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Author's Affiliation:
1. Department of Biology, College of Science, Qassim University, Qassim 51452 - Saudi Arabia.
2. Department of Basic Health Sciences, College of Applied Medical Sciences, Qassim University, Qassim 51452 - Saudi Arabia
3. Department of Matter Sciences, University Mohamed Khider, BP 145 RP, Biskra 07000 - Algeria.
4. Laboratory of Natural and Bio-active Substances, Faculty of Science, Tlemcen University, P.O. Box 119, Tlemcen 13000 - Algeria.
5. Department of Chemistry, College of Science, Qassim University, Qassim 51452 - Saudi Arabia.
6. Laboratoire de Caractérisations, Applications etModélisations des Matériaux, Faculté des Sciences de Tunis, Université Tunis El Manar, Tunis - Tunisia.
7. Organic Chemistry Laboratory, University Bayreuth, Universitätsstrasse 30, 95440 Bayreuth - Germany

***Corresponding Author:**
Bernhard Biersack
Email: bernhard.biersack@yahoo.com

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Antiparasitic Evaluation and In Silico Investigation of New Acyl Hydrazones from 3-Iodo-5-methoxy-4-propargyloxybenzaldehyde

Ibrahim S. Al Nasr[1], Waleed S. Koko[1], Tariq A. Khan[2], Ismail Daoud[3,4], Ridha Ben Said[5,6], Noureddine Amdouni[6], Rainer Schobert[7], Bernhard Biersack[7,*]

Abstract

Background: Potent and affordable antiparasitic drugs are required to meet current health problems caused by protozoal infectious diseases. 3-Iodo-5-methoxy-4-propargyloxyphenyl compounds are promising antiparasitic compounds.

Methods: Ten new and structurally simple acyl hydrazones were synthesized from 3-iodo-5-methoxy-4-propargyloxybenzaldehyde and analyzed. The antiparasitic activities against *Toxoplasma gondii* and *Leishmania major* cells were investigated and compared with host cell toxicities. ADMET (absorption, distribution, metabolism, excretion and toxicity) calculations and docking of the most promising derivatives in tubulin were performed.

Results: Considerable antiparasitic results and selectivities for parasite cells were discovered for certain acyl hydrazones. A new 3-fluorobenzoyl hydrazone was found highly active against *T. gondii* parasites yet weakly toxic to macrophages. Docking calculations suggest tubulin as a possible target. Moderate activities were observed for various compounds against *L. major* promastigotes. The relatively low toxicities observed for several compounds in macrophages indicate promising selectivity profiles. ADMET calculations supported the drug-like properties of the most active compounds.

Conclusion: A new 3-fluorobenzoyl hydrazone from 3-iodo-5-methoxy-4-propargyloxybenzaldehyde with promising activity against *Toxoplasma gondii* was identified. The strong selectivity for *Toxoplasma* parasites can lead to the development of improved antitoxoplasmal drug candidates in the future. Certain toxic effects on kidney cells might require further structural modifications of the drug candidates.

Introduction

Acyl hydrazones were developed as salient antibiotics for the therapy of perilous infections and showed antiparasitic activities [1, 2]. In terms of antiprotozoal activity, nifuroxazide and its 5-nitrothiophene analog were active against *Leishmania donovani* promastigotes [3]. Dynasore is another promising catechol-based acyl hydrazone example which interferes with cellular endocytosis and possesses antiviral activities [4, 5].

Alkyne-modified drug candidates can exert significant biological activities. Efavirenz is a prominent example of an acetylene-tagged antiviral drug, which inhibits HIV-1 reverse transcriptase and which is applied as a component of HAART, the highly active antiretroviral therapy, for HIV-1 patients [6]. Promising antiparasitic alkyne-bearing compounds are targeting toxoplasmosis (DHFR inhibitors) and leishmaniasis (acetogenins, chalcones, curcumins) [7-10]. The herbicide clodinafop-propargyl was found active against *Babesia* parasites, especially in combination with the antifungal azoles ketoconazole and clotrimazole [11]. It seems that the compound class of alkyne-substituted pesticides can be a treasure trove for the identification of antiparasitic compounds [12]. Moreover, 3-iodo-5-methoxy-4-propargyloxybenzonitrile was identified from a library screening as a tubulin-binding antileishmanial compound [13]. Recently, a 3,5-dimethoxy-4-propargyloxy-substituted naphthopyran was reported with tubulin-targeting and *c*-Myb inhibitory activities, and the propargyl moiety of this compound was successfully applied for cell localization studies using click chemistry reactions with azido-coumarins [14]. Thus, in the current study a 4-propargyloxybenzylidene moiety was chosen in order to obtain new acyl hydrazones with promising antiparasitic activities.

Protozoal parasites have emerged as a global health problem with millions of affected people. Toxoplasmosis is wide-spread and develops upon infection with apicomplexan *Toxoplasma gondii* parasites [15, 16]. The disease is associated with dangerous complications in immunocompromised patients which require a proper management in vulnerable people. The currently approved drugs comprise sulfadiazine, pyrimethamine, trimethoprim, and atovaquone. The current standard therapy for toxoplasmosis is a combination of the DHPS inhibitor sulfadiazine with the DHFR inhibitor pyrimethamine [17]. The identification of promising targets of the parasite and infected host is mandatory for the development of new antitoxoplasmal drugs [18].

Leishmaniasis is clinically manifested by cutaneous leishmaniasis/CL, mucocutaneous leishmaniasis/MCL, and visceral leishmaniasis/VL. With up to one million annual cases, CL is the most abundant leishmaniasis type leading to sore and stigmatizing lesions [19, 20]. A tremendous deficit of available inexpensive and safe drugs has led to the classification of leishmaniasis as a neglected tropical disease (NTD). Current treatment options include toxic antimonials and amphotericin B, for instance [20]. However, severe adverse effects and resistance formation confine their application, which requires new potent drug candidates for this NTD [21].

In this study, ten new hydrazones of 3-iodo-5-methoxy-4-propargyloxybenzaldehyde were prepared. *T. gondii* and *L. major* parasites were used to identify antiparasitic compounds which might be further developed as new antiprotozoals.

Methods

Chemistry

Commercially available chemicals were obtained from ABCR, Alfa Aesar, Sigma-Aldrich, and TCI. 3-Iodo-5-methoxy-4-propargyloxybenzaldehyde was synthesized following a modified literature protocol [22, 23]. Analytical machines applied for chemical analyses (i.e., melting point, NMR, IR, and ESI-MS machines) were mentioned elsewhere [14, 24]. The synthesis and characterization of the new compounds **2a–j** can be found in the supplemental online material of this article.

Antiparasitic activity testing *Toxoplasma gondii* drug evaluation

T. gondii tachyzoites (RH strain) were cultivated in Vero cells (ATCC® CCL81™, USA). *T. gondii* growth in the presence of compounds were studied as published previously [24]. IC_{50} values were determined to express antiparasitic activity (three independent experiments) [24].

Leishmania major drug evaluation

L. major promastigotes (isolated in 2016 from a Saudi patient) and amastigotes were used for compound testing according to previously published methods [24]. Antiparasitic activities were presented as IC_{50} values (n = 3). Handling of BALB/c mice (Pharmaceutical College, King Saud University, KSA) for parasite and macrophage isolation strictly followed the guidelines of the committee of research ethics, Deanship of Scientific Research, Qassim University, KSA (permission No. 20–03-20).

Cytotoxicity in host cells

The MTT assay was used to determine the toxicity of **2a–j** to Vero cells or murine macrophages according to a previous article [24]. IC_{50} values were calculated to express toxicities (three independent experiments).

Computational analyses Preparation of target and ligands

Compound **2a** and **2e** geometries were optimized by using DFT method (Density Functional Theory). The solvent (water) effect was considered using the CPCM [25]. The exchange Becke three-parameter Lee-Yang-Parr correlation (B3LYP) level of theory in conjunction with the base 6-311G(d, p) basis set already implemented in Gaussian 16 were used [26-28]. The optimized geometries of compound **2a** and **2e** are shown in Figure 1A. Both structures were converted into format .*mdb in order to use MOE-docking. The tubulin structure (PDB ID: 1SA0, resolution 3.58 Å) was downloaded from http://www.rcsb.org/pdb/ [29]. The binding location of colchicine (CN2) co-crystallized with tubulin was used to determine the binding cavity.

Molecular docking simulation

MOE software was used to predict the interactions between the colchicine binding site of tubulin and **2a** and **2e**. The applied docking protocol was published before [30, 31]. In detail, the default parameters Placement, Triangle Matcher; Rescoring 1, and London dG were used. To validate the method process, re-dock of the co-crystallized ligand (colchicine) with the binding site residues was employed ("Dock Option" in the MOE software). The RSMD value was less than 2.50 Å indicating an accurate and successful docking [32].

Results

The new acyl hydrazones 2a–j were prepared from 3-iodo-5-methoxy-4-propargyloxybenzaldehyde (1) and aromatic acyl hydrazides (Figure 1B). Starting compound 1 was obtained from 3-iodovanillin by Williams etherification with propargyl bromide and K_2CO_3 [22, 23]. The compounds were obtained as colorless or yellow solids in moderate to high yields. 2a was obtained as solid with solvated ethanol (equimolar). Only the yields of 2c and 2d were relatively low (29-30%) due to solubility issues during the work-up. ^{1}H and ^{13}C NMR spectra of compounds 2a–j displayed the characteristic propargyl, benzylidene and carbonyl signals. High-resolution mass spectra (HRMS) showed molecular peaks of the protonated target compounds confirming the molecular formulas of the compounds.

The effects on *T. gondii* parasites by 2a–j were initially evaluated (Table 1). Murine macrophages and Vero kidney cells were used to evaluate toxicity and parasite selectivity of the test compounds. The 3-fluorophenyl derivative 2e was the most active compound against *T. gondii* with an IC_{50} value of 1.11 µM, followed by the moderately active compounds 2a (IC_{50} = 11.6 µM), 2f (IC_{50} = 13.0 µM), 2d (IC_{50} = 13.7 µM), 2g (IC_{50} = 17.0 µM), and 2c (IC_{50} = 18.2 µM). 2e was markedly less active against macrophages (IC_{50} = 20.3 µM), indicating a considerable selectivity of this new acyl hydrazone for *T. gondii* (selectivity index/SI = 18.3, Table 1). However, toxic effects on Vero kidney cells were observed. Compounds 2j (IC_{50} = 33.7 µM), 2h (IC_{50} = 29.0 µM) and 2b (IC_{50} = 28.7 µM) were the least active derivatives. In terms of structure activity relationships (SARs), a halo-substitution of the phenyl ring at position 3 appeared to be favorable, and fluoro-residues (2d and 2e) were more beneficial than chloro (2f) and hydroxyl substituents (2b and 2c). Notably, the 3-hydroxy-2-naphthoyl compound 2a was much more active than the close salicyl analog 2b, which indicates a favorable effect of the naphthalene ring when compared with benzenes. Among the pyridine derivatives, the nicotinoyl/3-pyridyl 2g (IC_{50} = 17.0 µM) was more active than the isonicotinoyl/4-pyridyl 2h (IC_{50} = 29.0 µM), and 2g was the compound least harmful to Vero cells (IC_{50} = 30.3 µM). In addition, semicarbazide 2i (IC_{50} = 24.7 µM) was more active than its close thiosemicarbazide analog 2j (IC_{50} = 33.7 µM). It remains to be elucidated if the highly active compound 2e interacts with *T. gondii* tubulin. Protein import blocking in mitochondria upon targeting Tim44 interaction with Hsp70 might be an alternative mode of action, which was described for a close acyl hydrazone analog with a 5-nitrothienyl moiety [35].

ADMET

The SwissADME server (http://www.swissadme.ch/) was used for the calculation of a series of parameters (TPSA, nROT, MW, LogP, Number of hydrogen bond acceptors/nHA, and Number of hydrogen bonds donors/nHD) which were compared with the Lipinski, Veber and Egan rules [33]. In addition, the ADMETlab server (http://admet.scbdd.com/) was used for the calculation of the parameters [34]: absorption (Caco-2: Colon adenocarcinoma cells, HIA: Human intestinal absorption), distribution (PPB: Plasma protein binding, BBB: Blood–brain barrier), metabolism (CYP1A2 inhibition, CYP2C19 inhibition, CYP2D6 inhibition), excretion [T ½ (h): half-life, CL: Clearance] and human hepatotoxicity (H-HT).

Next, antileishmanial effects were studied using promastigotes and amastigotes of *L. major* (Table 1). Generally, 2a–j were more toxic to promastigotes than to amastigotes. In addition, all compounds 2a–j displayed a narrow activity range in the promastigotes, which hampers the identification of SARs. 2a (IC_{50} = 9.4 µM) and 2e (IC_{50} = 10.6 µM) were the most active compounds against promastigotes, but notably, 2a was the least active derivative against amastigotes (IC_{50} = 31.0 µM). Only 2d and 2h displayed IC_{50} values below 20 µM in the amastigotes, and, thus, showed activities against *Leishmania* amastigotes comparable with that

of a known 3-iodo-5-methoxy-4-propargyloxyphenyl analog [13]. The activities of the test compounds 2a–j against macrophages were not much different from those against amastigotes, but lower than those against promastigotes. Compound 2a displayed the highest selectivity (SI = 2.7) for promastigotes (Table 1). Semicarbazone 2i and thiosemicarbazone 2j showed moderate effects on *L. major* promastigotes and amastigotes. Half maximal inhibitory concentration (IC50) values from three independent experiments (means, SD ± 15%) after 72 h. 2 Values from [24].

To investigate the in silico interaction of compounds 2a and 2e, molecular docking into the colchicine-binding site of tubulin (PDB ID: 1SA0) was carried out and the results were compared with the data of co-crystallized colchicine (Figure 2, Table 2). Colchicine and compounds 2a and 2e show a similar conformation and occupy nearly the same position in the binding pocket of the colchicine-binding site. The score energy values of 2a and 2e indicate a high affinity for the colchicine-binding site based on various interactions (hydrogen bonds, hydrophobic interactions, etc.). Values of -6.044 and -6.205 kcal/mol were observed for 2a and 2e, respectively. Furthermore, the score of compound 2e was close to the one of colchicine (-7.730 kcal/mol) and indicates the formation of a complex with high stability by 2e.

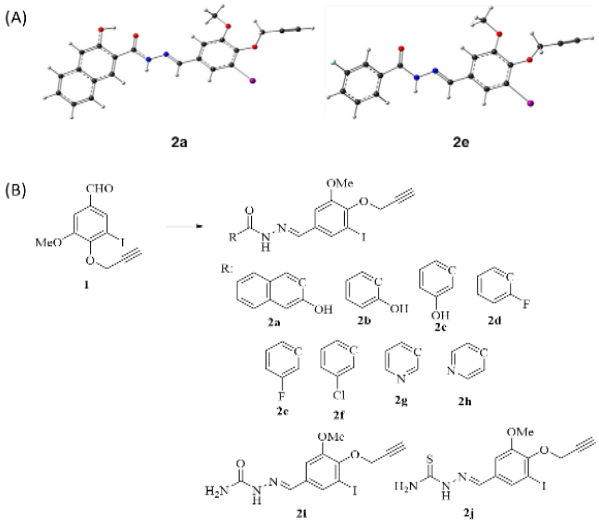

Figure 1: (A) Optimized geometries of compounds 2a and 2e which were obtained by DFT and applied for the molecular docking simulations with tubulin. (B) Chemical synthesis of compounds 2a–j from 1. The following reagents, solvent, and conditions were applied for the synthesis: Acyl hydrazide, EtOH, 78 °C, 2 h, 29-82%.

Activities					
Compd.	IC_{50} *T. gondii*	IC_{50} *L. major* promastigotes	IC_{50} *L. major* amastigotes	IC_{50} Vero	IC_{50} macrophages
2a	11.6	9.40	31.0	15.4	25.8
2b	28.7	11.6	29.8	18.0	25.3
2c	18.2	12.2	28.2	17.6	29.1
2d	13.7	11.7	19.2	12.8	16.6
2e	1.11	10.6	22.8	0.69	20.3
2f	13.0	13.2	20.9	13.2	19.4
2g	17.0	11.3	23.4	30.3	18.8
2h	29.0	13.1	17.2	26.9	18.2
2i	24.7	12.9	20.9	20.9	23.3
2j	33.7	15.2	22.9	28.8	21.1
ATO[2]	0.07	-	-	9.5	-
AmB[2]	-	0.83	0.47	-	8.1
SI values					
Compd.	Vero / *T. gondii*	Macrophages / *T. gondii*	Macrophages / promastigotes	Macrophages / amastigotes	
2a	1.3	2.2	2.7	0.8	
2b	0.6	0.9	2.2	0.5	
2c	1.0	1.6	2.4	1.1	
2d	0.9	1.2	1.4	0.8	
2e	0.6	18.3	2.1	0.9	
2f	1.1	1.5	1.5	0.9	
2g	1.8	1.1	1.7	0.8	
2h	0.9	0.6	1.4	1.1	
2i	0.8	0.9	1.8	1.2	
2j	0.9	0.6	1.4	0.9	
ATO	135	-	-	-	
AmB	-	-	9.8	17.2	

Table 1: Activity of test compounds 2a–j (IC50 values in µM) against Vero cells, macrophages, T. gondii cells, L. major promastigotes, and L. major amastigotes.1 Positive controls are atovaquone (ATO) and amphotericin B (AmB). SI (selectivity index) values of test compounds 2a–j for T. gondii cells (in comparison to Vero cells and macrophages), L. major promastigotes and amastigotes (in comparison to macrophages).

In detail, 2e establishes five hydrogen bonds with the binding site residues [36, 37]. Two strong conventional hydrogen bonds (O/ASN258-HD22, 2.57 Å; H/ASN258-O, 1.55 Å) and three carbonyl H-bonds (O/LYS352-HE3, 2.55 Å; H/ASN349-O, 2.78Å; H/ASN258-OD1, 2.25 Å) were formed. Two halogen interactions with MET259 and VAL238 were observed. 2e also formed eight hydrophobic interactions (two alkyl and six Pi-alkyl interactions). 2a established five strong hydrogen bonds with the binding site, two conventional H-bonds (H/ASN258-OD1, 1.82 Å; H/ASN258-O, 1.93 Å) and three other carbonyl H-bonds (O/ASN350-HA, 3.08 Å; H/LEU248-O, 3.00Å; H/LEU248-O, 3.09 Å). One Pi-Lone Pair interaction with ASN258 and nine hydrophobic interactions with the binding site (six alkyls and three Pi-alkyl interactions including CYS241) were found. Several studies showed that residues ASN258, LYS352 and CYS241 play an important role in the binding to the colchicine-binding site of tubulin and the inhibition of tubulin polymerization [38, 39].

Compounds	S-Score (kcal/mol)	Interactions between compounds and colchicine binding site residues of tubulin					
		Atom of compound	Receptor atoms	Receptor residues	Category	Type	Distance (Å)
2a	-6.044	H	OD1	ASN258	H-Bond	Conventional H-Bond	1.82
		H	O	ASN258	H-Bond	Conventional H-Bond	1.93
		O	HA	ASN350	H-Bond	Carbonyl H-Bond	3.08
		H	O	LEU248	H-Bond	Carbonyl H-Bond	3.00
		H	O	LEU248	H-Bond	Carbonyl H-Bond	3.09
		/	OD1	ASN258	Other	Pi-Lone Pair	2.82
		C	/	ALA250	Hydrophobic	Alkyl	3.64
		I	/	ALA316	Hydrophobic	Alkyl	3.79
		I	/	ALA354	Hydrophobic	Alkyl	4.06
		I	/	LEU248	Hydrophobic	Alkyl	4.73
		C	/	LEU252	Hydrophobic	Alkyl	5.34
		C	/	LEU255	Hydrophobic	Alkyl	4.36
		/	/	ILE347	Hydrophobic	Pi-Alkyl	4.40
		/	/	LEU248	Hydrophobic	Pi-Alkyl	5.44
		/	/	ILE347	Hydrophobic	Pi-Alkyl	4.79
2e	-6.205	O	HD22	ASN258	H-Bond	Conventional H-Bond	2.57
		O	HZ2	LYS352	H-Bond	Conventional H-Bond	1.55
		O	HE3	LYS352	H-Bond	Carbonyl H-Bond	2.55
		H	O	ASN349	H-Bond	Carbonyl H-Bond	2.78
		H	OD1	ASN258	H-Bond	Carbonyl H-Bond	2.25
		I	SD	MET259	Halogen	Halogen(Cl, Br,I)	3.52
		F	O	VAL238	Halogen	Halogen(Fluorine)	2.61
		I	/	MET259	Hydrophobic	Alkyl	4.31
		C	/	ILE347	Hydrophobic	Alkyl	4.84
		/	/	CYS241	Hydrophobic	Pi-Alkyl	4.40
		/	/	LEU242	Hydrophobic	Pi-Alkyl	5.23
		/	/	ALA250	Hydrophobic	Pi-Alkyl	4.89
		/	/	LEU255	Hydrophobic	Pi-Alkyl	4.65
		/	/	ALA316	Hydrophobic	Pi-Alkyl	5.45
		/	/	LYS352	Hydrophobic	Pi-Alkyl	4.23
Colchicine (CN2)	-7.730	O2	HG	CYS241	H-Bond	Conventional H-Bond	2.27
		O5	HD22	ASN258	H-Bond	Conventional H-Bond	2.75
		O5	HZ2	LYS352	H-Bond	Conventional H-Bond	1.96
		O6	HZ2	LYS352	H-Bond	Conventional H-Bond	2.31
		O5	HE3	LYS352	H-Bond	Carbonyl H-Bond	2.72
		H23	O	ASN258	H-Bond	Carbonyl H-Bond	2.88
		H24	O	VAL315	H-Bond	Carbonyl H-Bond	2.39
		H24	O	ASN350	H-Bond	Carbonyl H-Bond	2.80
		/	HD22	LEU255	Hydrophobic	Pi-Sigma	2.89
		/	/	LEU255	Hydrophobic	Alkyl	5.13
		/	/	ALA316	Hydrophobic	Pi- Alkyl	5.20
		/	/	LYS352	Hydrophobic	Pi- Alkyl	4.50
		/	/	CYS241	Hydrophobic	Pi- Alkyl	5.03
		/	/	ALA250	Hydrophobic	Pi- Alkyl	4.79

Table 2: Score energy and interactions of 2a, 2e and colchicine with tubulin (PDB ID: 1SA0).

Compd.	Physicochemical Property						Drug-likeness Rules		
	TPSA (Å²)	n-ROT	MW (g/mol)	MLog P / WLogP	n-HA	n-HD	Lipinski	Veber	Egan
	(0~140)	(0~11)	(100~500)	(0~5)	(0~10)	(0~7)			
2a	80.15	7	500.29	3.50 / 4.01	5	2	Accepted	Accepted	Accepted
2e	59.92	7	452.22	3.74 / 3.71	5	1	Accepted	Accepted	Accepted

Compd.	Absorption[1]		Distribution[1]		Metabolism[2]			Excretion[2]		Toxicity[2]	
	Caco-2 (cm/s)	HIA (%)	CNS (logPS)	BBB Penetration	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2D6 inhibitor	T ½ (h)	CL (mL/min/kg)	AMES Toxicity	H-HT
2a	1.752	91.63	-2.209	-0.676	Yes	Yes	No	0.130	2.489	Yes	No
2e	1.024	94.72	-2.407	-0.133	Yes	Yes	No	0.143	3.012	No	No

[1] SwissADME server; [2] ADMETlab server.

Table 3: Drug-likeness and physicochemical properties of 2a and 2e were determined using the SwissADME online server and did not violate Lipinski, Veber, and Egan rules. ADMET properties of 2a and 2e were calculated using the SwissADME and ADMETlab server and indicate amenable intestinal absorption with low CNS and liver toxicity.

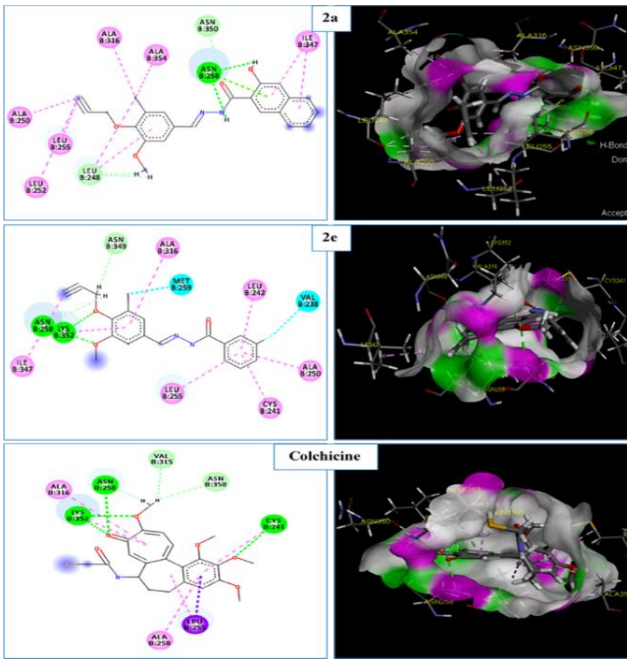

Figure 2: Docking images and interactions of 2a, 2e, and colchicine with tubulin's colchicine-binding site (1SA0).

To evaluate drug-likeness, the physicochemical properties of 2a and 2e were calculated using SwissADME (Table 3). The number of hydrogen bond acceptors of both compounds 2a and 2e is 5 (n-HA: (0~10)), and the number of hydrogen bond donors is <7 (n-HD: (0~7)).

The molecular weight of 2e is within the allowed interval (100 to 500 g/mol), while the molecular weight of 2a is exactly 500 g/mol. Besides, MLogP and WLogP values of 2a and 2e were <5. The flexibility of both compounds was indicated by nROTB values <11. All TPSA values are below 140 Å. Thus, all compounds fulfilled the criteria of the Lipinski, Veber, and Egan rules without violations, which suggests considerable oral bioavailability.

The pharmacokinetics parameters (ADMET, absorption, distribution, metabolism, excretion and toxicity) were determined with the servers ADMETlab (http://admet.scbdd.com/, accessed on 10th September 2023) and SwissADME (http://www.swissadme.ch/, accessed on 10th September 2023, Table 3). Caco-2 absorption of compounds 2a and 2e (> −5.15 cm/s) indicates a reasonable permeability. Furthermore, HIA values above 30% were observed, implying a considerable absorption in the gastrointestinal system. Moreover, these compounds showed positive responses to BBB parameters and are unable to penetrate the CNS according to their logPS values (-2< logPS <-3). 2a and 2e are CYP1A2 and CYP2C19 inhibitors, but do not inhibit CYP2D6. In addition, both compounds show average excretion clearance. Furthermore, the half-lives of both compounds were less than 3 h. While 2a is mutagenic, compound 2e is not. Both compounds show no risk of hepatotoxicity.

The antiparasitic testing of new acyl hydrazone derivatives including a 3-iodo-5-methoxy-4-propargyloxybenzylidene motif led to encouraging results. In particular, the 3-fluorophenyl derivative 2e exhibited promising activity against *T. gondii* cells, and, thus, can be considered for the development as a treatment option for toxoplasmosis infections. In addition, other apicomplexan parasite infection diseases such as malaria might be targeted by 2e. Vice versa, several antimalarial agents were already described which also showed high activity against *T. gondii* [40]. The alkynyl group of 2e can serve for cellular localization and target interaction studies by well-established click chemistry methods using suitable azido-substituted conjugation partners [14].

Molecular docking of 2e into tubulin led to interesting results and provided a first hint at a possible target for this compound. 2e shared crucial tubulin interactions with colchicine (H-bond with ASN258 and hydrophobic interaction with CYS241) which are required for tubulin polymerization inhibition [38, 39]. Tubulin binders of the colchicine binding site can induce cell death and were thoroughly studied for their anticancer activities [41]. Thus, 2e might also be a promising anticancer agent. However, tubulin and microtubules were also discussed as targets for leishmaniasis and toxoplasmosis therapy [42, 43].

ADMET calculations revealed some favorable drug-likeness properties of 2e which need to be confirmed in future laboratory experiments. Notably, 2e was only moderately toxic to macrophages, but its enhanced toxicity to renal cells needs to be investigated in more detail to evaluate its utility as an antitoxoplasmal drug candidate, and to find suitable strategies to circumvent renal toxicity. The currently applied drug sulfadiazine is inducing acute nephrotoxicity and thus combinations of 2e with sulfadiazine might enforce renal failure [44]. Proper formulations can solve the problem of severe adverse effects, and the nephrotoxicity of amphotericin B, for example, was eliminated by lipid and liposomal delivery systems [45].

Compound 2e was also among the most potent derivatives in promastigotes, however, the 3-hydroxy-2-naphthoic hydrazone 2a was more active than 2e, and less toxic to macrophages. Hence, 2a might be suitable for the development of improved antileishmanial

analogs in the future. Its structural analogy to the well-established endocytosis inhibitor dynasore might be a hint at possible structural modifications to increase antileishmanial activity [4].

In the docking studies, 2a showed a slightly lower affinity for tubulin than 2e. *L. major* cathepsin L-type cysteine proteases might also be possible drug targets of 2a given the structural similarity with bis-naphthyl-*N*-acylhydrazone inhibitors of these parasite enzymes [46]. The replacement of the naphthoyl moiety by a cinnamoyl moiety led to antileishmanial compounds with good selectivity profiles [47]. The optimized antileishmanial acyl hydrazone-based cysteine protease inhibitors LASSBio-1736 and LASSBio-1491 exhibited promising pharmacokinetics such as broad distribution and low drug clearance [48, 49]. In addition, acyl hydrazones can be valuable drug candidates for the therapy of Chagas disease caused by *T. cruzi* parasites [50]. Thus, compounds 2a and 2e can be suitable lead structures for the design of potent anti-infectives in the future.

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Author Contributions

Concept, I.S.A.N. and B.B.; methods, I.S.A.N., T.A.K., I.D., R.B.S. and B.B.; analysis, I.S.A.N., W.S.K., R.B.S. and B.B.; investigation, I.S.A.N., T.A.K., R.B.S. and B.B.; resources, I.S.A.N., W.S.K., T.A.K., I.D., R.S., R.B.S., and N.A.; software, I.D. and R.B.S.; original manuscript writing, B.B.; manuscript correction and editing, I.S.A.N., W.S.K., T.A.K., I.D., R.S., R.B.S. and N.A.

Conflict of Interest

The authors confirm that there is no conflict of interest.

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