European Journal of Medicinal Chemistry 94 (2015) 56–62

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of isocryptolepine analogues and their structure–activity relationship studies as antiplasmodial and antiproliferative agents

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ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form
23 February 2015
Accepted 24 February 2015
Available online 26 February 2015

Keywords:
Indoloquinoline alkaloid
Azide rearrangement
Isocryptolepine
Antiplasmodial
Antiproliferative
Cytotoxicity

ABSTRACT

Novel isocryptolepine analogues have been conveniently synthesized and evaluated for antimalarial and antiproliferative activities. We have found 3-fluoro-8-bromo-isocryptolepine (**1n**) to have the highest activities against chloroquine-resistant K1, chloroquine-sensitive 3D7, and chloroquine- and mefloquine-resistant SKF58 and SRIV35 strains. Several fluorine-substituted analogues (**1b**, **1n**, and **1q**) also showed excellent selectivities while maintaining good to excellent activities against all four *Plasmodium falciparum* strains. Additionally, antiproliferative properties of isocryptolepine derivatives against HepG2, HuCCA-1, MOLT-3 and A549 cancer cell lines are reported for the first time in this study. 2-Chloroisocryptolepine (**1c**) and benzo-fused-2-chloroisocryptolepine (**1i**) showed significant bioactivities whereas several novel fluorinated compounds and 2-chloro-8-bromoisocryptolepine (**1f**) displayed excellent selectivities.

1. Introduction

Malaria and cancer have been two major health issues affecting lives of millions worldwide. Malaria is a major tropical parasitic disease concentrated in both developing and industrialized countries. It has been estimated that in 2012 malaria caused up to 627,000 deaths from an estimated 207 million people infected [1]. Many factors contributing to the wide-spread of malaria include poor control of disease-borne mosquitos, changes in global climate, poor living environments and drug-resistance of several malarial strains, the latter of which has been the main factor which prevents it from being fully eradicated [1,2]. While malaria is a major infectious illness affecting lives of millions, for non-communicable diseases, cancer is among the leading and most dreaded causes of death. According to WHO, 14 million new cases of cancer were diagnosed and around eight million people died from cancer in 2012 [3].

Isocryptolepine (**1a**) is a natural alkaloid isolated from the root of the West and Central African plant *Cryptolepis sanguinolenta* [4] which has been used traditionally to treat a range of illnesses, including diabetes, fungal infection, pain and inflammation, urinary tract and upper respiratory tract infections and malaria [5–11]. In particular, in antimalarial studies, isocryptolepine has shown potential to be developed into a new antimalarial agent. Recently, studies have surfaced which demonstrated the mechanism of cytotoxicity of a related alkaloid, cryptolepine, suggesting a plethora of new indications for this type of structures [12]. Particularly, Wietrzyk and Inokuchi recently reported the cytotoxicities of 6-amino-substituted 11*H*- and 11-methyl-indolo[3,2-c]quinoline derivatives [13]. This has inspired us to investigate both antimalarial and antiproliferative properties of new isocryptolepine analogues.

The core structure of isocryptolepine is an indolo[3,2-*c*]quinoline which can be assembled *via* several published methods [14–19]. These methods included transition metal-catalyzed cross-coupling reactions starting from haloquinoline and haloaniline derivatives [14,15,18]. These syntheses suffered from the lack of

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http://dx.doi.org/10.1016/j.ejmech.2015.02.047
0223-5234/

diversity in commercially available starting materials and the use of transition metal catalysts which may contaminate the products. Murray and co-workers [18] reported that halogen-substituted isocryptolepine analogues, obtained *via* the direct halogenation of the parent isocryptolepine, provided improved antimalarial activities and selectivities. However, the preparation of such analogues by the direct halogenation was severely limited by low regioselecitivities and yields.

Our research group demonstrated that isocryptolepine (**1a**) could be efficiently synthesized in four steps from benzyl azide (**2a**; $R^1 = R^2 = R^3 = H$) and *N*-phenylsulfonyl indole (**3a**; Ar = Ph, $R^4 = R^5 = R^6 = H$) *via* the key TfOH-promoted arylmethyl azide rearrangement reaction [20]. The synthetic sequence provided compound **1a** in 65% overall yield without the need for metal catalysts and harsh reaction conditions (Scheme 1). One could envision that this synthetic route would allow a rapid construction of a library of diverse isocryptolepine analogues simply by starting from both starting materials (**2** and **3**) with various and available structures with pre-defined substitution patterns for further investigation of their biological activities (see Scheme 2).

In this work, we report a library of new isocryptolepine analogues and the investigation of their *in vitro* antiplasmodial activities against four *Plasmodium falciparum* strains, including chloroquine-sensitive, chloroquine-resistant, and mefloquine-resistant strains, and their *in vitro* antiproliferative activities against four primary cancer cell lines as well as the cytotoxicity against a normal human lung cell line for the determination of their selectivity indices. In addition, isocryptolepine analogues with various fluorine substituents were also investigated due to the well-known properties of fluorine substituents in modifying the biological and physicochemical properties of medicinal agents [21].

2. Results and discussion

2.1. Chemistry

In a recently reported preparation of isocryptolepine, our group has utilized a TfOH-promoted rearrangement of arylmethyl azide (**2**) and subsequent interception and annulation of the resulting *N*-aryliminium ion intermediate with a protected indole (**3**) as the key step in obtaining the core cis-fused *N*-tetracyclic structure of the natural product (**4**). DDQ oxidation of compound **4** then provided the fully aromatic tetracycle **5**. The arylsulfonyl group of compound **5** was subsequently removed under the basic conditions (to **6**), followed by the *N*-methylation reaction [22] at the quinoline nitrogen to arrive at the isocryptolepine analogues (**1**). This synthetic sequence has opened an opportunity for us to conveniently and easily synthesize other analogues of isocryptolepine, starting from various arylmethyl azides (**2**) and protected indoles (**3**), both of which are widely available *via* a short synthesis and/or from commercial sources.

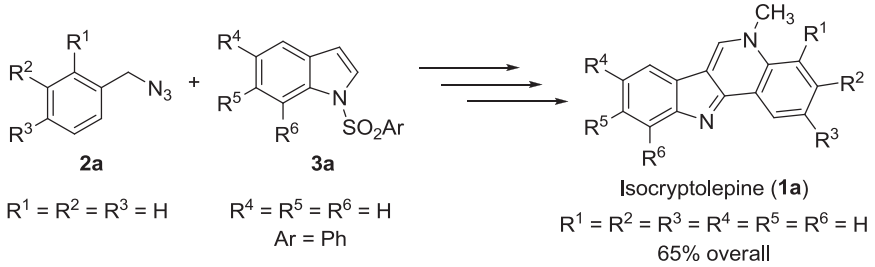
A number of isocryptolepine analogues have been evaluated previously for their antiplasmodial and antiproliferative activities. Murray and co-workers prepared and studied several halogen-substituted analogues for their antiplasmodial activities [18]. The study revealed that inclusion of halogen atoms in the structures could significantly increase the activities of these compounds. Fluorine is particularly of interest since it is well-known in the literature that the presence of fluorine atoms or groups in medicinal compounds can lead to improved biological and physicochemical properties which may be suitable for developing into active pharmaceutical ingredients [21]. Additionally, the studies of the effect of other types of substituents besides halogen atoms are rare in the literature. Based on the scarcity of available structure–activity relationship (SAR) information, we prepared new analogues of isocryptolepine starting from ten arylmethyl azides (**2a**–**2j**) and seven *N*-protected indoles (**3a**–**3g**) as shown in Fig. 1. We were able to successfully prepare a library of nineteen isocryptolepine analogues, including the parent compound (**1a**) and the previously reported **1f**, whose inclusions in this study were for comparison purposes. Compound **1f** was prepared previously by Murray and co-workers [18] and was shown to possess potent antiplasmodial activities against *P. falciparum* 3D7 and W2mef strains with high selectivity index over the normal mouse embryonic fibroblasts (3T3) cell line. The yields of each step of our syntheses are summarized in Table 1.

Of the nineteen isocryptolepine analogues (**1a**–**1s**), seventeen (**1a**–**1q**) were directly prepared from arylmethyl azides **2a**–**2j** and *N*-protected indoles **3a**–**3g**. For the remaining two analogues (**1r**–**1s**) the cis-fused *N*-tetracyclic compounds **4b** and **4m**, obtained from the rearrangement/annulation reactions between indole **3d** and azides **2b** and **2f**, respectively, were first electrophilically chlorinated [23] to give compounds **4r** and **4s** which were subsequently subjected to the remaining reactions in the synthetic sequence (entries 18–19, Table 1) as shown in Scheme 3.

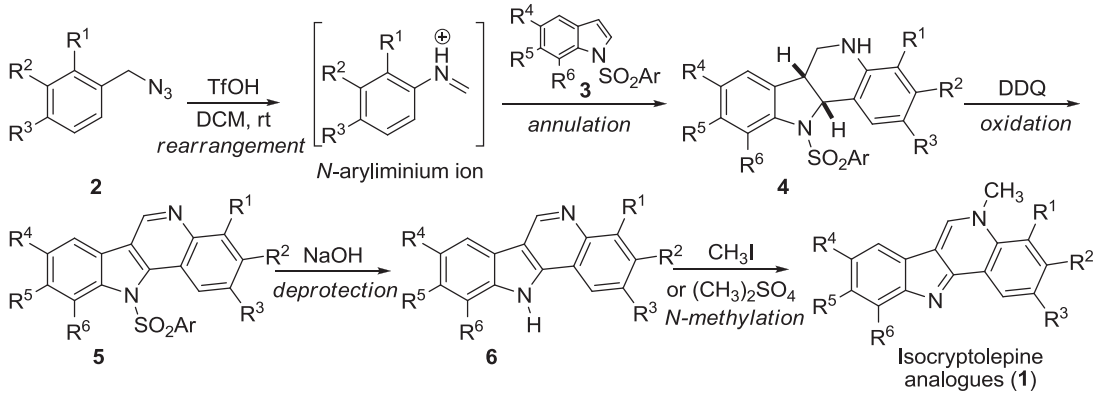
2.2. Biological evaluation

2.2.1. In vitro antiplasmodial assays

In the *in vitro* antiplasmodial assays, the twelve isocryptolepine analogues, as free bases, were subjected to the two standard laboratory *P. falciparum* isolates, chloroquine-sensitive (CQ-S) clone 3D7 and chloroquine-resistant (CQ-R) clone K1. The compounds were also tested against the two recently adapted isolates, SKF58 and SRIV35, which were collected from patients in Srisaket and Kanchanaburi provinces in Thailand in 2013, respectively. Both isolates are chloroquine-resistant (CQ-R) and mefloquine-resistant (MQ-R) phenotypes. In these studies, chloroquine, mefloquine, quinine, and artesunate were employed as positive controls for all strains. The compounds were also tested for their cytotoxicities against the normal human embryonic lung cell, MRC-5, for determination of their selectivity indices.



Scheme 1. Synthesis of isocryptolepine (**1a**) by Tummatorn and co-workers [20].



Scheme 2. Synthesis of isocryptolepine analogues (**1**). [a]Reagents and Conditions: (i) TfOH, CH_2Cl_2, rt, overnight; (ii) DDQ, CH_2Cl_2, rt, overnight; (iii) 2 M NaOH, MeOH, reflux, overnight; (iv) $(CH_3)_2SO_4$, CH_3CN, reflux, 3 h, then $NaHCO_3$, rt overnight, or CH_3I, $PhNO_2$, 130 °C in sealed tube, 1 h then 25% aq. NH_4OH, 80 °C in sealed tube, 1 h.

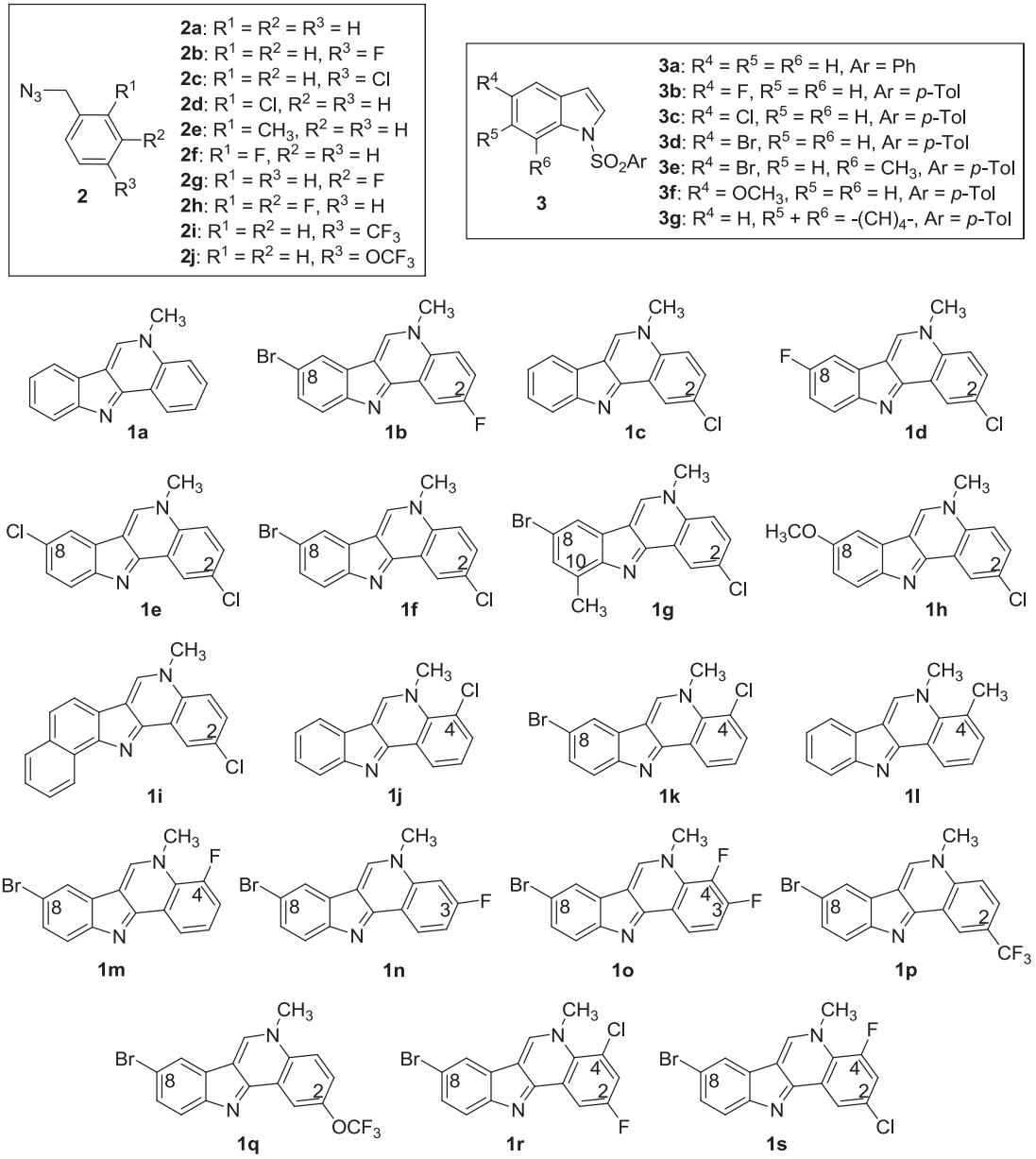
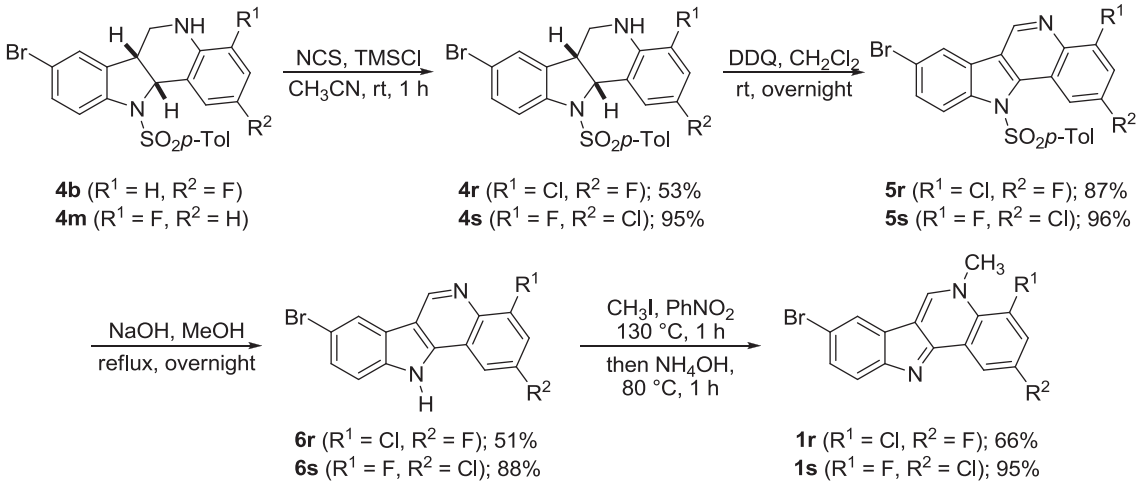


Fig. 1. Arylmethyl azides (**2**) and indoles (**3**) utilized in the preparation of isocryptolepine analogues **1a**–**1s**.

Table 1
Isolated chemical yields in the preparation of isocryptolepine analogues **1a**–**1s**.

Entry	Arylmethyl azide 2	Indole 3	Yields (%)				Isocryptolepine analogues
			Rearrangement/annulation	Oxidation	Deprotection	*N*-Methylation	
1	**2a**	**3a**	94	94	95	78[a]	**1a**
2	**2b**	**3d**	86	92	97	92	**1b**
3	**2c**	**3a**	65	96	90	68	**1c**
4	**2c**	**3b**	73	80	91	98	**1d**
5	**2c**	**3c**	63	78	95	61	**1e**
6	**2c**	**3d**	55	82	90	58	**1f**
7	**2c**	**3e**	56	90	90	92	**1g**
8	**2c**	**3f**	54	78	70	73	**1h**
9	**2c**	**3g**	33	78	>99	80	**1i**
10	**2d**	**3a**	75	76	80	48	**1j**
11	**2d**	**3d**	55	79	70	57	**1k**
12	**2e**	**3a**	71	70	89	67	**1l**
13	**2f**	**3d**	98	90	93	96	**1m**
14	**2g**	**3d**	25	76	99	73	**1n**
15	**2h**	**3d**	56	76	74	63	**1o**
16	**2i**	**3d**	51	90	55	95	**1p**
17	**2j**	**3d**	72	97	87	99	**1q**
18	–	–	–	87	51	66	**1r**[b]
19	–	–	–	96	88	93	**1s**[b]

[a] *N*-Methylation was performed using $(CH_3)_2SO_4$ in refluxing CH_3CN, followed by treatment with $NaHCO_3$.
[b] Isocryptolepine analogues **1r** and **1s** were prepared from tetracycles **4b** and **4m**, respectively, *via* electrophilic chlorination reaction. Please refer to text and Scheme 3 for details.



Scheme 3. Syntheses of isocryptolepine analogues **1r** and **1s** starting from tetracycles **4b** and **4m**.

As shown in Table 2, the parent compound **1a** was found to have lower activities when compared to other analogues tested. The previously reported compound **1f** showed decent activities against all four malarial strains. However, several of the new analogues displayed improved activities over **1f**. For example, compounds **1b**, **1e**, **1g**, **1i**, **1n**, and **1q** were all found to have better activities than compound **1f** for strains K1, 3D7, and SKF58, while analogues **1e**, **1g**, **1i**, **1n**, and **1q** also displayed better activities than analogue **1f** for SRIV35. Most notably, compound **1n** was found to have the best activities in all four strains with IC_{50} values 61.8, 37.9, 92.4, and 83.0 nM for K1, 3D7, SKF58, and SRIV35 strains, respectively. Compound **1n** also displayed a better activity than quinine in 3D7 (37.9 nM vs. 80 nM) and SKF58 (92.4 nM vs. 199 nM), and a better activity than both chloroquine and quinine against K1 (61.8 nM vs. 131 nM of chloroquine and 113 nM of quinine), and SRIV35 (83.0 nM vs. 145 nM of chloroquine and 431 nM of quinine). For SKF58, which is both chloroquine- and mefloquine-resistant, seven new compounds, **1b**, **1d**, **1e**, **1g**, **1i**, **1n**, and **1q**, were all found to show better activities than quinine. And for the choroquine- and mefloquine-resistant SRIV35, analogues **1g**, **1i 1n**, and **1q** displayed better activities than chloroquine while the new analogues **1b**–**1e**, **1h**–**1i**, **1m**–**1n**, and **1q** all showed better activities than quinine. The anti-malarial assays revealed the notable role of fluorine in improving the activities of the isocryptolepine analogues. In particular, compound **1n**, which contains 3-fluoro substituent, and compound **1q**, which contains 4-OCF_3 substituent, showed the most potent activities in all four plasmodial strains tested. It also showed better activities than quinine in all four strains and better activities than chloroquine in K1 and SRIV35.

Analogues **1a**–**1s** were next tested against the normal human embryonic lung cell, MRC-5, to determine their toxicity in the normal cell and thus to gauge their selectivity performance, which was expressed as the selectivity index (SI) (Table 2). The IC_{50} in MRC-5 of compound **1f** was determined to be more than 144,667 nM (see footnote d in Table 2), and thus leading to high SI values for this compound as shown in Table 2, which was in good

Table 2
In vitro antiplasmodial activities of isocryptolepine analogues **1a**–**1s** against four *P. falciparum* strains.

Analogues[a]	MRC-5 IC_{50} (nM)	K1 (CQ-R)[b]		3D7 (CQ-S)[b]		SKF58 (CQ-R and MQ-R)[b]		SRIV35 (CQ-R and MQ-R)[b]	
		IC_{50} (nM)	SI[c]	IC_{50} (nM)	SI[c]	IC_{50} (nM)	SI[c]	IC_{50} (nM)	SI[c]
1a	8352 ± 5640	778.6 ± 86.5	10.72	585.0 ± 50.1	14.27	410.0 ± 4.8	20.37	462.8 ± 16.5	18.04
1b	121944 ± 12577	187.6 ± 25.8	650.00	119.3 ± 13.7	1022.13	132.4 ± 5.0	921.00	211.3 ± 12.4	577.09
1c	10423 ± 2849	320.9 ± 19.6	32.47	221.3 ± 12.1	47.09	228.0 ± 10.5	45.70	216.9 ± 34.2	48.04
1d	4039 ± 1124	287.5 ± 23.8	14.05	165.8 ± 1.3	24.37	176.9 ± 18.7	22.84	176.2 ± 11.0	22.93
1e	25169 ± 7537	228.5 ± 21.9	110.15	172.2 ± 11.2	146.17	129.7 ± 15.6	194.06	155.8 ± 4.5	161.55
1f	>144667[d]	232.3 ± 41.9	>622.77	181.8 ± 30.1	>795.76	162.0 ± 1.6	>893.02	171.2 ± 3.5	>845.04
1g	5429 ± 362	145.0 ± 18.8	37.79	95.0 ± 4.9	57.68	92.6 ± 5.0	59.18	86.7 ± 1.6	63.21
1h	3100 ± 404	380.2 ± 14.9	7.52	211.4 ± 51.2	13.53	207.4 ± 52.5	13.79	208.7 ± 17.1	13.70
1i	2620 ± 126	190.3 ± 15.3	24.07	136.3 ± 18.8	33.60	133.6 ± 6.7	34.28	126.3 ± 5.3	36.26
1j	12447 ± 5286	>2000	–	>2000	–	>2000	–	>2000	–
1k	3154 ± 376	>2000	–	>2000	–	>2000	–	>2000	–
1l	39544 ± 10069	1738.5 ± 102.1	22.21	1569.4 ± 39.9	24.60	1569.4 ± 17.6	24.60	1569.4 ± 47.3	24.60
1m	15327 ± 709	295.9 ± 40.2	51.80	178.0 ± 25.8	86.11	243.1 ± 32.5	63.05	235.2 ± 58.8	65.17
1n	26279 ± 20837	61.8 ± 9.1	425.23	37.9 ± 9.9	693.38	92.4 ± 17.6	284.40	83.0 ± 17.3	316.61
1o	>72014[e]	1353.0 ± 70.7	>53.23	290.2 ± 132.3	>248.15	9865 ± 125.8	>7.30	>2000	–
1p	>131866[f]	1368.1 ± 325.8	>96.39	579.3 ± 82.4	>227.63	1223.7 ± 256.8	>107.76	929.2 ± 104.2	>141.91
1q	86633 ± 19200	67.9 ± 5.5	1275.89	57.7 ± 4.5	1501.44	108.4 ± 12.8	799.20	107.7 ± 15.4	804.39
1r	108000 ± 1556	>2000	–	>2000	–	>2000	–	>2000	–
1s	>137509[g]	1355.6 ± 255.2	101.44	757.8 ± 82.4	>181.46	1404.5 ± 152.8	97.91	1400.8 ± 320.4	>98.16
Chloroquine	–	131	–	18	–	76	–	145	–
Mefloquine	–	14	–	20	–	55	–	65	–
Quinine	–	113	–	80	–	199	–	431	–
Artesunate	–	3.5	–	4.2	–	10	–	4.1	–

[a] Analogues were tested as free bases.
[b] CQ-R = chloroquine-resistant, CQ-S = chloroquine-sensitive, MQ-R = mefloquine-resistant.
[c] SI = selectivity index = cytotoxicity in MRC-5/activity in malarial strain.
[d] At the maximum concentration of 50 μg/mL or 144667 nM, only 40.45% cytotoxicity on average in MRC-5 was observed.
[e] At the maximum concentration of 25 μg/mL or 72014 nM, only 32.60% cytotoxicity on average in MRC-5 was observed.
[f] At the maximum concentration of 50 μg/mL or 131866 nM, only 35.85% cytotoxicity on average in MRC-5 was observed.
[g] At the maximum concentration of 50 μg/mL or 137509 nM, only 34.00% cytotoxicity on average in MRC-5 was observed.

agreement with the data reported previously by Murray. In the current study, analogues **1b, 1n**, and **1q** were generally found to display excellent selectivities, especially analogues **1n** and **1q** showed *significantly lower* IC_{50} values than compound **1f** in all malarial strains. This again showed the important role of fluorine in improving the selectivities of isocryptolepine analogues over the previously reported compounds.

It could be concluded from these results that the presence of fluorine substituents in the quinoline sub-structure of isocryptolepine seemed to give a vast improvement both in activities and selectivities over compound **1f**. The structure-activity information obtained in this study should provide a platform for further development of antimalarial agents with both better activity and selectivity.

2.2.2. *In vitro antiproliferative assays*

Nineteen isocryptolepine analogues were for the first time subjected to *in vitro* antiproliferative assays in their free base form. The assays were performed against four primary cancer cell lines, including HepG2 (human liver carcinoma), HuCCA-1 (human cholangiocarcinoma), MOLT-3 (human acute lymphoblastic leukemia), and A549 (human lung adenocarcinoma). The selectivity indices (SI) of these compounds were also determined against the toxicity in the normal human embryonic lung cell, MRC-5. HepG2, HuCCA-1, A549, and MRC-5 assays were performed using MTT method while MOLT-3 assay was performed using XTT method. The positive controls against these cell lines were performed using either doxorubicin or etoposide or both which are common positive controls for these assays. The results of these assays are summarized in Table 3.

From Table 3, several of the isocryptolepine analogues were found to show better activities than the parent compound (**1a**). Specifically, compound **1c**, having only one chlorine substituent at C-2 position, showed lowest IC_{50} values in HepG2 (0.64 μM) and MOLT-3 (0.52 μM) cell lines while compound **1i**, with an additional benzo-fused structure, displayed the best activity with the lowest IC_{50} value (1.55 μM) in HuCCA-1. Compound **1i** also showed the best cytotoxicity in A549 cell line (1.20 μM), which was comparable to that of isocryptolepine (**1a**) (1.21 μM).

The selectivity performance, expressed as the selectivity index (SI), of these compounds in cancer cells was next studied in a similar fashion to that in the antimalarial study. As shown in Table 3, compound **1f** displayed a good selectivity profile, and was generally better than the parent compound (**1a**) due to its relatively low toxicity to MRC-5 (IC_{50} > 144.67 μM), while maintaining a comparable potency, especially in MOLT-3 cell line. Analogue **1b** was also found to display good selectivity in all four cancer cell lines and was found to have better selectivity than isocryptolepine (**1a**). The 2-fluoro and 2-chloro substituents (in **1b** and **1f**, respectively) seemed to help in improving the selectivity without compromising much of the activities. In addition, fluorinated analogues also seemed to generally display better selectivity profiles in all cell lines. For example, in HuCCA-1 cell line analogues **1p**–**1s** all showed higher selectivities than others while also possessing relatively potent antiproliferative properties.

The cytotoxicity results in cancerous cells showed that the activities of these analogues could be improved by substituting the hydrogen atom at C-2 position of isocryptolepine (**1a**) with a chlorine atom as seen in the improved activities of compound **1c** in HepG2 and MOLT-3 cell lines. For HuCCA-1, an addition of the benzo-fused moiety in the molecule, as seen in compound **1i**, was found to be effective in increasing the activity of the analogue while in A549 cell line, this difference was not obvious. In terms of the toxicity to the normal cell, analogues with various fluorine substituents were found to help in reducing its cytotoxicity against MRC-5 while maintaining its relatively high toxicity in all other cell

Table 3
In vitro cytotoxic activities of isocryptolepine analogues **1a**–**1s** against four primary cancer cell lines and one normal lung cell line.

Analogues[a]	MRC-5 IC_{50} (μM)	HepG2		HuCCA-1		MOLT-3		A549	
		IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI[b]
1a	8.35 ± 5.64	0.73 ± 0.09	11.41	1.81 ± 0.18	4.62	0.73 ± 0.09	11.41	1.21 ± 0.27	6.93
1b	121.94 ± 12.58	3.65 ± 1.22	33.45	14.98 ± 5.97	8.14	0.88 ± 0.12	138.41	13.22 ± 0.71	9.23
1c	10.42 ± 2.85	0.64 ± 0.04	16.35	6.45 ± 0.79	1.62	0.52 ± 0.11	19.86	1.50 ± 0.08	6.95
1d	4.04 ± 1.12	0.81 ± 0.14	5.00	9.48 ± 0.67	0.43	0.74 ± 0.04	5.48	7.90 ± 1.31	0.51
1e	25.17 ± 7.54	7.90 ± 1.49	3.18	13.25 ± 7.72	1.90	0.93 ± 0.03	27.07	30.25 ± 5.21	0.83
1f	>144.67[c]	4.83 ± 0.84	>29.95	30.79 ± 6.38	>4.70	0.75 ± 0.09	>192.89	7.03 ± 1.10	>20.58
1g	5.43 ± 0.36	1.39 ± 0.81	3.94	6.15 ± 2.36	0.89	1.17 ± 0.06	4.69	2.34 ± 0.01	2.35
1h	3.10 ± 0.40	2.19 ± 0.74	1.31	10.41 ± 5.47	0.28	2.90 ± 0.51	0.99	6.98 ± 2.43	0.41
1i	2.62 ± 0.13	0.76 ± 0.22	6.04	1.55 ± 0.11	2.96	1.55 ± 0.28	2.96	1.20 ± 0.07	3.82
1j	12.45 ± 5.29	2.62 ± 0.71	4.74	21.00 ± 10.92	0.59	1.27 ± 0.15	9.76	8.59 ± 1.56	1.45
1k	3.15 ± 0.38	2.55 ± 0.20	1.24	4.92 ± 0.00	0.64	0.78 ± 0.09	4.04	5.50 ± 0.19	0.57
1l	39.54 ± 10.07	2.60 ± 0.53	14.86	16.56 ± 0.28	2.33	2.92 ± 0.57	13.21	11.12 ± 0.03	3.47
1m	15.33 ± 0.71	3.22 ± 0.17	4.76	4.48 ± 0.11	3.42	1.09 ± 0.13	14.06	6.17 ± 1.72	2.48
1n	26.28 ± 20.84	7.41 ± 0.86	3.55	10.20 ± 3.29	2.58	1.26 ± 0.24	20.86	7.97 ± 5.07	3.30
1o	>72.01[d]	-[h]	-[h]	42.69 ± 7.63	>1.68	17.35 ± 3.15	>4.15	24.96 ± 0.14	>2.89
1p	>131.87[e]	75.61 ± 21.52	>1.74	7.44 ± 6.19	>17.72	34.96 ± 13.78	>3.77	105.43 ± 12.48	>1.25
1q	86.63 ± 19.20	8.20 ± 6.84	10.56	7.01 ± 1.18	12.36	2.60 ± 0.42	33.32	8.46 ± 2.50	10.24
1r	108.00 ± 1.56	3.47 ± 0.47	31.12	8.00 ± 3.54	13.50	-[h]	-[h]	35.89 ± 7.32	3.01
1s	>137.51[f]	8.76 ± 7.37	>15.70	11.39 ± 5.15	>12.07	55.66 ± 5.60	>2.47	-[h]	-[h]
Etoposide	–	40.47 ± 3.72	–	–	–	0.11 ± 0.01	–	–	–
Doxorubicin	>91.99[g]	0.40 ± 0.07	>229.98	2.39 ± 0.15	>38.49	–	–	0.98 ± 0.06	>93.87

[a] Analogues were tested as free bases.
[b] SI = selectivity index = cytotoxicity in MRC-5/cytotoxicity in cancer cell line.
[c] At the maximum concentration of 50 μg/mL or 144.67 μM, only 40.45% cytotoxicity on average in MRC-5 was observed.
[d] At the maximum concentration of 25 μg/mL or 72.01 μM, only 32.60% cytotoxicity on average in MRC-5 was observed.
[e] At the maximum concentration of 50 μg/mL or 131.87 μM, only 35.85% cytotoxicity on average in MRC-5 was observed.
[f] At the maximum concentration of 50 μg/mL or 137.51 μM, only 34.00% cytotoxicity on average in MRC-5 was observed.
[g] At the maximum concentration of 50 μg/mL or 91.99 μM, only 37.13% cytotoxicity on average in MRC-5 was observed.
[h] IC_{50} and selectivities of these compounds could not be determined.

lines. The data provided by the current study has shown for the first time that these isocryptolepine analogues could be effective agents in killing several cancer cells.

3. Conclusion

We have synthesized and evaluated nineteen isocryptolepine analogues for *in vitro* antiplasmodial activities against K1, 3D7, SKF58, and SRIV35 *P. falciparum* strains and have identified 8-bromo-3-fluoro-isocryptolepine (**1n**) to have much better activities against all four strains than both the parent compound (**1a**) and the previously reported 8-bromo-2-chloroisocryptolepine (**1f**). In addition, we have shown several fluorine-substituted analogues to be much less toxic against normal human lung cells, MRC-5, than the parent compound while maintaining their relatively potent bioactivities, especially in compounds **1n** and **1q**. Moreover, for the first time these analogues' *in vitro* activities against four primary cancer cell lines, HepG2, HuCCA-1, MOLT-3, and A549, were systematically evaluated and two new analogues (**1c** and **1i**) were shown to have better activities in these cell lines than the parent isocryptolepine (**1a**) and the previously reported analogue **1f**. In terms of their selectivity, several novel compounds with fluorine substituents seemed to display better *in vitro* selectivity than the parent isocryptolepine. The structure–activity relationship profile of isocryptolepine analogues obtained from this study, as well as the convenient and metal-free preparation of the compounds, will assist in facilitating the future antimalarial and antiproliferative drug discovery and development effort.

Acknowledgments

This work was financially supported by Thailand Toray Science Foundation (TTSF), Chulabhorn Research Institute (CRI), Chulabhorn Graduate Institute (CGI), and Center of Excellence on Environmental Health and Toxicology (EHT), Ministry of Education, for which we are grateful. We also would like to thank Ms. Pakamas Intachote, Ms. Busakorn Saimanee, and Ms. Suchada Sengsai for their assistance in evaluating cytotoxicity of the compounds.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.02.047.

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