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Synthesis of Isocryptolepine Derivative with Biological Evaluation

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Abstract

The successful synthesis of isocryptolepineanalogs with favorable yields necessitates both mono- and di-substitutions. Through a systematic structure-activity relationship (SAR) investigation, a set of indole[3,2-c] quinolones was generated. This investigation revealed significant findings: the methyl groups addition on the N11 position, various substitution moieties (F, Cl, Br, (-CH4) Me, (-OCH3) MeO, and NO2) at the C2 position, and side chain modifications of x-aminoalkylamines on C6 position. In-vitro, anti-plasmodial against the chloroquine-susceptible strain RKL-2 was examined. Notably, PN-6, identified as 11-benzoyl-2-fluoro-11H-indolo[3,2-c]-quinolin-6-yl) amino)propyl)3-phenyl urea exhibited the lowest IC50 (0.46 nM/antimalarial) and the highest selectivity index. The halogenated derivative emerged as the most effective compound. The introduction of diversesubstitutions at different positions elucidated varying degrees of antiplasmodial activity. Substitutions at the N11 and C2 positions, as well as modifications to the side chains at the C6 spot, demonstrated significant impacts on the efficacy of the synthesized compounds. These findings underscore the importance of strategic molecular design in optimizing the anti-malarial potential of isocryptolepine derivatives. Within our study on novel isocriptolepine compounds, these show great promise as malaria lead candidates

Keywords: Isocryptolepine, Plasmodium falciparum, Malaria, Antimalarial activity, Chloroquine-resistant, Chloroquine-sensitive

Introduction

Malaria and cancer are globally prevailing deadly diseases that cause millions of deaths per annum The medication is committed to the fight against malaria, a blood disease that is caused by the plasmodial protozoans. Although P. falciparum infections account for the bulk of malaria-related fatalities, there are five Plasmodium strains identified to infect humans: P. vivax, P. ovale, P. knowlesi, P. malariae form elsewhere in the world [1-3]. Malaria: despite the world community's strong position against the disease, WHO estimates that there were 229 million cases in 2019, with a death toll of

409,000, according to the [1]. The marathon for the scientist is the fast-running resistance strains that develop across all malaria-endemic regions. This is one of the major obstacles that prevent malaria eradication. Sub-Saharan Africa reports 90% of all the malaria cases [1]. This region is jeopardized as its main saving measures and the available treatments depend on money. The malaria zones then used to be stable in the areas near the tropics but then it started expanding to other areas due to global outreach. The outcome of this, based on the prognosis, will be the shift towards warm temperatures on a global scale, and a greater risk of becoming infected for the human population settled in those regions. At this moment, the most efficient therapeutic ways of coping with malaria after the chloroquine-resistant strains of P. falciparum became widespread are artemisinin-based treatments [6]. Yet, as recently as 2008, the first reports of a resistance mechanism based on artemisinin were observed in Cambodia [7]. Subsequently, this resistance spread rapidly, with hundreds of cases confirmed from 30 different countries within the span of just ten years. Considering this alarming trend, it becomes imperative to prioritize the development of medical therapies that are not only effective but also financially accessible to populations in need. Addressing the challenge of antimalarial drug resistance requires not only the discovery of new therapeutic options but also ensuring their affordability and accessibility to vulnerable populations. This emphasizes the importance of sustainable and affordable healthcare solutions to combat the global burden of malaria. In addition to other illnesses, malaria is an infectious disease whereas cancer is the highest 2nd leading cause of death accounting for almost a sixth of all deaths globally. Cancer influences individuals, families, and societies not only in mortality rates but also emotionally and economically. The WHO data shows that 18.1 million was the total number of newly diagnosed cases of cancer and 9.6 million deaths were from cancer in 2018 [1]. An interdisciplinary approach, creative research, and patient-oriented healthcare systems adapted to numerous populations are needed to fight this disease. Cancers of the lung, breast, colorectum, prostate, and stomach are the five most diagnosed varieties. Although there are some the anti-cancer treatments that are effective, the people who suffer from such kind of immune-system-suppression conditions are common. The next step after treatment of cancer is the development of “nosocomial infections” (infections caused by staphylococcus aureus that are drug-resistant) in immunocompromised individuals, thereby increasing the nosocomial disease burden as a whole [9]. The fact that multidrug-resistant (even notably difficult to be treated) biofilms pooling up the epidemic may sustain makes everything so much harder. There is a link between prolognatal bacterial complications and cancer cases with a WHO estimation that 13% of all malignancies worldwide are associated with these infections [1]. In recent research that came up, it was seen that the formation of microbial biofilm in the body was associated with the development of several types of cancer. [12-15]. The incapability of some anticancer drugs to effectively target biofilm discretely in its early stages is indicating a crucial obstacle to cancer cure. Biofilms are highly complex structures made by cancerous cells that guarantee cell entities protection and resistance to commonly used treatments, thus the disease can progress and overcome the conventional therapy. Consequently, it is of the utmost importance that the process of anticancer drug development is focused on the discovery of those agents that are capable of hindering biofilm development. If the new drugs are created, they would be targeted at the cellular processes that lead to the formation of biofilms, thus neutralizing and preventing cancer cells from aggregating and building these protective shields. The anticancer agents will work more effectively because of the disruption of biofilm anatomy. In addition, these drugs can prevent resistance to traditional anticancer medications as well. Cancer research towards the design and development of such drugs can be vital in terms of combating this issue of cancer treatment. Placing

the prevention of biofilm formation on the top, scientists could certainly raise therapeutic results and then give an alternative for cancer patients.

In this case, the biodiversity in natural products has been proven as a valuable source of lead molecules that can be used in therapeutic research and development [16-18]. Estimations show that about 40% of drugs that the US FDA has authorized by December 2020 originate from natural sources as statistics tell: a big proof of the importance of nature in drug production. To establish new medication candidates, it is still important to research and analyze compounds of natural sources as well as some derivatives of the semi-synthetic form [19]. Further, it is quite active in the fields of materials chemistry and the dye industry besides being the basis of the pharmaceutical industry where quinoline moiety is one of the most versatile building blocks. Even more so since quite a lot of natural compounds have quinoline nuclei which possess a great number of biological activities and show promising effects as a medicinal target for diseases. [1,20]

Materials and Methods

Materials

All reagents and diluents were of analytical and microbiological grade and purchased.

Methods

General method for synthesis of Isocryptolepine derivatives

Step-1: Synthesis of Isatin

Substituted m-anisidine, m-taulidine, and 3-chloroaniline (1ML) in a 250ML, spherical conical flask mounted with both a stirrer and a thermometer and a water cover of 1 ml and HCl of 1 ml was then used. The following solvent, placed into the mixture; it was whisked mechanically and then it consisted of additions salts; anhydrous sodium sulphate solution of 0.13 mole, chloral hydrate solution of 0.1.1 mole of water, and hydroxylamine hydrochloride solution of 0.13 mole water. The suspension was stirred with a mechanical bar until about 1000C was reached during these 6 hours; around 940C the solids showed a yellowish color and the observation time was about 30 minutes. The cooling phase was conducted with stirring for a one-minute reaction, followed by an overnight settling period. The resulting solids were then gathered, rinsed with water, and subsequently dissolved in a 10 ml solution of 1% sodium hydroxide. In the end, a minute quantity of boundary black-coloured solid solids was not dissolved, it was purified by filtration and washed with 10ml of 1% sodium hydroxide solution. The started filtrate and washings were acidified with 2 ml of the distilled concentrated hydrochloric acid to stop overflowing, letting the reaction vessel fill to the brim. With 10 ml of water, those were made to look like ordinary clear water. Then the start reaction happened and a white and puffy precipitate, taking notice of forming, was run together with water, dried, and screened.

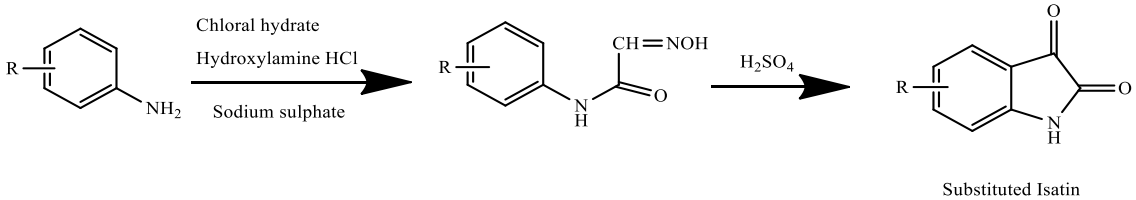


Fig.1: Synthetic scheme for Isatin synthesis

Step 2: Chlorination of 2-AminoBenzylamine

The solution was prepared from 2-Amino benzylamine (1.00 g), potassium iodide (1.03 g), and hydrogen peroxide (0.66 ml) by dissolving them with methanol (5 mL) and water (15 mL). Following this, the resultant blend underwent treatment with a mild hydrochloric acid solution (9.5 mmol) at ambient temperature for 40 to 45 minutes, with stirring sustained, further 2-3 hrs. Subsequently, thinned the blend with H2O (15 mL) & subjected to three extractions using dichloromethane (25 mL each). Finally, isolation of the organic layer, & solvent elimination are carried out at low pressure in order to yield the desired product.

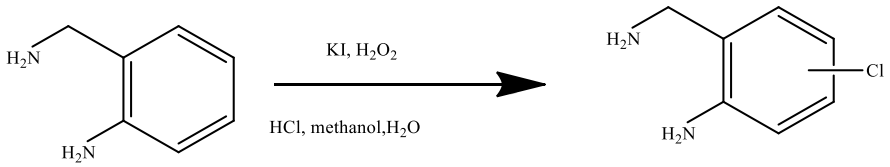


Fig.2: Chlorination of 2-AminoBenzylamine

Step 3: Synthesis of intermediates

Chlorinated Aminobenzylamine and substituted Isatin in equal quantity 1gm each, taken in round bottom flask containing 10ml acetic acid and then the reaction mixture was refluxed about 7h. Thin Layer Chromatography was done to examine the chemical reaction.

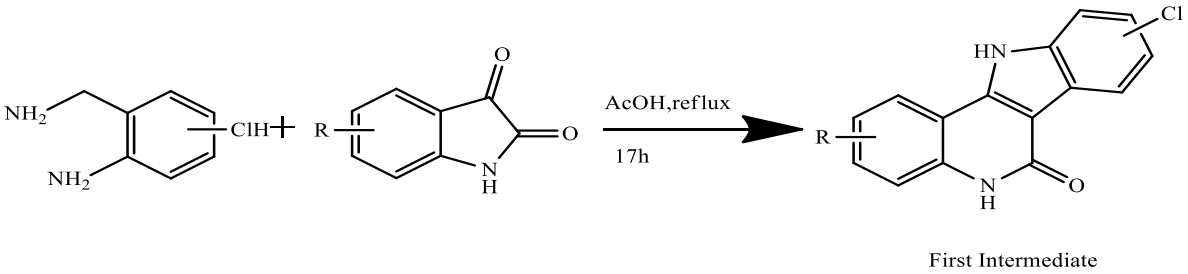


Fig.3: Chlorination of 2-AminoBenzylamineR_1= H/2-F/2-Cl/2-Br/2-Me/2-MeO

Step 4: Dehydrative chlorination with $POCl_3$

Chlorination of first intermediate was carried by adding 5ml of phosphoryl chloride to the reaction mixture and then the reaction permitted to reflux at 130°C, duration of 8h.

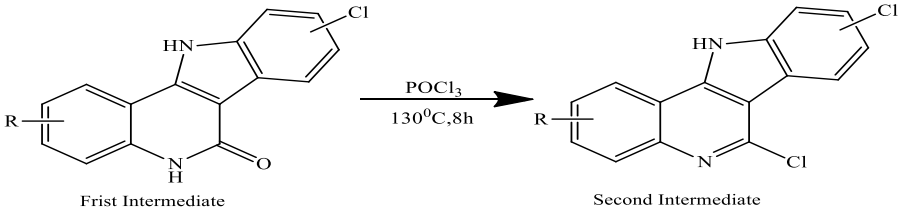


Fig.4: Dehydrative chlorination with $POCl_3$

Step 5: Amine addition

Amino groups were introduced at C-13 by ArSN reaction with various amines. Various amines such as 4-(3-amino propylmorpholino), N-methyl piperazino, N-ethyl piperazino, Benzyl amino, N-butyl amino, 3-hydroxy aniline, 4-hydroxy aniline, 4-bromo aniline and 4-fluro aniline 1g introduced at R1 position by heating the reaction mixture at 90-140°C for 4h.

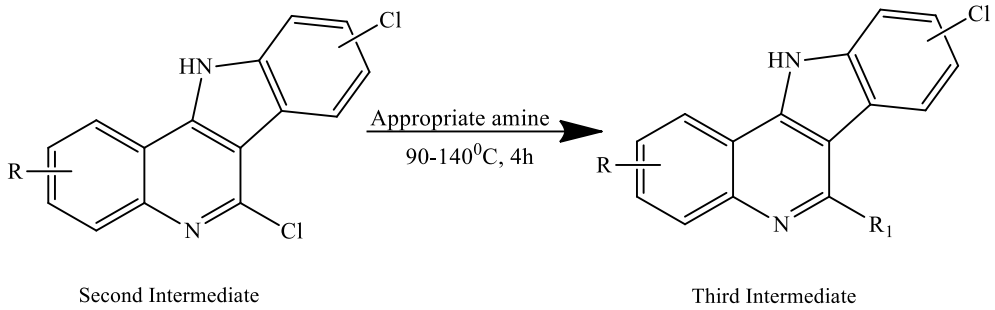


Fig.5: Amine addition

R_2=1-(6-(methylamino) hexyl)-3-phenylurea,1-(3-(methylamino) propyl)-3-phenylthiourea,1-(3-(methylamino) propyl)-3-phenylurea,1-(2-methyl-3-(methylamino)propyl)-3-phenylurea,1-(2,2-dimethyl-3-(methylamino)propyl)-3-phenylurea,N-(3-(methylamino)propyl)methanesulfonamide,1-(3-(methylamino)propyl)-3-phenylurea

Step 6: - addition of benzoyl chloride

Reaction conditions: intermediate third react with benzoyl chloride catalyst, reaction temperature:15°C. "Addition order: indole was added to the reaction mixture containing benzoyl chloride and catalyst."

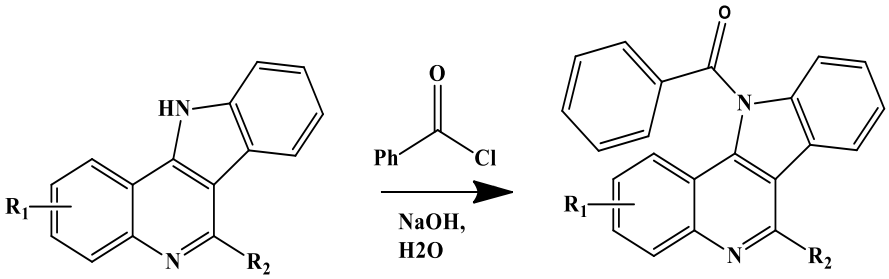


Fig.6: Addition of benzoyl chloride

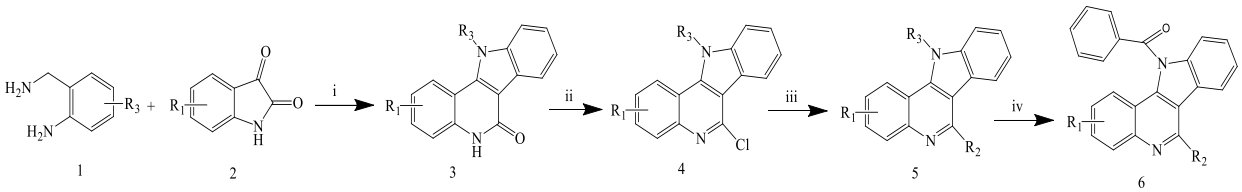


Fig.7: The overall synthetic scheme for the synthesis of Isocryptolepine analogues

(Bergman et al., 2003)

(i) Acetic acid, reflux, 8-20h.

(ii) $POCl_3$, 130°C, 8h.

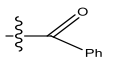
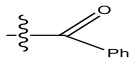
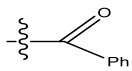
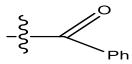
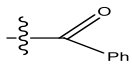
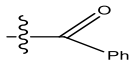
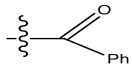
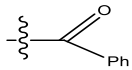
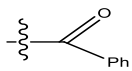
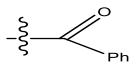
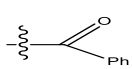
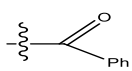
(iii) Appropriate amines, 90-140°C, and 20 min-4h

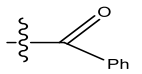
- R_1= H/2-F/2-Cl/2-Br/2-Me/2-MeO
- R_2= 1-(6-(methylamino) hexyl)-3-phenylurea, 1-(3-(methylamino) propyl)-3-phenylthiourea, 1-(3-(methylamino) propyl) -3-phenylurea, 1-(2-methyl-3-(methylamino) propyl) -3-phenylurea, 1-(2,2-dimethyl-3-(methylamino) propyl)-3-phenylurea, N-(3-(methylamino) propyl) methanesulfonamide, 1-(3-(methylamino)propyl)-3-phenylureaR_3= Benzoyl chloride

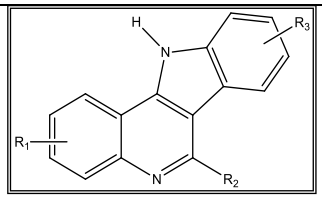
Selection of compounds for synthesis

Thirteen Isocryptolepineanalogs were painstakingly designed for synthesis and these compounds were modelled to have specific molecular modifications with the aim of finding their suitable biological activities. This subset, as presented in Table 1, contains numerous structural changes, ranging from modifications at different places to changes in the side chains. By employing selective and creative combination of active ingredients, malaria researchers bridge a gap to the discovery of new compounds that exhibit better antiplasmodial activities, thus leading to the evolution of new treatments against the malaria disease.

Table 1: List of synthesized thirteen compounds.

S.N.	Compound name	R_1	R_2	R_3	Molecular formula
1	PN-1	2F	N1-methylpropane-1,3-diamine		$C_{25}H_{21}FN_4O$
2	PN-2	H	1-(3-(methylamino)propyl)-3-phenylurea		$C_{35}H_{33}N_5O_2$
3	PN-3	H	1-(3-(methylamino)propyl)-3-phenylthiourea		$C_{32}H_{27}N_5OS$
4	PN-4	H	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{27}N_5O_2$
5	PN-5	2F	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{26}FN_5O_2$
6	PN-6	1Br	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{26}BrN_5O_2$
7	PN-7	2Br	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{26}BrN_5O_2$
8	PN-8	$2NO_2$	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{26}N_6O_4$
9	PN-9	2Cl	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{26}ClN_5O_2$
10	PN-10	2Cl	1-(2-methyl-3-(methylamino)propyl)-3-phenylurea		$C_{33}H_{28}ClN_5O_2$
11	PN-11	2Cl	1-(2,2-dimethyl-3-(methylamino)propyl)-3-phenylurea		$C_{34}H_{30}ClN_5O_2$
12	PN-12	H	N-(3-(methylamino)propyl) methanesulfonamide		$C_{26}H_{24}N_4O_3S$

13	PN-13	H	1-(3-(methylamino)propyl)-3-phenylurea		$C_{32}H_{27}N_5O_2$



Experimental

We did not use the water or water-based products we had commercially obtained further and washed again before we used them. As usual, in case it is not differently specified, the column chromatography was performed by employing the descent solvent "n-hexane/ethyl acetate as the eluent" in a silica row gel column containing 230-400 mesh capacity. Unmentioned was whether DMSO-d6 was from separately when taking 1 H NMR spectra by Bruker DRX-400 (400 MHz FT NMR spectrometer). The internal reference was TMS, which was used to calculate chemical shifts (d ppm). Prior to NMR analysis, the samples were dissolved in DMSO-d6 to ensure proper solubility and consistency in the spectra. Subsequently, 1H NMR spectra were attained by employing "Bruker DRX-400 (400 MHz FT NMR spectrometer)", with TMS as the internal reference for calculating chemical shifts (d ppm). The mental points were found with the help of the tube method of Thiele through these unknown zones of liquid acids. With the Thiel dynode, we were able to produce the EI-MS spectra both on the WATERS Q-TOF Micro-mass (LC-MS) and the SAIF/CIL chromatograph, respectively. High resolution MS data (brUmeSkymicrOTOF II-SKA) were obtained after CHP (HPLC) purity level was determined. By HPLC, Quinolin-6-amine (1 equiv.) was taken off completely in 1 mL THF and 1.2 equiv. of isothiocyanate was added drop by drop in the solution and stirred for four to six hours at room temperature. TLC ensured the reaction against violence was cut. We let the reaction mixture go off and dried up after the reaction was through. This was followed by subsequent methods of NMR and mass spectrometry analysis. n-hexane-AcOEt (7:3) hydrochloric acid was used as the developing solution in Thin-Layer chromatography for crude purification compound mixture and obtain pure products the analytes were separated into their respective components utilizing UV3000's Separations Module. UV detectors that have analytical technology as a carrying agent medium were examined. 1 milliliter per minute of flow rate was chosen and 1: 10% 100% B was performed gradient in 35 minutes. To the two solvents (A & B), 0.1% of HCOOH (formic acid) was added.

Strategy is through the merging of PN-1 whose core is 6-NH2 indole [3, 2 - c] quinoline & that of the 6-chloroidolo (3,2-c), which is its structure. The reaction starts when quinoline 4 which is about 0.4 mmol is heated with an excess of the amine, a round 2.0 mmol, within a temperature range between 90-100°C for 20 minutes to 24 hours. Another step is the inclusion of a critical checkpoint after the final material preparation is done. To promote the reaction, top the optimum environment stirring temperature and reaction time are vital. With their help, the expected product PN-1 will be formed in an ordered manner. Moreover, the excess of amine component employed during the reaction facilitates completion of the reaction and increase the product yield of the target 6-aminoindolo[3,2-c]-quinolines. Firstly, this is the strategic approach that not only simplifies the synthetic procedure yet and guarantee that the reproduction and the scalability of PN-1 synth for the next steps research and application.

PN-1: "(6-((3-aminopropyl) amino)-2fluoro-11H-indolo [3,2-c] quinoline 11-yl) (phenyl) methanone (PN-1)." Yield: 59%, MP: 230-232°C;1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m,

2H); d 8.43 (d, J = 8 Hz, 1H), 8.02 (dd, J = 9.60, 3.00 Hz, 1H), 7.67(dd, J = 9.00, 5.32 Hz, 2H), 7.44 (m, 1H), 7.37 (m, 1H), 7.34 (m, 1H), 7.08 (s, 1H), 3.76 (d, J = 5.20 Hz, 2H), 2.73 (t, J = 6.20 Hz, 2H), 1.81 (m, 2H)
"HPLC purity 99.6%. HRMS (ESI) calculated for C25H21FN4O [M+H] +Exact Mass: 412.46, got 412.49."

PN-2: "1-(3-(2-Methoxy-11H-indolo[3,2-c] quinolin-6-ylamino) propyl)-3-phenylurea (PN-2)."Yield: 60%, melting point: 140-143°C; 1H NMR d 7.98-7.96 (m, 2H),7.59 7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.58 (d, J = 3.70 Hz, 1H), 8.42 (d, J = 7.5o Hz, 1H), 7.76 (s, 1H), 7.63 (d, J = 7.80 Hz, 2H), 7.47-7.36 (m, 3H), 7.27(s, 1H), 7.22 (t, J = 7.80 Hz, 2H), 7.14 (d, J = 9 Hz, 1H), 6.89 (d, J = 7.40 Hz, 1H), 6.59 (m, 1H), 6.36 (d, J = 5 Hz, 1H), 3.89 (s, 3H), 3.74 (d, J = 5 Hz, 2H), 3.23 (m, 2H), 1.83 (dd, J = 5.6, 3.70 Hz, 2H)
"HPLC purity 94.7%. HRMS (ESI) calculated for C33H29N5O3 [M+H]+ Exact Mass: 543.23; 543.23; 543.23."

PN-3:"11-(3-((11-benzoyl-11H-indolo[3,2-c]quinolin-6-yl)amino)propyl)-3phenylthiourea(PN-3)."Yield:72%; melting point: 150-152°C 1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H); 9.64 (s, 1H), 8.43 (d, J = 8 Hz, 1H), 8.24 (d, J = 7.60 Hz, 1H), 8.01 (m, 1H), 7.66 (d, J = 8.30 Hz, 1H), 7.53 (m, 1H), 7.47-7.36 (m, 4H), 7.26 (d, J = 21.00, 15.40, 7.50 Hz, 4H), 7.11 (t, J = 7.60 Hz, 1H), 6.68(m, 1H), 3.66 (d, J = 12.2, 6.10 Hz, 2H), 3.69 (s, 2H), 2.01 (m, 2H)
"HPLC purity 99.4%. HRMS (ESI) calculated for C32H27N5OS [M+H] + Exact Mass: -529.19 (found -529.20)"

PN-4: "1-(3-((11-benzoyl-11H-indolo[3,2-c] quinolin-6-yl) amino)propyl)-3-phenylurea." Yield: 75%; melting point:152-154°C1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H),8.61 (s, 1H), 8.49 (d, J = 7.90 Hz, 1H), 8.28 (d, J = 7.90 Hz, 1H), 7.73 (d, J = 8.30 Hz, 1H), 7.68 (d, J = 8 Hz, 1H), 7.48 (m, 3H), 7.43 (t, J = 7.50 Hz, 1H), 7.29 (dt, J = 19.3, 7.50 Hz, 2H), 7.24 (t, J = 7.10 Hz, 2H), 6.90 (dd, J = 10.7, 4 Hz, 1H), 6.79 (t, J = 5 Hz, 1H), 6.39 (t, J = 5.40 Hz, 1H), 3.82 (dd, J = 11.8, 5.70 Hz, 2H), 3.32 (dd, J = 11.9, 5.80 Hz, 2H), 1.93 (dd, J = 12.5, 6.20 Hz, 2H); 37.3, 30.9. HPLC purity 98.1%. HRMS (ESI) calculated for C32H29N5O2 [M+H] + Exact Mass: A drop from 513.24 to 513.22.

PN-5: "11-(3-((11-benzoyl-2-fluoro-11H-indolo[3,2-c] quinolin-6-yl)amino)propyl)-3-phenylurea (PN-5)." Yield: 73%, melting point: 205-207°C; 1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H),8.57 (s, 1H), 8.47 (d, J = 8 Hz, 1H), 8.02 (dd, J = 9.6, 2.9 Hz, 1H), 7.72 (dd, J = 9.1, 5.4 Hz, 1H), 7.67 (d, J = 8.10 Hz, 1H), 7.43 (d, J = 7.60 Hz, 3H), 7.34 (dd, J = 10.4, 7.50 Hz,1H), 7.31-7.28 (m, 1H), 7.25 (q, J = 5.50 Hz, 2H), 6.79 (dd, J = 11.3, 4.30 Hz, 1H), 6.79 (s, 1H), 6.33 (s, 1H), 3.71 (q, J = 6.50 Hz, 2H), 3.21 (q, J = 6.60 Hz, 2H), 1.91-1.85 (m, 2H)
"HPLC purity 98.4%. HRMS (ESI) calculated for C32H26 FN5O2 [M+H] + Exact Mass: 531.23. Apart from that, I saw 521.24."

PN-6: "1-(3-((11-benzoyl-2-fluoro-11H-indolo [3, 2-c] quinolin-6-yl)amino)propyl)-3-phenylurea (PN-6)." Yield: 76%, melting point: 225-227°C; 1 H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.57 (s, 1H), 8.51 (d, J = 8 Hz, 1H), 7.99 (d, J = 8.20 Hz, 1H), 7.72 (d, J = 8.30 Hz, 1H), 7.50 (d, J = 7.50 Hz, 1H), 7.48-7.41 (m, 3H), 7.37 (m, 2H), 7.22 (t, J = 7.50 Hz, 2H), 6.96 (s, 1H), 6.88 (m, 1H), 6.32 (s, 1H), 3.78 (d, J = 6.20 Hz, 2H), 3.27 (d, J = 6.20 Hz, 2H), 1.89 (m, 2H).

HPLC purity 99.8%. HRMS (ESI) calculated for C32H26 BrN5O2 [M+H] + Exact Mass: 591.13, then 591.15.

PN-7: "1-(3-((11-benzoyl-2-bromo-11H-indolo[3,2-c]quinolin-6-yl)amino)propyl)-3-phenylurea(PN-7)." Yield: 76%, melting point: 123-125°C; 1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.57 (s, 1H), 8.48 (m, 2H), 7.66 (d, J = 8.10 Hz, 1H), 7.61 (d, J = 8.80 Hz, 1H), 7.56 (dd, J = 8.8, 2.30 Hz, 1H), 7.43 (t, J = 8 Hz, 3H), 7.31 (t, J = 7.50 Hz, 1H), 7.24 (t, J = 7.70 Hz, 2H), 6.87 (t, J = 7.30 Hz, 2H), 6.34 (s, 1H), 3.71 (d, J = 6.20 Hz, 2H), 3.27 (d, J = 6.20 Hz, 2H), 1.87 (m, 2H)
HPLC purity 91.3%. HRMS (ESI) calculated for C32H26 BrN5O2 [M+H]+ Exact Mass: 592,349 592, 50

PN-8: "1-(3-((11-benzoyl-2-methyl-11H-indolo[3,2-c]quinolin-6yl)amino)propyl)phenylurea (PN-8)." Yield: 76%, melting point: 142-144°C; 1H NMR d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.57 (s, 1H), 8.43 (d, J = 7.80 Hz, 1H), 8.04 (s, 1H), 7.62 (dd, J = 17.8, 8.20 Hz, 2H), 7.43 (d, J = 7.60 Hz, 2H), 7.39 (t, J = 7.40 Hz, 1H), 7.31 (d, J = 8.30 Hz, 1H), 7.24 (t, J = 7.30 Hz, 1H), 7.21 (t, J = 7.60 Hz, 2H), 6.82 (t, J = 7.20 Hz, 1H), 6.61 (d, J = 5 Hz, 1H), 6.31 (d, J = 5.10 Hz, 1H), 3.76 (d, J = 5.90 Hz, 2H), 3.27 (d, J = 6 Hz, 2H), 2.47 (s, 3H), 1.91-1.84 (m, 2H)
HPLC purity96.3%. HRMS (ESI) calculated d for C33H29 N5O2[M+H] + Exact Mass: $ 527.23; 527.26.

PN-9: "1-(3-((11-benzoyl-2-nitro-11H-indolo[3,2-c]quinolin-6-yl)amino)propyl)-3-phenylurea(PN-9)." Yield: 60%, melting point: 270-272°C; 1H NMR, d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 9.33 (d, J = 2.60 Hz, 1H), 8.58 (s, 1H), 8.54 (d, J = 8 Hz, 1H), 8.23 (dd, J = 9.2, 2.60 Hz, 1H), 7.71 (dd, J = 17.8, 8.60 Hz, 2H), 7.47 (t, J = 7.60 Hz, 1H), 7.42 (dd, J = 13.2, 6.50 Hz, 3H), 7.32 (t, J = 7.50 Hz, 1H), 7.24 (t, J = 7.90 Hz, 2H), 6.86 (t, J = 7.10 Hz, 1H), 6.38 (t, J = 5.80 Hz, 1H), 3.83 (q, J = 6.40 Hz, 2H), 3.26 (q, J = 6.40 Hz, 2H), 1.92-1.85 (m, 2H)
HPLC purity 95.6%. HRMS (ESI) calculated for C32H26 N6O4[M+H]+ Exact Mass: 558.20; 558.20.

PN-10:-1-(3-((11-benzoyl-2-chloro-11H-indolo[3,2-c]quinolin-6-yl)amino)propyl)-3-phenylurea (PN-10).Yield: 74%; melting point: 123-125°C 1H NMR, d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.58 (s, 1H), 8.49 (d, J = 7.90 Hz, 1H), 8.34 (d, J = 2.40 Hz, 1H), 7.64 (m, 2H), 7.46 (dd, J = 14.9, 8.10 Hz, 4H), 7.33 (t, J = 7.20 Hz, 1H), 7.25 (t, J = 7.80 Hz, 2H), 6.88 (t, J = 7 Hz, 2H), 6.34 (t, J = 5.40 Hz, 1H), 3.78 (m, 2H), 3.328 (m, 2H), 1.88 (m, 2H).
HPLC purity 99.8%. HRMS (ESI) calculated for C32H26 ClN6O2 [M+H] + Exact Mass: 547.18, see 547.20.

PN-11: "1-(3-((11-benzoyl-2-chloro-11H-indolo[3,2-c]quinolin-6-yl)amino)-2-methylpropyl)-3-phenylurea(PN-11)." Yield: 78%, mp: 143- 145°C; 1H NMR C33H28 ClN5O2,d 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), 8.58 (s, 1H), 8.52 (d, J = 8 Hz, 1H), 8.32 (d, J = 2.50 Hz, 1H), 7.67-7.63 (m, 2H), 7.45-7.40 (m, 4H), 7.33-7.27 (m, 1H), 7.24-7.17 (m, 2H), 6.93 (t, J = 6.10 Hz, 1H),6.89-6.85 (m, 1H), 6.42 (t, J = 6.10 Hz, 1H), 3.69 (dd, J = 12.6, 6.60 Hz, 1H), 3.62-3.55 (m, 1H), 3.18 (qd, J = 13.7, 7.80 Hz, 2H), 2.10 (dd, J = 12.7, 6.80 Hz, 1H), 0.96 (d, J = 6.80 Hz, 3H)
HPLC purity100%. HRMS (ESI) calculated for C33H28 ClN5O2 [M+H] + Exact Mass: 561.19 was stated 561.19.

PN-12:"1-(3-((11-benzoyl-2-chloro-11H-indolo[3,2-c] quinolin-6-yl) amino)-2,2 dimethylpropyl)-

3-phenylurea(PN-12)." Yield: 65%, melting point: 138-140°C; 1H NMRd 7.98-7.96 (m, 2H), 7.59-7.55 (m, 1H), 7.49-7.45 (m, 2H), d 8.71 (s, 1H), 8.65 (d, J = 8 Hz, 1H), 8.34 (d, J = 2 Hz, 1H), 7.70-7.64 (m, 2H), 7.46 (dd, J = 17.4, 8.20 Hz, 4H), 7.34 (t, J = 7.50 Hz, 1H), 7.24 (t, J = 7.90 Hz, 2H), 7.04 (s, 1H), 6.90 (t, J = 7.40 Hz, 1H), 6.62 (t, J = 6.10 Hz, 1H), 3.68 (d, J = 6.20 Hz, 2H), 3.11 (d, J = 6.20 Hz, 2H), 0.95 (s, 6H).
HPLC purity 98.0%. HRMS (ESI) calculated for C34H30 ClN5O2 [M+H] + Exact Mass: 575.21, @$575.21.

Biological evaluation

ANTIMALARIAL SCREENING

In the Chemical Synthesis Lab Research Institute (CSLRI) located near Bhopal, Madhya Pradesh, all newly produced chemicals were tested for any antimalarial activity. Through the 96-well micro-titre plates, a few modifications were introduced into the micro-assay and these plates were used for the in vitro antimalarial test. The Pf strain cultures were kept in RPMI 1640 culture medium, which was supplemented 10% hematin-activated human serum, 25% HEPES, 1% D-glucose, and 0.23% sodium bicarbonate. The synchronization of the P. falciparum cultures was achieved by treating with 5% D-sorbitol, resulting in ring stage parasites only. The hemolysis experiment attempted to determine the plasma ring parasitemia at 3% hematocrit by taking 200 µl of RPMI-1640 medium and staining it to obtain the current parasitemia (rings). Furthermore, the RBCs were kept at a uniform maturity of 50%. Standard stock solutions were prepared at 5 mg/ml in DMSO, and dilutions were made in a culture medium. Initial sample concentrations ranging from 0.4 to 100 µg/ml were prepared by adding 20 µl of the diluted sample to every test plate well. Further, following the incubation at 37°C of cultured plates in candle jars sterilized by a fan. Following the incubation phase, the plates were observed under the microscope for any traces of parasitemia or RBC abnormalities, and the outcomes were documented for further analysis. Thin blood smears were prepared from all wells after 36-40 hours of incubation, diluted, stained, and evaluated microscopically.
The critical measurement was the minimal inhibitory concentration (MIC) at which all schizonts were eliminated. Chloroquine served as the standard antimalarial agent. The results were reported as MICs and the number of rings, trophozoites, and schizonts per 100 parasites was counted after 38-hour incubation. Additionally, inhibition at maturation was quantified by the relative counts.

Result and Discussion

Table 2: fig.5.25: Evaluation of synthesized compounds for their antimalarial activity against the chloroquine and quinine sensitive strain RKL-2.

S. No.	Compound ID	IC_{50} (µg/ml)
1	PN-1	0.94
2	PN-2	0.56
3	PN-3	1.34
4	PN-4	0.59
5	PN-5	0.80
6	PN-6	0.46
7	PN-7	0.49
8	PN-8	1.26
9	PN-9	0.94
10	PN-10	0.76

11	PN-11	0.73
12	PN-12	0.74
13	PN-13	1.36
14	Chloroquine	0.020
15	Quinine	0.268

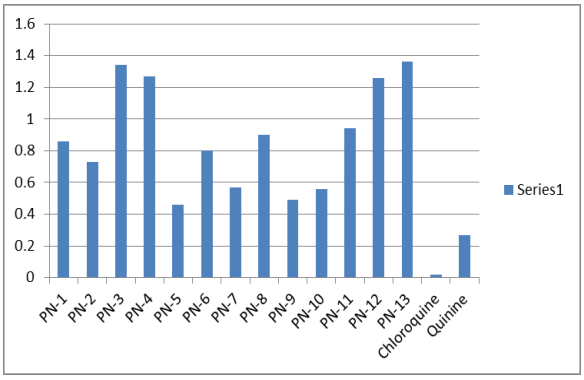


Fig 8: IN-VITRO ANTIMALARIAL SCREENING:

Conclusion

The antimalarial activity was carried out at all the synthesized compounds were screened for antimalarial activityin the pinnacle biomedical laboratory research institute (PBRI) and the results were expressed in IC50 µg/ml (Table 5.4). all the synthesized Isocryptolepine derivatives were found to be active in in-vitro antimalarial activity ranges from 0.46µg/ml to 1.37µg/ml. Compound, PN-2, PN-4, PN-11 and PN-12 were found to exhibit moderate in vitro antimalarial activity in comparison of chloroquine and quinine. The IC50 values of compounds PN-6, PN-7, PN-2, PN-4, and PN-11 were found to be 0.46, 0.49, 0.56, 0.59 and 0.73 µg/ml respectively.

The antimalarial activity results show that certain structural modifications can enhance the activity. Bromine substitution and methoxy substituents appear to improve antimalarial potential. However, it is noticeable that the most active PN-6, PN-7, have bromine substitution, and PN-2 have methoxy substituent in quinoline ring they show increased activity. Compound PN-8 which have methyl substitution on quinoline ring was found to be less active than having methoxy substitution PN-2 which justify that the methoxy group provide the hydrophobic character and helpful to bind the PDB hydrophobic pocket. And there was a greater activity of the urea derivatives PN-4 against the CQS strain. Less successful were the sulfonyl derivatives PN-13. The structure-activity relationships revealed provide direction for further optimization of antimalarial activity.

References

1. KS, Le, Tan KN, Abrigtsen M, Helgeland IUT, Lauga C, Matringe T, EK, Kennedyn JH, VM Avery, MO Sydnes. Biomimetic Assembly and Assessment of the Tetracyclic System of Isocryptolepine and Isomers Concerning Antimalarial, Antiproliferative, and Antimicrobial Activities.Molecules.2021 May 30;26(11):3268.
2. World Health Organization.World Malaria Report. 2020. Available online: https://www.who.int/publications/i/9789240015791 (accessed on 11 January 2021).
3. Collins FH, Paskewitz SM. Malaria: immediate and long-term prospects of control.Annual review of entomology.1995 Jan;40(1):195-219.

4. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ, D'Alessandro U. Quinine, an old anti-malarial drug in a modern world: function in the treatment of malaria.Malaria journal.2011 Dec;10(1):1-2.
5. G Van Baelen G, Hostyn S, Dhooghe L, Tapolcsányi P, Mátyus P, Lemière G, Dommisse R, Kaiser M, Brun R, Cos P, Maes L. Structure-activity relationship of antihelmintic and cytotoxic indoloquinoline alkaloids, and their related tricyclic and bicyclic analogs.Bioorganic& medicinal chemistry.2009 Oct 15;17(20):7209-17.
6. Tse EG, Korsik M, Todd MH. Antimalarial medications the past , present and future.Malaria journal.2019 Dec;18(1):1-21.
7. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Epidemiological proof of artemisinin resistance in western Cambodia. The New England Journal of Medicine.2008 Dec 11;359(24):2619-20.
8. Amato R, Pearson RD, Almagro-Garcia J, Amaratunga C, Lim P, Suon S, Sreng S, Stalker J, Miotto O, Fairhurst RM. Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: a review of the genetic history.The Lancet Infectious Diseases.2018 Mar 1;18(3):337-45.
9. Drenkard E. Microbial resistance to antimicrobials in biofilms produced by Pseudomonas aeruginosa.Microbes and infection.2003 Nov 1;5(13):1213-9.
10. Teng CP, Zhou T, Ye E, Liu S, Koh LD, Low M, Loh XJ, Win KY, Zhang L, Han MY. Efficient photo-induced necrosis of both multidrug and biofilm-forming bacteria using NIR-absorbing gold nanocrosses.Advanced healthcare materials.2016 Aug;5(16):2122-30.
11. Rizzato C, Torres J, Kasamatsu E, Camorlinga-Ponce M, Bravo MM, Canzian F, Kato I. It might be that biofilm formation plays a role in the cancer development with special reference to Helicobacter pylori infection.Frontiers in Microbiology.2019 Apr 29;10:846.
12. Johanson CH, Dejea CM, Edler D, Hoang LT, Santidrian AF, Felding BH, Ivanisevic J, Cho K, Wick EC, Hechenbleikner EM, Uritboonthai W. The metabolism, in turn, links bacterial biofilms and colon cancer progression.Cell metabolism.2015 Jun 2;21(6):891-7.
13. Drewes JL, White JR, Dejea CM, Fathi P, Iyadorai T, Vadivelu J, Roslani AC, Wick EC, Mongodin EF, Loke MF, Thulasi K. Meta-analysis of high-resolution bacterial 16S rRNA gene profiles and biofilm status points common cohorts of colorectalNPJ biofilms and microbiomes.2017 Nov 29;3(1):34.
14. Lee KH. Discovering and developing drugs from a natural product-based chemotherapy using a medicinal chemistry approach.Journal of natural products.2010 Mar 26;73(3):500-16.
15. Newman DJ, Cragg GM. Natural products as novel therapies over the course of nearly 4 decades from 01 January 1981 to the present day.Journal of natural products.2020 Mar 12;83(3):770-803.
16. Braccia AB, Heredia DA, Larghi EL, Kaufman TS. Neocryptolepine (cryprotackieine), a unique bioactive natural product: Isolation, synthesis, and biological mechanism of action. The European Journal of Organic Chemistry.2014 Dec;2014(36):7979-8003.
17. Sydnes MO. The advance in the construction of antimalarial indoloquinoline natural products and analogues synthesis. Fields of Natural Products Chemistry.2020 Jan 1;64:59-84.
18. Sydnes MO. A Synthetic Approach to Indoloquinoline Derivatives Synthesis. Heterocyclic Substituents; Attanasi, OA, Merino, P, Spinelli, D., Eds.2019:201-19.
19. Wang N, Wicht KJ, Imai K, Wang MQ, Ngoc TA, Kiguchi R, Kaiser M, Egan TJ, Inokuchi T. Synthesis, β-haematin inhibition, and in vitro antimalarial testing of isocryptolepine analogues: SAR studies of indolo [3,2-c] quinoline with various classes of substituents at C2, C6, and N11.Bioorganic & medicinal chemistry.2014 May 1;22(9):2629-42.

20. Aroonkit P., Thongsornkleeb C., Tummatorn J., Krajangsri S., Mungthin M., Ruchirawat S. The synthesis of isocryptolepine analogues to evaluate SAR studies for their antiplasmodial and antiproliferative activities. European Journal of Medicinal Chemistry.2015 Apr 13;94:56-62.
21. Kola I, Landis J. Whether or not the pharmaceutical industry can reduce the dropout rates remains to be seen.Nature reviews Drug discovery.2004 Aug 1;3(8):711-6.
22. Jnckers TH, Van Miert S, Cimanga K, Bailly C, Colson P, De Pauw-Gillet MC, HeuvelHvd, Claeys M, Lemière F, Esmans EL, Rozenski J. Neocryptlepine-derived new synthesis, cytoxicity, and antipalasmodial and antitrypanJournal of Medicinal Chemistry.2002 Aug 1;45(16):3497-508.
23. El Sayed I, Van der Veken P, Steert K, Dhooghe L, Hostyn S, Van Baelen G, Lemière G, Maes BU, Cos P, Maes L, and Joossens J. Synthesis of a neocryptolepine-derived aminoalkylamino-substituted molecule showing antiplasmodialactivity.Journal of medicinal chemistry.2009 May 14;52(9):2979-88.
24. EH K, Di L. Pharmaceutical profiling in drug discoveryDrug discovery today.2003 Apr 1;8(7):316-23.
25. Wong RP, Salman S, Ilett KF, Siba PM, Mueller I, & Davis TM. Desbutyl-lumefantrine is a metabolite of lumefantrine displaying considerably pronounced in vitro antimalarial activity. This metabolite might be able to determine success of artemether-lumefantrinetreatment.Antimicrobial agents and chemotherapy.2011 Mar;55(3):1194-8.
26. Fox SA, Loh SS, Dharmarajan AM, Garlepp MJ. In a study using murine malignant mesothelioma cells, it was found that Bcl-xL expression is transcriptionally downregulated by cisplatin treatment and TNF-α (tumor necrosis factor-alpha) exposure. Extending to biochemical and biophysical scientific communications.2005 Nov 25;337(3):983-91.