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New antiprotozoal agents: Their synthesis and biological evaluations

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ABSTRACT

Here we report identification of new lead compounds based on quinoline and indenoquinolines with variable side chains as antiprotozoal agents. Quinolines **32**, **36** and **37** (Table 1) and indenoquinoline derivatives **14** and **23** (Table 2) inhibit the in vitro growth of the *Trypanosoma cruzi*, *Trypanosoma brucei*, *Trypanosoma brucei rhodesiense* subspecies and *Leishmania infantum* with IC_{50} = 0.25 μM. These five compounds have superior activity to that of the front-line drugs such as benznidazole, nifurtimox and comparable to amphotericin B. Thus these compounds constitute new 'leads' for further structure–activity studies as potential active antiprotozoal agents.

Neglected tropical diseases (NTD)[1] include Chagas' disease (American trypanosomiasis[2]), human African trypanosomiasis HAT (sleeping sickness)[3] and leishmaniasis.[4] These are parasitic diseases caused by the parasitic protozoan's *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* (*T. brucei*) and *Leishmania* species, respectively. It is a serious health problem of today mainly in tropical countries and in Central and South American continent causing two million deaths per year.[5,6] The present treatment is not very effective in the chronic phase and has toxicity, side effects[7–15] and parasite resistance.[10–12,16–21]

Thus, there is a considerable potential in developing novel approaches for antitrypanosoma and antileishmania drugs. Molecular modeling,[22] enzymatic[23] and crystallographic studies[24] on tipifarnib (**1**, EC_{50} = 4 nM, Fig. 1) and its analogues (compounds **2** and **3**)[22,25–27] have shown role of quinoline and side chain in its biological activity. In these studies, the X-ray structure of cocrystal of compound **2** with *T. brucei* CYP51, has elegantly shown that the quinolone together with its imidazole ring side chain was coordinated with heme iron whereas the phenyl ring attached to quinolone occupying an additional CYP51 active-site cavity. Qunoline as a pharmacophore against *T. brucei* and *T. cruzi* is also interesting because tafenoquine (**3**, Fig. 1) is known to act on unique target such as cytochrome *c* reductase. As a part of our research project on antitubercular drug discovery, we have screened a library of 39 compounds based on a quinoline and indenoquinolines with various side chains for antiprotozoal activity, which have also shown anti-TB activity.[28–32] We have thus identified five compounds (**14**, **23**, **32**, **36** and **37**) that have shown excellent in vitro antitrypanosomal and antileishmanial activity as low as IC_{50} = 0.25 and 0.40 μM, respectively, which is superior to frontline drugs benznidazole[33] (IC_{50} = 3.66 μM), nifurtimox[34] (IC_{50} = 1.8 μM) and comparable to amphotericin B[35] (IC_{50} = 0.25 μM). The diverse structures of these active compounds further suggest that both quinoline and side chain variations are important for antiprotozoal activity.

Following the literature procedures compound **6** was prepared through functionalization of 4-OH of 6-bromo-2-(trifluoromethyl)quinolin-4-ol, **4**[36] (Scheme 1). Compound **4** was brominated by using PBr_3 in DMF to give 4,6-dibromo compound **5**[37] which was treated with strong base LDA followed by benzaldehyde in dry THF to obtain the desired compound **6**. To achieve the target compound **9** (Scheme 2), compound **7**[29] was treated with *m*-(trifluoromethyl) benzene sulfonyl chloride in presence of dry pyridine to give sulfonamide **8**. Carbonyl group of sulfonamide **8** was reduced by $NaBH_4$ to give hydroxy derivative **9**. To accomplish the synthesis of compounds **11**, **12**, **14** and **15** (Scheme 3),

Abbreviations: CC_{50}, concentration of inhibitor resulting in 50% parasite growth inhibition; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDC·HCl, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride; Et_3N, triethylamine; EtOH, ethanol; MeOH, methanol; IC_{50}, concentration of inhibitor resulting in 50% inhibition; SI, Ratio of CC_{50} value/IC_{50}; NMR, nuclear magnetic resonance; SAR, structure–activity relationship; PFT, protein farnesyltransferase; PPA, polyphosphoric acid.

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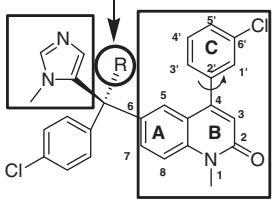
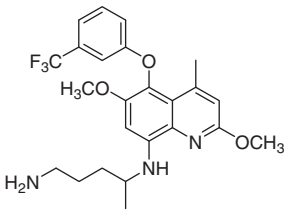
R = NH_2 binds to mammalian PFT *via* farnesyl diphosphate
R = OCH_3 binds to *T.cruzi* 14DM



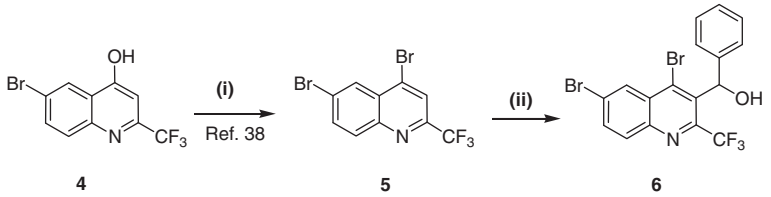
1: R = NH_2, Tipifarnib (EC_{50} = 4 nM) *T. cruzi protein farnesyltransferase (PFT); human (hPFT* IC_{50}= 0.7 nM)

2: R = OCH_3 (EC_{50} = 0.6 nM) *T. cruzi (PFT); human (hPFT* IC_{50}>5000 nM)

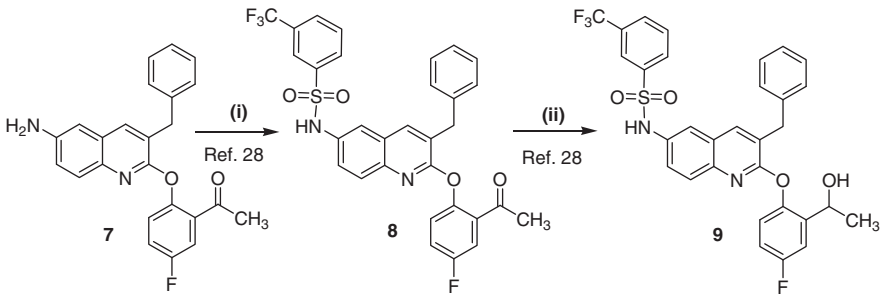


3: Tafenoquine (IC_{50} = 5.6 μM) against *L. donovani* *Target: mitochondrial dysfunction through cytochrome c reductase*

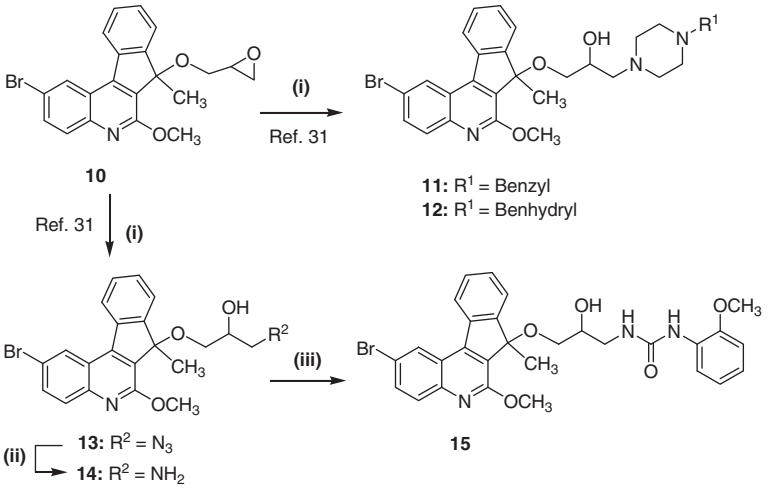
Figure 1. Quinoline based antiparasitic lead molecules (**1**–**3**) under clinical trials.



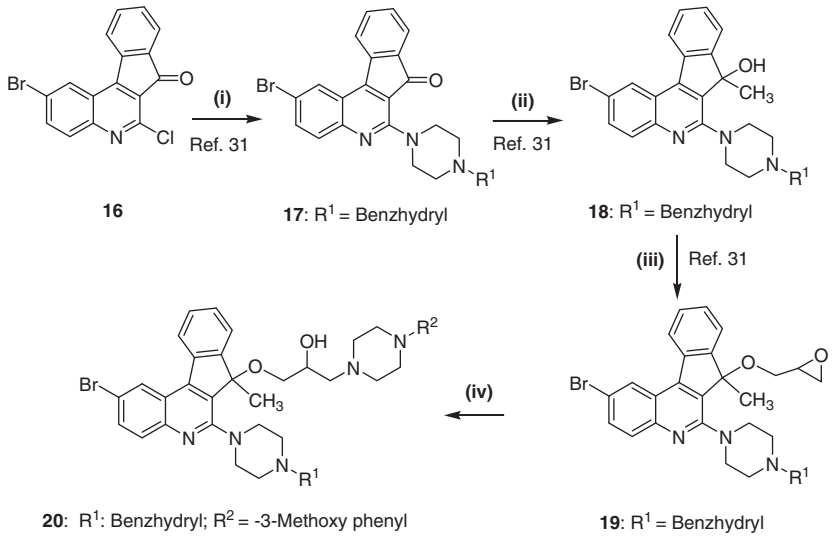
Scheme 1. Reagents and conditions: (i) dry DMF, PBr_3 at 0 °C then at rt, 4 h, 82%; (ii) LDA, dry THF, 30 min, PhCHO, −78 °C, 2 h, 16%.



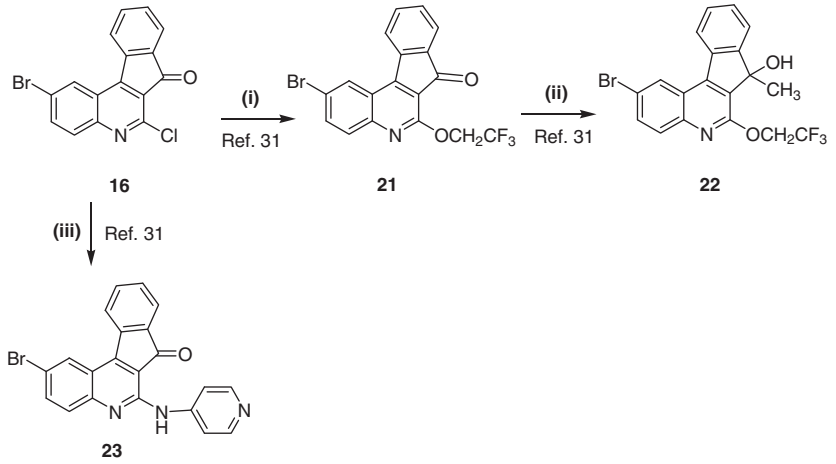
Scheme 2. Reagents and conditions: (i) 3-(trifluoromethyl)benzene-1-sulfonyl chloride, dry pyridine, rt, 12 h, 54%; (ii) $NaBH_4$, EtOH–THF (2:1, 6 mL), rt, 2 h, 53%.



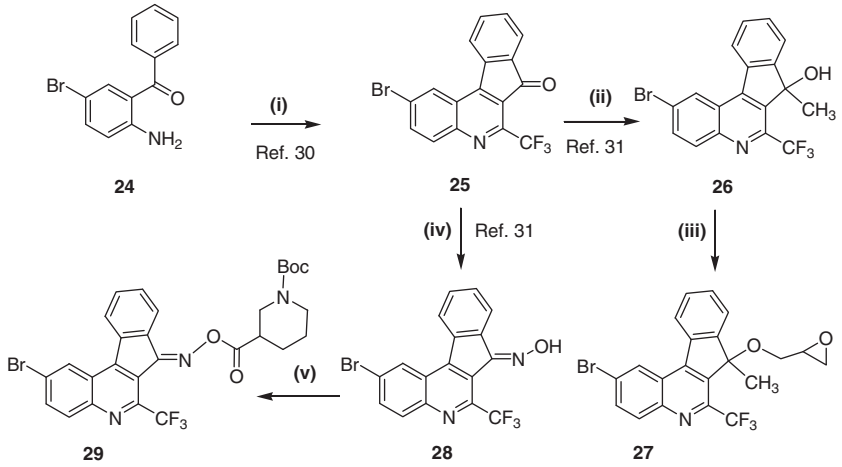
Scheme 3. Reagents and conditions: (i) *iso*-propanol, 1-benzyl piperazine for **11**, 1-benzhydryl piperazine for **12**, and NaN_3 for **13**, reflux, 12 h, (**11**, 26%; **12**, 27% and **13**, 61%); (ii) dry THF, PPh_3, reflux, 15 h, 58%; (iii) dry DCM, 2-methoxyphenyl isocyanate, dry Et_3N, 0 °C–rt, 1 h, 9%.



Scheme 4. Reagents and conditions: (i) dry pyridine, 1-benzhydryl piperazine, reflux, 8 h, 71%; (ii) dry THF, MeMgI, rt, 5 h, 82%; (iii) dry DMF, NaH, *epi*-chlorohydrin, 12 h, 78%; (iv) *iso*-propanol, *m*-methoxyphenyl piperazine, reflux, 12 h, 38%.



Scheme 5. Reagents and conditions: (i) dry DMF, 2,2,2-trifluoroethanol, NaH, rt, 15 h, 65%; (ii) dry THF, MeMgI, rt, 14 h, 58%; (iii) dry pyridine, 4-aminopyridine, reflux, 8 h, 87%.



Scheme 6. Reagents and conditions: (i) ethyl 4,4,4-trifluoro-3-oxobutanoate, PPA, 150 °C, 11 h, 45%; (ii) dry THF, MeMgI, 5 °C–rt, 5 h, 71%; (iii) dry DMF, NaH, rt, *epi*-chlorohydrin, 10 h, 51%; (iv) EtOH:H_2O (2:1), $NH_2OH{\cdot}HCl$, NaOH, 0 °C 15 min then 90 °C, 8 h, 94%; (v) dry DMF, DMAP, EDC·HCl, Boc-nipecotic acid, rt, 3 h, 28%.

Table 1
In vitro activity (IC_{50} μM) of compounds against *T. cruzi* (*T.c.*), *T. brucei brucei* (*T.b.*), *T. brucei rhodesiense* (*T.r.*) and *Leishmania infantum* (*L.inft.*)

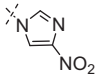
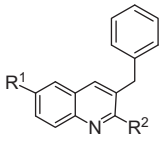
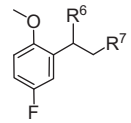
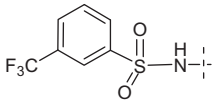
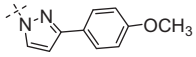
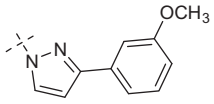
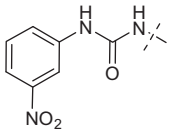
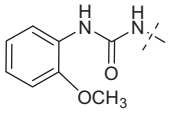
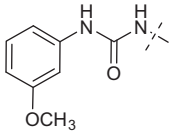
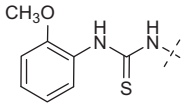
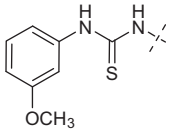
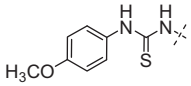
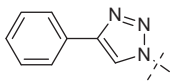
No	Structure				IC_{50} (μM)				CC_{50}	log *P*
	R^1	R^2	R^3	R^5	*T.c.*	*T.b.*	*T.r.*	*L.inft.*		
6	Br	CF_3	OH	Br	8.07	7.52	5.94	8.11	1.51	6.26
30	Br	OCH_3		H	0.46	32.69	8.43	25.40	30.83	4.52
31	Br	OCH_3		H	6.96	2.16	0.44	50.80	64.00	–
										
	R^1		R^6	R^7	*T.c.*	*T.b.*	*T.r.*	*L.inft.*	CC_{50}	log *P*
9			–	OH	3.70	2.18	1.46	8.11	8.00	7.69
32	NO_2			OH	0.25	2.18	1.81	2.52	31.44	–
33	NO_2			OH	2.00	2.01	1.22	2.16	8.10	–
34	NO_2			OH	64.00	2.12	0.71	20.32	64.00	–
35	NO_2			OH	6.01	2.40	2.08	8.00	64.00	–
36			–	OH	1.00	0.51	0.25	1.70	2.20	6.84
37			–	OH	2.89	0.65	0.25	3.17	6.21	6.72
38			–	OH	6.23	2.04	2.07	8.11	64.00	6.72
39			–	OH	4.33	1.79	0.53	5.28	16.00	7.86
40			–	OH	14.67	2.00	0.53	6.82	64.00	7.86
41			–	OH	5.81	2.09	1.31	8.11	42.33	7.86
42			–		1.93	2.04	2.03	20.32	64.00	7.91

Table 2
In vitro activity (IC_{50} μM) of conformationally locked compounds

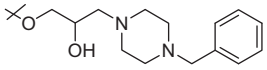
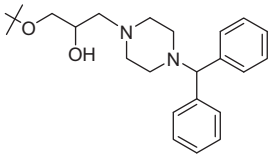
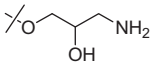
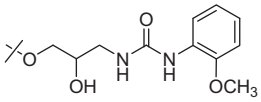
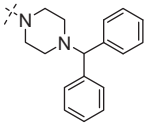
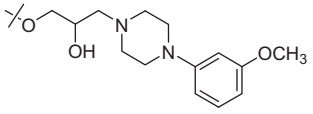
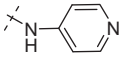
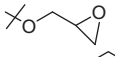
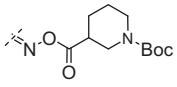
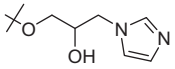
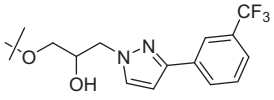
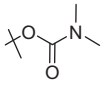
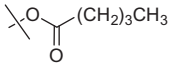
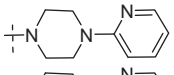
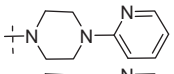
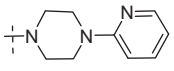
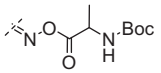
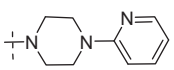
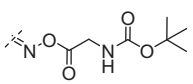
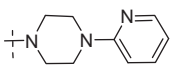
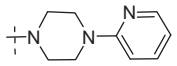
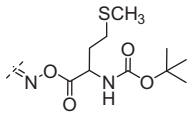
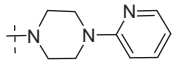
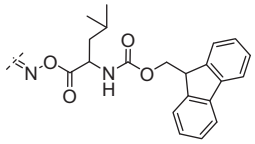
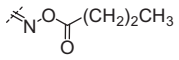
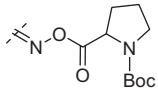
No.	Structure				IC_{50} (μM)				CC_{50}	log P
	R^1	R^2	R^3	R^4	*T.c.*	*T.b.*	*T.r.*	*L.inft.*		
11	Br	OCH_3		CH_3	7.25	2.24	1.99	9.51	5.63	5.85
12	Br	OCH_3		CH_3	5.85	2.04	1.64	8.11	17.55	7.56
14	Br	OCH_3		CH_3	0.47	0.25	0.25	0.40	1.36	3.46
15	Br	OCH_3		CH_3	2.50	8.23	1.88	9.51	5.31	4.84
20	Br			CH_3	64.0	2.03	0.86	30.05	61.44	–
22	Br	OCH_2CF_3	–OH	CH_3	6.92	8.23	3.23	8.00	19.84	5.71
23	Br		=O	–	0.25	0.56	0.25	5.08	0.75	4.64
27	Br	CF_3		CH_3	8.37	8.57	5.49	27.27	4.00	5.23
29	Br	CF_3		–	12.29	7.56	3.06	8.11	5.84	–
43	Br	OCH_3		CH_3	2.16	2.03	1.17	6.01	8.00	3.68
44	Br	OCH_3		CH_3	5.35	2.31	1.25	5.08	24.11	7.50
45	Br	OCH_3		CH_3	7.42	8.23	5.28	64.00	64.00	4.91
46	Br		–OH	CH_3	1.39	18.78	5.93	12.70	11.45	3.86
47	Br			CH_3	0.93	28.98	7.11	21.11	32.22	4.09
48	Br			CH_3	1.46	8.11	5.81	5.66	21.77	5.58
49	Br		=O	–	6.87	7.69	5.98	20.32	4.33	6.01
50	Br			–	7.01	8.17	2.61	9.51	64.00	6.40
51	Br			–	6.21	2.04	1.65	6.96	26.91	–
52	Br			–	6.82	6.05	1.20	5.08	64.00	–
53	Br			–	7.87	2.16	1.36	5.66	35.60	–

Table 2 (*continued*)

No.	Structure				IC_{50} (μM)				CC_{50}	log P
	R^1	R^2	R^3	R^4	*T.c.*	*T.b.*	*T.r.*	*L.inft.*		
54	Br			–	7.39	2.40	1.44	9.51	64.00	–
55	Br			–	7.52	7.33	1.52	3.17	13.59	8.30
56	Br			–	2.38	8.17	5.14	6.01	8.23	–
57	Br			–	7.38	7.31	4.33	12.70	8.00	–

compound **10**[30] served as a key intermediate. Oxirane **10** upon heating at reflux with appropriate piperazine(s) in *iso*-propanol gave the desired piperazine derivatives **11** and **12** as a diastereomeric mixture. Under similar conditions when oxirane **10** was reacted with NaN_3 gave azido compound **13**. Azide **13** was reduced by employing PPh_3 in THF (Staudinger reaction) to give amine **14** which was further converted to urea **15** by treating it with 2-methoxyphenyl isocyanate. Benzhydryl derivative **20** (Scheme 4) was prepared from 2-bromo-6-chloro-indeno[2,1-*c*]quinolin-7-one (**16**).[30] The C2-chloro group of chloroketone **16** was nucleophilically displaced by benzhydrylpiperazine to give ketone **17** which was subjected to Grignard reaction (MeMgI) to transform to the hydroxy derivative **18** in 82% yield. Compound **18** furnished oxirane **19** (78%) upon treatment with *epi*-chlorohydrin and NaH in dry DMF. Subsequently oxirane ring of **19** was opened by *m*-methoxyphenyl piperazine in *iso*-propanol to give compound **20**. Similarly C2-chloro of chloroketone **16**[30] was nucleophilically displaced by 2,2,2-trifluoroethanol to give ketone **21** (65%, Scheme 5). Compound **21** was treated with Grignard reagent (MeMgI) to obtain desired hydroxy compound **22**. Another target compound **23** (87%) was synthesized by reacting chloroketone **16** with the nucleophile 4-aminopyridine. To engineer the bioactivity we have functionalized C2 position with CF_3 group (as in compounds **27** and **29**): we started synthesis from 2-amino-5-bromobenzophenone **24** (Scheme 6).[38] Quinoline ring was constructed by treating compound **24** with ethyl 4,4,4-trifluoro-3-oxobutanoate in PPA at 150 °C to get C_2–CF_3 indeno[2,1-*c*]quinoline-7-ketone derivative **25**. Compound **25** was treated with Grignard reagent MeMgI to give hydroxyl derivative **26**. This was further treated with *epi*-chlorohydrin to get desired oxirane **27**. Compound **25** was converted to its corresponding oxime **28** (94%) by heating it with hydroxylamine hydrochloride in presence of NaOH. Oxime **28** was coupled with Boc-nipecotic acid to get the desired compound **29**.

The growth inhibition assays were performed against protozoans[39–41] including epimastigote form of *T. cruzi* (Tulahuen 2 strain), *T. brucei* subsp. brucei 427, *T. brucei* subsp. rhodesiense STIB900 and *L. infantum* (MHOM/MA (BE)/67) on new compounds **6**, **9**, **11**, **12**, **14**, **15**, **20**, **22**, **23**, **27**, **29** and the available library of quinoline derivatives (**30**,[28] **31**,[28] **32**–**42**,[29] **43**–**46**,[30] **47**,[32] **48**,[32] **49**,[30] **50**,[30] and **51**–**57**[32] (Tables 1 and 2). Results obtained are displayed in Tables 1 and 2 using benznidazole as the reference drug including toxicity values against Vero cells (Table 3).

The in vitro screening of the synthesized compounds enabled us to identify five compounds (**14**, **23**, **32**, **36** and **37**) with excellent activity in whole cell parasite culture (IC_{50} = 0.25 μM, details of the screening protocol can be found in SI). Despite a limited SAR that can be drawn from these molecules, it displays a clear potential of quinoline and indenoquinoline based compounds with various substituents for further exploration against parasites. Compound **30** (R^1 = OCH_3; R^3 = imidazole) has shown IC_{50} = 0.46 μM (Table 1) and SI 67 (Table 3) against *T. cruzi*, whereas compound **31** (R^3 = 4-nitro-imidazole) and **6** (R^3 = OH) resulted in relatively inferior activity with IC_{50} = 6.96 and 8.07 μM, respectively against *T. cruzi*. In compounds (**9** and **32**–**42**), position 6 of quinoline (Fig. 2) is substituted with nitro, urea, thiourea, thioamide and phenyl tetrazole whereas position 2 has fluorophenyl substitution. These chemical variations provided three active compounds (**32**, **36** and **37**) that have shown IC_{50} = 0.25 μM.

Compound **32** (R^1 = NO_2 and R^6 = imidazole) has been found to be most active against both *Trypanosoma* spp. (IC_{50} = 0.25–2.18 μM) and *L. infantum* (IC_{50} = 2.52 μM) with excellent SI >125 (CC_{50} = 31.44 μM). When imidazole group in compound **32** was substituted by piperidine (**33**), or by *p*-methoxyphenyl pyrazole (**34**), or by *m*-methoxyphenyl pyrazole (**35**), led to less active compounds than that of compound **32** (Table 1). Compound **32** having imidazole makes it more selective towards *T. cruzi* (SI = 126) and it was found to be 8, 256 and 24 times more potent than **33**–**35**, respectively against *T. cruzi*, thus, the imidazole moiety seems to play an important role in determining the antiparasitic activity. It is likely that the imidazole ring in compounds **30** and **32** enhance the cell-membrane permeability,[42,43] while the OH may form polar interactions with the target site.

Table 3
Selectivity index (SI, ratio of CC_{50} and IC_{50}) of compounds

#	*T.c.*	*T.b.*	*T.r.*	*L.inft.*
6	0.19	0.20	0.25	0.19
9	4.36	32.0	120.75	9.38
11	0.77	2.51	2.83	0.59
12	3.0	8.60	10.70	2.16
14	2.89	5.44	5.44	3.4
15	2.12	0.64	2.82	0.56
20	0.96	30.26	71.44	2.04
22	2.86	2.41	6.14	2.48
23	3	1.34	3	0.15
27	0.48	0.47	0.73	0.14
29	0.47	0.77	1.91	0.72
30	67.02	0.943	3.65	1.21
31	9.19	29.62	145.45	1.25
32	125.76	14.42	17.37	12.47
33	2.20	4.31	8.8	1.29
34	33.16	31.37	31.52	3.15
35	4.05	4.03	6.64	3.75
36	2.15	9.55	24.84	1.96
37	2.16	3.66	5.48	0.99
38	3.7	8.94	30.18	3.03
39	7.28	20.25	32.31	5.22
40	10.64	26.66	30.77	8.0
41	10.27	31.37	30.91	7.89
42	1	30.18	90.14	3.15
43	3.70	3.94	6.83	1.33
44	4.50	10.43	19.29	4.74
45	8.62	7.77	12.12	1
46	8.23	0.61	1.93	0.9
47	34.65	1.11	4.53	1.52
48	14.91	2.68	3.74	3.84
49	0.63	0.56	0.72	0.21
50	9.13	7.34	24.52	6.73
51	4.33	13.19	16.30	3.86
52	9.38	10.58	53.33	12.60
53	4.52	16.48	26.17	6.29
54	8.66	26.66	44.44	6.73
55	1.80	1.85	8.94	4.28
56	3.45	1	1.60	1.37
57	1.08	1.09	1.85	0.63

Interestingly, among the urea derivatives (**36**–**42**) compound **36** (R^1 = *m*-*nitro*-phenyl urea) showed significant activity against *Trypanosoma* spp. (IC_{50} = 0.25–1.0 μM) and *L. infantum* (IC_{50} = 1.70 μM). The decrease in *T. cruzi* activity for compounds **37**–**41** was ascribed to the presence of the amide moiety (urea or thiourea) with electron-donating methoxy group on the aryl ring, while compounds **36**–**42** showed good activity in the range of IC_{50} = 0.25–2.09 μM against *T. brucei* and *T. rhodesiense* (Table 1). Thiourea derivatives **39**–**41** are structural isomers having OCH_3 group at *ortho*-, *meta*- and *para*-positions, respectively. Their activities against *T. cruzi* show that *ortho*-OCH_3 (as in **39**) and *p*-OCH_3 (as in **41**) substitutions are preferred 2–3 times over its *m*-OCH_3 analog (as in **40**).

In addition to quinoline compounds we have also synthesized and screened indenoquinoline derivatives. In these compounds, a new ring D (Fig. 2) was constructed by covalently locking the C4 center of the quinoline moiety with the C2′ center of the phenyl ring (Fig. 2) with the aim to reduce the conformational flexibility across C2′–C4. This chemical construction would decrease the entropic penalty (as shown in general structure **II** from more flexible general structure **I**; Fig. 2) for the complex within the target protein, which may in turn give improved free energy of stabilization to the complex. Interestingly, compounds following this strategy have been found to be very active against *Mycobacterium tuberculosis*[31,32] and protozoal diseases.[44]

These chemical changes produced thirteen active compounds (IC_{50} <2 μM) (**11**, **12**, **14**, **15**, **23**, **43**, **44**, **46**, **47** and **52**–**55**). Compound **14** showed excellent activity in range of IC_{50} = 0.25–0.47 μM against *Trypanosoma* spp. and *Leishmania* (SI 3–5, Table 3). When the free amine in **14** was replaced by imidazole (**43**), benzhydryl piperazine (**12**), benzyl piperazine (**11**), *m*-trifluoromethylphenyl pyrazole (**44**), *o*-methoxyphenyl urea (**15**) or amide (**45**) antiparasitic activity decreased. This analysis suggests that hydrophobic nature (higher log*P*, Tables 1 and 2) or potential steric contribution led to less active compounds.

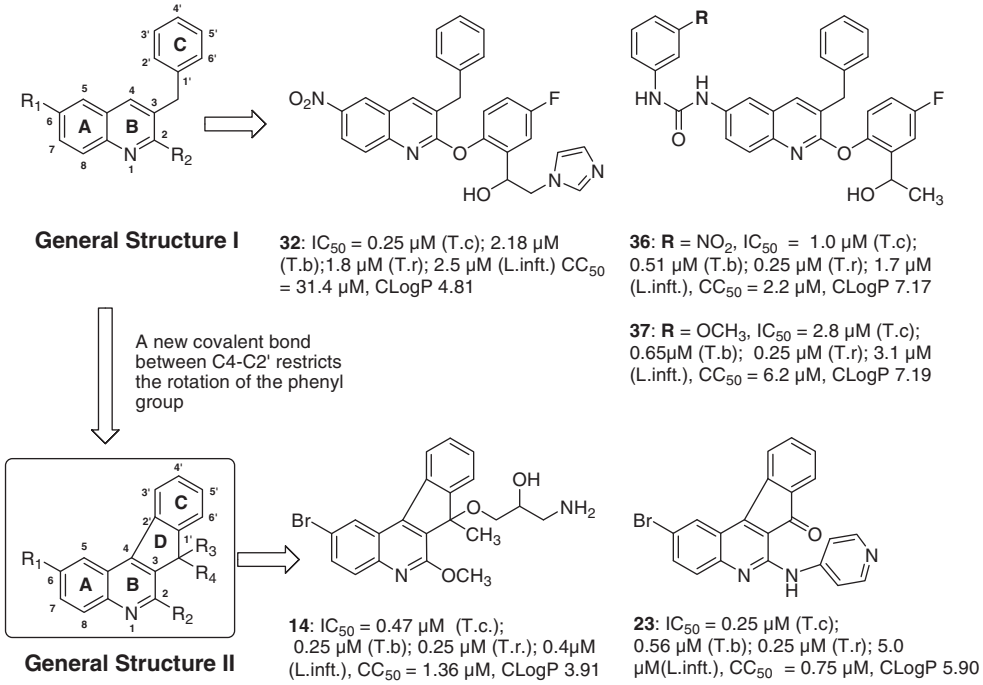


Figure 2. Most active five compounds (**14**, **23**, **32**, **36** and **37**) are shown with substituents R^1–R^5. See Tables 1 and 2 for structures of all compounds along with their antiprotozoal activity (IC_{50}) and cytotoxicity (CC_{50}).

Apart from this, replacement of the OCH_3 group at R^2 with 1*H*-imidazole (**46**–**48**) and alkyl esters (**47** and **48**) at R^3, produced active compounds against *T. cruzi* (IC_{50} = 0.93–1.46 μM, SI 8–35, Table 3). Based on this small library of compounds it can be suggested that heterocyclic aromatic amines (imidazole) as substituents are more selective to *T. cruzi* (**46**–**48**) whereas, piperazine substituents (**20**, **11** and **12**) make molecule active against *T. brucei* (Table 2).

Compounds **30**, **32** and **46**–**48** having an imidazole moiety showed very similar antiprotozoal activity, suggesting a critical role of the imidazole ring, because when imidazole in **30**, **32** and **46**–**48** was replaced by pyrazole derivatives **34**, **35** and **44** resulted in a drastic reduction in biological activity of the latter. For further exploration, R^2 was substituted with 2-pyridyl piperazine and other heterocyclic moieties and R^3 was replaced with various amino acids (**51**–**55**) and ester (**56**) of oxime (**50**). In these efforts, compound (**23**) having 4-aminopyridine was found to have excellent activity against *Trypanosoma* spp. (IC_{50} = 0.25 μM), whereas compounds substituted with 2-pyridinyl-piperazine (**49**–**55**), imidazole (**56** and **57**) and CF_3 (**29**) found to be less active.

However, these preliminary results based on limited number of compounds allow us to conclude that besides the common core of conformationally-constrained system, the imidazole ring may have a crucial role in modulating the binding to the target protein as displayed by their excellent inhibition concentration (IC_{50}).

In summary, quinoline derivatives (**32**, **36** and **37)** and conformationally-constrained indeno[2,1-*c*]quinoline derivatives (**14** and **23)** with various substituents presented here have shown excellent activity (IC_{50} = 0.25 and 0.40 μM) against *T. cruzi*, *T. brucei* and *L. infantum* in the whole cell parasite culture with low cytotoxicity in murine macrophage host cells and in diploid human fibroblasts MRC-5 cell line. These five compounds had better activity than the front-line drugs such as benznidazole, nifurtimox and comparable in activity to amphotericin B. Thus these active analogues may act as promising 'lead' compounds for further structure–activity studies. However, we know from the available compounds that quinoline in combination of appropriate side chain produced active compounds. Side chains substituted with electron withdrawing groups and small cyclic or acyclic amines were found to be more active compared to electron rich substituents. In this regard, beside the replacement of various polar groups in the molecular structures of these compounds, we foresee that further SAR and target enzyme studies are needed with more focused library following the trail of 'lead' molecules, which is in progress.

The Implication of this work is that the compounds with antiprotozoal activity of IC_{50} <5 μM, should be utilized for further lead optimization to come up with the best potential candidate. There are compounds which have been identified specific to *Trypanosoma* spp. and some are even active against all tested species of *Trypanosoma* spp. and *L. infantum*, which poses a challenge for further medicinal chemistry in order to explore that how the aspecific character of these compounds can be directed to one organism. The compounds of present study further suggest that a small number of molecules with extensive diversification are not appropriate to optimize the drug-like candidates but it provides a fast track procedure to identify the best possible type of compounds, where the more concentrated efforts can be made to develop the single-point diversified library to reach the drug like compound.

Many of the compounds reported here have shown impressive activity against sensitive strain H37Rv of *Mycobacterium tuberculosis*.[28–32] Therefore, these compounds may enable to hit multiple targets and that might provide new avenues for the treatment of protozoan diseases. This may impact in many ways: (i) such multiple action single-drug therapeutic against different protozoans may improve health of those infected with multiple parasites at a low cost, (ii) the risk of possible drug–drug interactions would be avoided in such multiple action single-drug therapeutic, (iii) in addition, drug potency and efficacy might be increased and thereby potential reduction of drug-resistance.

Acknowledgements

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Supplementary data

Supplementary data (electronic supplementary information available: Spectral data contains (^1H, ^{13}C NMR, COSY, HMQC and HMBC) for all new compounds including experimental and NMR assignments showing the purity and structural integrity. Biological assays are also given in SI) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.054.

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