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Synthesis and efficacy of pyrvinium-inspired analogs against tuberculosis and malaria pathogens

Vikas R. Gaikwad[a], Uttam B. Karale[a,b], Gokulapriya Govindarajalu[c], Navin Adhikari[c], E. Vamshi Krishna[b,d], Vagolu Siva Krishna[e], Sunil Misra[b,d], Dharmarajan Sriram[e], Puran Sigh Sijwali*[b, c], Haridas B. Rode*[a,b]

[a] Department of Organic Synthesis and Process Chemistry, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500 007, India

[b]Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh- 201 002, India

[c]CSIR-Centre for Cellular and Molecular Biology, Habsiguda, Uppal Road, Hyderabad-500007

[d]Department of Applied Biology, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500 007, India

[e]Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Shameerpet mandal, R.R. District, Hyderabad- 500 078, India

KEYWORDS: Pyrvinium analogs, malaria, antitubercular compounds, heterocyclic compounds

ABSTRACT:

Herein, we report the synthesis and evaluation of pyrvinium-based antimalarial and antitubercular compounds. Pyrvinium is an FDA approved drug for the treatment of pinworm infection, and it has been reported to have antiparasitic and antimicrobial activities. Pyrvinium contains quinoline core coupled with pyrrole. We replaced the pyrrole with various aryl or heteroaryl substituents to generate pyrvinium analogs. The profiling of these compounds against malaria parasite *P. falciparum* 3D7 revealed analogs with better antimalarial activity than pyrvinium pamoate. Compound **14** and **16** showed IC_{50} of 23 nM and 60 nM against *P. falciparum* 3D7, respectively. These compounds were also effective against drug-resistant malaria parasite *P. falciparum* Dd2 with IC_{50} of 53 nM and 97 nM, respectively. The cytotoxicity against CHO-K1, HEK and NRK-49F cells revealed better selectivity index for these new analogs compared to pyrvinium. Additionally, this series of compounds showed activity against *M. tuberculosis* H37Rv; particularly compounds **10**, **13**, **14** and **16** showed equipotent antitubercular activity to that of pyrvinium pamoate. The compounds **14** and **16** should be taken forward as leads for further optimization.

Infectious diseases affect human population at large, and the emergence of drug-resistant pathogens has further complicated treatment of these diseases, including malaria and tuberculosis. According to the WHO report, the estimated malaria cases jumped to 219 million in 2017 from 217 million in 2016. Globally, there were about 435000 deaths from malaria in 2017[1] and an estimated 1.3 million deaths were due to tuberculosis.[2] This scenario emphasizes the need of new drugs for these diseases. Subsequently, increased efforts in drug discovery resulted in potent leads targeting such diseases (figure 1). These leads include, antimalarial MK-4815, which selectively accumulates in infected blood cells and is highly effective against trophozoite/early schizont stages[3], the PfPI4K inhibitor UCT943[4] and the inhibitor of *Plasmodium* cytochrome bc1 complex ELQ-300[5]. The synthetic trioxolane OZ439[6], spiroindolone analogue Cipargamin[7], KAE609[7] and dihydroorotate dehydrogenase inhibitor DSM265[8] are under clinical evaluation.[9]

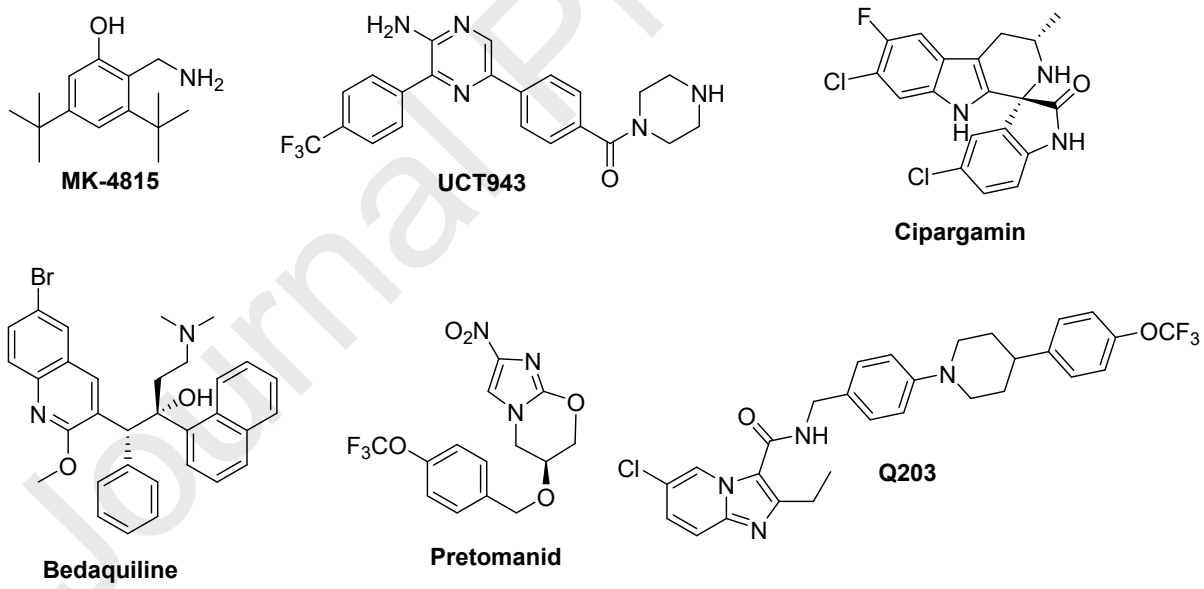


Figure 1: Structures of selected antimalarial and antitubercular agents

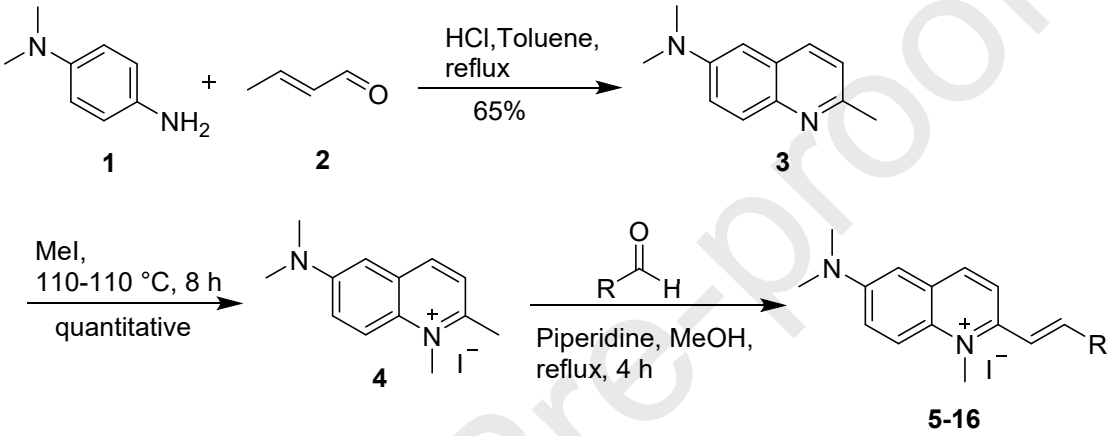
In the case of tuberculosis, imidazo[1,2-*a*]pyridine based Q203[10] and quinoline analogue TBAJ-876[11] and oxazolidinone TBI-223[12] are promising candidates. The diarylquinoline TBAJ-876 is in preclinical development.[11] Bedaquiline has been approved for the treatment

of multidrug-resistant (MDR) tuberculosis[13] while Pretomanid in combination with Linezolid and Bedaquiline has been approved in 2019 for extensively drug-resistant (XDR) tuberculosis or non-responsive MDR-TB patients.[14]

Pyrvinium is an FDA approved drug for the treatment of pinworm (*Enterobius vermicularis*) infection in humans[15-17]. Especially, pyrvinium pamoate salt in children was highly successful[18]. It is important to note that the treatment of oxyuriasis (thread worm infection) using a single dose of pyrvinium embonate[19] and pyrvinium pamoate[20] was a milestone discovery. It has antitubercular[21], antibacterial against *S. aureus*[22] and *C. albicans* biofilm inhibition[23] activities. Further, its antitumor activity against human pancreatic cancer cell line PANC-1 in SCID mice and nude mice, when administered orally, was successfully demonstrated.[24] Off note, Sullivan *et al.* reported its *in vitro* activity against *Entamoeba histolytica* with IC_{50} of 4-5 μM[25] while IC_{50} against *P. falciparum* 3D7 is in nanomolar concentration.[26] These multiple pharmacological activities of pyrvinium inspired us to synthesize its analogs to generate more potent inhibitors for infectious diseases like malaria and tuberculosis. This is in line with our efforts towards the development of lead compounds against infectious diseases.[27-32] With this background, herein, we report the pyrvinium analogs that showed potent activity against drug-resistant malaria and tuberculosis.

Pyrvinium has a quinoline core connected to pyrrole ring system. It is chemically tractable and we believed that its chemical modification could result in more potent inhibitors. We began the study by synthesizing pyrvinium. Two methods have been reported for the synthesis of pyrvinium. The J. E. Macdonald *et al.*'s route[33] for the synthesis of pyrvinium makes use of Paal-Knorr and Vilsmeier-Haack as key reactions, while the other route described by Jing *et al.*[34] makes use of 2-amino-5-(dimethylamino)benzaldehyde as key intermediate. Our strategy uses the synthesis of 2-methylquinoline from *N*,*N*-Dimethyl-1,4-phenylenediamine (**1**, scheme 1) using Döbner-Miller reaction. Briefly, the reaction between

1 and **2** in the presence of HCl afforded 2-methylquinoline **3**. The treatment of **3** with methyl iodide in a sealed tube gave quinaldinium salt **4** in quantitative yield. Finally, the condensation between **4** with various aldehydes in the presence of piperidine afforded quinolinium compounds **5-16** (scheme 1, table 1). This way, pyrvinium iodide (**5**) was obtained as dark red solid. The **5** was treated with sodium pamoate to afford pyrvinium pamoate (**5a**).



Scheme 1: Synthesis of quinolinium derivatives

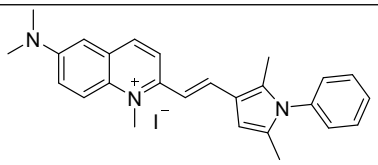
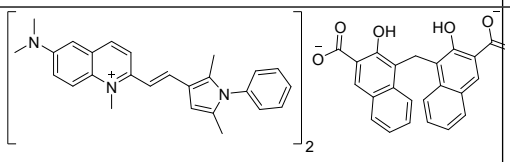
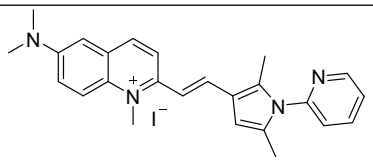
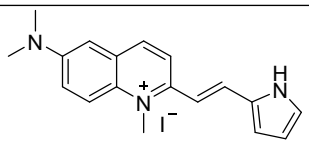
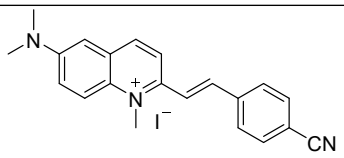
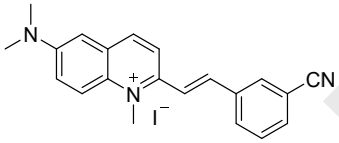
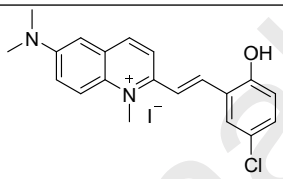
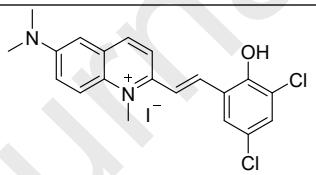
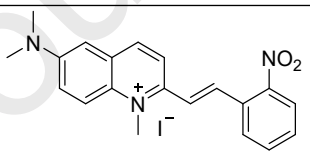
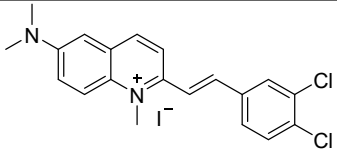
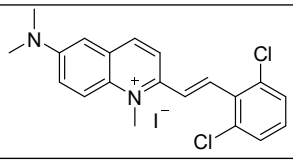
Thus synthesized compounds were screened for inhibition of *P. falciparum* 3D7 erythrocytic stage development and the results are shown in table 1. Earlier, Sullivan *et al*[26] and Tilley *et al.*[35] described nanomolar antimalarial activity of pyrvinium pamoate (**5a**) against *P. falciparum* 3D7. Sullivan *et al*[26] reported the IC_{50} value of 3 nM for pyrvinium pamoate (**5a**) whereas our screen showed IC_{50} of 342 nM (figure 2) against *P. falciparum* 3D7. This difference in IC_{50} value can be augmented to the differences in screening protocols (96 hours treatment in Sullivan *et al.*'s protocol[26] versus 50 hours in our protocol). To understand the influence of treatment duration, we assessed the effect of pyrvinium pamoate (**5a**) on parasite growth upon 96 hours treatment. A significant decrease in IC_{50} values for **5a** (5.04 ± 1.6 nM) upon 96 hours treatment was observed as compared to the 50 hours treatment (IC_{50}= 342 nM). This clearly indicates that the potency of pyrvinium pamoate increased with longer

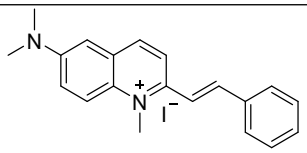
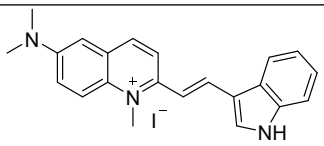
exposure time. Pyrvinium iodide (**5**) showed IC_{50} of 774 nM against *P. falciparum* 3D7, which is about twice the IC_{50} of pyrvinium pamoate. The *N*-pyridyl-pyrrole derivative **6** showed potent antimalarial activity with IC_{50} of 228 nM, whereas mono-substituted pyrrole **7** was less effective than pyrvinium iodide. This could be due to the hydrogen bond acceptor character of pyridine substituent of **6**. We then assessed non pyrrole quinolinium iodide derivatives **8**-**16** for inhibition of *P. falciparum* 3D7 erythrocytic stage development. As expected, compound **8** and **9** retained potent antimalarial activity likely due to the hydrogen bond acceptor character of nitrile substituent. The lower activity in case of compound **7**, **10** and **11** might augment to their hydrogen bond donor ability. Compound **12**, which has nitro substituent, showed lowest antimalarial activity with IC_{50} of 3.60 μM in the series. Important to note that the presence of hydrophobic substituents, like chloro, in compound **13** and **14** showed high antimalarial activity. Between these two, the positional isomer i.e. 2,6-dichlorophenyl (in case of **14**, IC_{50}= 23 nM) is preferred over the 3,4-dichlorophenyl (in case of **13**, IC_{50}= 87 nM) for the antimalarial activity. Compound **14** was found to be the most potent (IC_{50}= 23 nM) among all the derivatives against *P. falciparum* 3D7 erythrocytic stage development. Importantly, a significant decrease in IC_{50} values (2.37 ± 0.44 nM) for compound **14** upon 96 hours treatment was also observed. Even the compound with unsubstituted phenyl ring (compound **15**) retained potent antimalarial activity. Compound **16** which has indole substituent showed IC_{50} of 60 nM. When compared with compound **7** which has pyrrole, compound **16** showed better antimalarial activity which can be augmented to the presence of more hydrophobic indole substituent as compared to pyrrole substituent in compound **7**. Further, the effects of pyrvinium pamoate (**5a**) and compound **14** on *P. falciparum* 3D7 morphology were evaluated by treating the early ring stage parasites for 48 hours. The treatment caused growth arrest at the early trophozoite stage (figure 3), which could be due to inhibition of mitochondrial function as has been previously proposed[35]. To

find out if the inhibition was due to death or mere arrest of the parasite development, drug was washed off after 48 hours of treatment with pyrvinium pamoate (**5a**) or compound **14**, and parasites were cultured in normal medium for the next 48 hours. The control parasites (DMSO-treated) grew normally and showed about 12-fold multiplication at the end of 2nd cycle compared with the starting parasitemia (~2%). On the other hand, cultures of parasites treated with pyrvinium pamoate or compound **14** did not have any parasites at the end of 2nd cycle, indicating that these compounds exert parasiticidal effect. To investigate if these compounds are effective against the drug resistant *P. falciparum* strain Dd2, compound **14**, **16** and **5a** were assessed for inhibition of the erythrocytic development of Dd2 (Table 2). *P. falciparum* strain Dd2 is highly resistant to pyrimethamine and moderately resistant to Chloroquine as compared to the 3D7 strain. As expected Dd2 and 3D7 showed similar IC_{50} for artemisinin but the IC_{50} concentrations of pyrimethamine and Chloroquine were significantly higher for Dd2 than 3D7 (Table 1 and 2). All the three compounds tested had comparable potency against 3D7 and Dd2 parasites. To assess safety, these analogs were profiled against CHO-K1, HEK and NRK-49F cells (Table 1). The selectivity indices for these compounds were calculated using IC_{50} of compounds against CHO-K1 cells divided by IC_{50} of compounds against *P. falciparum* 3D7. All the compounds showed better selectivity index than **5a**. The potent antimalarial analogous **8**, **13**, **14, 15** and **16** showed more than 140 selectivity index, indicating their selective efficacy against the parasite. It is important to note that compound **8** showed highest selectivity index of >500 while the most potent compound **14** showed selectivity index of >340.

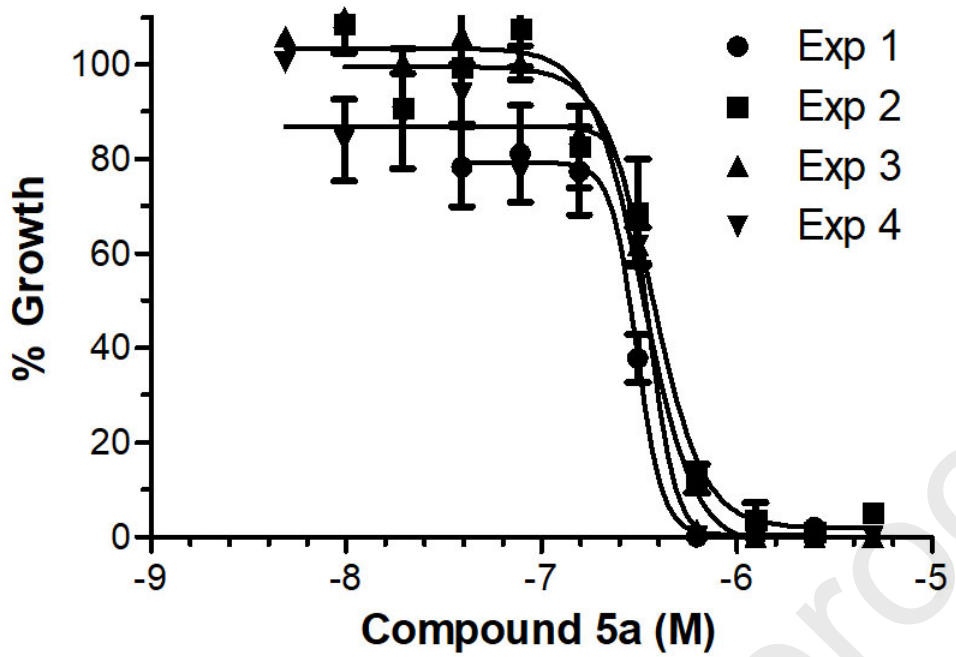
Table 1: Antimalarial activity against *P. falciparum* and toxicity on CHO-K1, HEK and NRK-49F cells

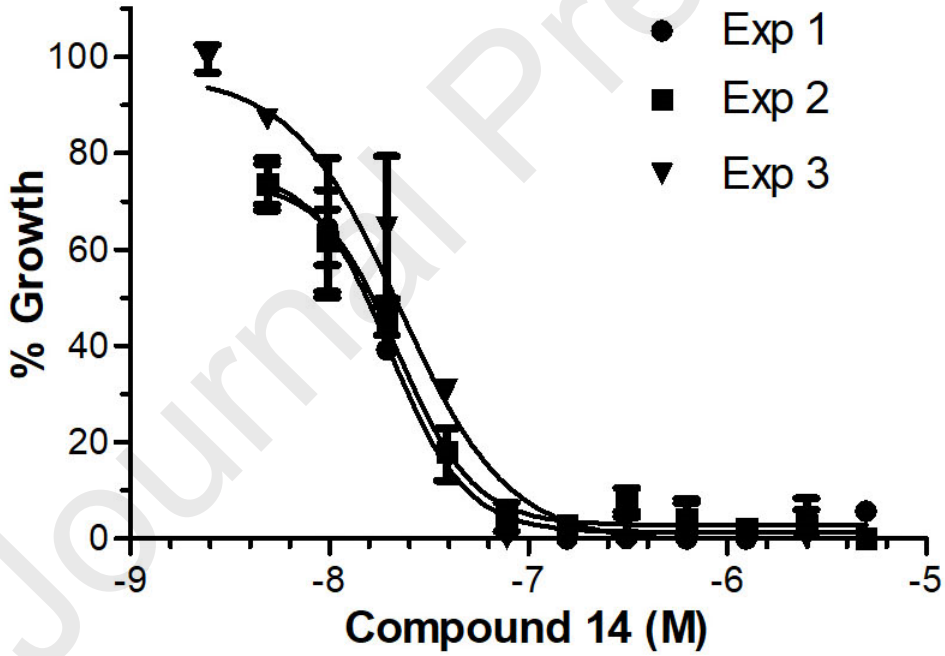
Comp. No.	Structure	IC_{50} (μM)[a]	IC_{50} (CHO-K1 , μM)[b]	IC_{50} (HEK, μM)[c]	IC_{50} (NRK-49F, μM)[d]	SI (IC_{50}[b]/ MIC[a])

5		0.774 ± 0.06	10.90 ± 0.06	15.98 ± 0.96	20.15 ± 2.4	14.0
5a		0.342 ± 0.03	3.50 ± 0.69	2.99 ± 0.12	3.02 ± 0.39	10.2
6		0.228 ± 0.03	15.15 ± 2.15	14.56 ± 0.98	12.67 ± 0.74	66.2
7		1.54 ± 0.18	77.84 ± 0.87	66.89 ± 1.69	75.98 ± 1.56	50.5
8		0.094 ± 0.02	52.57 ± 2.2	62.12 ± 1.36	50.23 ± 0.14	553.1
9		0.217 ± 0.01	87.62 ± 1.62	55.97 ± 1.02	59.45 ± 1.09	403.6
10		0.441 ± 0.04	30.33 ± 0.54	15.26 ± 0.56	11.99 ± 0.56	68.7
11		2.47 ± 0.25	54.69 ± 3.68	50.12 ± 1.02	49.09 ± 1.99	22.1
12		3.60 ± 0.14	73.86 ± 1.74	66.66 ± 0.25	69.52 ± 1.45	20.5
13		0.087 ± 0.006	12.44 ± 0.06	8.56 ± 0.12	11.29 ± 0.36	142.5
14		0.023 ± 0.002	7.92 ± 0.42	5.62 ± 0.23	5.23 ± 0.12	344.3

15		0.089 ± 0.01	41.64 ± 1.8	39.81 ± 1.2	42.15 ± 0.18	467.4
16		0.060± 0.001	11.36 ± 1.6	10.56 ± 0.12	11.29 ± 0.54	188.3
Pyrimethamine	-	0.011 ± 0.001	-	-	-	-
Artemisinin	-	0.025 ± 0.001	-	-	-	-
Chloroquine	-	0.008 ± 0.001	-	-	-	-

[a]Half maximal inhibitory concentration (IC_{50}) of the indicated compounds against the *P. falciparum* 3D7 erythrocytic development. The data is mean of three independent experiments for pyrvinium pamoate (**5a**) and its analogs (2 experiments for antimalarials), each done in duplicates. [b]Cytotoxicity (IC_{50}) of compounds against CHO-K1 cells. [c]Cytotoxicity (IC_{50}) of compounds against HEK cells. [d]Cytotoxicity (IC_{50}) of compounds against NRK-49F cells. Selectivity index (SI) is calculated by IC_{50} of compounds against CHO-K1 cells divided by IC_{50} of compounds against *P. falciparum* 3D7





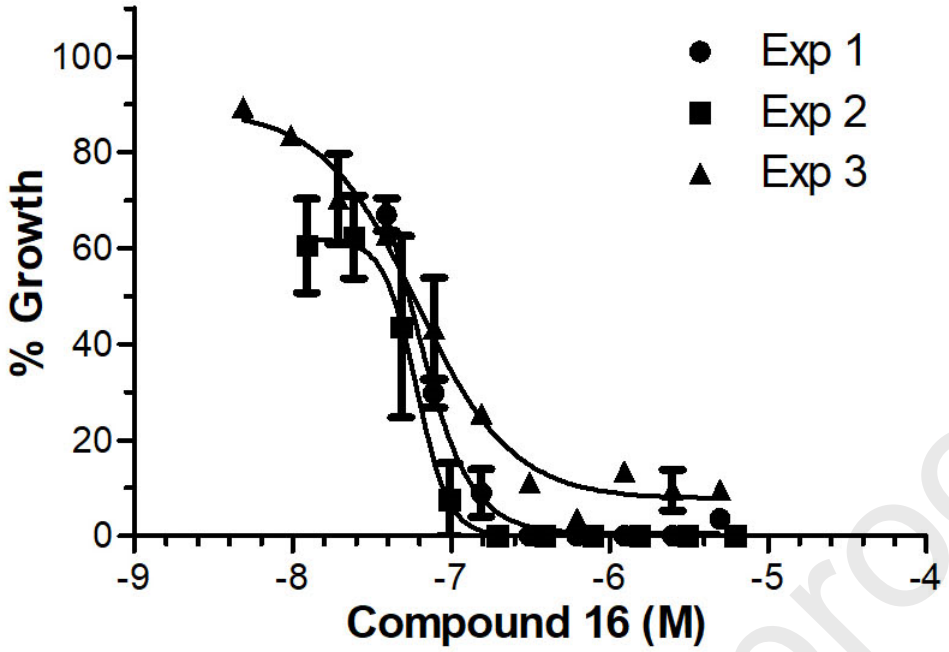


Figure 2: IC_{50} graphs. The plots show inhibition of *P. falciparum* 3D7 erythrocytic development (% Growth) at different concentrations of the compound **5a**, **14** and **16** (x-axis).

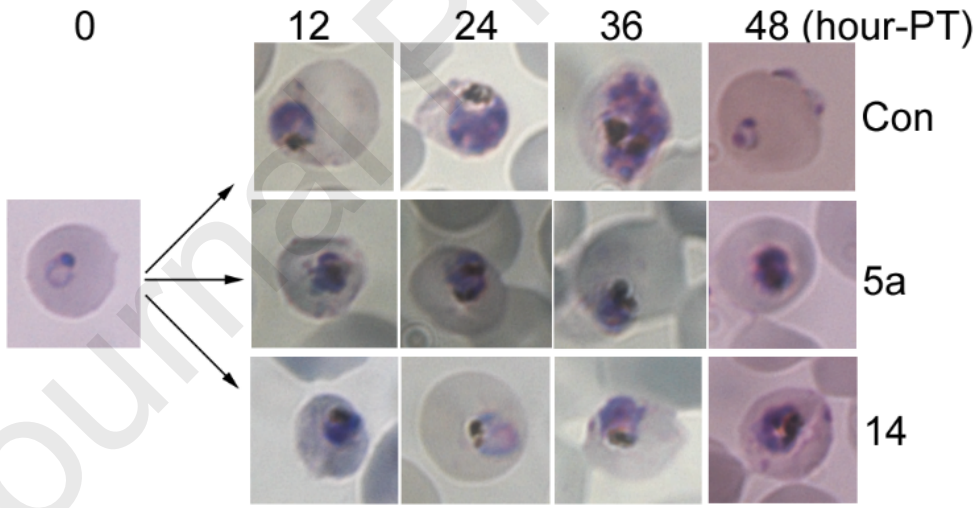


Figure 3: Effect of inhibitor treatment on parasite morphology. Early ring stage *P. falciparum* 3D7 parasites were treated with DMSO (Con, at 0.1%), **5a** (at 3X IC_{50} concentration) or the compound **14** (at 3X IC_{50} concentration) for 48 hours. Giemsa-stained smears of cultures were prepared before the treatment (0 hour) and at the indicated hours of post-treatment (hour-PT). The smears were observed under the 100x objective of a light microscope and images were taken. The morphologies of treated parasites are shown.

Table 2: Antimalarial activity of **5a**, **14** and **16** against *P. falciparum* Dd2. The data is mean of three independent experiments for pyrvinium pamoate (**5a**) and its analogs (2 experiments for antimalarials), each done in duplicates.

Compound/drug	IC_{50} (μM)
5a	0.219 ± 0.022
14	0.053 ± 0.004
16	0.097 ± 0.006
Pyrimethamine	85.0 ± 3.2
Artemisinin	0.021 ± 0.001
Chloroquine	0.0417 ± 0.004

Furthermore, compounds were evaluated for inhibition of *M. tuberculosis* H37Rv (table 3). The MABA assay (Microplate Alamar Blue Assay) was used for determining the MIC of these pyrvinium-inspired analogous (supporting information). Compounds **10**, **13**, **14** and **16** showed equipotent inhibition of *M. tuberculosis* H37Rv to that of **5a** with MIC of 3.12 μM. The compounds **10**, **13** and **14** have chlorophenyl substituent and indicate the possible role of hydrophilicity in their antitubercular activity. This way, the pyrrole ring of pyrvinium pamoate (**5a**) was successfully replaced by various chlorophenyl substituents. Compound **5** and **6** showed moderate antitubercular activity with MIC of 6.25μM against *M. tuberculosis* H37Rv. The cyanophenyl-substituted analogs **8** and **9** showed weak antituberculosis activity. Compound **15**, which has unsubstituted phenyl ring, found to be inactive against the tuberculosis bacteria. Further, the selectivity indices were measured for these compounds (table 3). The selectivity index was in the rage of 1 to 9. This indicate the need for further modifications of these compounds to generate more potent antitubercular compounds. Taking together, this series should be further explored for development of next generation pyrvinium-based chemotypes.

Table 3: Antitubercular activity of analogs and selectivity index

Compound No.	MIC (μM)	SI (IC_{50}[b]/MIC[a])
5	6.25	1.73
5a	3.12	1.12
6	6.25	2.42
7	25	3.11
8	50	1.05
9	25	3.50
10	3.12	9.72
11	25	2.18
12	50	1.47
13	3.12	3.98
14	3.12	2.53
15	>100	<0.41
16	3.12	3.64
Rifampicin	0.24	-

Minimum inhibitory concentration (MIC) of compounds against *M. tuberculosis* H37Rv was measured using MABA assay. The MIC was defined as the lowest concentration of compound that prevented the growth of *M. tuberculosis* H37Rv. The data is mean of two independent experiments, each done in duplicates. [b]Cytotoxicity (IC_{50}) of compounds against CHO-K1 cells (please refer table 1)

In summary, we have demonstrated new pyrvinium analogs as antimalarial and antitubercular agents. The study identified compound **14** and **16** with IC_{50} of 23 nM and 60 nM against *P. falciparum* 3D7, respectively. Of note, these compounds also showed activity against drug-resistant malaria parasite strain *P. falciparum* Dd2 with IC_{50} of 53 and 97 nM, respectively. The selectivity index of compound **14** and **16** for antimalarial activity was found to be more than 180 when compared with their toxicity against CHO-K1 cells. Importantly, these new analogs are more selective and potent in their antimalarial activity than pyrvinium pamoate. Furthermore, the analogs **10**, **13**, **14** and **16** were also found to be equipotent to that of pyrvinium pamoate against *M. tuberculosis* H37Rv.

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