Hindawi Publishing Corporation
Journal of Parasitology Research
Volume 2013, Article ID 716976, 8 pages
http://dx.doi.org/10.1155/2013/716976

Research Article

Direct Effect of Two Naphthalene-Sulfonyl-Indole Compounds on *Toxoplasma gondii* Tachyzoite

Qasem Asgari,[1,2] Hossein Keshavarz,[1] Mostafa Rezaeian,[1] Mohammad Hossein Motazedian,[2] Saeedeh Shojaee,[1] Mehdi Mohebali,[1] and Ramin Miri[3,4]

[1] *Department of Parasitology and Mycology, Faculty of Health, Tehran University of Medical Sciences, Tehran 14177613191, Iran*
[2] *Department of Parasitology and Mycology, Shiraz University of Medical Sciences, Shiraz, Iran*
[3] *Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[4] *Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

Correspondence should be addressed to Hossein Keshavarz; hkeshavarz@tums.ac.ir

Received 19 May 2013; Revised 22 July 2013; Accepted 1 August 2013

Academic Editor: Fabio Ribeiro Braga

Past studies have stated that the parasitostatic effect of IFN-γ is most likely due to the starvation of *Toxoplasma gondii* for tryptophan in the host cell. The aim of this study was to evaluate the direct effect of two new Naphthalene-Sulfonyl-Indole compounds as competitive molecules for tryptophan on viability and infectivity of *Toxoplasma* tachyzoites. Tachyzoites of RH strain were incubated in various concentrations (25–800 μM) of 1-(naphthalene-2-sulfonyl)-2,3-dihydro-1H-indole and 1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole for 1.5 hours. Then, they were stained by PI and analyzed by FACS. To evaluate the infectivity, 2×10^6 tachyzoites exposed to the concentrations mentioned above were intraperitoneally inoculated into five mice from each group. Also, naïve parasites and parasites exposed to DMSO (control) were inoculated in both groups of mice. The LD_{50} of 1-(naphthalene-2-sulfonyl)-2,3-dihydro-1H-indole was 62 μmol whilst the quantity of 1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole was more than 800 μmol. The infectivity of tachyzoites exposed to both of the compounds preserved and killed mice. No statistical correlation was seen between longevity of mice groups and different doses of the compounds. If we consider a well-organized transporter mechanism for indole compounds in the parasite, thus the designation of an antagonist that has indole groups can assist with the production of new drugs.

1. Introduction

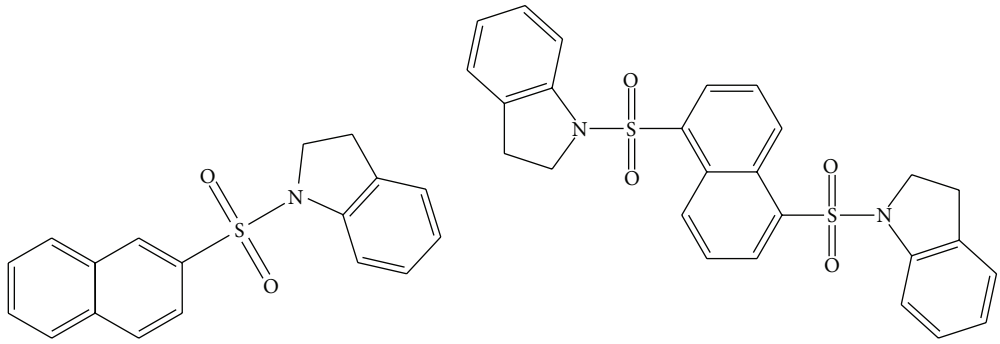
Toxoplasmosis is an infectious disease caused by the intracellular protozoon parasite *Toxoplasma gondii*. This parasite can infect nucleated cells of birds and mammals, including humans. In humans, it causes severe medical complications in the fetus or in immunocompromised individuals [1].

Combination therapy with pyrimethamine and sulfadiazine is the standard treatment for toxoplasmosis [2]. Sulphadiazine is a dihydropteroate synthase (DHPS) inhibitor, while pyrimethamine inhibits dihydrofolate reductase (DHFR). Both enzymes are fundamental for the biosynthesis of pyrimidines in *T. gondii* [3].

Long-term treatment with these drugs can result in megaloblastic anemia or myelosuppression; consequently, folate supplementation should be used. Folate deficiency or an increased folate requirement might trigger the onset of myelotoxicity due to the use of these drugs [4]. Also, neutropenia and the potential teratogenic effects of pyrimethamine during the first trimester of pregnancy have been proven [5].

Current toxoplasmosis treatment for pregnant women is based on the administration of spiramycin in order to decrease the risk of fetal transmission. Grujić et al. have shown that even when greatly reducing residual infection, no spiramycin regimen can completely eradicate the parasite [6]. The common side effects of this drug include skin rash, itching, abnormal bruising, and uncommon gastrointestinal bleeding [7, 8].

Atovaquone, a structural analog of protozoan ubiquinone, has been shown to have significant activity against the bradyzoite stage of *Toxoplasma* in both *in vitro* and *in vivo*



(a) (b)

Figure 1: Formula of Naphthalene-Sulfonyl-Indole compounds: (a) [1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole] and (b) 1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole.

assays [9–12]. Conversely, Pearson et al. have shown that patients who administered atovaquone favorably responded to treatment, and their visual acuity stabilized or improved [13]. Unfortunately, there is strong evidence that the targeted parasites are able to spontaneously develop drug resistance by mutation of amino acid residues located in or near the atovaquone-binding site on cytochrome *b* [14].

Consequently, it is imperative to conduct a study on new and efficient drugs that have minimal side effects.

In the parasite, essential nutrients, which must be acquired from its host cells, can be considered as potential drug targets. One of the known essential amino acids is tryptophan used for intracellular proliferation of the parasite [15]. The parasitostatic effect of IFN-γ most likely results from the starvation of *T. gondii* for tryptophan [16]. Tryptophan is transferred across the cell membrane through facilitated diffusion with the aid of adenosine triphosphate (ATP) and specific proteins.

Tryptophan is present in most proteins and has an indole functional group as a distinguishing structural characteristic. Asai et al. have shown that the indole compounds as inhibitors of nucleoside triphosphate hydrolase can prevent tachyzoite replication *in vitro* [17].

We undertook the present study to assess the direct effect of two Naphthalene-Sulfonyl-Indole compounds on the viability and infectivity of *Toxoplasma* tachyzoites.

2. Materials and Methods

2.1. Compounds. Compound A (Naphthalene-Sulfonyl-Indole). [1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole], Compound Number; MolPort-000-556-394, Molecular Formula; $C_{18}H_{15}NO_2S$, Molecular Weight: 309.4 (Figure 1).

Compound B (Naphthalene-Sulfonyl-diIndole). 1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole, Compound Number; MolPort-001-637-694, Molecular Formula; $C_{26}H_{22}N_2O_4S_2$, Molecular Weight: 490.6 (Figure 1).

2.2. Animals. A total of 100 inbred BALB/c mice were provided from Pasteur Institute, Tehran, Iran, at 6–8 weeks aged and 22–25 gram weight. Animals were kept in the Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran. The procedures of all trials and sacrifices were identical for all animals. During the experiments, from April to May 2012, the animals were housed in cages and maintained under controlled environmental conditions (21 ± 2°C, 65–70% Room Humidity and a balanced diet with an access to food and water ad libitum). The experiments were undertaken based on the guidelines of the laboratory animals in the research and teaching book [18].

2.3. Parasites. The virulent RH strain of *T. gondii* was obtained from Tehran University of Medical Sciences, Tehran, Iran. Tachyzoites of the RH strain of *T. gondii* were maintained by serial intraperitoneal passaging in BALB/c inbred mice. After 72 hours, 10^6 parasites inoculation in the mice, the tachyzoites were collected after repeated flushing of the peritoneal cavity by phosphate buffered Saline (PBS) at a pH of 7.2. Then, tachyzoites were harvested and centrifuged for 5 min at 200 g at room temperature to remove peritoneal cells and cellular debris. The supernatant was collected and centrifuged for 10 min at 1200 rpm (800 g) [19]. The pellet, enriched with parasite tachyzoites, was recovered with PBS and used in the experiments.

2.4. Extracellular Viability Assay. We dissolved the compounds in DMSO to obtain a final concentration of 10 mM. The final concentration of DMSO should not exceed 1%. Various concentrations (25, 50, 100, 200, 400, 800 μM) of the compounds were then prepared by the following: 2.5–80 μL of the final concentration was added to 920–997.5 μL of solutions that contained 2×10^6 tachyzoites per mL of PBS. Tachyzoites were incubated with either DMSO (as control) or the diluted compounds for 1.5 h at 4°C. Next, the tachyzoites were collected in Eppendorf tubes and incubated for 30 min at 4°C with 50 μg/mL propidium iodide (PI, Sigma Company, USA). After incubation, the parasites were kept on ice until analysis. Positive controls for PI staining were acquired by incubating parasites in the presence of 0.2% saponin. The cell suspension was transferred into polystyrene flow cytometry tubes (BD Falcon Company, USA). We performed data

Table 1: The mean of life duration (days) of mice groups exposed to compound A: Naphthyl-Sulfonyl-Indole [1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole].

Control	DMSO	Compound A 25 μMol	Compound A 50 μMol	Compound A 100 μMol	Compound A 200 μMol	Compound A 400 μMol	Compound A 600 μMol	Compound A 800 μMol
5.6	5.4	5.6	5.6	5.4	5	5.4	5.2	6

Table 2: The mean of life duration (days) of mice groups exposed to compound B: Naphthyl-Sulfonyl-diIndole or 1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole.

Control	DMSO	Compound B 25 μMol	Compound B 50 μMol	Compound B 100 μMol	Compound B 200 μMol	Compound B 400 μMol	Compound B 600 μMol	Compound B 800 μMol
5.4	5.2	5.8	5.6	5	5	5.8	5.2	6.6

acquisition and analysis, with a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, USA) and Cell Quest Pro software. A total 1000–30000 event was acquired in the region that had been previously established as corresponding to the parasites.

2.5. Tachyzoite Infectivity in Animals. A total of 2×10^6 tachyzoites exposed to the concentrations of the compounds mentioned above were intraperitoneally inoculated into five mice from each group. For the control, naive parasites and parasites exposed to DMSO were intraperitoneally inoculated in both groups of mice. If the mice died, their liver touch smears were Giemsa stained and observed under light microscopy for parasite detection.

2.6. Data Analysis. Data were analyzed using SPSS software (version 11.5, Chicago, USA) by the Mann-Whitney nonparametric test. $P < 0.05$ was considered statistically significant.

3. Results

The result of flow cytometry tests on tachyzoite of *Toxoplasma* exposed to DMSO, saponin as positive control, and different doses of [1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole] were shown in Figure 2. The LD_{50} of compound A [1-(naphthalene-2-sulfonyl)-2,3-dihydro-1H-indole] was 62 μmol whilst the quantity of compound B [1-[5-(2,3-dihydro-1H-indole-1-sulfonyl)naphthalene-1-sulfonyl]-2,3-dihydro-1H-indole] was more than 800 μmol. The mortality rate of tachyzoites exposed to the compounds was shown in Figure 3.

The infectivity of tachyzoites exposed to 25–800 μmol doses of both of the compounds was preserved and these tachyzoites were able to kill the mice. The mean longevity (days) of mice infected by intact tachyzoites and tachyzoites exposed to the compounds was in the range of 5-6/6 (Tables 1 and 2). No statistical correlation was seen between the life duration of mice groups and different doses of the compounds.

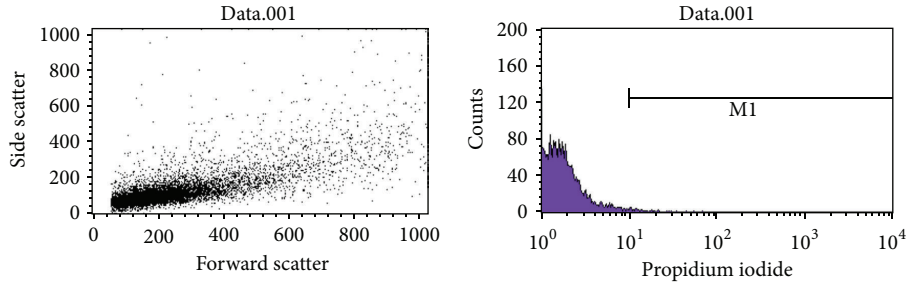
4. Discussion

The treatment of toxoplasmosis is difficult due to the toxic effects of available drugs and the fact that reinfection may occur rapidly, so the introduction of new antitoxoplasma drugs and vaccines seems essential. Only a commercial vaccine (S-48), which is an attenuated *T. gondii* tachyzoite form, has been successfully employed for animal use. Vaccination with the live parasite cannot be safely carried out in humans [20]. The general consensus is that tissue cysts are resistant to commonly used drugs in the treatment of *Toxoplasma* infection, including pyrimethamine, sulfadiazine, and atovaquone, either alone or in a combination form. The cyst is believed to protect the parasite from the host immune system and act as a barrier for antiparasitic compounds [21].

Several studies indicated that the resistance to acute infection in mice is greatly related to endogenous IFN-γ [22, 23]. The activation of host cells against *Toxoplasma* induced by IFN-γ is dependent on the tryptophan concentration [24]. Däubener et al. have revealed that induction of indole amine 2,3-dioxygenase (IDO) contributes in antiparasitic mechanisms induced in human brain's microvascular endothelial cells by IFN-γ and TNF-α, which indicates that a protective activity is mediated by IDO [25]. Däubener et al. (2002) has shown that the depletion in intracellular tryptophan levels induced by IDO is an important mechanism by which IFN-γ controls the intracellular replication of *T. gondii* tachyzoites in various types of human cells [25]. IFN-γ-mediated induction of IDO appears to be critical for resistance of brain against *T. gondii* in human being [26].

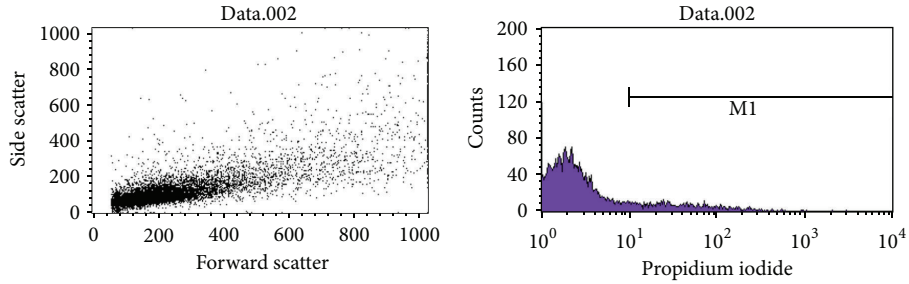
The local tryptophan-depletion in microenvironments are assumed to be caused by macrophages, which have a unique high-affinity tryptophan importing system. The existence of highly specific and efficient transport machinery for tryptophan in macrophages has previously been confirmed. Macrophages can import and degrade tryptophan even at very low exogenous concentrations [27]. Tryptophan is an essential amino acid for the parasite's survival and proliferation in the host cell [15]. This amino acid has an indole functional group. Since *T. gondii* is an auxotroph for tryptophan and acquires the needed nutrients from its host cells. So, indole compounds can probably be introduced as drug antagonists.

Camalexin (3-thiazol-2′-yl-indole) was first isolated from the leaves of *Camelina sativa* in response to an infection by *Alternaria brassicae* [28]. Tsuji et al. [29] have reported the synthesis of camalexin by *Arabidopsis thaliana*, which is accumulated in high levels after infection with an avirulent



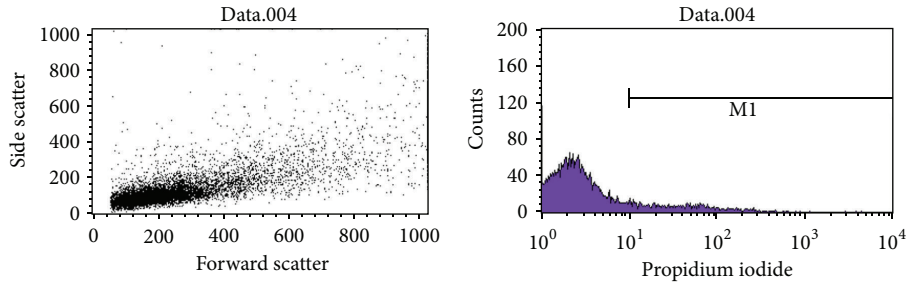
Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	1.79	1.53	101.05	1.35	1
M1	10, 9910	73	0.73	0.73	16.16	14.61	60.44	12.52	10

(a) Parasite



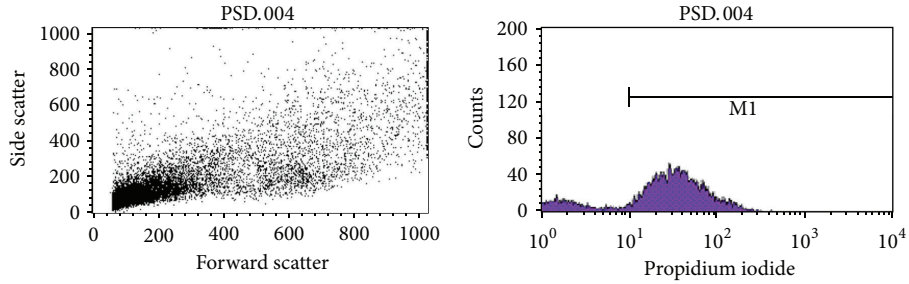
Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	10.66	3.00	481.39	2.15	1
M1	10, 9910	1387	13.87	13.87	62.11	36.45	203.17	31.62	25

(b) Dye Para



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	11.80	3.19	625.75	2.37	1
M1	10, 9910	1403	14.03	14.03	68.53	37.38	273.53	33.38	10

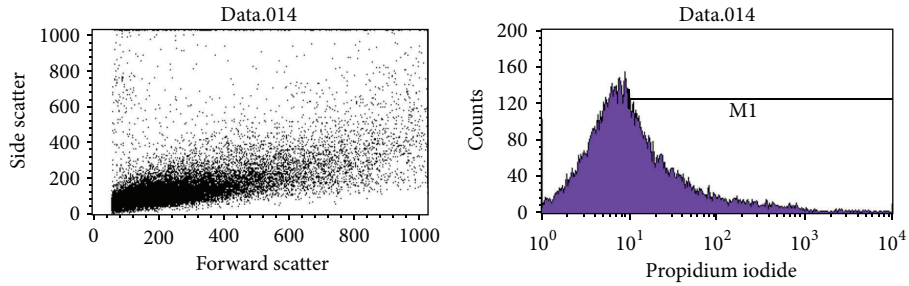
(c) DMSO



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	8816	100.00	29.39	37.10	20.62	97.29	28.39	1
M1	4, 9910	7363	83.52	24.54	44.08	34.27	80.66	33.68	28

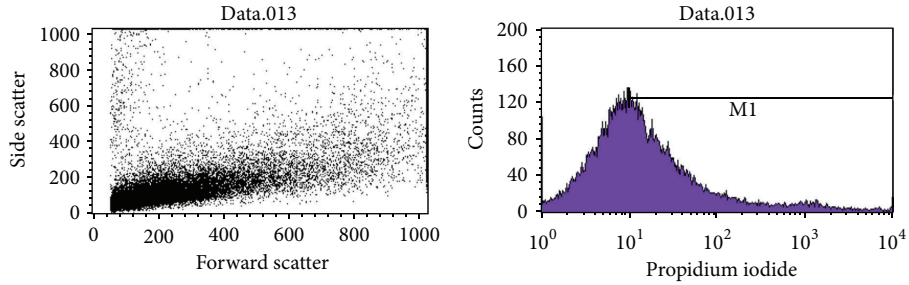
(d) Saponin

Figure 2: Continued.



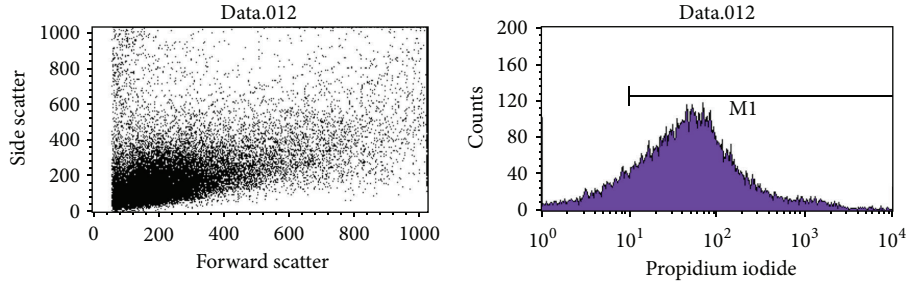
Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	48.00	10.91	724.55	8.82	1
M1	10, 9910	13600	45.33	45.33	99.43	29.92	514.81	20.91	10

(e) Com A. 25 μM



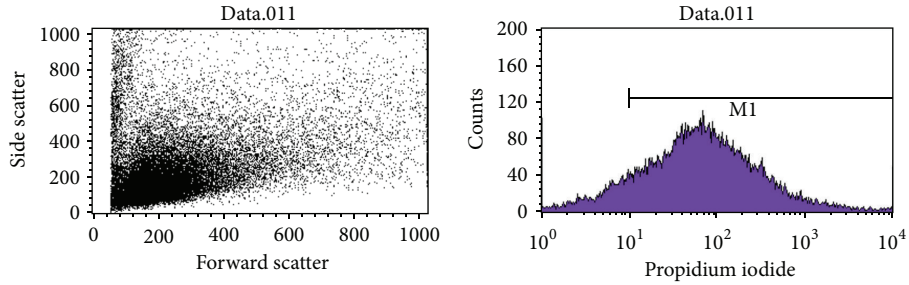
Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	94.58	14.21	572.80	11.14	1
M1	10, 9910	16656	55.52	55.52	166.03	34.22	433.17	22.67	10

(f) Com A. 50 μM



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	152.88	14.69	389.78	44.51	1
M1	10, 9910	25619	85.40	85.40	178.21	62.90	359.94	54.74	67

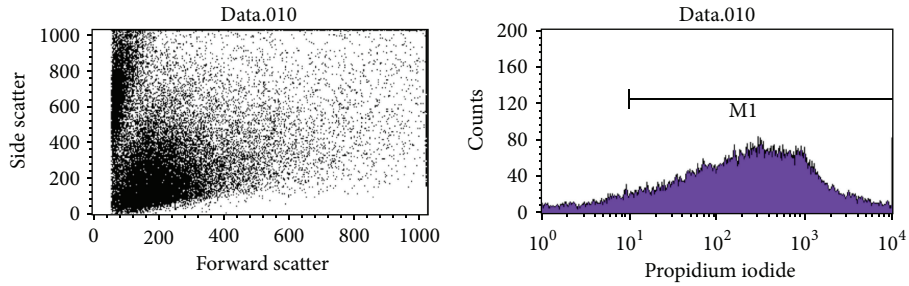
(g) Com A. 100 μM



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	212.45	60.47	356.80	62.08	1
M1	10, 9910	26684	88.95	88.95	238.19	83.57	335.88	73.65	68

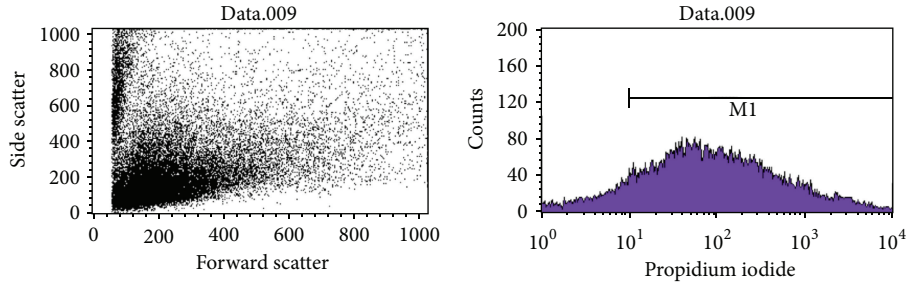
(h) Com A. 200 μM

Figure 2: Continued.



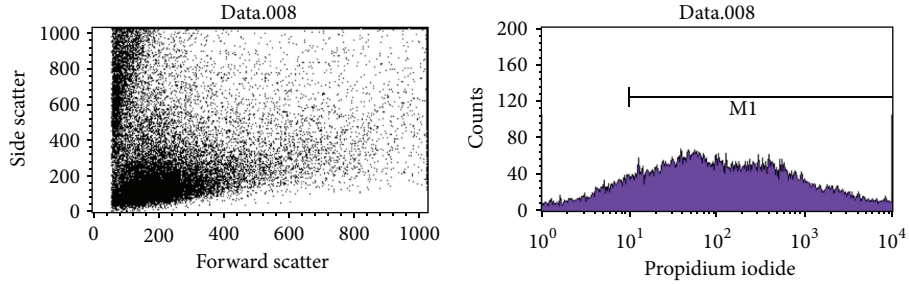
Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	642.33	153.49	204.08	210.97	1
M1	10, 9910	26852	89.51	89.51	717.21	248.79	190.48	266.55	9910

(i) Com A. 400 μM



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	350.88	72.44	264.80	71.69	1
M1	10, 9910	26121	87.07	87.07	402.34	115.05	244.91	96.47	39

(j) Com A. 600 μM



Marker	Left, right	Events	Gated (%)	Total (%)	Mean	Geo mean	CV	Median	Peak Ch
All	1, 9910	30000	100.00	100.00	686.60	99.55	252.93	91.40	1
M1	10, 9910	25955	86.52	86.52	792.88	167.21	232.63	138.24	9910

(k) Com A. 800 μM

Figure 2: The result of flow cytometry tests on tachyzoite of *Toxoplasma* exposed to DMSO, saponin as positive control and different doses of compound A.

strain of *Pseudomonas syringae*. Perhaps the indole ring of camalexin is derived from indole-3-glycerol phosphate group [28]. This is an intermediate molecule in biosynthesis of tryptophan [30, 31]. Another indole compound derived from tryptophan which is brassinin is provided from plants. The results of an investigation by Sellam et al. confirmed the antifungal effects of camalexin and brassinin at different developmental stages of both *Alternaria* species [32].

In this study, tachyzoites were exposed to the molecules while the parasites were intracellular and endured in the parasitophorous vacuoles (PV) of the host cells. The PV membrane is a permeable structure with a size prohibiting limit of ~1,300 Da [33]. Since *Toxoplasma* is auxotrophic for tryptophan and purine [15, 34], these pores may be used in receipt of the molecules using by host cytosolic ATP. An NTP hydrolase (NTPase) has been identified essential in the PV for tachyzoite replication within the host cells and may be partly responsible for this salvage process [35, 36]. It was shown that NTPases as new targets are still choices for chemotherapeutic measures against the disease. It seems that the enzyme is unique to the parasite and its activity appears to be imperative for the parasite's proliferation.

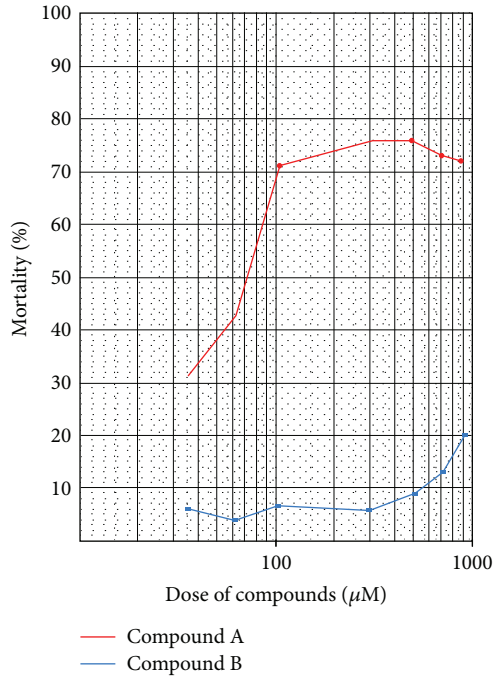


Figure 3: The mortality of tachyzoite of *Toxoplasma* exposed to compounds A and B.

Modification in indole and phenol has shown that these compounds have modest IC_{50}'s in the low micromolar ranges to inhibit *T. gondii* NTPases and prevent proliferation of tachyzoites [17]. Our study showed that the 1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole was effective on the viability of tachyzoites. These experiments were undertaken on exposed tachyzoites, not intracellular ones. It seems that this compound affects tachyzoites based on mechanisms previously described [17].

It was demonstrated that indole naphthyridinones as inhibitors of bacterial enoyl-ACP reductases is a key enzyme in type II fatty acid biosynthesis (FAS-II) pathway and is a valid antimicrobial choice too [37]. The fatty acid synthesis of apicoplast in *T. gondii* is essential for organelle biogenesis and the parasite survival. Apicoplast prokaryotic fatty acid synthesis is a type II one and has recently received particular attention. The FAS II pathway is a metabolic process fundamentally different from the analogous FAS I pathway in humans that was recommended as a therapeutic measure [38, 39]. The effective levels of 1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole on the viability of tachyzoites were less than other molecules. Our findings showed that the toxicity of compound A may not be related to the indole group. Alternatively, the membrane permeability of compound B may be low. In our study, the viability of tachyzoites exposed to different concentrations of 1-(naphthalene-2-sulfonyl)-2,3-dihydro-H-indole was different based on flow cytometry results and the survival of mice.

5. Conclusion

The presence of a well-organized transport system for indole compound within the parasite in conjunction with several effective mechanisms for the compound on *Toxoplasma* viability provide the chance for introduction of an antagonist material containing an indole group as a new drug.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

The authors would like to thank the Office of Vice-Chancellor for Research of the Tehran University of Medical Sciences, Tehran, Iran for financial support of this project. We express our appreciation to the staff of the Animal Laboratory Center, the Medicinal and Natural Products Chemistry Research Center, and the Central Laboratory of Shiraz University of Medical Sciences, Shiraz, Iran for the development of the experimental studies.

References

[1] J. P. Dubey, "History of the discovery of the life cycle of *Toxoplasma gondii*," *International Journal for Parasitology*, vol. 39, no. 8, pp. 877–882, 2009.

[2] J. G. Montoya and O. Liesenfeld, "Toxoplasmosis," *The Lancet*, vol. 363, no. 9425, pp. 1965–1976, 2004.

[3] A. C. Anderson, "Targeting DHFR in parasitic protozoa," *Drug Discovery Today*, vol. 10, no. 2, pp. 121–128, 2005.

[4] T. Mori, J. Kato, and S. Okamoto, "Pancytopenia due to pyrimethamine triggered by transplant-associated microangiopathy after allogeneic bone marrow transplantation," *Journal of Infection and Chemotherapy*, vol. 17, no. 6, pp. 866–867, 2011.

[5] B. Lipka, B. Milewska-Bobula, and M. Filipek, "Monitoring of plasma concentration of pyrimethamine (PYR) in infants with congenital *Toxoplasma gondii* infection–own observations," *Wiadomości parazytologiczne*, vol. 57, no. 2, pp. 87–92, 2011.

[6] J. Grujić, O. Djurković-Djaković, A. Nikolić, I. Klun, and B. Bobić, "Effectiveness of spiramycin in murine models of acute and chronic toxoplasmosis," *International Journal of Antimicrobial*, vol. 25, pp. 226–230, 2005.

[7] L. S. Ostlere, J. A. A. Langtry, and R. C. D. Staughton, "Allergy to spiramycin during prophylactic treatment of fetal toxoplasmosis," *British Medical Journal*, vol. 302, no. 6782, p. 970, 1991.

[8] E. Rubinstein and N. Keller, "Spiramycin renaissance," *Journal of Antimicrobial Chemotherapy*, vol. 42, no. 5, pp. 572–576, 1998.

[9] F. G. Araujo, J. Huskinson, and J. S. Remington, "Remarkable in vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of *Toxoplasma gondii*," *Antimicrobial Agents and Chemotherapy*, vol. 35, no. 2, pp. 293–299, 1991.

[10] F. G. Araujo, J. Huskinson-Mark, W. E. Gutteridge, and J. S. Remington, "In vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against the cyst form of *Toxoplasma gondii*," *Antimicrobial Agents and Chemotherapy*, vol. 36, no. 2, pp. 326–330, 1992.

[11] D. J. P. Ferguson, J. Huskinson-Mark, F. G. Araujo, and J. S. Remington, "An ultrastructural study of the effect of treatment with atovaquone in brains of mice chronically infected with the ME49 strain of *Toxoplasma gondii*," *International Journal of Experimental Pathology*, vol. 75, no. 2, pp. 111–116, 1994.

[12] J. Huskinson-Mark, F. G. Araujo, and J. S. Remington, "Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*," *Journal of Infectious Diseases*, vol. 164, no. 1, pp. 170–177, 1991.

[13] P. A. Pearson, A. R. Piracha, H. A. Sen, and G. J. Jaffe, "Atovaquone for the treatment of *Toxoplasma* retinochoroiditis in immunocompetent patients," *Ophthalmology*, vol. 106, no. 1, pp. 148–153, 1999.

[14] D. C. McFadden, S. Tomavo, E. A. Berry, and J. C. Boothroyd, "Characterization of cytochrome b from *Toxoplasma gondii* and Q(o) domain mutations as a mechanism of atovaquone-resistance," *Molecular and Biochemical Parasitology*, vol. 108, no. 1, pp. 1–12, 2000.

[15] W. Dai, H. Pan, O. Kwok, and J. P. Dubey, "Human indoleamine 2,3-dioxygenase inhibits *Toxoplasma gondii* growth in fibroblast cells," *Journal of Interferon Research*, vol. 14, no. 6, pp. 313–317, 1994.

[16] E. R. Pfefferkron, M. Eckel, and S. Rebhun, "Interferon-γ suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan," *Molecular and Biochemical Parasitology*, vol. 20, no. 3, pp. 215–224, 1986.

[17] T. Asai, T. Takeuchi, J. Diffenderfer, and L. D. Sibley, "Identification of small-molecule inhibitors of nucleoside triphosphate hydrolase in *Toxoplasma gondii*," *Antimicrobial Agents and Chemotherapy*, vol. 46, no. 8, pp. 2393–2399, 2002.

[18] C. K. Akins, S. Panicker, and C. L. Cunningham, *Laboratory Animals in Research and Teaching: Ethics, Care, and Methods*, APA, Washington, DC, USA, 2004.

[19] M. D. F. Ferreira-da-Silva, R. M. Rodrigues, E. F. de Andrade et al., "Spontaneous stage differentiation of mouse-virulent *Toxoplasma gondii* RH parasites in skeletal muscle cells: an ultrastructural evaluation," *Memorias do Instituto Oswaldo Cruz*, vol. 104, no. 2, pp. 196–200, 2009.

[20] E. Jongert, C. W. Roberts, N. Gargano, E. Förster-Wald, and E. Petersen, "Vaccines against *Toxoplasma gondii*: challenges and opportunities," *Memorias do Instituto Oswaldo Cruz*, vol. 104, no. 2, pp. 252–266, 2009.

[21] M. di Cristina, D. Marocco, R. Galizi, C. Proietti, R. Spaccapelo, and A. Crisanti, "Temporal and spatial distribution of *Toxoplasma gondii* differentiation into bradyzoites and tissue cyst formation in vivo," *Infection and Immunity*, vol. 76, no. 8, pp. 3491–3501, 2008.

[22] N. M. Silva, C. V. Rodrigues, M. M. Santoro, L. F. L. Reis, J. I. Alvarez-Leite, and R. T. Gazzinelli, "Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1," *Infection and Immunity*, vol. 70, no. 2, pp. 859–868, 2002.

[23] E. Y. Denkers, R. T. Gazzinelli, D. Martin, and A. Sher, "Emergence of NK1.1+ cells as effectors of IFN-γ dependent immunity to *Toxoplasma gondii* in MHC class I-deficient mice," *Journal of Experimental Medicine*, vol. 178, no. 5, pp. 1465–1472, 1993.

[24] E. R. Pfefferkorn, "Interferon γ blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 3, pp. 908–912, 1984.

[25] W. Däubener, B. Spors, C. Hucke et al., "Restriction of *Toxoplasma gondii* growth in human brain microvascular endothelial cells by activation of indoleamine 2,3-dioxygenase," *Infection and Immunity*, vol. 69, no. 10, pp. 6527–6531, 2001.

[26] Y. Suzuki, "Immunopathogenesis of cerebral toxoplasmosis," *Journal of Infectious Diseases*, vol. 186, supplement 2, pp. S234–S240, 2002.

[27] R. L. Seymour, V. Ganapathy, A. L. Mellor, and D. H. Munn, "A high-affinity, tryptophan-selective amino acid transport system in human macrophages," *Journal of Leukocyte Biology*, vol. 80, no. 6, pp. 1320–1327, 2006.

[28] L. M. Browne, K. L. Conn, W. A. Ayer, and J. P. Tewari, "The camalexins: new phytoalexins produced in the leaves of Camelina sativa (cruciferae)," *Tetrahedron*, vol. 47, no. 24, pp. 3909–3914, 1991.

[29] J. Tsuji, E. P. Jackson, D. A. Gage, R. Hammerschmidt, and S. C. Somerville, "Phytoalexin accumulation in arabidopsis thaliana during the hypersensitive reaction to Pseudomonas syringae pv syringae," *Plant Physiology*, vol. 98, no. 4, pp. 1304–1309, 1992.

[30] B. P. H. J. Thomma, I. Nelissen, K. Eggermont, and W. F. Broekaert, "Deficiency in phytoalexin production causes enhanced susceptibility of Arabidopsis thaliana to the fungus Alternaria brassicicola," *Plant Journal*, vol. 19, no. 2, pp. 163–171, 1999.

[31] E. Glawischnig, "Camalexin," *Phytochemistry*, vol. 68, no. 4, pp. 401–406, 2007.

[32] A. Sellam, B. Iacomi-Vasilescu, P. Hudhomme, and P. Simoneau, "In vitro antifungal activity of brassinin, camalexin and two isothiocyanates against the crucifer pathogens Alternaria brassicicola and Alternaria brassicae," *Plant Pathology*, vol. 56, no. 2, pp. 296–301, 2007.

[33] J. C. Schwab, C. J. M. Beckers, and K. A. Joiner, "The parasitophorous vacuole membrane surrounding intracellular *Toxoplasma gondii* functions as a molecular sieve," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 2, pp. 509–513, 1994.

[34] J. D. Schwartzman and E. R. Pfefferkorn, "*Toxoplasma gondii*: purine synthesis and salvage in mutant host cells and parasites," *Experimental Parasitology*, vol. 53, no. 1, pp. 77–86, 1982.

[35] L. D. Sibley, I. R. Niesman, T. Asai, and T. Takeuchi, "*Toxoplasma gondii*: secretion of a potent nucleoside triphosphate hydrolase into the parasitophorous vacuole," *Experimental Parasitology*, vol. 79, no. 3, pp. 301–311, 1994.

[36] V. Nakaar, B. U. Samuel, E. O. Ngo, and K. A. Joiner, "Targeted reduction of nucleoside triphosphate hydrolase by antisense RNA inhibits *Toxoplasma gondii* proliferation," *Journal of Biological Chemistry*, vol. 274, no. 8, pp. 5083–5087, 1999.

[37] M. A. Seefeld, W. H. Miller, K. A. Newlander et al., "Indole naphthyridinones as inhibitors of bacterial enoyl-ACP reductases FabI and FabK," *Journal of Medicinal Chemistry*, vol. 46, no. 9, pp. 1627–1635, 2003.

[38] J. Mazumdar, E. H. Wilson, K. Masek, C. A. Hunter, and B. Striepen, "Apicoplast fatty acid synthesis is essential for organelle biogenesis and parasite survival in *Toxoplasma gondii*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 35, pp. 13192–13197, 2006.

[39] S. Smith, A. Witkowski, and A. K. Joshi, "Structural and functional organization of the animal fatty acid synthase," *Progress in Lipid Research*, vol. 42, no. 4, pp. 289–317, 2003.