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Synthesis of metergoline analogues and their evaluation as antiplasmodial agents†

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A series of compounds based on metergoline were synthesized and evaluated *in vitro* for their antiplasmodial activity against the chloroquine-sensitive NF54 strain of the malaria parasite *Plasmodium falciparum*. These compounds were also screened for their cytotoxicity towards a mammalian cell line. Some of the compounds exhibited superior antiplasmodial activity with a good selectivity index relative to metergoline.

Introduction

Malaria caused by plasmodia species continues to pose serious mortality and morbidity problems. The World Health Organization has estimated that in 2010, there were 219 million documented cases of malaria. The disease killed between 660 000 and 1.2 million people in that year, many of whom were children in Africa.[1] Of the plasmodia species known to cause malaria, *Plasmodium falciparum* is the most problematic, mainly due to its high prevalence and virulence.[2] Currently, there are no vaccines available for malaria. For this reason, control relies on chemotherapy and insect control measures. Antimalarial drug treatment failure due to emergence of resistance toward standard antimalarial agents remains a major contributor to the burden of malaria.[3] Thus, the search for novel, structurally diverse, and affordable drugs with novel mechanisms of action has become critical in the quest to control and potentially eradicate malaria.

Since development of new drugs is a lengthy and costly undertaking, one of the recognised strategies to discover new therapies against certain diseases is to reposition, repurpose or find new uses for drugs that are already used for other indications.[4,5] A number of drugs have been repositioned for the treatment of malaria in the past few decades.[4] Chemical structures of some selected drugs that have been repositioned for the treatment of malaria are shown in Fig. 1.

On the other hand the potential of natural products as a source of antimalarial drugs has been ably demonstrated by the antimalarial drugs quinine and artemisinin from *Cinchona officinalis* and *Artemisia annua*, respectively, along with the well documented use of plant material in traditional medicines for the treatment of the disease.[6,7] The aforementioned encouraged us to investigate the potential of metergoline, a semi-synthetic ergoline alkaloid derived from the ergoline backbone, which occurs naturally in lower fungi of the genus *Claviceps* (Fig. 2).[8] Metergoline is used clinically in disorders associated with hyperprolactinemia, in the inhibition of lactation and in the prophylaxis of migraine headaches.[9] Metergoline has been documented to have *in vitro* antifungal activity,[10,11] and has been investigated in the management of psychiatric disorders like

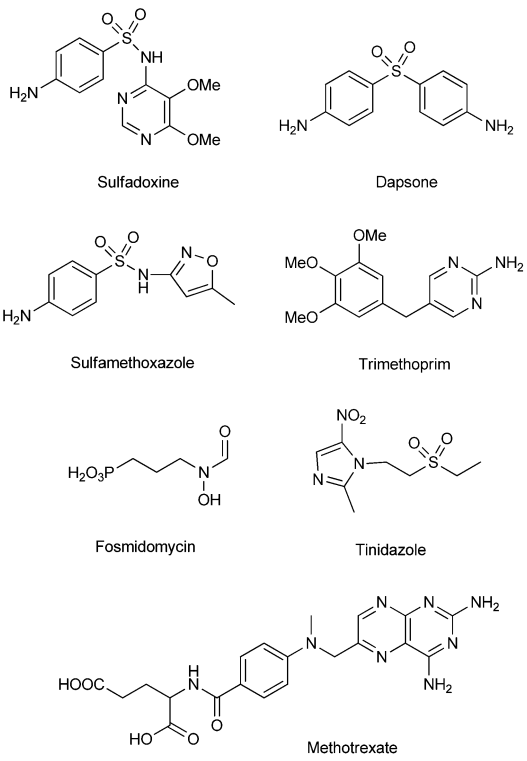

Fig. 1 Selected drugs that have been repositioned for the treatment of malaria.

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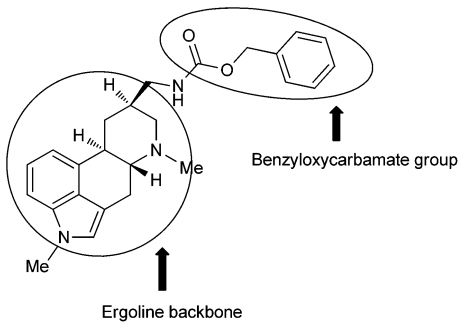


Fig. 2 Structure of metergoline.

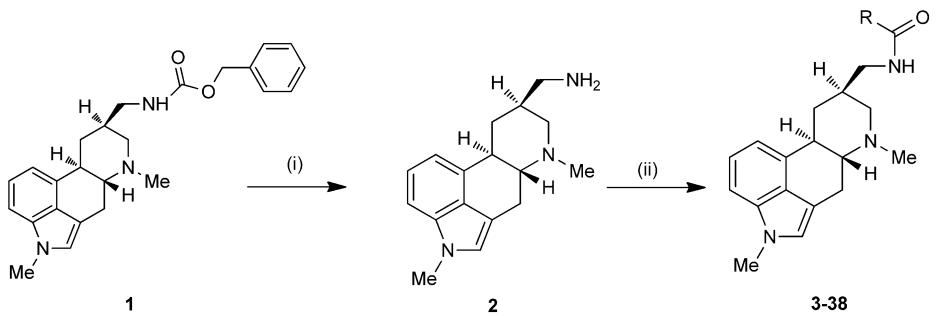
obsessive compulsive disorder, carbon dioxide induced anxiety and premenstrual syndrome.[9,12–14] Recently, metergoline was identified as a novel chemosensitizer to tackle transporter-mediated chloroquine resistance in *P. falciparum*.[15] In addition quantitative High Throughput Screen (HTS) for delayed death inhibitors of the malaria parasite plasmid on a National Institute of Health (NIH) molecular library also found metergoline to have antiplasmodial activity with an IC_{50} value of 7.4 μM.[16]

The human system profile of metergoline is well known as it is already in a clinical use for the treatment of other indications.[9–14] This makes metergoline a promising new antiplasmodial scaffold for repositioning *via* medicinal chemistry approaches. In view of this, we explored the potential of this compound as an antiplasmodial agent by performing structural modifications aimed at improving selective antiplasmodial activity.

Result and discussion

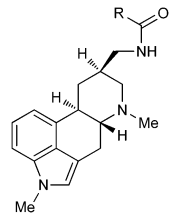
In the present study, the ergoline backbone of metergoline was retained while the benzyloxycarbamate group was replaced with various amide groups. The first step towards structural modification of metergoline involved the removal of the benzyloxycarbamate group (Fig. 2). This was achieved by the catalytic cleavage of commercially available metergoline **1** by hydrogenation using Pd/C as a catalyst in methanol at room temperature to afford the key amine intermediate **2** (Scheme 1). EDCI-mediated coupling of amine **2** with various carboxylic acids afforded target compounds in reasonable yields (Table 1). Compounds **13**, **14**, **28**, **33** and **35** were synthesized by reacting amine **2** with respective acid chlorides in DCM. Substituted aromatic, heterocyclic aromatic as well as aliphatic carboxylic acids (acyclic and cyclic) were used in these reactions. The selection of the aromatic substituent was mainly based on the lipophilic (π) and electronic (σ) considerations as defined by Craig's plot.[17] In the present study, –F, –Cl, –Br, I, $–CF_3$, $–OCF_3$ and $–NO_2$ substituents were selected to represent electron-withdrawing (+σ) and hydrophobic (+π) substituents; –CN, $–COCH_3$ and $–SO_2Me$ were selected for their electron-withdrawing and hydrophilic (+σ/−π) properties, while –OMe group was representative of electron-donating and hydrophilic (−σ/−π) substituent; –SMe, –Et, –*n*Pr and $–NMe_2$ represented electron-donating and hydrophobic (−σ/+π) substituents. All target compounds were purified using column chromatography and fully characterized by a range of analytical and spectroscopic techniques (see ESI†). All the synthesized compounds were evaluated for *in vitro* antiplasmodial activity against the chloroquine-sensitive (CQS) NF54 strain of *P. falciparum*, and for cytotoxicity against a mammalian cell-line, Chinese Hamster Ovarian (CHO) cell line (Table 1). CQ and artesunate were used as the reference drugs.

Compound **2** obtained after the removal of benzyloxycarbamate group from metergoline **1** (Scheme 1), showed activity (IC_{50}, 3.6 μM) comparable to that of metergoline (IC_{50}, 3.7 μM) (Table 1), suggesting that the benzyloxycarbamate group of metergoline may not contribute to the observed antiplasmodial activity. Reaction of amine **2** with various carboxylic acids yielded amides with varying activities. Most of the amides exhibited micromolar potencies against the chloroquine-sensitive NF54 strain of *P. falciparum*, with 28 of the 36 compounds having IC_{50} values between 0.5 μM and 20 μM (Table 1). First of all, we evaluated the effect on antiplasmodial activity of aromatic amides substituted at the 4-position of the phenyl ring. Unsubstituted aromatic amide derivative **3** was found to have poor activity as compared to metergoline with an IC_{50} value of 24.4 μM. Substitution with electron-withdrawing and hydrophobic groups at 4-position of the phenyl ring yielded compounds with varying activities (Table 1, compounds **4–10**). Introduction of a fluorine atom (**4**) and $–OCF_3$ group (**8**) at the *para*-position of the phenyl ring resulted in reduced activity. However, introduction of bromo (**5**), iodo (**6**), trifluoromethyl (**7**), nitro (**9**) and chloro (**10**) groups at the *para*-position resulted



Scheme 1 *Reagents and conditions*: (i) MeOH, H_2, 10% Pd/C (50% moisture), RT, 16 h. (ii) RCOOH, EDCI, HOBt, DCM, RT, 3 h or RCOCl, DCM, RT, 24 h.

Table 1 Yields, *in vitro* antiplasmodial activity of compounds against NF54 strains of *P. falciparum*, their cytotoxicity against CHO cell line and selectivity index (SI)



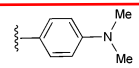
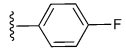
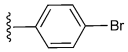
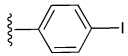
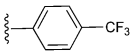
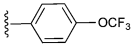
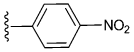
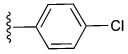
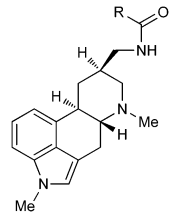
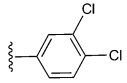
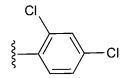
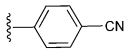
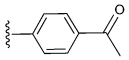
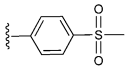
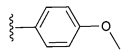
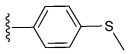
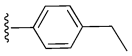
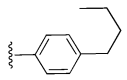
Compound	R	Yields (%)	Antiplasmodial activity NF54 IC_{50}, μM (μg ml^{-1})	Cytotoxicity[a] (CHO) IC_{50},μM (μg ml^{-1})	SI[b]	Compound	R	Yields (%)	Antiplasmodial activity NF54 IC_{50}, μM (μg ml^{-1})	Cytotoxicity[a] (CHO) IC_{50},μM (μg ml^{-1})	SI[b]
1	Metergoline		3.7 (1.5)	16.6 (6.7)	4.5	**22**		78	33.8 (14.1)	132.5 (55.2)	4.0
2	Amine	95	3.6 (1.0)	26.1 (7.0)	7.0	**23**		45	17.4 (6.5)	162 (60.6)	9.0
3		27	24.4 (9.1)	27.0 (10.1)	1.0	**24**		23	17.5 (6.6)	161 (60.2)	9.0
4		28	12.5 (4.9)	7.3 (2.9)	0.6	**25**		50	11.4 (4.3)	112 (42.1)	10
5		53	2.6 (1.2)	5.1 (2.3)	2.0	**26**		51	8.4 (3.2)	163.5 (61.4)	20
6		46	3.0 (1.5)	5.5 (2.7)	2.0	**27**		35	27.4 (10.3)	ND	
7		43	2.7 (1.2)	4.9 (2.2)	2.0	**28**	CH_3	43	199 (62.0)	ND	
8		23	4.2 (1.9)	12.8 (5.8)	3.0	**29**	CH_2CH_3	67	40.3 (13.1)	ND	
9		37	0.5 (0.2)	5.0 (2.1)	11	**30**	$(CH_2)_2CH_3$	71	32.1 (10.9)	ND	
10		35	1.3 (0.5)	4.3 (1.7)	3.0	**31**	$(CH_2)_3CH_3$	91	18.7 (6.6)	105 (37.1)	6.0
11		66	14.6 (5.9)	25.0 (10.2)	2.0	**32**	$C(CH_3)_3$	50	89.1 (31.5)	ND	
12		63	4.9 (2.0)	4.7 (1.9)	1.0	**33**	$(CH_2)_4CH_3$	52	28.6 (10.5)	ND	

Table 1 (*Contd.*)



Compound	R	Yields (%)	Antiplasmodial activity NF54 IC_{50}, μM (μg ml^{-1})	Cytotoxicity[a] (CHO) IC_{50},μM (μg ml^{-1})	SI[b]	Compound	R	Yields (%)	Antiplasmodial activity NF54 IC_{50}, μM (μg ml^{-1})	Cytotoxicity[a] (CHO) IC_{50},μM (μg ml^{-1})	SI[b]
13		53	4.8 (2.1)	3.8 (1.7)	1.0	**34**	$(CH_2)_5CH_3$	49	9.1 (3.5)	8.9 (3.4)	1.0
14		41	8.3 (3.7)	7.5 (3.3)	1.0	**35**	$(CH_2)_6CH_3$	53	3.9 (1.6)	4.6 (1.8)	1.0
15		52	0.6 (0.2)	103.6 (41.3)	188	**36**	$(CH_2)_8CH_3$	64	5.2 (2.2)	86.6 (36.7)	17
16		41	12.3 (5.1)	151.9 (63.1)	12	**37**		63	4.3 (1.6)	143.6 (52.5)	33
17		44	14.2 (6.4)	221.5 (100)	16	**38**		41	1.23 (0.5)	16.6 (6.3)	13
18		41	8.4 (3.4)	7.4 (3.0)	1.0		CQ		5.2 ± 0.6 ng ml^{-1}		
19		31	4.7 (2.0)	6.0 (2.5)	1.0		Artesunate		2.8 ± 0.6 ng ml^{-1}		
20		37	4.3 (1.7)	5.6 (2.2)	1.0		Emetine			0.05 ± 0.01	
21		38	2.0 (0.9)	14.5 (6.2)	7.0						

[a] ND = not determined; CQ = chloroquine. [b] SI (selectivity index) = IC_{50}CHO/IC_{50}NF54.

in improved antiplasmodial activities as compared to metergoline **1** (Table 1). In this series, 4-NO_2 substituted amide derivative **9** was found to be most active with IC_{50} value of 0.5 μM and selectivity index value 11.

In addition, the activity of 4-chloro-substituted derivative (**10**) was also compared with its regioisomers, 2-chloro (**11**), 3-chloro (**12**) as well as 3,4-dichloro (**13**) and 2,4-dichlorosubstituted (**14**) amide derivatives and compound **10** was found to be the most active among them with an IC_{50} value of 1.3 μM. These results clearly indicated the preference for a *para*-substituted phenyl ring over the *ortho*-, *meta*- and disubstituted phenyl rings. Derivatives substituted at the *para*-position with electron-withdrawing and hydrophilic groups like –CN, –COMe and –SO_2Me were also synthesized. The 4-CN substituted derivative **15** showed superior activity with an IC_{50} value of 0.6 μM and demonstrated the best selectivity index value of 188 (Table 1). However, introduction of –COMe (**16**) and –SO_2Me (**17**) at 4-position of the phenyl ring resulted in decreased activity. Introduction of an electron-donating and hydrophilic –OMe group at the *para*-position of the phenyl ring yielded amide derivative **18** with reduced activity (IC_{50} = 8.4 μM, Table 1). However, introduction of an electron-donating and hydrophobic groups at the *para*-position of the phenyl ring yielded compounds with varying activities (**19–22**). Amide derivatives substituted with –SMe (**19**), –Et (**20**) and –$N(CH_3)_2$ (**22**) groups were found to be less active as compared to metergoline **1** but introduction of –*n*Bu group (**21**) resulted in improved activity (IC_{50} = 2.0 μM). SAR studies concerning the various substitution patterns around the phenyl nucleus suggested that most compounds with hydrophobic substituents (**5–10**, **12–14** and **19–21**, Table 1) were found to be generally more active than those derivatives with hydrophilic substituents (**16–18**, Table 1) with the exception of the most active compound **15**. Thus, the electronic properties of the substituents did not have a significant impact on the antiplasmodial activity of the compounds. Within the context of cytotoxicity, compounds which had the phenyl ring substituted with hydrophobic groups showed significant cytotoxicity on the CHO cell line and therefore displayed a low selectivity index. The activity of these compounds could presumably be due to their cytotoxicity. The best combination of activity and selectivity was found with compound **15** having an activity of 0.6 μM and a selectivity index of 188. Heterocyclic aromatic amides, nicotinamide (**23**), isonicotinamide (**24**), picolinamide (**25**), pyrazine-2-carboxamide (**26**) and pyrimidine-4-carboxamide (**27**) derivatives showed IC_{50} values between 8.4 and 27.4 μM (Table 1). These results indicate that heterocyclic aromatic systems are not tolerated for this class of compounds.

The effect of aliphatic amides with varying carbon chain lengths on antiplasmodial activity was also established and it was observed that derivatives **28** to **36** (Table 1) showed reduced antiplasmodial activity as compared to metergoline **1**. As the chain length increases, activity also increases and octanamide derivative **35** was found to be the most active with an IC_{50} value of 3.9 μM, which is comparable to metergoline. The effect of branched carbon chain amide (**32**) on antiplasmodial activity was also evaluated and found to be detrimental to activity (IC_{50}, 89.1 μM). However, cyclopentyl amide derivative **37** showed activity similar to metergoline (IC_{50}, 4.3 μM) and a much better selectivity index value of 33 (Table 1). Heterocyclic alkyl amide **38** showed better activity (IC_{50}, 1.2 μM) as compared to metergoline, alkyl as well as cycloalkyl amides. Here the heteroatom might be playing an important role in maintaining the favorable interactions required for antiplasmodial activity.

From a structure–activity relationship viewpoint, a few observations can be made. It is clear that aromatic amide derivatives (**3–22**) have a much greater impact on antiplasmodial activity compared to aliphatic amide derivatives (**28–36**). These results suggest that the aromatic moiety is necessary to maintain the favourable interactions that maybe responsible for antiplasmodial activity. Among aromatic amide derivatives, *para*-substituted derivative (**10**) is more active compared to *-ortho*, *-meta* as well as disubstituted amide derivatives (**11–14**). Among aliphatic amide derivatives, cyclic aliphatic amide derivative (**37**) was found to be more active and less cytotoxic as compared to acyclic aliphatic amide derivatives (**28–36**). Heterocyclic aliphatic amide **38** was found to be the second most active derivative, reflecting the favourable interactions of such systems required for antiplasmodial activity.

In conclusion, a series of compounds have been synthesized with antiplasmodial activity through a drug repositioning approach. Structural modifications were performed on metergoline with aromatic, heteroaromatic, aliphatic and aliphatic cyclic amides replacing the benzyloxycarbamate group. Some of the analogues exhibited improved antiplasmodial activity relative to metergoline against the chloroquine-sensitive (CQS) NF54 strain of *P. falciparum*. Compound **15**, with the cyano substituent in the 4-position of the phenyl ring was found to be the most active and with the highest selectivity index. Overall this work demonstrates the potential of metergoline as a promising potential novel antimalarial template for repositioning purposes.

Acknowledgements

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