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Synthesis of some Newer Pyrimidine Derivatives as Antimalarial Agents

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ABSTRACT: Five compounds were synthesized using the synthetic scheme that was derived from the works of Panda and Ram. The synthesis of 3, 4-dihydropyrimidine was performed by using substituted benzaldehyde, urea and ethylacetoacetate as depicted in the section below by Biginelli condensation. The hydrazide derivative of the dihydropyrimidine-2-one was synthesized by the treatment of the product of step 1 by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid. The triazolo derivatives were synthesized by the condensation of the hydrazide derivative, ammonium acetate and an aldehyde in the presence of acetic acid. The product precipitated on neutralization with ammonia solution. Alkylation of the pyrimidine derivatives obtained in the step 3 was accomplished by the treatment with alkyl halide and K_2CO_3 in presence of DMF. The compounds were obtained in the yield of 68 to 80% and were off white to pale yellow in color. All the compounds were soluble in water and DMSO, slightly soluble in methanol and insoluble in chloroform.

IndexTerms **– Docking, Pyrimidine, Uracil, Thymidine, Cytosine and Antimalarial activity**

INTRODUCTION

Pyrimidine is a very important class of molecule as uracil, thymidine and cytosine; three of the five bases found in the nucleotides are pyrimidine derivatives[7]. Being an integral part of DNA and RNA, the pyrimidines have diverse pharmacological actions. Due to these diverse biological properties the pyrimidine derivatives have been extensively studied. Pyrimidine was first isolated in 1899 by Gabriel and Colman. Since then the pyrimidine nucleus has been a center of attraction for biologists and chemists. Pyrimidines are compounds belonging to the class of diazines, i.e., ring systems derived from benzene by replacement of two of the ring carbon atoms by nitrogen[8]. Pyrimidines are the most important member of all the diazines as this ring occurs in many compounds vital to living system. They are non-basic, stable, colorless and soluble in water. The pKa value of pyrimidine is 1.3. The drop in the basicity is believed to be the consequence of destabilization of the N-1 protonated cations by the inductive electron withdrawal by the second nitrogen.The chemistry of pyrimidine is widely studied. The introduction of alkyl groups in pyrimidine enhances the basicity. The literature revealed that various synthetic methods and various reactions were reported for pyrimidines.

RESEARCH ENVISAGED

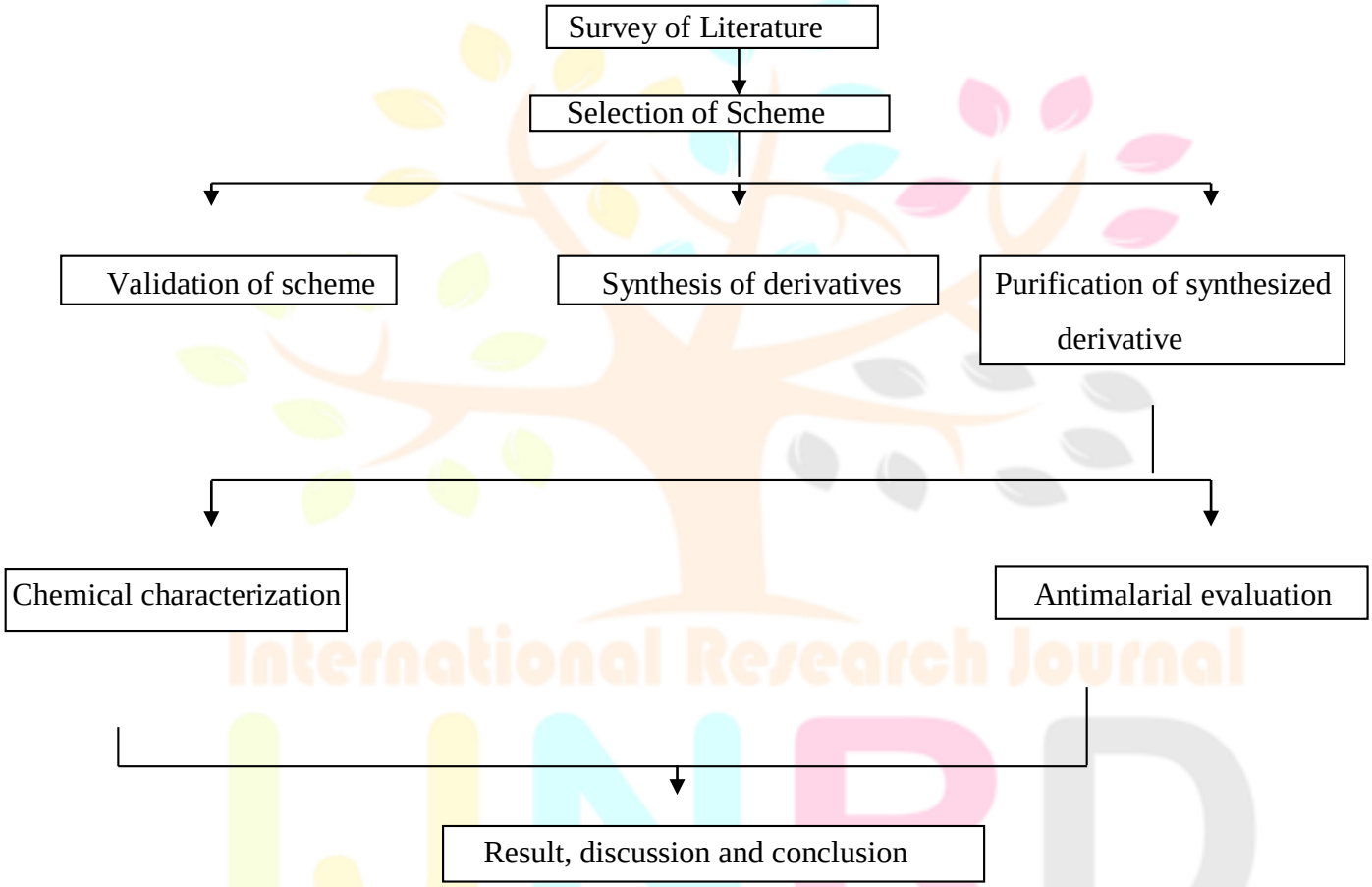
As revealed from the SAR studies that the substitution of alkyl groups at nitrogen of pyrimidine, the overall toxicity of the molecule increases, it could be beneficial to alkylate N-position of pyrimidine and make

substitutions at various other positions on the pyrimidine nucleus. Also substitution at 5- position of the pyrimidine nucleus renders the molecule to be anti-malarial.

Hence it was decided to synthesize a few pyrimidine derivatives and evaluate the anti- malarial activity of the compounds using *in vitro* method.

PLAN OF WORK

Biginelli condensation as a route for the synthesis of pyrimidine by conventional refluxing is by far the simplest method. The 5- position of the pyrimidine nucleus could well be converted to a thiadiazolo or triazolo substituted one by utilizing the scheme obtained from the literature published by Panda *et al*. The alkylation at N-3 postion of pyrimidine nucleus can be accomplished by the method reported by Ram *et al*. The *in vitro* anti-malarial efficacy of the compounds, can be tested against plasmodium in culture medium.



RESEARCH METHODOLOGY

Materials And Methods: All the chemicals and reagents used were of laboratory grade and were purchased from E Merck Ltd., Ranbaxy fine chemicals, Sigma-Aldrich and Spectrochem, India. All the solvents used were dried and purified as and when required. The melting points reported here-in are uncorrected and were determined in open capillaries using melting point apparatus. All the reactions were monitored by thin layer chromatography (TLC), which was performed on pre-coated silica gel G plates and the spots were visualized by exposure to UV light. A few pyrimidine derivatives were synthesized using Biginelli condensation of ethylacetoacetate, urea and 4-chlorobenzaldehyde. The steps adapted in the synthesis of the pyrimidine derivatives are depicted in the scheme below adapted from the schemes reported by Mishra et al[64].

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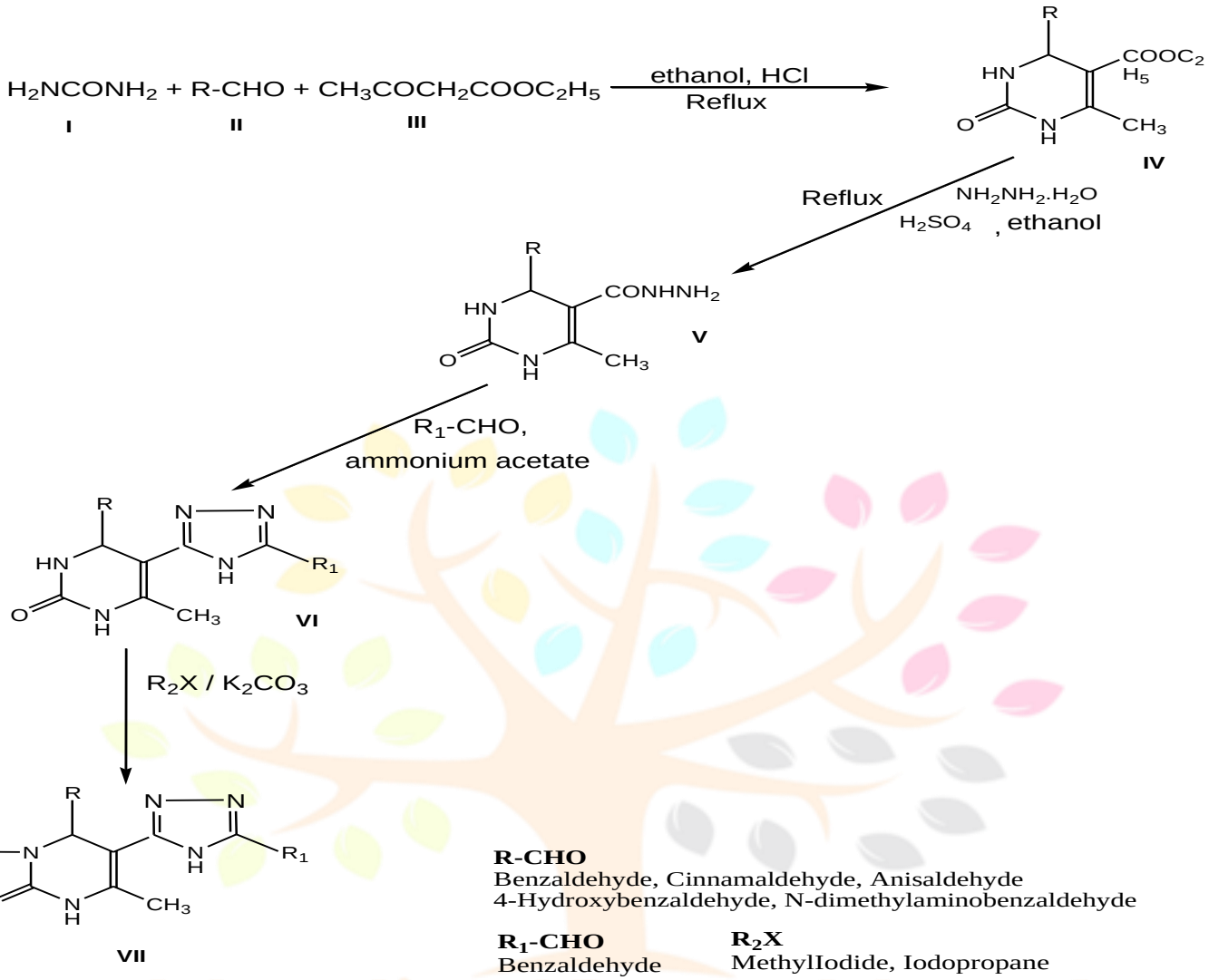
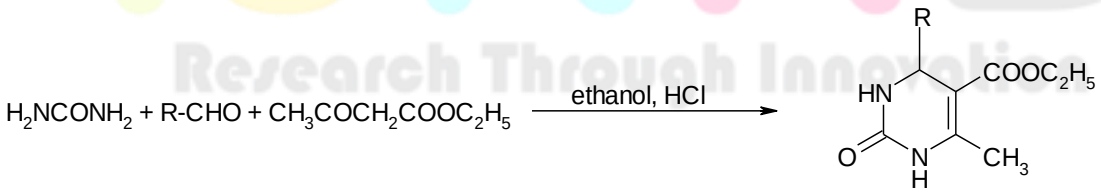


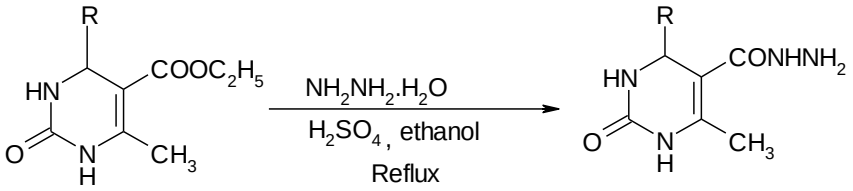
SYNTHESIS OF PYRIMIDINE DERIVATIVES

Step 1: Synthesis of 4-phenyl-5-carboethoxy-6-methyl-3, 4-dihydropyrimidine-2- one. The synthesis of 3, 4-dihydropyrimidine was performed by using substituted benzaldehyde, urea and ethylacetoacetate as depicted in the section below by **Biginelli condensation**.



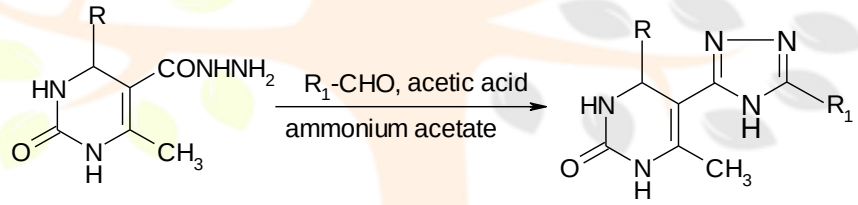
0.5 moles of urea (**I**), 0.75 moles of ethylacetoacetate (**III**) and 0.5 moles of appropriate benzaldehyde (**II**) were mixed in 25 mL of ethanol. Catalytic amount of concentrated hydrochloric acid (5 drops) was added to the mixture and the mixture was refluxed until the completion of the reaction (approximately 3 hours). On cooling, a solid separated which was filtered and recrystallized using ethanol to give the product **IV.** Completion of the reaction was monitored by TLC.

Step 2: Synthesis of 4- phenyl -5-carboxyhydrazide-6-methyl-3, 4-dihydropyrimidine-2-one. The hydrazide derivative of the dihydropyrimidine-2-one was synthesized by the treatment of the product of step 1 by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid.



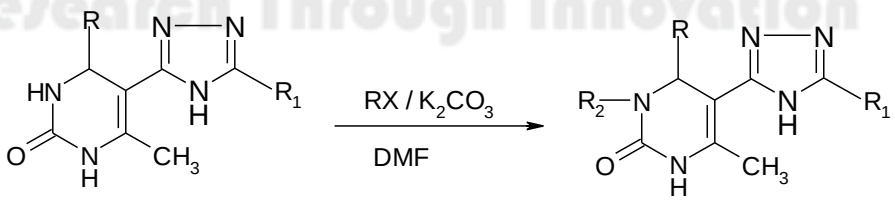
To 0.1 mole of the product **IV** in 20 mL ethanol, 0.1 mole of hydrazine hydrate was added. To the mixture, catalytic amount of concentrated sulfuric acid was added. The mixture was refluxed until the completion of the reaction (approximately 2 hours). On cooling, a solid separated, which was recrystallized from ethanol to give the product **V.**

Step 3: Synthesis of 4- phenyl -5-(2'-substituted-1', 3', 4'-triazolo)-6-methyl-3, 4-dihydropyrimidine-2-one. The triazolo derivatives were synthesized by the condensation of the hydrazide derivative, ammonium acetate and an aldehyde in the presence of acetic acid. The product precipitated on neutralization with ammonia solution.



To 0.1 mole of product **V** in 20 mL acetic acid, a pinch of ammonium acetate was added, followed by the addition of 0.1 mole of Benzaldehyde solution. The mixture was stirred for 24 hours at room temperature. After 24 hours, the reaction mixture was neutralized with ammonia solution, to give a solid, which was recrystallized from ethanol to give the product **VI.**

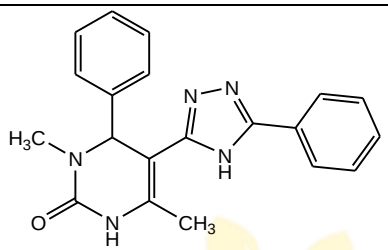
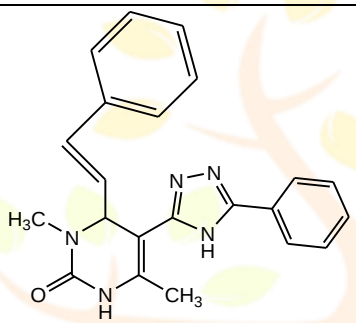
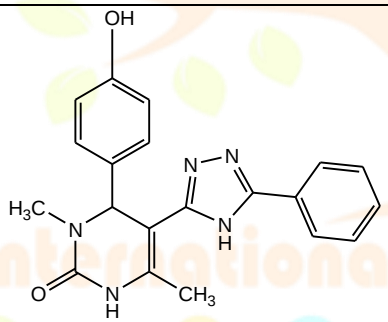
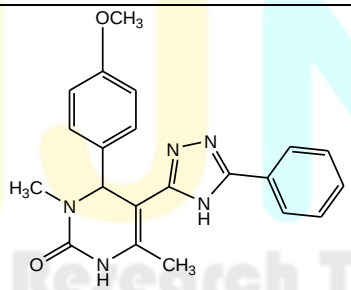
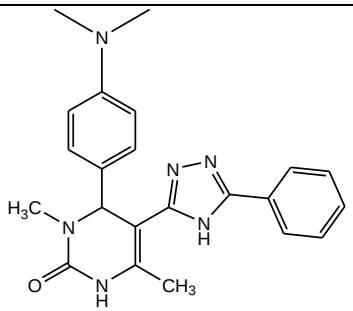
Step 4: Synthesis of the N-substituted compound. Alkylation of the pyrimidine derivatives obtained in the step 3 above was accomplished by the treatment with alkyl halide and K_2CO_3 in presence of DMF.



A mixture of 2.17 mmoles of **VI**, 4.35 mmoles of K_2CO_3 and 4.48 mmoles of the methyl iodide in 6 mL of DMF was stirred for 4 hours at room temperature. The mixture was then diluted with water and the solid was filtered off and was recrystallized from ethanol to give the final product **VII**.

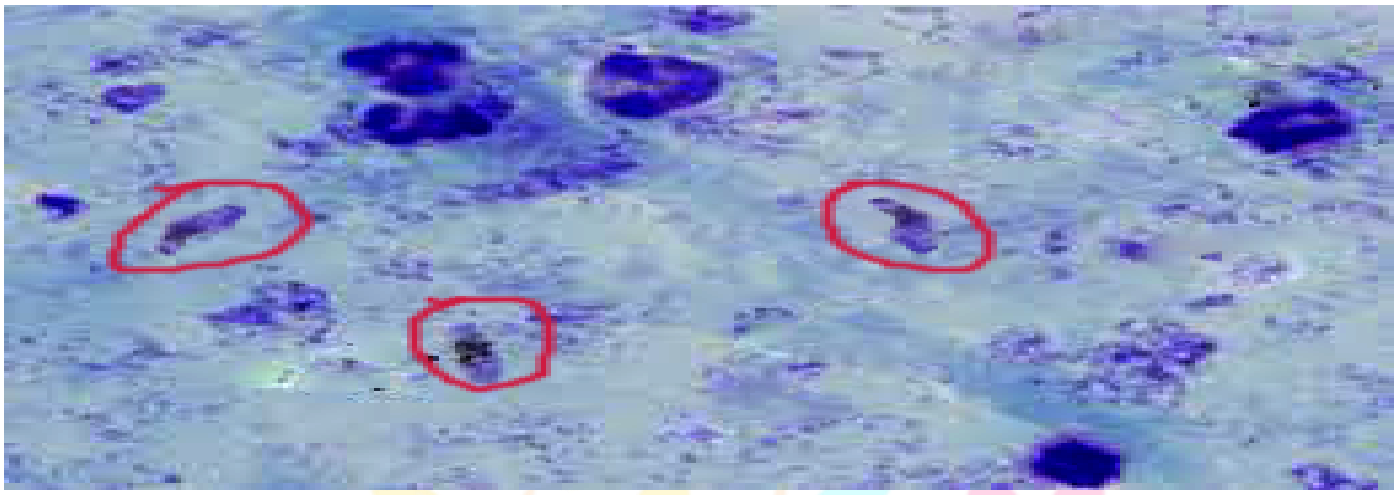
Five compounds were synthesized using the synthetic scheme depicted in the previous chapter. The compounds were coded as **SAP1** to **SAP5** and the solubility, melting point, retention factor and yield of the obtained product was determined.

Precentage yield of synthesized compounds

Compound code	Structure	Molecular Formula	% Yield	Rf value	Melting Point (°C)
SAP1		$C_{20}H_{19}N_5O$	**72**	**0.83**	**155-160**
SAP2		$C_{22}H_{21}N_5O$	**76**	**0.56**	**171-174**
SAP3		$C_{20}H_{19}N_5O_2$	**80**	**0.44**	**159-162**
SAP4		$C_{21}H_{21}N_5O_2$	**62**	**0.59**	**167-170**
SAP5		$C_{22}H_{24}N_6O$	**68**	**0.37**	**173-176**

Antimalarial activity :- The antimalarial action of the synthesized compounds was determined in vitro by culturing the plasmodium in RPMI medium and determining the IC_{50} as 50% inhibition in parasitemia. The

stained RBCs were observed under high resolution microscope and counted. The number of parasitized RBCs (infected with plasmodium) was counted as well as the total number of RBCs counted was also recorded. The percent parasitemia was calculated using the previously mentioned formula



Giemsa stained culture of parasite culture

EC_{50} value of the test compounds against *Plasmodium falciparum in vitro*

Compound Code	EC_{50} (µg/mL)
SAP1	>100
SAP2	50
SAP3	20
SAP4	20
SAP5	>100

The results revealed that of the five compounds only two compounds **SAP3** and **SAP4** were able to reduce the parasitemia effectively.

Antimalarial action

Calculation of *in vitro* percent parasitemia is a widely accepted method for the assessement of antimalarial action of compounds. The EC_{50} values were calculated using this method and it was found that the presence of a functional group capable of hydrogen bonding at para-position of the aromatic ring at C-4 of pyrimidine (**SAP3**- OH & **SAP4**-OCH_3) was able to increase the antimalarial potential of the compounds.

Summary and Conclusion

In an effort to develop new potential molecules for the betterment of the health condition, a series of pyrimidine derivatives were synthesized. Literature is flooded with various pyrimidine derivatives possessing

a vivid variety of activities. The activities like anti-malarial, antimicrobial, anti-cancer, anti-epileptic etc. are exhibited by the pyrimidine analogs. The objective of the present investigation was to synthesize few pyrimidine derivatives and evaluate them for their antimalarial potential using in vitro method for percent parasitemia.

SUMMARY

Five compounds were synthesized using the synthetic scheme that was derived from the works of Panda and Ram. The synthesis of 3, 4-dihydropyrimidine was performed by using substituted benzaldehyde, urea and ethylacetoacetate as depicted in the section below by **Biginelli condensation**. The hydrazide derivative of the dihydropyrimidine-2-one was synthesized by the treatment of the product of step 1 by hydrazine hydrate in presence of ethanol with catalytic amount of concentrated sulfuric acid. The triazolo derivatives were synthesized by the condensation of the hydrazide derivative, ammonium acetate and an aldehyde in the presence of acetic acid. The product precipitated on neutralization with ammonia solution. Alkylation of the pyrimidine derivatives obtained in the step 3 was accomplished by the treatment with alkyl halide and K_2CO_3 in presence of DMF.

The compounds were obtained in the yield of 68 to 80% and were off white to pale yellow in color. All the compounds were soluble in water and DMSO, slightly soluble in methanol and insoluble in chloroform. In ^{1}H-NMR spectra protons of pyrimidine and benzene ring were observed between 8-7 ppm the methyl protons were found at 1.2 - 3ppm. IR spectra of the synthesized compound exhibited stretching and bending vibrations for N=H, C=O, C=C, C-C, CH_2, C-H.

The EC_{50} values were calculated using *in vitro* method and it was found that the presence of a functional group capable of hydrogen bonding at para-position of the aromatic ring at C-4 of pyrimidine (**SAP3**- OH & **SAP4**-OCH_3) was able to increase the antimalarial potential of the compounds.

CONCLUSION

The results obtained led to the conclusion that the activity of the pyrimidine derivatives as anti-malarial agents is affected the type of the substituent at the 4-position of the pyrimidine nucleus. The substitution of the electron withdrawing group on the para position of the 4-position pyrimidine ring substitutent led to significant anti-malarial activity. The scheme can be extended in the future by utilizing bioisoteric principles for the synthesis of newer derivatives. A complete series of 20 or more compounds would be very much beneficial in performing QSAR studies and optimizing the molecule as a lead molecule for the treatment of malaria.

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