A Divergent SAR Study Allows Optimization of a Potent 5-HT$_{2c}$ Inhibitor to a Promising Antimalarial Scaffold

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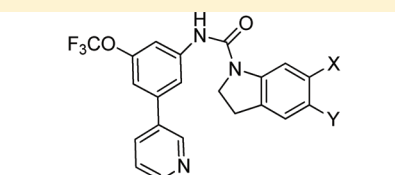
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Ⓢ Supporting Information

ABSTRACT: From the 13 533 chemical structures published by GlaxoSmithKline in 2010, we identified 47 quality starting points for lead optimization. One of the most promising hits was the TCMDC-139046, a molecule presenting an indoline core, which is well-known for its anxiolytic properties by interacting with serotonin antagonist receptors 5-HT$_2$. The inhibition of this target will complicate the clinical development of these compounds as antimalarials. Herein, we present the antimalarial profile of this series and our efforts to avoid interaction with this receptor, while maintaining a good antiparasitic potency. By using a double-divergent structure–activity relationship analysis, we have obtained a novel lead compound harboring an indoline core.

Compound	TCMDC-139046 (1)	27
selectivity (3D7/5HT$_{2c}$)	0.62	71.4

KEYWORDS: indoline, malaria, Tres Cantos Antimalarial set, divergent SAR, open-innovation

Malaria is a major global disease caused by parasites of the genus *Plasmodium*, which are transmitted to people when female anopheles mosquitoes feed on human blood. The reasons for the mortality and morbidity are varied; however, probably the most remarkable is the existence of *Plasmodium falciparum*-resistant strains to nearly all standard antimalarial drugs.[1] As a consequence, there is an urgent requirement for novel antimalarial drugs, and numerous programs from public and private institutions or public–private partnerships are being developed.[2,3]

The criteria for new antimalarial drugs are very demanding; first, the drug must be safe and efficacious.[4] Then, the profile of a new molecule should be better than any of the existing drugs, and it should be affordable (less than $1/treatment for an adult), should be active against resistant strains, and preferably should exert its antimalarial effects through a new mode of action. Moreover, with the eradication strategy in mind, one component of the profile should ideally include activity against sexual stages and mosquito stages of the parasite lifecycle or also be effective against hypnozoites (the hard-to-control dormant liver stages of this parasite).

In 2010, St. Jude's Children's Research Hospital,[5] Novartis,[6] and GlaxoSmithKline[7] published the structures of thousands of compounds that inhibit parasite intraerythrocytic growth. This represents a big step change in the number of hits available for drug discovery programs (the three sets of compounds are available for download from the Chembl-NTD database: http://www.ebi.ac.uk/chemblntd).

Very recently, we published our initial strategy to mine the GSK set, the TCAMS (Tres Cantos Antimalarial Set), which includes the identification of quality starting points to start lead optimization.[8] Different mining approaches have already been published; however, our priority was to identify quality starting points suitable for the discovery of oral bioavailable drugs. These criteria have dictated the mining and filtering processes that we used. This is just one approach for mining TCAMS and one that may deprioritize compounds that could represent perfectly good starting points if a different mining or filtering process is carried out. Authors also included a top-five favorites series. The efforts for one of this series, the cyclopropyl carboxamides, have been already published.[9,10] In this communication, we intend to update our efforts in exploring one of the other four series, the indolines.

The primary hit for this series, TCMDC-139046 (1) (Figure 1), is a compound that shows a good in vitro antimalarial activity with IC$_{50}$ values of 80, 40, and 25 nM against *P.falciparum* 3D7A at 48, 72, and 96 h assays, respectively. This indicates that compound **1** kills the parasite in the first generation, and it does not present a delayed death phenotype. When parasite cultures are treated with this compound (at a concentration of 10-fold IC$_{50}$ value), the erythrocytic cycle is stopped, and pyknotic forms are visible after 24 h of exposure (Figure 1). The appearance of treated parasites is similar to phenotypes produced in the parasites by chloroquine or artemisinin treatments (both are fast-killing antimalarials), and these are very different to the phenotypes produced by

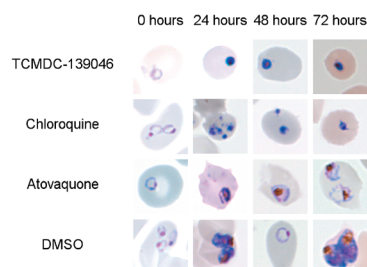|	TCMDC-139046 (1)
Pf IC50 nM (3D7A,HB3, Dd2)	80, 97, >1000
5-HT$_{2c}$ (µM)	0.05
Selectivity 3D7/5HT$_{2c}$	0.6
Cytotoxicity (µM) (HepG2,L1210,Neuro 2A, H9C2(2-1),MDCK)	7, 3.2, >12.5, >12.5 and >12.5
Cli (mL/min·G) (mouse, human)	<0.3 , <0.3
Protein binding HSA, mouse plasma (%)	97.8%, 99.8%
P450	2C9 (2 µM), 2D6 (13 µM), 3A4 and 2C19 (> 50 µM)
hERG (µM)	>16
eXP panel	Low impact in 40 of 42 assays
clogP	6.17
LogCHI^{12} pH 2, 7.4, 10.5	3.09, 4.22, 4.18
Solubility (µg/mL) PBS/FaSSIF/FeSSIF/SGF	<0.1/<0.1/16.7/1550
Mw	497.4
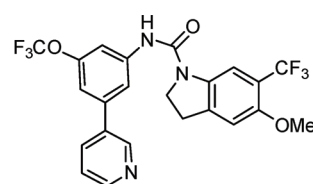
TCMDC-139046 (1)

Figure 1. Complete profile[12] of TCMDC-139046 as a representative compound of the indoline series showing antimalarial activity, cytotoxicity, affinity for 5HT$_{2c}$, in vitro DMPK profile, and chemical profile (table on the left,). The structure of TCMDC-139046 and the effects on the parasites are shown on the right.

atovaquone (bc1 complex inhibitor),[11,12] which stops the cell cycle in the trophozoite stage. In vitro efficacy was also determined using *P. falciparum* strains HB3 (moderately resistant to pyrimethamine) and Dd2 (chloroquine- and pyrimethamine-resistant). Compound **1** retains its in vitro activity against *P. falciparum* HB3 (IC$_{50}$ value 90 nM); however, a more than 10-fold decreased is observed against multidrug-resistant strain Dd2 (IC$_{50}$ > 1 µM).

This compound showed a good metabolic stability in vitro when it was tested in mouse and human microsome assays.[12] To determine the cytotoxicity profile, it was evaluated using five different mammalian cell lines [HepG2, L1210, Neuro2A, H9c2(2-1), and MDCK].[12] Results showed TOX$_{50}$ values around 10 µM, probably due to its moderate solubility in aqueous media (less than 0.1 µg mL^{-1} in PBS).[12] This representative compound has been profiled in a secondary pharmacology panel of assays relevant for clinical development, resulting inactive in 40 of 42 assays, showing that the main issue of this series is their high affinity for the 5-hydroxytryptamine subtype 2c (5HT$_{2c}$) receptor (Figure 1).

The 5HT receptors are part of the G-protein-coupled receptor (GPCR) superfamily and are classified into seven main types, 5-HT$_{1-7}$. They can be localized in both the peripheral and the central nervous system (CNS), and they mediate in numerous physiological functions. The serotonin 5-HT$_2$ receptor family is comprised of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ subtypes. These are grouped together on the basis of their primary structure, secondary messenger system, and operational profile.[13−15] In fact, sequence analysis of these receptors has revealed an approximately 80% of similarity, so it is not surprising that compounds with affinity for one of the receptors also bind with high affinity to the others. 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors are widely distributed in the peripheral (also can be found in the CNS but lower quantity), while the 5-HT$_{2C}$ receptor has been found only in the CNS.

A complete structure−activity relationship (SAR) study was published 12 years ago[16,17] presenting the biarylcarbamoylindo-lines **2** and the bispyridyl ether carbamoylindolines **3** (Figure 2) as very promising novel and selective 5-HT$_{2C}$ receptor inverse agonists, with antidepressant and anxiolytic properties. This
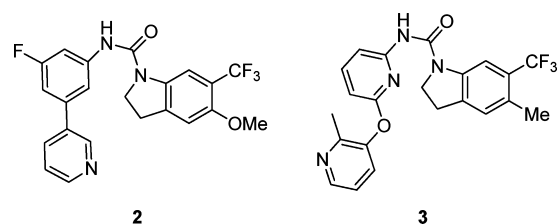

Figure 2. 5-HT$_{2c}$ inhibitor hits.

work is justified by the significant number of therapeutic opportunities for the treatment of different CNS disorders such as depression,[18] schizophrenia,[18,19] migraine,[20] and Parkinson's disease[21] offered by 5-HT$_{2C}$ antagonism.

Both hits can be related easily to our lead compound **1**, especially compound **2** (IC$_{50}$ 5-HT$_{2C}$ = 0.041 µM), which presents a biarylcarbamoyl-5-methoxy-6-trifluoromethylindo-line in the right-hand side and a fluorine atom in the aniline ring directly attached to the urea instead of a trifluoromethoxy group (compound **1**). This potent inhibition of the 5-HT$_{2c}$ receptors can complicate the clinical development of this series as antimalarials, so we wondered: would it be possible to reduce the high human 5-HT$_{2c}$ affinity while increasing the antimalarial activity?

To face this challenge, we designed a double SAR study aimed at determining which features can drive to a more potent antimalarial in both in *P. falciparum* 3D7 and *P. falciparum* Dd2, while avoiding interaction with 5-HT$_{2c}$ receptors. In addition, the optimization should lead to a compound with an improved physicochemical profile because **1** presents a poor developability profile (high cLog P and low solubility in aqueous media, Figure 1).

If we insert the indoline scaffold A (Figure 3) in the Chembl-NTD compound structure search page (https://www.ebi.ac.uk/chemblntd/compound/structure_home), 76 hits are obtained with potencies 0.040−1.3 µm in 3D7 at 72 h, but no clear SAR conclusions can be achieved. We decided to start our analysis using classical SAR techniques by studying the aniline moiety by testing if the lipophilic trifluoromethoxy can be
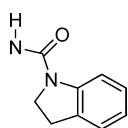
Figure 3. Scaffold A.

replaced for a more desirable group such as F, H, CF$_3$, or Br (Table 1, entries 2, 3, 4, and 5).

However, the trifluoromethoxy was shown to be the best substitution because the rest of the groups tested presented a reduced antimalarial activity, and moreover, a notable increase in the 5-HT$_{2c}$ affinity was observed. In fact, the results suggested that small substituents, or even no substitution, at this position drive to compounds with high affinity to 5-HT$_{2c}$ while the antiplasmodial potency, and consequently selectivity, can be achieved with bulky substituents. In addition, all of them lose potency when they are tested against *P. falciparum* Dd2.

Next, we drove our attention to the pyridyl ring (R$_2$). When we changed the pyridyl ring for a H, Me, and Br (Table 1, entries 6, 7, and 8), a loss in the antiplasmodial activity was observed. The same result was obtained when this moiety was substituted for different aromatic entities such as phenyl ring and related heterocycles (Table 1, entries 9–12). Although no great improvements were obtained in terms of potency, we realized that the pyridyl ring can be substituted by a pyrimidyl, which presents a slight improvement in the physicochemical

profile (clog P = 5.25). However, following the docking analysis of compound **2** described by Bromidge et.al.,[16,17] where in the proposed binding site, the 3-pyridyl ring occupies a quite deep lipophilic pocket that can be occupied by the pyrimidine ring or related aromatic moieties, a drastic change in this part of the molecule would be needed to decrease the 5-HT$_{2c}$ selectivity.

So, finally, we focused our efforts in the indoline ring where a wide variety of substituents are allowed based on the diversity found in the TCAMS. That gave us confidence of the real chances for getting selectivity while maintaining antiplasmodial activity plus improving the druglike properties. Searching the literature, we found three main opportunities to avoid 5-HT$_{2c}$ affinity in the indoline core: unsubstituted indolines, introduction of substituents at position 7, and introduction of polar groups.[15,16]

We started by synthesizing the unsubstituted indoline **14** where we saw that an increase in the antimalarial activity was achieved [low tens of nanomolar in both 3D7 and Dd2 (Table 2, entry 2)], but also, we achieved a selectivity ratio of 125. Moreover, no less important is the fact that this change drove us to a more attractive compound in terms of physicochemical properties (cLog P = 4.9), although it showed a weak metabolic stability [in vitro clearance[12] mouse/human, 6.2/1.0 mL/min g]. This issue was solved by just introducing a fluorine atom at position 6 (compound **15**, Table 2, entry 3; in vitro clearance mouse/human, 2.1/0.5 mL/min g).[12] The substitution by Cl, Br, and methylene (Table 2, entries 4–6) resulted in loss of

Table 1. SAR Analysis on the Left-Hand Side of the Molecule
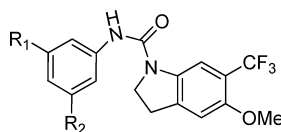
Entry	Compound	R1	R2	IC$_{50}$ (µM) *Pf* 3D7A	IC$_{50}$ (µM) *Pf* Dd2	ratio *Pf* Dd2/*Pf* 3D7A	IC$_{50}$ (µM) 5-HT$_{2c}$	selectivity (3D7/5HT$_{2c}$)
1	1	OCF$_3$	Py	0.08	>1	>13	0.05	0.62
2	2	F	Py	0.43	2.12	>8	0.01	0.02
3	4	H	Py	4.45	N.D.	N.D.	0.0007	0.0001
4	5	CF$_3$	Py	0.14	0.81	>9	0.01	0.07
5	6	Br	Py	0.16	1.10	6	0.01	0.06
6	7	OCF$_3$	H	>5	N.D.	N.D.	N.D.	N.D
7	8	OCF$_3$	Me	>5	N.D.	N.D.	N.D.	N.D
8	9	OCF$_3$	Br	4.84	N.D.	N.D.	0.39	0.08
9	10	OCF$_3$	Ph	2.26	N.D.	N.D.	N.D.	N.D.
10	11	OCF$_3$	(pyrimidinyl)	0.03	>1	>33	N.D.	N.D.
11	12	OCF$_3$	(6-Me-pyridinyl)	0.29	0.80	2.75	N.D.	N.D.
12	13	OCF$_3$	(pyrrolyl)	1.07	N.D.	N.D.	N.D.	N.D.
13	Chloroquine			0.01	0.71	71	N.D.	N.D.
14	Pyrimethamine			0.02	>40	>2000	N.D.	N.D.
15	Artemisinin			0.02	0.02	1	N.D.	N.D.

Table 2. SAR Analysis on the Right-Hand Side of the Molecule
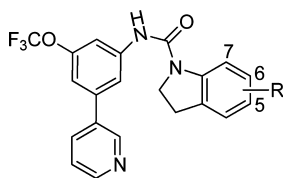
Entry	Compound	R5	R6	R7	IC$_{50}$ (μM) *Pf* 3D7A	IC$_{50}$ (μM) *Pf* Dd2	Ratio *Pf* Dd2/*Pf* 3D7A	IC$_{50}$ (μM) 5-HT$_{2c}$	selectivity (3D7/5HT$_{2c}$)
1	1	OMe	CF$_3$	H	0.08	>1	>13	0.05	0.62
2	14	H	H	H	0.01	0.02	2	1.25	125
3	15	H	F	H	0.01	0.01	1	0.6	60
4	16	H	Cl	H	0.15	N.D.	N.D.	N.D.	N.D.
5	17	H	Br	H	0.19	0.64	3	N.D.	N.D.
6	18	H	Me	H	0.03	N.D.	N.D.	N.D.	N.D.
7	19	Me	Cl	H	0.02	0.14	6	N.D.	N.D.
8	20	F	H	H	0.01	N.D.	N.D.	N.D.	N.D.
9	21	Me	H	H	0.01	0.04	3	N.D.	N.D.
10	22	CF$_3$	H	H	0.02	N.D.	N.D.	N.D.	N.D.
11	23	H	H	F	0.41	1.07	3	>40	>97
12	24	H	OMe	H	0.09	N.D.	N.D.	0.12	1.33
13	25	H	CH$_2$OH	H	0.40	N.D.	N.D.	0.18	0.45
14	26	H	⟨piperazine⟩	H	0.49	N.D	N.D.	>40	>80
15	27	Cl	⟨piperazine⟩	H	0.14	0.21	2	10	71.4

activity as compared with 14 and 15. Neither combination Cl/Me (Table 2, entry 7) nor monosubstitution at position 5 (Table 2, entries 8−10) resulted in any improvement.

Although compounds 14 and 15 were satisfactory to our purpose, we tested the two other hypotheses, so compound 23 (table 2, entry 11), which presents a fluorine in position 7, was prepared. This compound showed a good selectivity window although a loss in potency against both 3D7A and Dd2 was detected. Finally, analogues 24, 25, 26, and 27 presenting polar groups were prepared to test the third hypothesis. As expected, better selectivity was obtained, highlighting the piperidine derivatives 26 and 27 where no interaction with 5-HT$_{2c}$ was detected (Table 2, entry 12−15) and the activity against multidrug resistance strains Dd2 was maintained.

As result of the SAR analysis, compound 27 has shown to be the most promising compound, demonstrating that a divergent SAR between in vitro antimalarial activity and affinity for 5-HT$_{2c}$ is possible. This compound has not lost significant antimalarial potency against the 3D7 sensitive strain as compared to our initial hit 1, it is active against *P. falciparum* Dd2 (chloroquine- and pyrimethamine-resistant), and treated parasites retain the same phenotypic effect (supporting the same mode of action).[12] Compound 27 was tested in vivo against *P. berghei*. Compound 27 was administered by the oral route at 50 mg/kg, once a day for two consecutive days. In this assay, compound 27 inhibited parasitemia at day 4 after infection by 35% with respect to vehicle group. However, despite this weak in vivo efficacy, this new derivative has been able to reduce the initial number of viable parasites in a culture,

from 10^5 to less than 10 parasites, when this culture was treated during 72 h at 10-fold the IC$_{50}$ concentration. This result is similar to cultures treated at bioequivalent concentrations of chloroquine or artemisinin. Compound 27 presents a compatible profile with oral absorption in terms of physicochemical properties (CHI log *D* pH 6.5/7.4 3.9/4.2)[12] and in vitro intrinsic clearance (mouse/human, 4.0/1.5 mL/min g). Compound 27 was tested as 5-HT$_{2c}$ agonist showing a selectivity (3D7/5HT$_{2c}$) > 70.

In conclusion, the SAR analysis reported in this paper describes our strategy to convert a well-known 5-HT$_{2c}$ serotonin receptor inhibitor into a promising antimalarial lead compound presenting a potent whole cell activity, fast killing profile, and no cross-resistance with chloroquine- and pyrimethamine-resistant strains. The next milestone will be to improve its in vivo efficacy.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization data for the synthesis of all compounds and phenotypes for assays for compounds 14, 27, and 25. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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