学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Studies on Design, Synthesis, and Biological Evaluation of Indoloquinolines of Antimalarial and Anticancer Activities

抗マラリアおよび抗ガン活性を有するインドロキノリン分子の設計、合成および活性評価に関する研究

学位論文の要旨 Abstract of Thesis

The World Malaria Report 2013 summarizes that an estimated 3.4 billion people were at risk of malaria and there were 627,000 malaria deaths worldwide in 2012. Accordingly, exploring new, safe, and effective drugs are urgently needed. The natural alkaloid indoloquinolines from *Cryptolepis sanguinolenta* have been extensively studied due to their promising antimalarial activity and potent intercalation property with DNA double helix. The author thus paid her attention to modify the indoloquinoline scaffolds and evaluate biological activities of their derivatives as antimalarial and anticancer agents.

In this thesis the author has engaged with five research topics toward the design, synthesis and evaluation of biological activity of indoloquinoline derivatives.

(1) Synthesis and in Vitro Testing of Antimalarial Activity of 6-Methylindolo[2,3-b]quinolines

The author synthesized a serious of 6-methyl-5*H*-indolo[2,3-*b*]quinoline and evaluated their antiplasmodial activity against *P. falciparum* (NF54), cytotoxicity toward L6 cells, and *β*-haematin inhibition activity. The antimalarial activities of the compounds bearing alkylamino or *ω*-aminoalkylamino stubstituents at C11 were increased compared to the 11-non (alkylamino) derivatives **1**. Introduction of methyl group at N6 was not favorable for antimalarial activity. The urea and thiourea derivatives did not favor antimalarial activity, while the cytotoxicity was improved against normal cells.

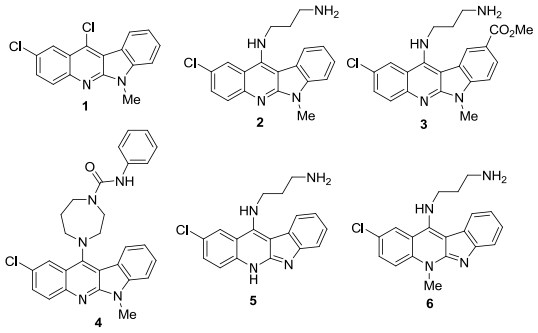
Table 1. Antimalarial activity of neocryptolepine derivatives against NF54.

Compound	(L6 cells) IC_{50} nM	(NF54) IC_{50} nM	SI (L6/NF54)	Compound	(L6 cells) IC_{50} nM	(NF54) IC_{50} nM	SI (L6/NF54)
1	68565.9	32739.0	2090	**4**	117141.0	317	369.5
2	1606	113	14.2	**5**	3482.0	27.7	125.7
3	1732	86	20.2	**6**	268.6	11.8	22.8

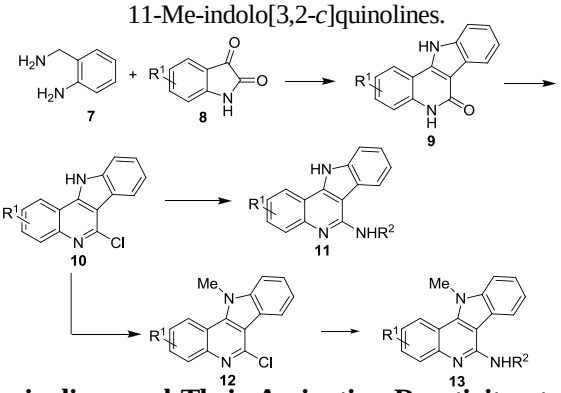
Compound **4** showed the lowest cytotoxicity. And the ester group at C9 position much enhanced the antimalarial activity of 6-methyl-5*H*-indolo[2,3-*b*]quinoline derivative. Compound **3** showed the highest antimalarial activity against NF54. Furthermore, a linear correlation between the *β*-haematin inhibition activity

and biological activity was found only for those compounds with basic amino side chains. The strong correlation between antimalarial activity and physic-chemical parameters is observed for all of compounds of 6-Methyl-6H-indolo[2,3-*b*]quinoline structure.

Figure 1. Neocryptolepine derivatives.



Scheme 1. Synthesis of 11*H*-6-chloro-indolo[2,3-*b*]quinolines 11-Me-indolo[3,2-*c*]quinolines.



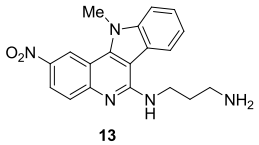
(2) Regioselective *N*-Methylation of Indolo[3,2-c]quinolines and Their Amination Reactivity at the C-6 Position

The author synthesized 11-Me-indolo[3,2-*c*]quinolines by regioselective N-methylation, and the introduction of an alkylamino group at the C-6 position. The methylation of 6-chloro-indolo[3,2-*c*]quinoline **10** with NaH-MeI proceeded at the N11, forming **12** (**Sheme 1**). Finally, amination reactivity at the C6 was assigned in the order of **10** > 11-methylated **12**.

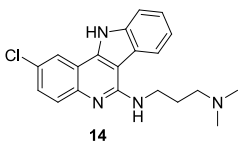
(3) Synthesis and in Vitro Antiproliferative Activity of 6-Amino-substituted 11H- and 11Me-indolo[3,2-c]quinolines

Author synthesized a series of 11*H*- and 11Me-indolo[3,2-*c*]quinoline derivatives with various alkylamino groups at C6 under varying substituents at C2. The antiproliferative activities of these compounds *in vitro* were tested against MV4-11 (human leukemia), A549 (non-small cell lung cancer) and HCT116 (colon cancer) and BALB/3T3 (normal murine fibroblasts). The methyl group introduced at *N*-11 was significantly efficacious regarding the antiprolifeative activity. All the *N*-11 methylated compounds significantly increased the antiproliferative activity. Compound **13** was the most active in the MV4-11 cell line, and Antiproliferative also exhibited a selective activity against A549, HCT116, and BALB/3T3 cell line. Author studied interactions of 1*H*- and 11Me- indolo[3,2-*c*]quinolines with DNA. The binding constants of **14** and **15** were 1.05×10^6 L/mol and 4.84×10^6 L/mol.

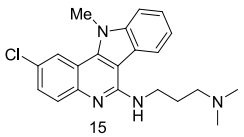
Figure 2. Activity of indolo[3,2-*c*]quinoline derivatives.



IC_{50} = 0.052±0.014 μM (MV4-11)
IC_{50} = 0.112±0.069 μM (A549)
IC_{50} = 0.007±0.004 μM (HCT116)
IC_{50} = 0.083±0.020 μM (BALB/3T3)



IC_{50} = 0.052±0.014 μM (MV4-11)



IC_{50} = 0.076±0.030 μM (MV4-11)
IC_{50} = 0.572±0.305 μM (A549)
IC_{50} = 0.098±0.022 μM (HCT116)
IC_{50} = 0.818±0.079 μM (BALB/3T3)

(4) Synthesis, β-Haematin Inhibition and in Vitro Antimalarial Testing of Isocryptolepine Analogues

Author synthesized a series of indolo[3,2-*c*]quinolines, whose side chains at the C6 position constituted with *ω*-aminoalkylamines, then the terminal amino group were modified to ureide group. Subsequently, the effect of of substituents such as 2-F, 1-Br, 2-Br, 2-Me, 2-MeO, 2-NO_2 at the C1 or C2 positions was examined. All the indolo[3,2-*c*]quinoline derivatives showed potent antimalarial activity against the CQS strain (NF54) and the CQR strain (K1) *in vitro*. The 2-chloro- substituded derivative **17** was the most effective, and urea derivatives **18** had increased activity against the CQS strain (NF54). The compounds **19** and **20** containing branched methyl groups in the lariat exhibited much higher cytotoxicity values, and antimalarial activity

increased especially against the CQR strain (K1). *In vivo* testing of **20** showed a reduction in parasitaemia on day 4, with an activity of 38%. And the linear correlation analysis revealed that there were three contributing factors, namely water solubility, hydrophilic surface area, and *β*-haematin inhibition that influence biological activity of this series against CQS (NF54) parasites.

Figure 3. Indolo[3,2-*c*]quinoline derivatives.

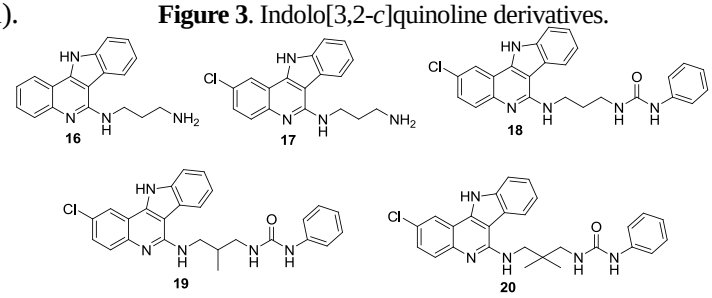


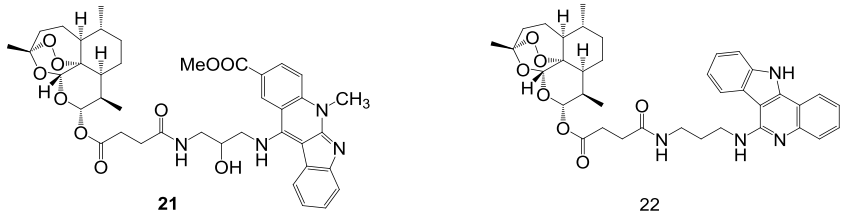
Table 2. Antimalarial activity of indolo[3,2-*c*]quinolines against NF54.

NO.	L6 cells	NF54	SI[a]	K1	SI[a]	RI[b]	*β*-Haematin Inhibition
	IC_{50} nM	IC_{50} nM	L6/NF54	IC_{50} nM	L6/K1	K1/NF54	μM
Isocryptolepine	1190			780	1.5		
10	192956	10209.7	18.9	NT[d]			>1000
16	626.8	13.7	45.8	82.7	7.6	6.0	116.3
17	1120.7	6.2	180.8	67.7	16.6	10.9	12.4
18	1152.6	2.4	480.3	53.7	21.5	22.4	23.9
19	4105.3	10.9	376.6	17.5	234.6	1.6	14.6
20	4004.4	10.6	377.8	16.9	235.9	1.6	11.7
Chloroquine		9.4		209.5		22.3	30-33

(5) Synthesis and Evaluation of Artemisinin-Indoloquinoline Hybrids as Antimalarial and Anticancer Drug Candidaes

The hybrids of artemisinin from indolo[2,3-*b*]quinoline, **21**, and indolo[3,2-*c*]quinoline, **22,** were synthesized and screened for antiplasmodial activity against two different strains (CQS: NF54 and CQR: K1) and the cytotoxicity activity against normal L6 cells. All hybrids showed the decreased cytotoxity and the increased antimalarial activity. Furthermore, these hybrids showed improving *β*-haematin inhibition activity. The highest antimalarial activity among the prepared hybrids was **21** with IC_{50} value of 1.48 nM against CQS (NF 54) strains and of 0.40 nM against CQR (K1) strains.

Figure 4. Artemisinin-indoloquinoline hybrids.



Summary:

(1) The presence and position of Me group at either N5 or N6 on neocryptolepine core are highly efficacious for low cytotoxicity and antimalatial activity.

(2) Potent antimalal and anticancer agents have been discovered by introduction and modification of substituents on isocryptolipine core.

(3) Significantly potent antimalarial agents were prepared by hybridization of artemisinin with indoloquinolines. The hybrids showed promising activity against the CQ-resistance *Plasmodia* parasites.