Tetrahedron 64 (2008) 11802–11809

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline: two new unnatural isoquinoline isomers of the cryptolepine series

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ARTICLE INFO

Article history:
Received 1 July 2008
Received in revised form 8 August 2008
Accepted 26 August 2008
Available online 4 October 2008

Keywords:
Malaria
Plasmodium falciparum
Cryptolepines
Palladium
Buchwald–Hartwig reaction
C(sp^2)–H activation
Suzuki reaction
Nitrene insertion

ABSTRACT

11*H*-indolo[3,2-*c*]isoquinoline has been synthesized in two steps starting from 4-bromoisoquinoline and 2-bromoaniline via a selective Buchwald–Hartwig reaction followed by a Pd-catalyzed intramolecular direct arylation involving C(sp^2)–H activation. The synthesis of 7*H*-indolo[2,3-*c*]isoquinoline was achieved by a combination of a Suzuki reaction with an intramolecular nitrene insertion reaction starting from 4-bromoisoquinoline and {2-[(2,2-dimethylpropanoyl)amino]phenyl}boronic acid. Selective methylation of the tetracyclic skeletons yielded the title compounds 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline, which have never been described in the literature before.

1. Introduction

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* and transmitted by the *Anopheles* mosquito. Among the four pathogenic species, *Plasmodium falciparum* is the most dangerous one. Each year, at least 300 million acute cases of malaria are estimated to occur globally. Although malaria can be prevented and treated, it still kills more than one million people each year.[1] A major problem is the increasing resistance of the parasite against the currently available drugs, hence the continuous need for new and more efficient antiplasmodial drugs.

Although the synthetic drugs (e.g., chloroquine, halofantrine and mefloquine) still prove their usefulness in prevention and/or therapy, the World Health Organization (WHO) now strongly recommends to switch to combination therapy to avoid build up of resistance and clinical failure. Besides synthetic drugs, several natural products have shown antiplasmodial activity. Among the most potent is quinine, isolated from the *Cinchona* bark and artemisinin, isolated from the leafy portions of *Artemisia annua*.[2,3] The above mentioned synthetic drugs are currently used in combination with the artemisinin derivatives artesunate and artemether.

In Central and West African traditional folk medicine, a decoction of the root of the plant *Cryptolepis sanguinolenta* is used to treat fevers, including those caused by malaria. Several alkaloids have been isolated, such as cryptolepine[4a] (5-methyl-5*H*-indolo[3,2-*b*]quinoline) (**1**), neocryptolepine[4b,c] (cryptotackieine, 5-methyl-5*H*-indolo [2,3-*b*]quinoline) (**2**) and isocryptolepine[4d,e] (cryptosanguinolentine, 5-methyl-5*H*-indolo[3,2-*c*]quinoline) (**3**) (Fig. 1). The benzo-β-carboline (5-methyl-5*H*-indolo[2,3-*c*]quinoline), for which we have adopted the name isoneocryptolepine) (**4**), surprisingly has not yet been found in nature. Recently, we developed an efficient synthetic strategy for this 'missing' isomer and for its 7*H*-indolo[2,3-*c*]quinoline core. The methodology used for the synthesis of 7*H*-indolo[2,3-*c*]quinoline is based on the combination of a selective Buchwald–Hartwig reaction with a selective Pd-catalyzed intramolecular direct arylation reaction.[5,6]

Molecules **1**–**4** are isomeric indoloquinolines which possess an interesting antiplasmodial activity. The 'missing' isomer

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doi:10.1016/j.tet.2008.08.116

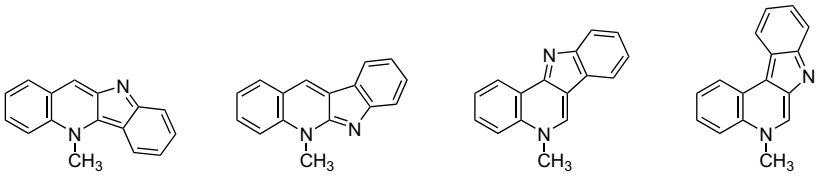


Figure 1. Cryptolepine (**1**), neocryptolepine (**2**), isocryptolepine (**3**) and isoneocryptolepine (**4**).

isoneocryptolepine (**4**) is approximately two times less active (K1 strain of *P. falciparum*, resistant to chloroquine and pyrimethamine) than cryptolepine (**1**) (the most active compound of the quartet), but it is also four times less cytotoxic (L6 cells), resulting in the best selectivity index (cytotoxicity/antiplasmodial activity ratio) of the four indoloquinolines (Table 4).[7]

The selectivity index of **4** stimulated us to synthesize new unnatural indoloquinoline isomers. Here we describe the synthesis of two indoloisoquinoline isomers; 6-methyl-6*H*-indolo[3,2-*c*]-isoquinoline (**5**) and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline (**6**), which are the isoquinoline analogues of, respectively, **3** and **4** (Fig. 2).

2. Results and discussion

Previously, our laboratory synthesized isocryptolepine (**3**) and isoneocryptolepine (**4**) via the combination of a selective Buchwald–Hartwig reaction with a Pd-catalyzed direct arylation reaction.[5,6,8] Therefore, we attempted to prepare 11*H*-indolo[3,2-*c*]isoquinoline (**7**) via a similar reaction sequence starting from commercially available 4-bromoisoquinoline (**8**) and 2-bromoaniline (**9**) (Tables 1 and 2). The selective amination of **8** with **9** (Table 1) was first executed under the reaction conditions previously used for the amination of 3-bromoquinoline with **9** [2.5 mol % $Pd_2(dba)_3$/5.5 mol % XANTPHOS (9,9-dimethyl-4,5-bis(diphenylphosphino)-9*H*-xanthene) catalyst, 1.2 equiv amine, 3 equiv Cs_2CO_3, dioxane, reflux]. In the rest of the article these reaction conditions will be mentioned as 'standard conditions'.[5,6] After 27 h of reflux, complete conversion of **8** was still not obtained (Table 1, entry 1). The use of a stronger base such as K_3PO_4 did not provide a better result (Table 1, entry 2). Doubling the catalyst loading allowed an almost complete conversion in 27 h (Table 1, entry 3). An additional increase of the reaction time to 48 h gave essentially the same isolated yield (Table 1, entry 4).

Applying the Pd-catalyzed intramolecular direct arylation reaction conditions developed earlier for the ring closure of *N*-(2-bromophenyl)quinolin-3-amine on *N*-(2-bromophenyl)isoquinolin-4-amine (**10**) led to the formation of 11*H*-indolo[3,2-*c*]isoquinoline (**7**) (Table 2).[5] A catalyst loading of 10 mol % was not sufficient to drive the reaction to completion within 16 h. A double loading (20 mol %) gave full conversion in the same reaction time and the indoloisoquinoline **7** could be isolated in 78% (Table 2, entries 1 and 2). More, recently we reported on the high temperature Pd-catalyzed intramolecular direct arylation using microwave irradiation. Among several other brominated substrates,

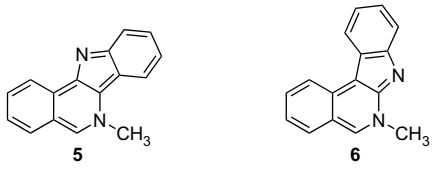
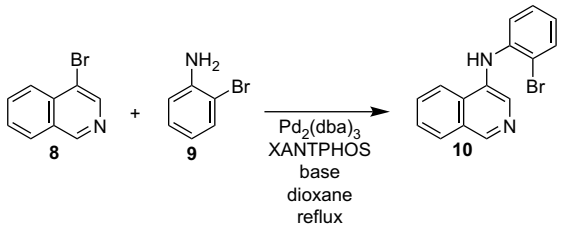


Figure 2. Target indoloisoquinolines: 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline (**5**) and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline (**6**).

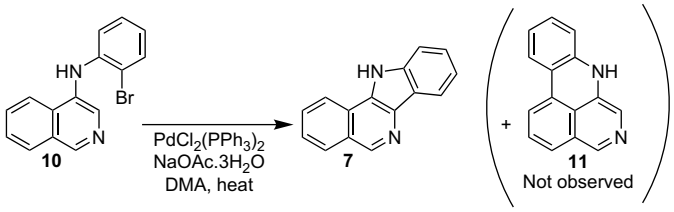
Table 1
Selective Buchwald–Hartwig reaction of 4-bromoisoquinoline (**8**) with 2-bromoaniline (**9**)



Entry	$Pd_2(dba)_3$ (mol %)	XANTPHOS (mol %)	Base	Time (h)	Recovered **8** (%)	Yield[a] **10** (%)
1	2.5	5.5	Cs_2CO_3	27	20	61
2	2.5	5.5	K_3PO_4	27	25	64
3	5.0	11.0	Cs_2CO_3	27	7	74
4	5.0	11.0	Cs_2CO_3	48	8	72

[a] *x* mol % $Pd_2(dba)_3$, *y* mol % XANTPHOS, 3 mmol **8**, 3.6 mmol **9**, 9 mmol base, 12 mL dioxane, reflux.

Table 2
Pd-catalyzed intramolecular direct arylation of **10** under conventional and microwave heating

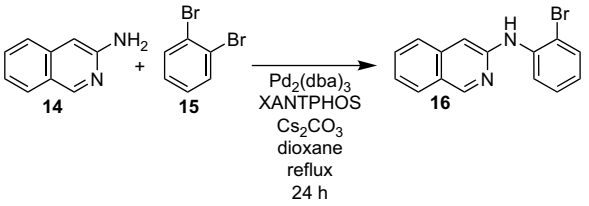


Entry	$PdCl_2(PPh_3)_2$ (mol %)	Temperature (°C)	Time	Yield **7** (%)
1	10.0	130	88 h	65[a]
2	20.0	130	16 h	78[a]
3	1.0	180	10 min	71[b]
4	1.0	200	10 min	79[b]

[a] *x* mol % $PdCl_2(PPh_3)_2$, 0.6 mmol **10**, 1.47 mmol NaOAc·$3H_2O$, 10 mL DMA, oil bath.
[b] *x* mol % $PdCl_2(PPh_3)_2$, 0.6 mmol **10**, 1.47 mmol NaOAc·$3H_2O$, 1 mL DMA, μW.

N-(2-bromophenyl)quinolin-3-amines could be smoothly cyclized in only 10–30 min using a low catalyst loading (1 mol %) at a high reaction temperature (180–200 °C).[6,9] The standard protocol uses 1 mol % $PdCl_2(PPh_3)_2$, 0.6 mmol substrate, 1.47 mmol NaOAc·$3H_2O$ and 1 mL DMA at 180 °C (μW) for 10 min. Applying these conditions on **10** gave 71% of **7** and a recovery of 8% of starting material (Table 2, entry 3). Increasing the reaction temperature to 200 °C did drive the reaction to completion in 10 min and an isolated yield of 79% was obtained (Table 2, entry 4). Besides **7**, theoretically isomeric 7*H*-pyrido[3,4,5-*gh*]phenanthridine (**11**) can also be formed but we have never observed it. Compound **7** has already been synthesized by Hajós and co-workers using a Suzuki–nitrene insertion approach.[10] They started from the

Table 3
Buchwald–Hartwig reaction of isoquinolin-3-amine (**14**) with 1,2-dibromobenzene (**15**)



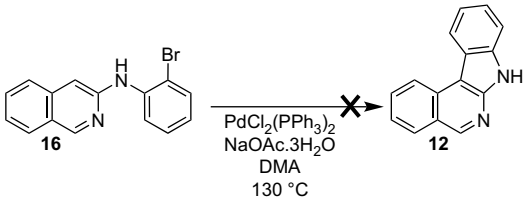
Entry	$Pd_2(dba)_3$ (mol %)	XANTPHOS (mol %)	Yield **16** (%)
1	2.5	5.5	56[a]
2	2.5	5.5	62[b]

[a] *x* mol % $Pd_2(dba)_3$, *y* mol % XANTPHOS, 1 mmol **15**, 1.2 mmol **14**, 3 mmol Cs_2CO_3, 10 mL dioxane, reflux, 24 h.
[b] 1.2 mmol **15** and 1 mmol **14** were used.

N-oxide of *N*-(2-isoquinolin-3-ylphenyl)-2,2-dimethylpropanamide. Our approach is shorter with respect to the number of steps and the overall yield is higher (78% vs 43%).

Secondly, the synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via a selective Buchwald–Hartwig reaction followed by a Pd-catalyzed intramolecular direct arylation reaction starting from commercially available isoquinolin-3-amine (**14**) and 1,2-dibromobenzene (**15**) was attempted. The amination reaction occurred fairly well under standard conditions yielding 56% of **16** after 24 h of reflux (Table 3, entry 1). TLC analysis showed that there was only a small fraction of unconverted **15** left.

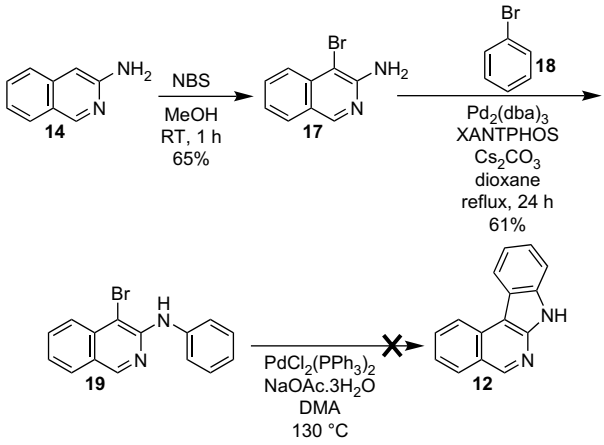
Before further optimizing the Buchwald–Hartwig reaction, the intramolecular Pd-catalyzed direct arylation reaction of **16** was tested. No reaction product **12** could be observed after 48 h of reflux under conventional heating using a 20 mol % catalyst loading (Scheme 1). Only substrate as well as dehalogenated starting material was found.



Scheme 1. Approach no. 1: synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via a Pd-catalyzed intramolecular direct arylation of **16**.

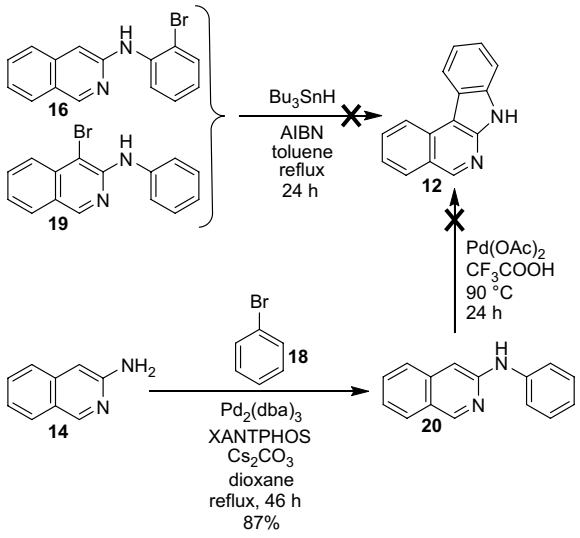
In order to achieve cyclization via Pd-catalysis we attempted to move the bromine atom leaving group from the benzene ring to the isoquinoline ring (Scheme 2). The required 4-bromoisoquinolin-3-amine (**17**) could be obtained by bromination of **14** with NBS followed by a selective Buchwald–Hartwig reaction of **17** with bromobenzene (**18**) under standard conditions. An isolated yield of 61% of 4-bromo-*N*-phenylisoquinolin-3-amine (**19**) was obtained after 24 h of reflux (Scheme 2). Unfortunately, also on this substrate the subsequent Pd-catalyzed intramolecular direct arylation reaction failed and the main product found was dehalogenated starting material.

After two defeated attempts based on C–H activation, we changed tack. To obtain our target molecule **12**, a radical cyclization reaction using tributyltinhydride (Scheme 3) was attempted.[11] Compound **16** as well as **19** was subjected to a radical C–C bond formation reaction using AIBN as initiator. Unfortunately, no cyclization reaction occurred. After 24 h of reflux only substrate and dehalogenated starting material could be found. As a second



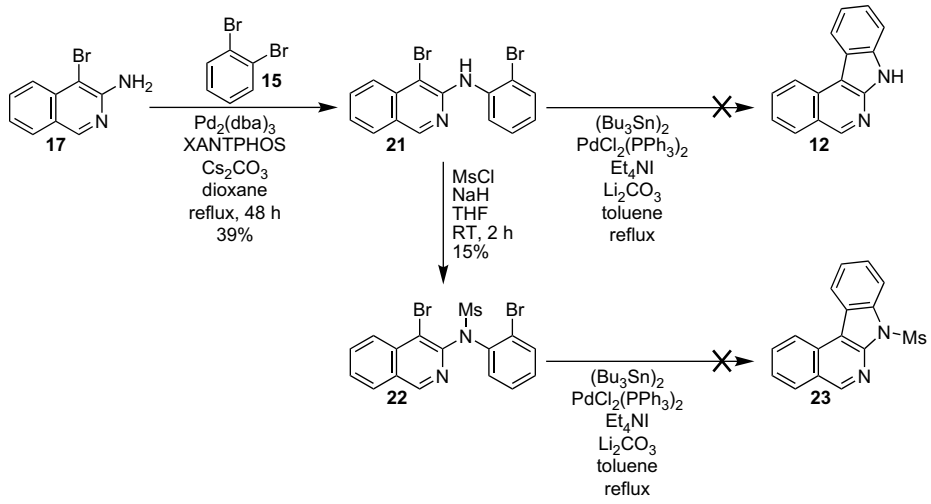
Scheme 2. Approach no. 2: synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via a Pd-catalyzed intramolecular direct arylation of **19**.

alternative strategy we tried ring closure by oxidative cyclization (Scheme 3).[12] The required substrate **20** could easily be obtained in a good yield (87%) via a Buchwald–Hartwig reaction of **14** with **18** under standard conditions. The aimed cyclization reaction was unfortunately again not successful.

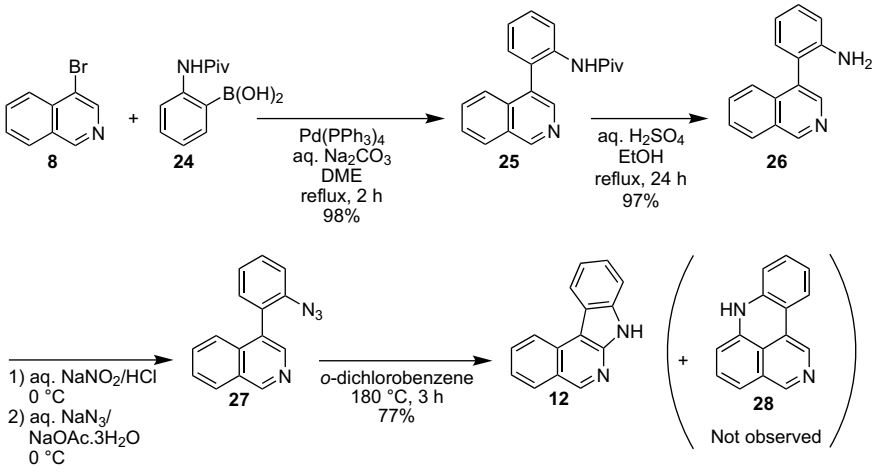


Scheme 3. Approach no. 3 and 4: synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via a radical and oxidative cyclization of **16**, **19** and **20**, respectively.

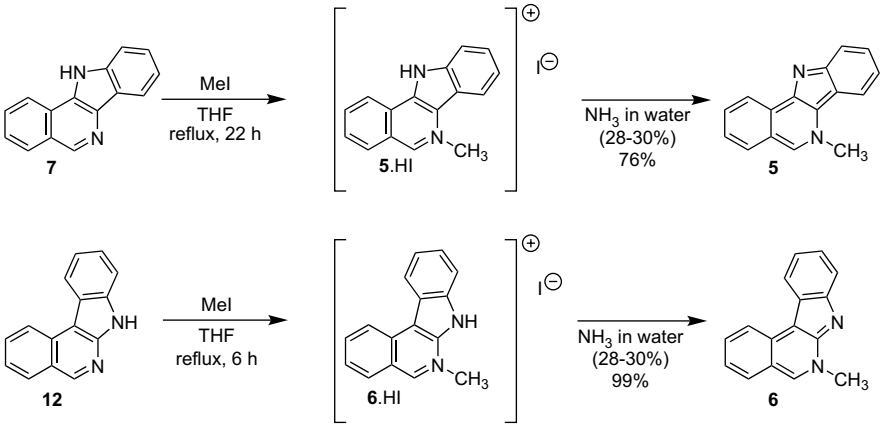
The Stille–Kelly reaction, a Pd-catalyzed intramolecular biaryl coupling of aryl halides or aryl triflates in the presence of distannanes, was the fifth protocol we tried in order to obtain **12** (Scheme 4).[13] In contrast to the previous methodologies where the presence of one bromine atom was sufficient, a system with two bromine atoms in the same molecule was needed. The substrate for this kind of cyclization (**21**) was synthesized via a selective Pd-catalyzed amination reaction of **17** with **15** under standard conditions, only changing the catalyst loading to 5 mol % $Pd_2(dba)_3$. The resulting yield (39%) was not high, but we decided to test the possibility to synthesize **12** via Stille–Kelly cyclization on **21** before considering optimizing the Buchwald–Hartwig reaction. Unfortunately, also in this case no reaction product was formed in the Stille–Kelly reaction on **21**. Based on results of Iwaki and co-workers, the nitrogen atom of the *N*-phenylisochinolin-3-amine entity of **21** was protected with a mesyl-group but also this substrate (**22**) did not allow cyclization (Scheme 4).[14]



Scheme 4. Approach no. 5: synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via a Stille–Kelly reaction of **21** and **22**.



Scheme 5. Synthesis of 7*H*-indolo[2,3-*c*]isoquinoline (**12**) via intramolecular nitrene insertion of **27**.



Scheme 6. Selective *N*-6 methylation of **7** and **12**.

In a last attempt to obtain the indoloisoquinoline **12** we tried to combine a Suzuki reaction with an intramolecular nitrene insertion reaction (Scheme 5). This protocol has been used by us and others to obtain other indolo-fused ring systems.[5,10,15–17] Suzuki reaction of 4-bromoisoquinoline (**8**) with {2-[(2,2-dimethylpropanoyl)-amino]phenyl}boronic acid[18] (**24**) under Gronowitz conditions[19] yielded *N*-(2-isoquinolin-4-ylphenyl)-2,2-dimethylpropanamide (**25**) in 98% yield. Acid hydrolysis of pivalamide **25** in 40% aq H_2SO_4/ethanol gave 2-(isoquinolin-4-yl)aniline (**26**) in a very good yield (97%). Subsequent diazotization of the amine **26** followed by

Table 4
Antiprotozoal activity (IC_{50}, μM), cytotoxicity (IC_{50}, μM) and selectivity index (cytotoxicity/antiplasmodial activity ratio) of compounds **1**–**4**[7] and **5**–**6**

Compound	*P. falciparum* K1	*T.b. rhodesiense*	*T. cruzi*	*L. donovani*	Cytotoxicity (L6 cells)	Selectivity index SI
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	
1	0.12±0.02	0.60±0.07	0.22±0.07	2.68±0.89	1.12±0.07	9.3
2	2.61±0.67	2.23±0.82	2.01±1.30	49.5±3.7	3.24±0.04	1.2
3	0.78±0.30	0.52±0.11	1.27±0.78	39.1±11.5	1.19±0.26	1.5
4	0.23±0.04	6.48±0.76	21.0±0.2	75.4±2.4	4.32±0.04	18.8
5	0.04±0.01	3.33	20.66	4.78	1.31±0.85	32.8
6	0.68±0.13	0.72	1.66	15.54	1.48±0.72	2.1
Chloroquine[a]	0.24±0.04					
Melarsoprol[a]		0.010±0.003				
Benznidazole[a]			1.25±0.28			
Miltefosine[a]				0.26±0.08		
Podophyllotoxin[a]					0.010±0.002	

[a] Positive control.

introduction of the azido group via a S_N1 reaction on the diazonium salt gave 4-(2-azidophenyl)isoquinoline (**27**). Finally, thermal decomposition of the azide **27** in boiling 1,2-dichlorobenzene yielded the target indoloisoquinoline **12** in a good yield (77%). The mechanism of this reaction presumably occurs via the formation of a nitrene (formed from the azide), which formally inserts into the C3-H bond.[20,21] Interestingly, the intramolecular nitrene insertion is C-3 regioselective since no C-5 ring closed product (7*H*-pyrido[3,4,5-*kl*]acridine (**28**)) was found.

For the selective *N*-6 methylation of **7** and **12** the conditions we previously reported for the selective methylation of 10-chloro-7*H*-indolo[2,3-*c*]quinoline and 9-trifluoromethyl-7*H*-indolo[2,3-*c*]-quinoline were used (CH_3I, THF, reflux; then 28–30% NH_3 in H_2O) (Scheme 6).[6,22] In previous selective methylation reactions toluene was used.[5,6] The use of THF instead of toluene facilitated the work-up and also allowed the selective methylation of **7** and **12** since the formed hydroiodide salts **5**·HI and **6**·HI immediately precipitated, hereby avoiding dimethylation. A reaction time of 22 h was necessary to obtain full conversion of **7** to **5**·HI and a reaction time of 6 h to obtain **6**·HI. The free bases **5** and **6** could be obtained from **5**·HI and **6**·HI in, respectively, 76% and 99% yield after an acid–base extraction using ammonia in water (28–30%).

Antiprotozoal activity and cytotoxicity data of the new indoloisoquinolines **5** and **6** are outlined in Table 4. Although **5** is almost six times more active against *P. falciparum* than isoneocryptolepine (**4**), **5** as well as **6** are around three times more cytotoxic than **4**. These data result in a selectivity index (SI) for **5** which is almost two times better than the one for lead compound **4** and a SI for **6** which is nine times worse. Because of the poor selectivity against any of the parasites, the relatively high cytotoxicity (which also may explain at least in part the higher activity of **5**) and the rather moderate or low selectivity index of **5** and **6**, respectively, both compounds cannot be considered as promising new leads for further exploration.

3. Conclusions

In conclusion, we synthesized two new unnatural isoquinoline isomers of the cryptolepine series: 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline (**5**) and 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline (**6**). To the best of our knowledge these compounds have never been synthesized before. 11*H*-Indolo[3,2-*c*]isoquinoline (**7**) was obtained in 57% overall yield via a selective Buchwald–Hartwig – Pd-catalyzed intramolecular direct arylation reaction starting from 4-bromoisoquinoline and 2-bromoaniline. Selective *N*-6 methylation of 11*H*-indolo[3,2-*c*]isoquinoline (**7**), gave indoloisoquinoline **5**. The synthesis of 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline (**6**) was not so straightforward. The selective Buchwald–Hartwig – Pd-catalyzed intramolecular direct arylation reaction starting from two different substrates did not work and an alternative synthesis strategy was searched. Neither a radical cyclization, nor an oxidative cyclization, nor a Stille–Kelly reaction was successful. Finally, 7*H*-indolo[2,3-*c*]isoquinoline (**12**) could be synthesized using a combination of a Suzuki reaction with an intramolecular nitrene insertion reaction in a 73% overall yield. Selective *N*-6 methylation gave the second target compound 6-methyl-6*H*-indolo[2,3-*c*]isoquinoline (**6**). Biological evaluation of the indoloisoquinolines **5** and **6** revealed that these compounds possess antiplasmodial activity, but are relatively high in cytotoxicity and possess a moderate (compound **5**) or low (compound **6**) selectivity.

4. Experimental

4.1. General

All melting points were determined on a Büchi apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer Avance 400 in the solvent indicated with TMS as an internal standard. All coupling constants are given in hertz and chemical shifts are given in parts per million. The assignment of the ^1H NMR signals of all products is based on 2D NMR techniques (COSY, NOESY, HMQC and HMBC). In compounds **10**, **16**, **19**, **20**, **21**, **22**, **25** and **26** the aryl substituent is numbered with primes and the isoquinoline core without. For mass spectrometric analysis, samples were dissolved in CH_3OH containing 0.1% formic acid and diluted to a concentration of approximately 10^{-5} mol/L. Injections (1 mL) were directed to the mass spectrometer at a flow rate of 0.7 mL/min (CH_3OH and 0.1% formic acid), using a Kontron HPLC system. Mass spectrometric data were acquired on an AQA Navigator mass spectrometer (ThermoQuest, Finigan) equipped with an ApCI ionisation interface. The AQAMax voltage was set to 20 V, the corona voltage to 3.5 kV and the probe temperature to 250 °C. Nitrogen gas was used for nebulation. Mass spectra were acquired by summing the spectra in the elution plug. In positive ion mode, the protonated molecule $[M+H]^+$ was recorded. Accurate mass data were acquired on a Q-TOF 2 (Micromass) mass spectrometer equipped with a Nanomate (Advion, Ithaca, NY) nano-electrospray source in LC-mode. Cone voltage (approx. 35 V) and ESI voltage (approx. 1.7 kV) were optimized on one compound and used for all others. For the determination of the accurate mass of the molecular ion $[M+H]^+$, a solution of polyethylene glycol 300 in CH_3OH/H_2O with 1 mmol ammonium acetate, was added just before the mass spectrometer (at a rate of 1 mL/min) to the mobile phase. The calculated masses of PEG ions ($[M+H]^+$ and $[M+NH_4]^+$) were used as lock mass. 4-Bromoisoquinoline, XANTPHOS, $PdCl_2(PPh_3)_2$, Cs_2CO_3 (99%) and NaH (assay 55–65%) were obtained from Sigma–Aldrich and used as such. Isoquinolin-3-amine,

2-bromoaniline, 1,2-dibromobenzene, bromobenzene, NBS, MsCl, THF (99.85%, water <50 ppm, extra dry over molecular sieve), DME, DMA, 1,2-dichlorobenzene, MeI, $Pd(OAc)_2$ and $Pd_2(dba)_3$ were obtained from Acros and used as such. For the Buchwald–Hartwig reaction freshly distilled dioxane (dried over sodium benzophenone) was used. Flash column chromatography was performed on Kieselgel 60 (ROCC, 0.040–0.063 mm).

4.2. General procedure for Buchwald–Hartwig reaction

*4.2.1. N-(2-Bromophenyl)isoquinolin-4-amine (**10**)*

A round-bottomed flask was charged with $Pd_2(dba)_3$ (0.137 g, 0.150 mmol, 5 mol %) and XANTPHOS [9,9-dimethyl-4,5-bis(diphenylphosphino)-9*H*-xanthene] (0.191 g, 0.330 mmol, 11 mol %) followed by dry dioxane (12 mL) (freshly distilled). The mixture was flushed with N_2 for 10 min. Meanwhile, in another round-bottomed flask 4-bromoisoquinoline (**8**) (0.624 g, 3.0 mmol), 2-bromoaniline (**9**) (0.619 g, 3.6 mmol) and Cs_2CO_3 (2.932 g, 9.0 mmol) (Aldrich, 99%) were weighed. To this mixture, the Pd-catalyst solution was added and the flask was subsequently flushed with N_2 for 5 min. The resulting mixture was heated at reflux (oil bath temperature: 110 °C) for 27 h under magnetic stirring. After cooling down to room temperature dichloromethane (25 mL) was added and the suspension was filtered over a pad of Celite® and rinsed with dichloromethane (125 mL). The solvent was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel using CH_2Cl_2/EtOAc (95:5) as the eluent yielding **10** in 74%.

Red/brown crystals; mp 94–96 °C; δ_H ($CDCl_3$): 9.11 (s, 1H, H-1), 8.54 (s, 1H, H-3), 8.04 (dd, *J*=7.9, 1.1 Hz, 1H, H-8), 7.96 (dd, *J*=8.4, 0.9 Hz, 1H, H-5), 7.71 (ddd, *J*=8.2, 6.9, 1.2 Hz, 1H, H-6), 7.65 (ddd, *J*=8.1, 6.9, 1.2 Hz, 1H, H-7), 7.57 (dd, *J*=7.9, 1.5 Hz, 1H, H-3′), 7.09 (ddd, *J*=8.2, 7.3, 1.5 Hz, 1H, H-5′), 6.80 (dd, *J*=8.2, 1.5 Hz, 1H, H-6′), 6.75 (ddd, *J*=7.9, 7.3, 1.5 Hz, 1H, H-4′), 6.28 (br s, 1H, NH); δ_C ($CDCl_3$): 149.1, 142.6, 137.5, 132.9, 132.2, 131.8, 130.5, 129.3, 128.3, 128.1, 127.7, 121.6, 121.0, 115.6, 111.5; HRMS (ESI) for $C_{15}H_{12}N_2Br$ $[M+H]^+$: calcd 299.0184, found 299.0184.

*4.2.2. N-(2-Bromophenyl)isoquinolin-3-amine (**16**)*

$Pd_2(dba)_3$ (0.023 g, 0.025 mmol, 2.5 mol %), XANTPHOS (0.032 g, 0.055 mmol, 5.5 mol %), 1,2-dibromobenzene (**15**) (0.283 g, 1.2 mmol), isoquinolin-3-amine (**14**) (0.144 g, 1.0 mmol), Cs_2CO_3 (0.978 g, 3.0 mmol) and dry dioxane (10 mL) (freshly distilled). Reaction time=24 h. Eluent: CH_2Cl_2 (100%); yield 62%; green solid; mp 103–104 °C; δ_H ($CDCl_3$): 9.00 (s, 1H, H-1), 7.85 (dd, *J*=8.2, 0.9 Hz, 1H, H-8), 7.81 (dd, *J*=8.2, 1.5 Hz, 1H, H-3′), 7.63–7.59 (m, *J*=8.4, 8.0, 1.5 Hz, 2H, H-5 and H-6′), 7.56 (ddd, *J*=8.4, 6.6, 1.2 Hz, 1H, H-6), 7.35 (ddd, *J*=8.2, 6.6, 1.3 Hz, 1H, H-7), 7.31 (ddd, *J*=8.1, 7.3, 1.5 Hz, 1H, H-4′), 7.21 (s, 1H, H-4), 6.93 (br s, 1H, NH), 6.89 (ddd, *J*=8.0, 7.3, 1.5 Hz, 1H, H-5′); δ_C ($CDCl_3$): 152.0, 150.8, 139.2, 138.4, 133.1, 130.7, 128.2, 127.7, 125.4, 125.1, 124.3, 122.9, 119.2, 114.4, 101.3; HRMS (ESI) for $C_{15}H_{12}N_2Br$ $[M+H]^+$: calcd 299.0184, found 299.0184.

*4.2.3. 4-Bromo-N-phenylisoquinolin-3-amine (**19**)*

$Pd_2(dba)_3$ (0.023 g, 0.025 mmol, 2.5 mol %), XANTPHOS (0.032 g, 0.055 mmol, 5.5 mol %), 4-bromoisoquinolin-3-amine (**17**) (0.223 g, 1.0 mmol), bromobenzene (**18**) (0.188 g, 1.2 mmol), Cs_2CO_3 (0.978 g, 3.0 mmol) and dry dioxane (4 mL) (freshly distilled). Reaction time=20 h. Eluent: CH_2Cl_2 (100%); yield 61%; shiny yellow powder; mp 127 °C; δ_H ($CDCl_3$): 8.91 (d, *J*=0.6 Hz, 1H, H-1),[23] 7.97 (ddd, *J*=8.7, 1.7, 0.9 Hz, 1H, H-5), 7.84 (d, *J*=8.2 Hz, 1H, H-8), 7.67 (ddd, *J*=8.6, 6.8, 1.3 Hz, 1H, H-6), 7.56 (dd, *J*=8.6, 1.1 Hz, 2H, H-2′), 7.39–7.33 (m, *J*=8.6, 7.5, 1.0 Hz, 3H, H-3′ and H-7), 7.14 (br s, 1H, NH), 7.07 (tt, *J*=7.3, 1.2 Hz, 1H, H-4′); δ_C ($CDCl_3$): 150.0, 148.7, 140.5, 136.9, 131.8, 129.0, 128.1, 125.4, 124.4, 124.0, 122.7, 120.2, 100.3; HRMS (ESI) for $C_{15}H_{12}N_2Br$ $[M+H]^+$: calcd 299.0184, found 299.0184.

*4.2.4. N-Phenylisoquinolin-3-amine (**20**)*

$Pd_2(dba)_3$ (0.092 g, 0.10 mmol, 5 mol %), XANTPHOS (0.127 g, 0.22 mmol, 11 mol %), isoquinolin-3-amine (**14**) (0.288 g, 2.0 mmol), bromobenzene (**18**) (0.377 g, 2.4 mmol), Cs_2CO_3 (1.955 g, 6.0 mmol) and dry dioxane (12 mL) (freshly distilled). Reaction time=46 h. Eluent: CH_2Cl_2 (100%); yield 87%; greenish/yellow powder; mp 102–103 °C; δ_H ($CDCl_3$): 8.93 (s, 1H, H-1), 7.82 (dd, 1H, *J*=8.3, 0.9 Hz, H-8), 7.58–7.51 (m, *J*=8.4, 8.3, 6.1, 1.6, 1.2 Hz, 2H, H-5 and H-6), 7.41–7.28 (m, *J*=8.3, 6.1, 1.8 Hz, 5H, H-2′, H-3′ and H-7), 7.20 (s, 1H, H-4), 7.09 (tt, *J*=7.0, 1.5 Hz, 1H, H-4′), 6.94 (br s, 1H, NH); δ_C ($CDCl_3$): 152.0, 151.9, 141.0, 138.7, 130.6, 129.4, 127.7, 125.2, 124.5, 123.6, 122.7, 120.1, 99.1; HRMS (ESI) for $C_{15}H_{13}N_2$ $[M+H]^+$: calcd 221.1079, found 221.1076.

*4.2.5. 4-Bromo-N-(2-bromophenyl)isoquinolin-3-amine (**21**)*

$Pd_2(dba)_3$ (0.046 g, 0.05 mmol, 5 mol %), XANTPHOS (0.064 g, 0.11 mmol, 11 mol %), 4-bromoisoquinolin-3-amine (**17**) (0.2231 g, 1.0 mmol), 1,2-dibromobenzene (**15**) (0.283 g, 1.2 mmol), Cs_2CO_3 (0.978 g, 3.0 mmol) and dry dioxane (12 mL) (freshly distilled). Reaction time=48 h. Eluent: Heptane/CH_2Cl_2 (70:30); yield 39%; greenish/yellow powder; mp 108–109 °C; δ_H ($CDCl_3$): 8.94 (s, 1H, H-1), 8.42 (dd, 1H, *J*=8.3, 1.5 Hz, H-3′), 8.01 (ddd, *J*=8.6, 1.7, 0.8 Hz, 1H, H-5), 7.86 (br d, *J*=8.1 Hz, 1H, H-8), 7.77 (br s, 1H, NH), 7.70 (ddd, *J*=8.6, 6.8, 1.3 Hz, 1H, H-6), 7.59 (dd, *J*=8.0, 1.5 Hz, 1H, H-6′), 7.41 (ddd, *J*=8.2, 6.8, 1.1 Hz, 1H, H-7), 7.34 (ddd, *J*=8.3, 7.4, 1.5 Hz, 1H, H-4′), 6.90 (ddd, *J*=8.0, 7.3, 1.6 Hz, 1H, H-5′); δ_C ($CDCl_3$): 149.8, 148.1, 138.5, 136.9, 132.5, 131.9, 128.0, 128.0, 125.6, 124.7, 124.5, 122.8, 120.1, 113.8, 101.8; HRMS (ESI) for $C_{15}H_{11}N_2Br_2$ $[M+H]^+$: calcd 376.9289, found 376.9278.

4.3. General procedure for the Pd-catalyzed intramolecular direct arylation reaction

4.3.1. Conventional heating

*4.3.1.1. 11H-Indolo[3,2-c]isoquinoline (**7**).* A round-bottomed flask was charged with $Pd(PPh_3)_2Cl_2$ (0.084 g, 0.12 mmol, 20 mol %), *N*-(2-bromophenyl)isoquinolin-4-amine (**10**) (0.180 g, 0.6 mmol) and $NaOAc \cdot 3H_2O$ (0.200 g, 1.47 mmol) followed by DMA (10 mL). The mixture was flushed with Ar for 5 min and then stirred at 130 °C under Ar atmosphere for 16 h. Subsequently, the mixture was evaporated to dryness in vacuo. The crude product was purified via flash column chromatography on silica gel (the residue was brought on column mixed with silica) using CH_2Cl_2/EtOAc (97:3) as the eluent yielding **7** in 78%.

Yellow/orange powder; mp >300 °C; δ_H (DMSO-d_6): 12.33 (br s, 1H, NH), 9.13 (s, 1H, H-5), 8.52 (dd, *J*=8.2, 0.9 Hz, 1H, H-1), 8.28 (d, *J*=8.2 Hz, 1H, H-4), 8.25 (d, *J*=7.8 Hz, 1H, H-7), 7.91 (ddd, *J*=8.2, 6.9, 1.2 Hz, 1H, H-2), 7.71 (ddd, *J*=8.2, 7.0, 1.1 Hz, 1H, H-3), 7.70 (dd, *J*=8.1, 0.9 Hz, 1H, H-10), 7.50 (ddd, *J*=8.2, 7.1, 1.2 Hz, 1H, H-9), 7.32 (ddd, *J*=7.8, 7.1, 0.9 Hz, 1H, H-8); δ_C (DMSO-d_6): 144.5, 138.5, 133.5, 129.8, 128.5, 127.2, 126.4, 126.1, 125.4, 123.4, 122.6, 121.0, 119.7, 119.2, 111.8; HRMS (ESI) for $C_{15}H_{11}N_2$ $[M+H]^+$: calcd 219.0922, found 299.0922.

4.3.2. Microwave irradiation

*4.3.2.1. 11H-Indolo[3,2-c]isoquinoline (**7**).* A microwave vial of 10 mL was charged with *N*-(2-bromophenylisoquinolin-4-amine) (**10**) (0.180 g, 0.6 mmol) and $NaOAc \cdot 3H_2O$ (0.200 g, 1.47 mmol). Subsequently, the vial was flushed with Ar for 1 min. Then, 1 mL of a stock solution[†] of catalyst (1 mol %) in DMA was added via

[†] Preparation of the stock solution of catalyst: $PdCl_2(PPh_3)_2$ (0.211 g, 0.03 mmol) was dissolved in 5 mL of DMA. Next, the mixture was flushed with Ar for 5 min and subsequently stirred until the catalyst was completely dissolved.

a syringe and the resulting mixture was stirred and flushed with Ar for an additional 2 min. Next, the vial was sealed with an Al crimp cap with a septum and heated at 200 °C in a CEM Discover microwave apparatus. The set power was 100 W and the total heating time was 10 min. After the reaction vial was cooled down to room temperature using a propelled air flow, it was opened and the content was poured into a round-bottomed flask. The vial was rinsed with methanol (50 mL) and the combined organic phase was evaporated to dryness. Finally, the crude product was purified via flash column chromatography on silica gel (the residue was brought on column mixed with silica) using CH_2Cl_2/EtOAc (97:3) as the eluent yielding the pure compound in 79%. The characterization data of it were identical to those described in Section 4.3.1.

4.4. 4-Bromoisoquinolin-3-amine (17)

To a round-bottomed flask filled with isoquinolin-3-amine (**14**) (0.144 g, 1.0 mmol) a solution of NBS (0.208 g, 1.17 mmol) in MeOH (5 mL) was added dropwise. After the addition was complete, the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the crude reaction product was dissolved in EtOAc (20 mL) and poured into a separating funnel. An equal amount of water was added. The water layer was extracted three more times (3×20 mL EtOAc) before the combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure and the reaction product was purified by recrystallization from water yielding **17** in 65%.

Pale yellow powder; mp 120–122 °C; δ_H ($CDCl_3$): 8.78 (d, J=0.6 Hz, 1H, H-1),[23] 7.91 (ddd, J=8.6, 1.7, 0.9 Hz, 1H, H-5), 7.81 (d, J=8.2 Hz, 1H, H-8), 7.67 (ddd, J=8.6, 6.8, 1.3 Hz, 1H, H-6), 7.33 (ddd, J=8.2, 6.8, 1.7 Hz, 1H, H-7), 5.18 (br s, 2H, NH_2); δ_C ($CDCl_3$): 151.7, 150.3, 137.1, 132.0, 128.1, 124.7, 123.8, 123.6, 97.7; HRMS (ESI) for $C_9H_8N_2Br$ $[M+H]^+$: calcd 222.9871, found 222.9876.

4.5. *N*-(4-Bromoisoquinolin-3-yl)-*N*-(2-bromophenyl)methanesulfonamide (22)

A solution of 4-bromo-*N*-(2-bromophenyl)isoquinolin-3-amine (**21**) (0.378 g, 1.0 mmol) in THF (5 mL) was added dropwise to a suspension of NaH (assay 55–65%) (0.166 g) in THF (5 mL). The mixture was stirred for 1 h at room temperature. Mesyl chloride (0.16 mL, 2.0 mmol) was added in one aliquot to the mixture and stirring at room temperature was continued for two more hours. Then the solvent was evaporated under reduced pressure and the crude reaction product was dissolved in EtOAc (100 mL) and poured into a separating funnel. After this, 100 mL of water was added. The organic layer was then washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the reaction product was purified by flash column chromatography on silica gel using CH_2Cl_2/heptane (60:40) as the eluent yielding **22** in 15%.

Yellow powder; mp 197–198 °C, decomp.; δ_H ($CDCl_3$): 9.11 (d, 1H, J=0.7 Hz, H-1),[23] 8.22 (ddd, 1H, J=8.6, 1.7, 0.8 Hz, H-5), 8.12 (dd, J=8.2, 1.6 Hz, 1H, H-3′), 7.98 (br d, J=8.1 Hz, 1H, H-8), 7.78 (ddd, J=8.6, 6.9, 1.3 Hz, 1H, H-6), 7.66 (ddd, J=8.1, 6.9, 1.1 Hz, 1H, H-7), 7.57 (dd, J=8.0, 1.5 Hz, 1H, H-6′), 7.42 (ddd, J=8.2, 7.4, 1.6 Hz, 1H, H-4′), 7.21 (ddd, J=8.0, 7.4, 1.7 Hz, 1H, H-5′), 3.59 (s, 3H, CH_3); δ_C ($CDCl_3$): 149.5, 147.1, 137.8, 137.5, 136.6, 134.3, 131.9, 129.9, 128.5, 128.3, 127.6, 127.4, 127.0, 123.0, 116.1, 42.6; HRMS (ESI) for $C_{16}H_{13}N_2O_2Br_2S$ $[M+H]^+$: calcd 454.9064, found 454.9061.

4.6. *N*-(2-Isoquinolin-4-ylphenyl)-2,2-dimethylpropanamide (25)

4-Bromoisoquinoline (**8**) (0.832 g, 4.0 mmol) was dissolved in DME (24 mL). $Pd(PPh_3)_4$ (0.231 g, 0.2 mmol) was added and the solution was subsequently stirred for 10 min under a N_2 atmosphere. Next, {2-[(2,2-dimethylpropanoyl)amino]phenyl}boronic acid (**24**) (1.105 g, 5.0 mmol) and aq Na_2CO_3 (10%, 4 mL) were added. The mixture was magnetically stirred and heated at reflux in an oil bath (oil bath temperature: 110 °C) under an inert atmosphere (N_2) for 2 h. After cooling the reaction mixture to room temperature, water (60 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (3×60 mL). The combined organic phase was dried over $MgSO_4$, filtered and subsequently evaporated to dryness. Finally, the residue was purified via flash column chromatography on silica gel using CH_2Cl_2/EtOAc (90:10) as the eluent yielding **25** in 98%.

Off white to pale yellow powder; mp 148 °C; δ_H ($CDCl_3$): 9.40 (s, 1H, H-1), 8.51 (s, 1H, H-3), 8.27 (d, J=8.3 Hz, 1H, H-6′), 8.16 (dd, J=7.0, 2.0 Hz, 1H, H-8), 7.79–7.71 (m, 2H, H-6 and H-7), 7.62 (dd, J=7.0, 1.8 Hz, 1H, H-5), 7.54 (ddd, J=8.1, 7.2, 2.0 Hz, 1H, H-5′), 7.34 (dd, J=7.6, 1.7 Hz, 1H, H-3′), 7.30 (td, J=7.4, 1.1 Hz, 1H, H-4′), 6.97 (br s, 1H, NH), 0.77 (s, 9H, CH_3); δ_C ($CDCl_3$): 176.2, 152.9, 143.5, 136.4, 134.3, 131.5, 130.7, 129.5, 129.2, 128.2, 128.1, 128.0, 126.9, 124.7, 124.4, 121.8, 39.4, 27.0; HRMS (ESI) for $C_{20}H_{21}N_2O_1$ $[M+H]^+$: calcd 305.1654, found 305.1655.

4.7. 2-(Isoquinolin-4-yl)aniline (26)

2,2-Dimethyl-*N*-(2-isoquinolin-4-ylphenyl)propanamide (**25**) (0.609 g, 2.0 mmol) was dissolved in ethanol (150 mL). Next, aq H_2SO_4 (40%, 150 mL) was added dropwise. The obtained mixture was stirred and refluxed in an oil bath (oil bath temperature: 130 °C) for 24 h. Subsequently, the pH of the reaction mixture was adjusted to 8–9 with 28–30% NH_4OH under cooling in an ice bath. Next, the aqueous phase was extracted with $CHCl_3$ (3×100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness. Finally, the residue was purified via flash column chromatography on silica gel using CH_2Cl_2/EtOAc (70:30) as the eluent yielding **26** in 97%.

Yellow/orange viscous oil: δ_H ($CDCl_3$): 9.31 (s, 1H, H-1), 8.50 (s, 1H, H-3), 8.08 (dt, J=7.9, 1.2 Hz, 1H, H-8), 7.72–7.64 (m, 3H, H-5 and H-6 and H-7), 7.30 (ddd, J=8.1, 7.4, 1.6 Hz, 1H, H-5′), 7.15 (ddd, J=7.6, 1.7, 0.3 Hz, 1H, H-3′), 6.90 (td, J=7.5, 1.1 Hz, 1H, H-4′), 6.86 (ddd, J=8.0, 1.2, 0.3 Hz, 1H, H-6′), 3.13 (br s, 2H, NH_2); δ_C ($CDCl_3$): 151.4, 144.6, 142.1, 135.0, 131.5, 131.4, 131.3, 129.7, 128.4, 128.3, 128.0, 125.4, 121.4, 118.6, 115.7; HRMS (ESI) for $C_{15}H_{13}N_2$ $[M+H]^+$: calcd 221.1079, found 221.1076.

4.8. 7*H*-Indolo[2,3-*c*]isoquinoline (12)

2-(Isoquinolin-4-yl)aniline (**26**) (0.419 g, 1.9 mmol) was dissolved in aq HCl (37%, 20 mL) and the mixture cooled to 0 °C using an ice bath. Subsequently, ice cooled aq $NaNO_2$ (0.4 M, 11 mL) was added dropwise keeping the temperature below 3 °C. The mixture was stirred for 1.5 h at 0 °C. Ice cooled aq NaN_3/NaOAc solution (4.0 mmol NaN_3 and 26.6 mmol NaOAc·$3H_2O$ in 10 mL water) was added dropwise keeping the temperature below 3 °C. Next, the mixture was stirred for another hour at 0 °C. The reaction mixture was neutralized with saturated aq Na_2CO_3 while keeping the temperature below 3 °C and subsequently extracted with EtOAc (5×100 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was dissolved in 35 mL of 1,2-dichlorobenzene and flushed with Ar. The mixture was stirred and heated in an oil bath at 180 °C for 3 h under Ar atmosphere. After cooling down to room temperature, the solvent was removed under reduced pressure. Finally, the obtained residue was purified via flash column chromatography on silica gel using CH_2Cl_2/EtOAc (90:10) as the eluent yielding **12** in 77%.

Pale yellow needles; mp 259–260 °C; δ_H (DMSO-d_6): 12.21 (br s, 1H, NH), 9.20 (s, 1H, H-5), 8.73 (br dd, 1H, J=8.4, 0.8 Hz, H-1), 8.56 (d, J=8.0 Hz, 1H, H-11), 8.27 (br d, J=8.1 Hz, 1H, H-4), 7.93 (ddd, J=8.4, 6.9, 1.3 Hz, 1H, H-2), 7.66 (dt, J=8.1, 0.8 Hz, 1H, H-8),

7.59 (ddd, J=8.2, 6.9, 1.0 Hz, 1H, H-3), 7.48 (ddd, J=8.1, 7.2, 1.1 Hz, 1H, H-9), 7.36 (ddd, J=7.9, 7.2, 1.1 Hz, 1H, H-10); δ_C (DMSO-d_6): 151.0, 148.3, 137.7, 132.4, 131.7, 129.9, 125.3, 124.6, 124.0, 122.8, 122.6, 121.6, 120.5, 112.3, 105.6; HRMS (ESI) for $C_{15}H_{11}N_2$ $[M+H]^+$: calcd 219.0922, found 219.0923.

4.9. General procedure for the selective methylation of indoloisoquinolines 7 and 12

*4.9.1. 6-Methyl-6H-indolo[3,2-c]isoquinoline (**5**)*

In a round-bottomed flask 11*H*-indolo[3,2-*c*]isochinoline (**7**) (0.109 g, 0.5 mmol), dry THF (7.5 mL) and CH_3I (3 mL) were heated at reflux under N_2 atmosphere (oil bath temperature: 80 °C) for 22 h with magnetic stirring. Then the solvent was evaporated to dryness under reduced pressure and the crude product was mixed with silica gel and purified via flash column chromatography on silica gel using CH_2Cl_2/MeOH (90:10) as the eluent yielding 6-methyl-6*H*-indolo[3,2-*c*]isoquinoline hydroiodide (**5**·HI) as a yellow powder. To obtain the free base, **5**·HI was brought in a mixture of CH_2Cl_2 (100 mL) and 28–30% ammonia in water (100 mL). The organic phase was separated and the aqueous phase was subsequently extracted with CH_2Cl_2 (6×50 mL). The combined organic phase was dried over $MgSO_4$, filtered and evaporated to dryness to yield **5** in 76%.

Bright red/orange powder; mp 208–210 °C, decomp.; δ_H (DMSO-d_6): 9.05 (s, 1H, H-5), 8.82 (dd, J=8.4, 1.1 Hz, 1H, H-1), 8.32–8.26 (m, 2H, H-4 and H-7), 7.97 (ddd, J=8.4, 6.9, 1.2 Hz, 1H, H-2), 7.82 (dt, J=8.2, 0.9 Hz, 1H, H-10), 7.75 (ddd, J=8.2, 6.8, 1.2 Hz, 1H, H-3), 7.41 (ddd, J=8.4, 6.7, 1.2 Hz, 1H, H-9), 7.16 (ddd, J=8.1, 6.9, 1.1 Hz, 1H, H-8), 4.86 (d, J=0.5 Hz, 3H, CH_3); δ_C (DMSO-d_6): 151.4, 142.3, 131.9, 131.5, 128.8, 128.6, 126.3, 124.4, 124.3, 123.5, 122.2, 120.5, 118.5, 117.2, 117.1, 45.7; HRMS (ESI) for $C_{16}H_{13}N_2$ $[M+H]^+$: calcd 233.1079, found 233.1076.

*4.9.2. 6-Methyl-6H-indolo[2,3-c]isoquinoline (**6**)*

7*H*-Indolo[2,3-*c*]isoquinoline (**12**) (0.109 g, 0.5 mmol), dry THF (7.5 mL) and CH_3I (3 mL). Reaction time=6 h. Eluent: CH_2Cl_2/MeOH (90:10); yield 99%; bright red powder; mp 91–92 °C; δ_H (DMSO-d_6): 9.35 (s, 1H, H-5), 8.68 (d, J=8.6 Hz, 1H, H-1), 8.54 (d, J=8.0 Hz, 1H, H-11), 8.19 (d, J=8.3 Hz, 1H, H-4), 7.91 (ddd, J=8.6, 6.7, 1.3 Hz, 1H, H-2), 7.79 (dt, J=8.2, 0.9 Hz, 1H, H-8), 7.44 (ddd, J=8.2, 6.9, 1.1 Hz, 2H, H-3 and H-9), 7.24 (ddd, J=7.9, 7.0, 1.0 Hz, 1H, H-10), 4.55 (s, 3H, CH_3); δ_C (DMSO-d_6): 151.2, 149.3, 140.2, 134.1, 134.0, 130.7, 125.0, 123.6, 122.8, 122.3, 122.2, 118.9, 118.6, 118.3, 113.4, 41.7; HRMS (ESI) for $C_{16}H_{13}N_2$ $[M+H]^+$: calcd 233.1079, found 233.1076.

4.10. Antiprotozoal evaluation and cytotoxicity

Antiplasmodial activity was determined against the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine), using a modified [^{3}H]-hypoxanthine incorporation assay as described before.[24] Chloroquine was used as positive control. Activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani* and cytotoxicity against rat skeletal myoblasts (L6 cells) were evaluated as described before.[24] Melarsoprol was used as positive control against *T.b. rhodesiense*, benznidazole against *T. cruzi*, miltefosine against *L. donovani* and podophyllotoxin for cytotoxicity. Results are expressed as IC_{50} in μM±standard deviation (SD) (n=3).

Acknowledgements

Gitte Van Baelen would like to thank the IWT-Flanders (The Institute for the promotion of Innovation by Science and Technology in Flanders) for a Ph.D. scholarship. The authors acknowledge financial support from the University of Antwerp (GOA BOF UA, NOI BOF UA) and the Flemish Government (Impulsfinanciering van de Vlaamse Overheid voor Strategisch Basisonderzoek PFEU 2003) for financial support. The authors would like to thank R. Brun of the Swiss Tropical Institute for biological evaluation of the compounds. C.M. thanks the FWO-Flanders for a Ph.D. scholarship.

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