sheets). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Jeol-JNM-GX 400-MHz spectrometer, at the University of Konstanz, Germany, using TMS as an internal standard; chemical shifts are expressed as δ and DMSO-d_6 as solvent. Mass spectra were recorded on Finnigan MAT 312 spectrometer, in m/z (rel. %) Elemental analysis was carried out at the Microanalytical Center at the University of Konstanz, Germany.

Synthesis of 3-methylquinoxaline-2(*1H*)-one

Ethyl pyruvate (5.1 ml, 0.05 mol) and 2-aminoaniline (5.0 g, 0.05 mol) were dissolved in ethanol (80 mL), containing few drops of HCl (4 N), in a beaker and covered with a glass. The mixture was pulse-irradiated in a domestic microwave oven (with an emitted power of 400 W) for a total of 10 minutes and the clear solution was left to cool to give silvery white crystal of the quinoxalinone (6.3 g, 79 %). ^{1}H-NMR (DMSO-d_6): δ 2.41 (CH_3), 7.27 (2H, m, aromatic-H), 7.47 (1H, t, aromatic-H), 7.69 (1H, d, J = 7.7, aromatic-H), 12.32 (1H, s, N-H). ^{13}C-NMR (DMSO-d_6): δ 20.46 (CH_3), 115.16, 122.90, 127.80, 129.17, 131.61, 131.87, 154.87, 159.11. Mass spectrum (m/z (rel. int.)): 160 (M^+, 98.5%), 132 ([*M*- C=O]$^+$, 100%), 105 (34%), 104 (37%), 90 (43%), 77 (35%), 63 (46%).

Synthesis of 3'-methyl-1'*H*,3*H*-spiro[furo[2,3-*b*]quinoxaline-2,2'-quinoxaline] 1'

1.08 ml (0.012 mol) of oxalyl chloride was added to a solution of 3-methylquinoxalin-2-one 2.0 g (0.012 mol) in benzene (60 mL) and the resulting mixture was refluxed for 15 h. The solvent was removed by distillation and the black residue was extracted with chloroform. The crude chloroform extract was subjected to column chromatography (silica gel, toluene/ethyl acetate 2:1) to give the pure product **1** (0.98 g, 54 % yield), m.p. 269-270 °C. ^{1}H-NMR (DMSO-d_6): δ 1.41 (s, 3H, CH_3), 3.28 (1H, d, J = 18.5, C-H-3), 3.82 (1H, d, J = 18.5, C-H-3), 7.08-7.23 (3H, m, aromatic-H), 7.44 (1H, t, J = 7.4, aromatic-H), 7.57 (1H, t, J = 7.48, aromatic-H), 7.70 (1H, d, J = 8.0, aromatic-H), 7.81 (1H, d, J = 7.9, aromatic-H), 8.50 (1H, d, J = 7.1, aromatic-H), 10.93 (1H, s, N-H). ^{13}C-NMR (DMSO-d_6): δ 23.53 (CH_3), 36.72 (CH_2), 62.21 (spiro-C), 115.64, 119.87, 122.78, 124.20, 124.30, 125.00, 126.28, 127.92, 128.87, 128.98, 137.93, 140.39, 150.15, 152.08, 168.35. Mass spectrum (m/z (rel. int.)): 302 (M^+, 72%), 287 ([*M* - Me]$^+$, 100%), 273 (20%), 259 ([*M*- Me - C=O]$^+$, 53%), 131 (18%), 130 (20%), 102 (18%), 51 (17%). Anal. Calc. for $C_{18}H_{14}N_4O$ (302.33): C 71.51, H 4.67, N 18.53; found: C 71.38; H 4.79, N 18.30.

Virtual screening by molecular docking with *P. falciparum* transketolase and ADME prediction

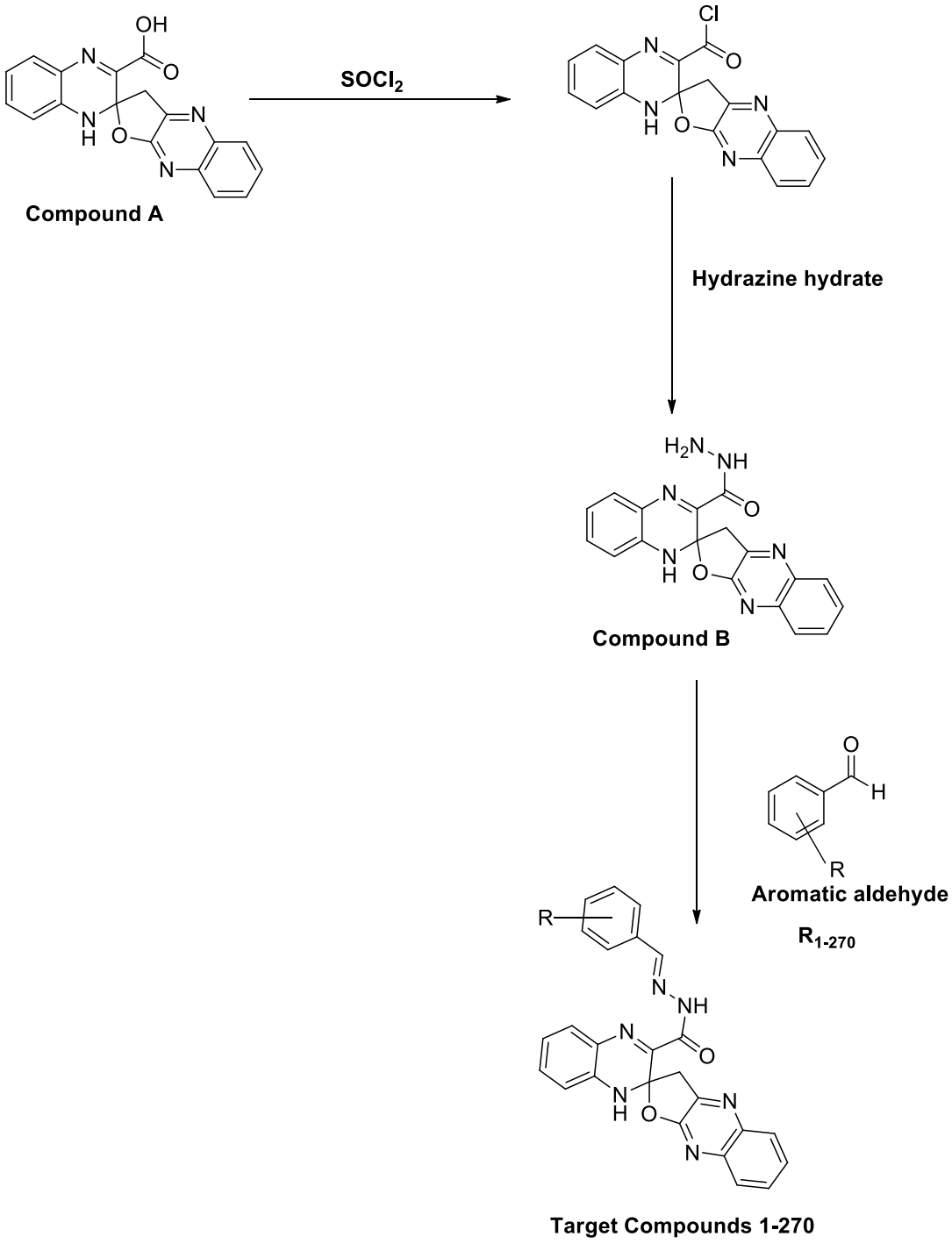
The 3D structure of the PfTk was obtained by homology modelling and optimized by molecular dynamics simulation [17]. The protein structure saved as a pdb file was used as target protein in the virtual screening. The virtual screening was done using different filters beginning with molecular docking followed by a drug-likeness assessment and finally ADME predictions. The molecular docking of the modelled compounds was done with a vina based

perl script on an ubuntu machine. The 3D structures of the compounds were prepared and minimized using the Chem3D Ultra software. After which they were docked with the transketolase protein from *P. falciparum.* The co-factor (thiamine pyrophosphate) of the transketolase was also docked with the protein as reference as well as a synthetic analog of the cofactor (name of analog) and a known inhibitor (oxythiamine). The cutoff binding energy was set as -7.1 kcal/mol and reduced the number of compounds screened from 270 to 170 with the compounds having binding energy ranging from -7.7 to -10.3 kcal/mol. The co-ordinates of the remaining 170 compounds were submitted on the preADMET server to determine their druglikeness using the Lipinski's rule of five to prune down further after which the remaining compounds were resubmitted on the server to predict their pharmacokinetic properties which was eventually used to zero in on the few compounds selected for further study. The two precursors (compound A and compound B) were also docked with the target protein and assessed for drug-likeness and ADME properties for comparison with target compounds.

3. Results

In the present work, the starting material, 3-methylquinoxalin-2-one was prepared from the condensation of ortho-phenylenediamine with ethyl pyruvate. Its mass spectrum showed a molecular ion peak m/z at 160.0, while the IR spectrum revealed the presence of carbonyl group at 1668 cm $^{-1}$. The ^{1}H-NMR spectrum showed signals assigned for aromatic protons, one N-H and one CH_3 protons, while the ^{13}C-NMR spectrum showed nine signals as expected for the structure. 3'-methyl-1'*H*,3*H*-spiro[furo[2,3-*b*]quinoxaline-2,2'-quinoxaline] (**1'**) was easily prepared through the reaction of 3-methylquinoxalin-2-one with oxalyl chloride in benzene. The molecular structure of the spiro compound **1'** was interpreted from its FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometric analysis.

The mass spectrum showed a molecular ion peak m/z at 302.1, which agrees with the suggested structure. The ir spectrum of the product showed absence of carbonyl amide expected around 1680 cm$^{-1.}$ The ^{1}H-NMR of the spiro product **1'** exhibited a singlet signal assigned to NH group at δ = 10.93, a methyl group as a singlet signal at δ 1.41, in contrast to the starting quinoxalinone with the methyl signal showing at δ 2.41 and the aromatic protons at δ = 7.05–8.49 ppm.



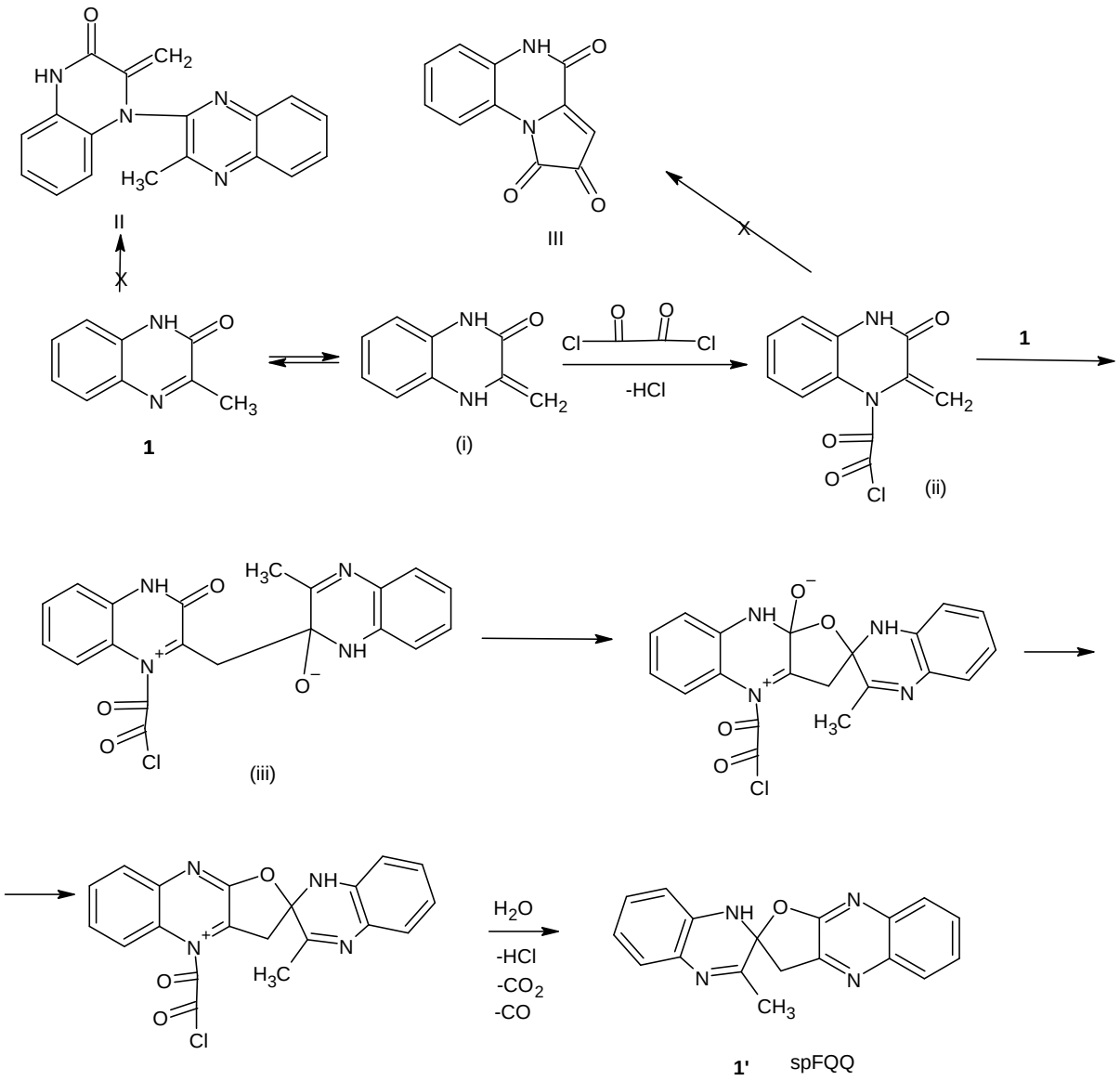
Scheme 1. Suggested synthetic route to the target compound

The ^{13}C-NMR of the starting material revealed 9 signals as expected, while the product **1'** revealed 18 signals, suggesting dimerization reaction. The mass spectrum of **1'** showed the molecular mass to be 302 indicating that two molecules of the starting material reacted, with loss of a water molecule. In the ^{13}C-NMR, the carbonyl amide signal, which appeared at δ 159.11 in the starting material, disappeared in the product, with appearance of new signals at δ 36.7 (CH_2) and 62.21 (spiro C). The diastereotopic CH_2 protons appeared as doublet of doublet at δ 3.82 and 3.28, similar to those of spiro[1-benzofuran- 2,1'-cyclohexanes] [18].

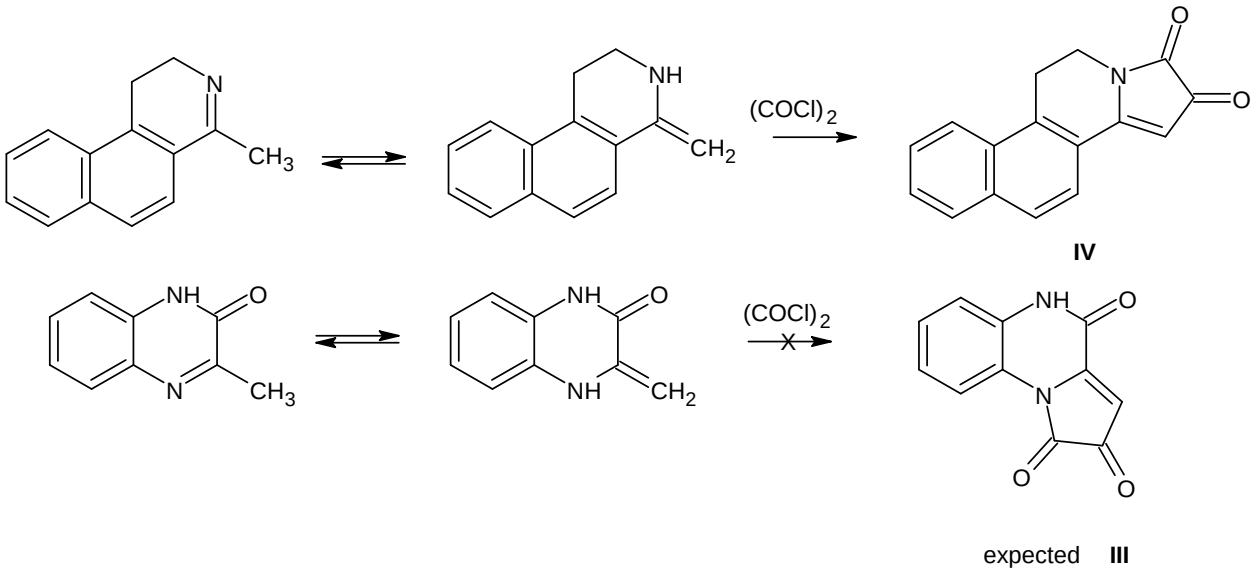
The synthetic pathway to the modelled compounds is shown in Scheme 1, while the results of the virtual screening are summarized in Table 1.

4. Discussion

A major concern with the control of malaria is the emergence of resistance to artemisinin and its derivatives and other antimalarial drugs. Hence the urgent need to develop new antimalarials. The suggested mechanism in support the formation of the spiro compound **1'** is as shown in Scheme 2. Presumably the reaction proceeded via the N-acylation of the enamine tautomer of the starting material, 3-methylidene-3,4-dihydroquinoxalin-2(1H)-one (**i**) to give the N-(oxo)acetyl chloride (**ii**), which then reacted with another molecule of the starting material **1** at the carbonyl portion of the lactam functional group to give the 'onium dihydroquinoxlin-oxide (**iii**). This, with appropriate orientation, underwent cyclization, followed by loss of water, carbon monoxide and dioxide and HCl to give the isolated compound **1'.** The dimer **II,** with the same molecular mass as **1',** was not observed. Heterocyclic compounds containing the methylimine group, -N=C-CH_3, in the ring, for example, 4-methyl-1,2-dihydrobenzo[f]isoquinoline, are known to react with oxalyl chloride to give a fused 1,2-dioxo five-membered ring product (**IV**), with the reaction going via the 4-methylidene tautomer (Scheme 3) [19]. A similar reaction was expected between the starting material, 3-methylquinoxalin-2-one, and oxalyl chloride to give pyrrolo[1,2-a]quinoxaline-1,2,4(5H)-trione **III**. However, compound **III** was not observed (ir and nmr evidence), as the hydrogen of the methylene O=C-CH=C(3) group expected as a 1H singlet signal at around δ 6.0 in the ^{1}H NMR was missing and no carbonyl signals were observed in the ^{13}C-NMR.



Scheme 2. The Suggested mechanism for the synthesis of 3'-methyl-1'*H*,3*H*-spiro[furo[2,3-*b*]quinoxaline-2,2'-quinoxaline] **1'.**



Scheme 3. The reaction of 4-methyl-1,2-dihydrobenzo[f]isoquinoline with oxalyl chloride [19].

Virtual Screening

After the molecular docking and selecting the 170 compounds with high binding affinity and submitting to the preADMET server, 112 compounds were found to be druglike – the others had two violations (molecular weight and log P). The remaining 112 compounds were also submitted for pharmacokinetic property predictions on the preADMET server. The blood brain barrier penetration, Human Intestinal Absorption, cell permeability, plasma protein binding and interactions with some cytochrome P450 enzymes were predicted (see Table 1). All the compounds have similar profiles for BBB, PPB, and HIA therefore the major filter used for pruning down was the cell permeability. The two models used were Caco2 and MDCK models of which the variation in the caco2 was not as distinct as that of the MDCK even though the observed trend was similar. Thus, compounds with medium permeability (less than 25 nm/sec is low permeability while a value of between 25-500 nm/sec is medium permeability) were selected. 21 compounds were selected which were then assessed for their interactions with cytochrome P450 enzymes in order to select the compounds that would be least prone to rapid metabolism during the first pass mechanism. All the compounds that were predicted to be substrates of CYP3A4 were dropped while those ones that were weak substrates/non-substrates were selected which reduced the number of compounds from 21 to 12. And of the 12, three of them are inhibitors of CYP3A4 as well as being weak substrates which may cause

Table 1: Summary of results from virtual screening

Name of Aldehyde	Code	BA‡ (-kcal/mol)	BBB	Caco2 nm/sec	CYP 3A4 i*	CYP 3A4 S**	HIA (%)	MDCK nm/sec	Pgp	PPB (%)
Compound 1′	**A**	7.9	0.898	16.02	Non	Non	97.47	267.72	I	99.99
Hydrazide intermediate	**B**	8.4	0.521	6.495	Non	Non	92.76	171.88	Non	89.56
1-naphthaldehyde	**5**	10.3	0.074	8.86	I	W	96.78	43.48	I	93.5
Picolinaldehyde	**18**	8.9	0.216	5.48	Non	S	95.43	90.31	Non	100.00
3-chlorobenzaldehyde	**22**	8.8	0.151	21.75	Non	W	96.22	56.77	I	93.21
3-cyclohexene-1-carboxaldehyde	**33**	8.5	0.137	4.66	Non	W	95.32	84.11	Non	96.04
m-tolualdehyde	**34**	8.9	0.136	6.62	Non	W	95.73	63.98	I	99.73
4-ethylbenzaldehyde	**49**	8.6	0.161	9.24	Non	W	95.86	48.92	I	98.28
3-cyanobenzaldehyde	**56**	8.8	0.168	2.67	Non	W	95.77	43.95	I	100.00
3-methoxysalicylaldehyde	**57**	9.1	0.067	19.83	Non	S	95.53	30.48	Non	98.01
3,5-dimethylbenzaldehyde	**61**	8.7	0.200	7.82	Non	S	95.86	55.14	I	98.87
3,4-dimethylbenzaldehyde	**65**	8.8	0.167	9.18	Non	S	95.86	28.22	I	99.34
3-methoxybenzaldehyde	**74**	8.7	0.067	19.84	Non	S	95.53	30.48	Non	98.01
2-allyloxybenzaldehyde	**82**	9.5	0.182	35.31	I	W	95.94	94.25	Non	93.33
3-ethoxybenzaldehyde	**93**	8.6	0.073	20.07	I	S	95.62	44.08	Non	97.13
4-nbutylbenzaldehyde	**105**	8.5	0.470	13.99	I	W	96.11	58.83	I	95.62
3-(4-methoxy-phenyl)-propionaldehyde	**130**	8.6	0.036	19.02	Non	S	95.75	40.30	I	95.82
3-(3-chloro-phenyl)-propionaldehyde	**142**	8.8	0.133	23.01	Non	W	95.46	63.99	I	91.56
Cuminaldehyde	**184**	8.6	0.218	11.40	Non	W	95.99	46.96	I	97.09
Trans-cinnamaldehyde	**185**	8.9	0.217	11.40	Non	W	95.99	46.96	I	97.09
5-ethynyl-1,3-benzenedicarboxaldehyde	**213**	9.3	0.079	10.32	I	S	95.70	38.62	I	96.91
m-phthaldehyde	**245**	8.6	0.129	9.728	Non	S	95.56	26.65	Non	99.19
4-methylbenzaldehyde	**264**	8.7	0.105	7.91	Non	W	95.73	32.33	I	99.99

*** CYP3A4_i = Inhibitor to CYP3A4**

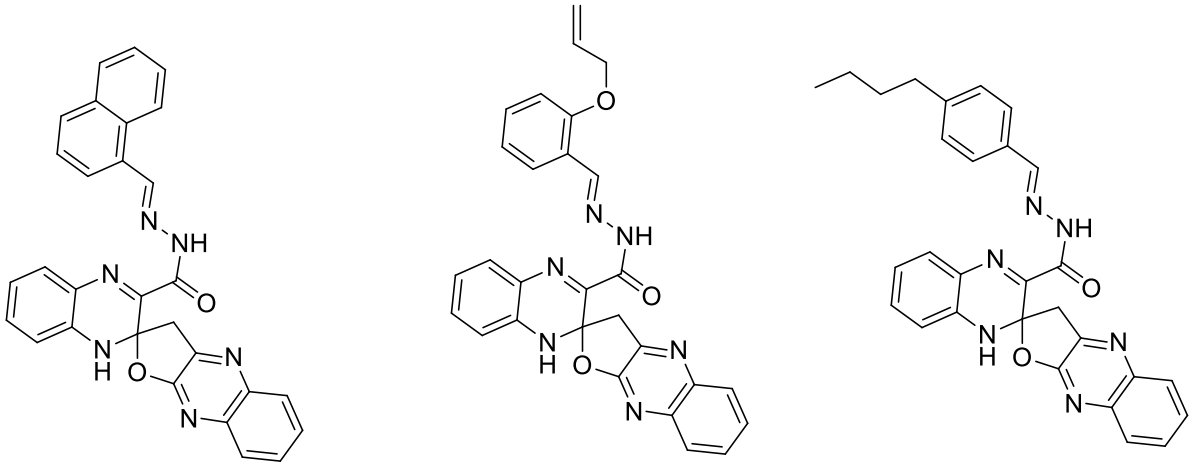
****CYP3A4 S = Substrate of CYP3A4**

‡ BA = Binding Affinity

the compounds and or their metabolites to be better able to survive the first-pass metabolism and be more bioavailable at the site of action. These three (compounds 5, 82 and 105) are deemed to be the compounds with the best pharmacokinetic profile apart from having high binding affinity for the target protein and likely to be antimalarial agents (Figure 3). Figure 4 shows the two-dimensional representations showing interactions (dotted lines) of the portions of the proposed compounds 5 and 105 with protein side chains.

These compounds along with the other nine (colour-coded blue in Table 1) would be synthesized and tested for their antimalarial properties. The precursors, Compound A and

Compound B would also be tested for antimalarial properties along with other synthesized compounds.



Compound 5 **Compound 82** **Compound 105**

Figure 3: The Structures of the three compounds with the best pharmacokinetic profiles. These are the three colour-coded as green in Table 1.

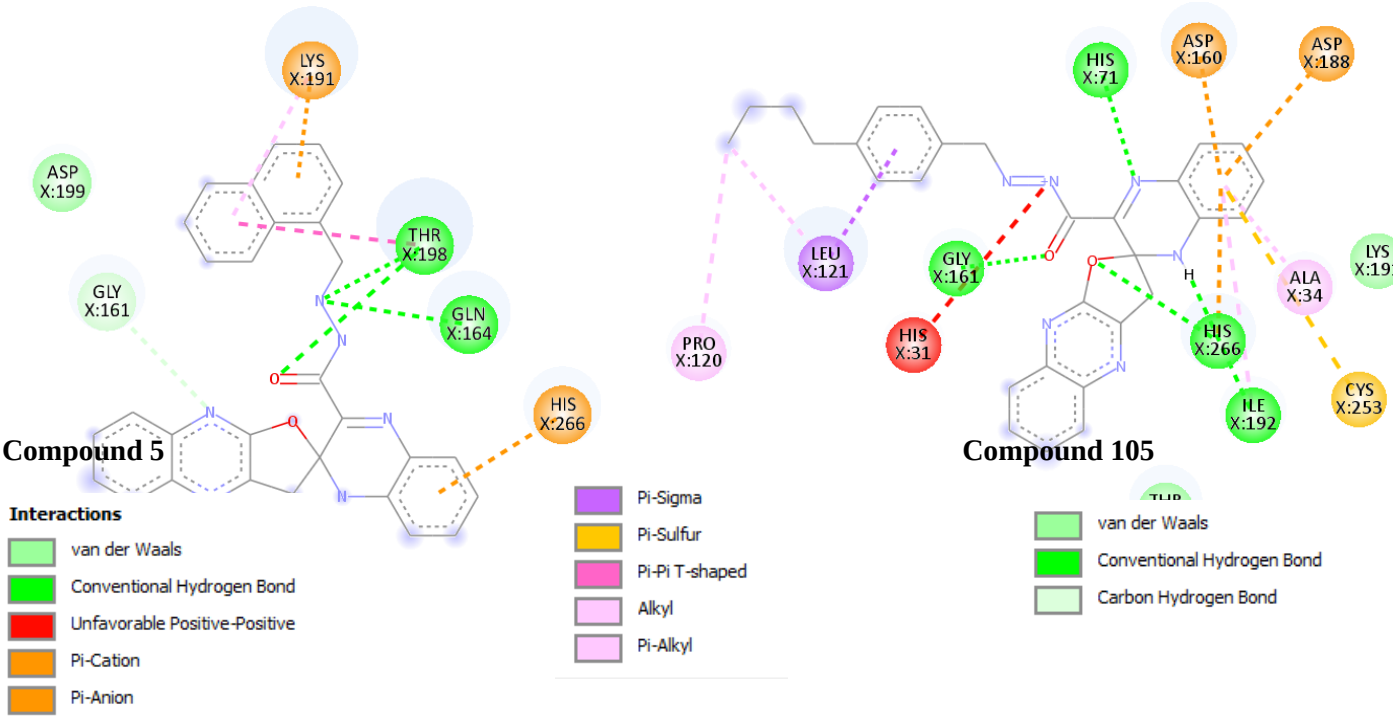


Figure 4: 2D representations showing interactions (dotted lines) of the portions of the proposed compounds with protein side chains. These two compounds appear to have a lot of hydrophobic interactions within the binding pocket occupied.

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