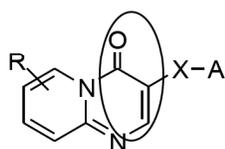


5. Summary

The existing armamentarium of antimalarial drugs is not sufficient to combat malaria, primarily because of resistance developed by the parasite. The problem got further compounded due to non-enrichment of antimalarial drug inventory. Unfortunately an effective antimalarial vaccine also could not be developed due to fast and constant mutations in the parasite genome. All these factors led to a woefully thin antimalarial drug development pipeline with little chemical diversity.

Genome mapping of the malaria parasite offered a number of attractive drug targets including plasmepsins and falcipains, the enzymes involved in parasite metabolism and in providing nutrients to the parasite to meet its energy requirements. There exist a great potential in the exploration of cysteine proteases falcipain inhibitors. Falcipains 2 and 3 especially were more attractive targets as these enzymes degrade haemoglobin to small sized peptides in the acidic environment of the vacuole and degrade the membrane proteins of the host cells and help the parasite rupture the host cells in the alkaline environment. So, inhibition of FP-2/3 enzymes could effectively arrest the growth of the parasite and control their progression in the host system.

A large number of peptidic and non-peptidic motifs like chalcones, (thio) semicarbozones, hydrazides, heterocycles, oxiranes and succinates have been reported in the literature to exhibit FP-2/3 inhibitory activity. Unfortunately none of such compounds have crossed the clinical phase of evaluation as antimalarial agents. There are some reports of some heterocyclic and polycyclic compounds exhibiting FP-2/3 inhibiting activities. Taking the basic information from literature about the enzyme inhibitors into consideration, it was planned to synthesize pyrido[1,2-*a*]pyrimidin-4-one class of compounds (**M**).



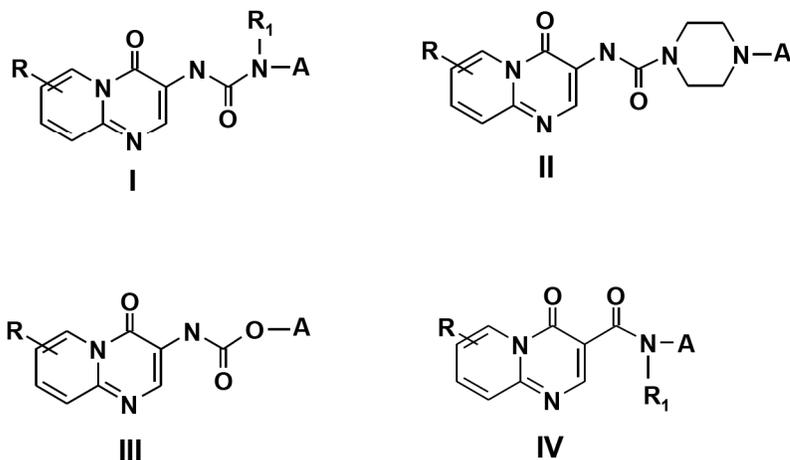
X = Urea, carbamate, amide
 A = Alkyl/aryl/heteroaryl
 R = H, CH₃, Cl, 4-C₆H₄OCH₃

(**M**)

It is worth noting that the circled pharmacophore may act as Michael acceptor for reversible/irreversible binding to the thiol nucleophile present in the FP-2/3 enzymes in the P1 binding pocket. While the groups, X and R were selected in such a way that compounds could exhibit better binding profile in the P2 and P3

binding pockets of the falcipain enzyme that could translate in to enhanced potency of the compounds.

It was planned to synthesize the given four (**Series-I to IV**) of compounds and to evaluate them against FP-2 enzyme in the *in vitro* tests. FP-3 enzyme having a high degree of homology to FP-2 was presumed to react with all such compounds which would prove to be powerful inhibitors of FP-2.



Where:

R = H, 8-CH₃, 7-Cl, 4-C₆H₄OCH₃

R₁ = H, Alkyl

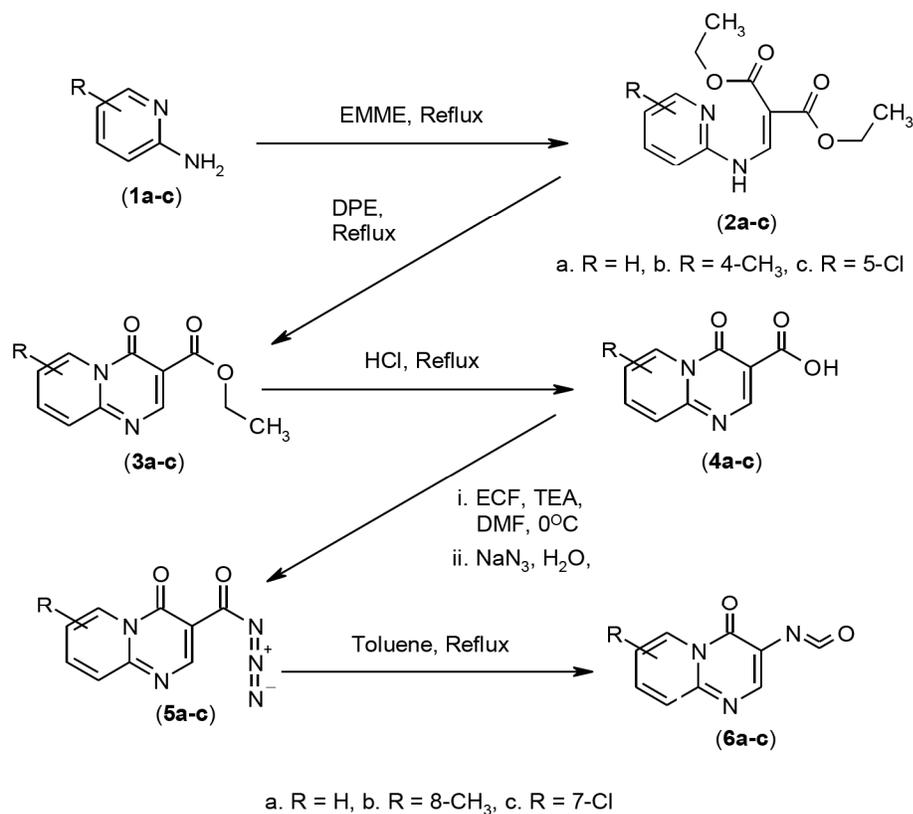
A = Alkyl/heteroaryl or alkyl

All of the derivatives of the given four (**Series-I to IV**) were synthesized by following either of the **Schemes 1-6** and the compounds were characterized on the basis of their spectral data. Synthetic methods for preparing these new chemical entities, spectral (IR, PMR and Mass spectrometry) data and biological activities of these compounds have been discussed in detail in this thesis work.

The acids (**4a-c**) and the isocyanates (**6a-c**) are the key intermediates in the preparation of the targeted compounds. Their synthesis was accomplished by following the key steps i) Gould and Jacob reaction ii) cyclization, iii) hydrolysis and iv) Curtius rearrangement reaction. Synthesis of these intermediates is accomplished by following the reaction sequence as outlined in the **Scheme 1**.

2-Aminopyridines (**1a-c**) by Gould and Jacob coupling reaction with diethyl ethoxymethylenemalonate yielded (**2a-c**), which on cyclization by

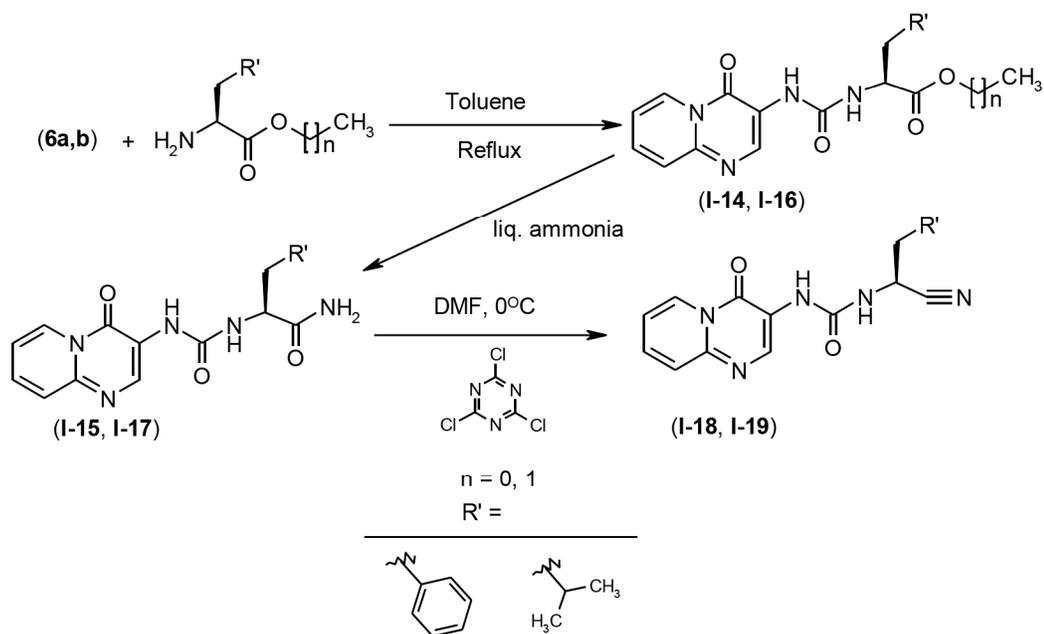
refluxing in diphenyl ether resulted into pyrido[1,2-*a*]pyrimidin-4-one monoesters (**3a-c**). The esters (**3a-c**) on acid catalyzed hydrolysis offered free acids (**4a-c**). The acids (**4a-c**) when reacted with ethyl chloroformate in presence of triethylamine yielded anhydrides which on further *in situ* reaction with sodium azide yielded the acid azides (**5a-c**). The azides (**5a-c**) offered isocyanate derivatives (**6a-c**) (**Scheme 1**) by refluxing them in toluene.



Scheme 1

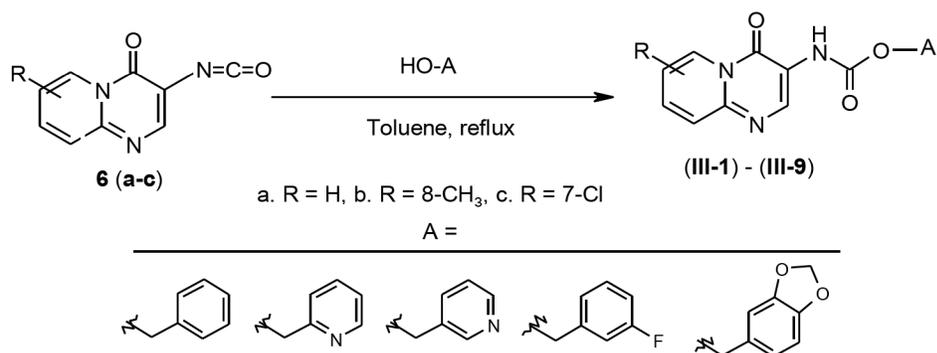
The ureas **Series-I** (**I-1** to **I-34**) were prepared by coupling of suitable isocyanate (**6a-c**) with amine derivatives of type R_1NHA in toluene under reflux conditions following the procedure as depicted in **Scheme 2** or **Scheme 3**.

While piperazine-urea analogs of the **Series-II** (**II-1** to **II-11**) were synthesized by coupling of isocyanate (**6a-c**) with suitably substituted piperazine derivatives under reflux conditions in toluene as depicted in **Scheme 2**.



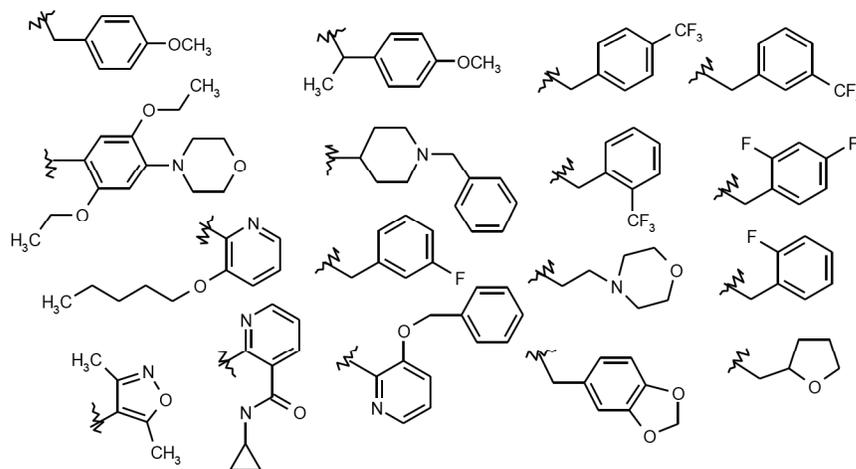
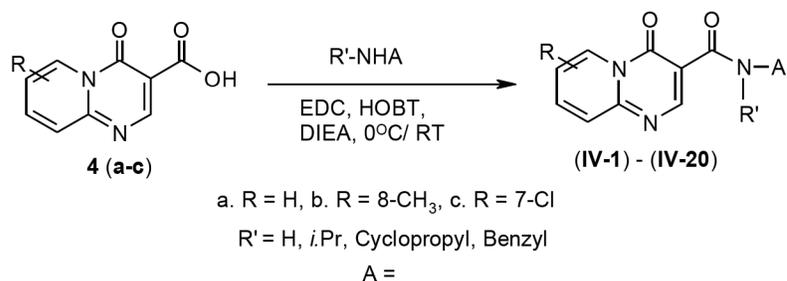
Scheme 3

Synthesis of the carbamate derivatives of **Series-III** (**III-1** to **III-9**) have been accomplished by reaction of the isocyanate (**6a-c**) and suitable alcohol derivative (**A-HO**) in toluene under reflux conditions following the procedure as outlined in **Scheme 4**.



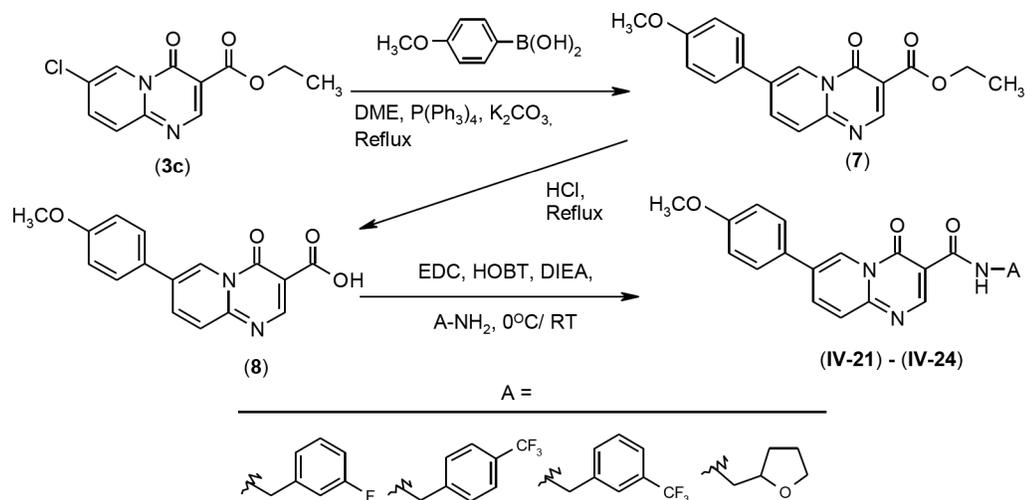
Scheme 4

Synthesis of the amide derivatives of **Series IV** (**IV-1** to **IV-26**) have been accomplished by reacting the acid (**4a-c**) with suitable amine ($R'-NHA$) using EDC as a coupling agent as outlined in **Scheme 5**.



Scheme 5

In order to study the hydrophobic binding interactions by increasing the steric bulk of the group attached to the B-ring (i.e. pyrido) of the pyridopyrimidine ring, some additional compounds (**IV23 - IV24**) were synthesized. The 7-chloro ester (**3c**) was reacted with *p*-methoxyphenylboronic acid through Suzuki coupling offering the ester (**7**) which on acid hydrolysis yielded the acid (**8**). The acid derivative (**8**) was coupled with suitable amines to get the targeted compounds (**IV23 - IV24**) as depicted in **Scheme 6**.



The synthesised compounds were submitted for *in vitro* FP-2 inhibition evaluation. Compounds were evaluated for their inhibitory activity against recombinant FP-2 using Cbz-Phe-Arg-AMC as a fluorogenic substrate. Preliminary screening was performed at 10 μM concentration. An equivalent concentration of DMSO was used as negative control and the irreversible standard inhibitor of clan CA family C1 cysteine proteases (papain family), namely E-64 was used as positive control. Assays were performed to determine the percentage inhibition of the enzyme at a concentration of 10 μM . FP-2 activity is assessed by cleavage of the fluorogenic substrate Cbz-Phe-Arg-AMC releasing the fluorescent AMC group. Hence decrease in fluorescence intensity in a sample represents inhibition of enzyme activity. The IC_{50} values were determined for those compounds only which showed more than 40 % inhibition at 10 μM concentration. The FP-2 enzyme inhibition data of the synthesised compounds shows that the pyrido[1,2-*a*]pyrimidin-4-ones show FP-2 inhibition in micromolar range. Based on the result of evaluated compounds a broad structure activity relationship could be deduced for this series of the compounds. Urea moiety has the potential to provide potent FP-2 inhibitors as the carbamate and carboxamide derivatives are much less active in comparison to the substituted ureas.

Ethylmorpholine (**I-26**, IC_{50} **6.93 μM**) and methoxyethyl (**I-21**, IC_{50} **6.36 μM**) urea derivatives proved to be the most potent compounds. 2-Thiophenethyl (**I-8**, IC_{50} **14.27 μM** ; **IV-23**, IC_{50} **21.16 μM** ; **I-27**, IC_{50} **23.35 μM**), acetamidoethyl

(**I-4**, IC₅₀ **16.08 μM**) and 4-methoxyphenylpiperazine (**II-5**, IC₅₀ **18.87 μM**) were the other groups which gave active compounds when attached to the other end of the urea moiety. 2-Pyridylpiperazine is another moiety which offered quite active compounds (**II-8**, IC₅₀ **12.16 μM**; **II-1**, IC₅₀ **14.84 μM**).

In general 8-methyl and 7-chloro substituted derivatives offered more potent compounds indicating that increasing the steric bulk of the group attached to the B-ring (i.e. pyrido) is likely to provide more potent FP-2 inhibitors. An electron rich environment at the end of the carbon chain attached to the urea nitrogen also seems to be conducive for better activity as indicated by the compounds having morpholine, 2-thiophenyl, 2-pyridyl, 4-methoxyphenyl, acetamidoethyl and 2-methoxyethyl groupings. But bulky groupings (**II-7**, benzhydrylpiperazine and **I-9**, 4-morpholinopropyl) may not offer potent derivatives.

With the availability of biological activity for compounds the current work has proved the potential of pyrido[1,2-*a*]pyrimidin-4-one derivatives as potent FP-2 inhibitors. Based on the activity data of the synthesized compounds it could be claimed that pyrido[1,2-*a*]pyrimidin-4-one can serve as a lead series for future investigations. By appropriate structural optimizations the potency of this series of compounds can be improved substantially against FP-2 enzyme which in turn can serve to be potential antimalarial agents. Further optimization of the lead structures and biological screening is in progress in the laboratory.