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FULL PAPER

Dual Parasiticidal Activities of New Phthalimides: Synthesis and Biological Profile against *Trypanosoma cruzi* and *Plasmodium falciparum*

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Abstract: Chagas disease and malaria are two Neglected tropical diseases (NTDs) that prevail in tropical and subtropical conditions in 149 countries. Chagas is also present in Europe, the United States and, Australia due to the immigration of asymptomatic infected individuals. In the absence of an effective vaccine, the control of both diseases relies on chemotherapy. However, the emergence of parasite drug resistance is rendering currently available drugs obsolete. Hence, it is crucial to develop new molecules. Phthalimides, thiosemicarbazones, and 1,3-thiazoles nucleus have been used as platform types to obtain antiplasmodial and anti-*Trypanosoma cruzi* agents. Here we present the synthesis of 24 phthalimido-thiosemicarbazones (**3a-x**) and 14 phthalimido-thiazoles (**4a-n**) and the corresponding biological activity against *T. cruzi, Plasmodium falciparum,* and cytotoxicity against mammalian cell lines. Some of these compounds showed potent inhibition of *T. cruzi* at low cytotoxicity concentrations in RAW 264.7 cells. The most active compounds, **3t** (IC_{50}= 3.60 μM), **3h** (IC_{50}= 3.75μM), and **4j** (IC_{50}= 4.48 μM), were more active than the control drug Benznidazole (IC_{50}= 14.6 μM). Overall, the phthalimido-thiosemicarbazone derivatives were more potent than phthalimido-thiazoles derivatives against *T. cruzi*. Flow cytometry assay showed that compound **4j** was able to induce necrosis and apoptosis in trypomastigotes. Analysis by scanning electron microscopy showed that *T. cruzi* trypomastigote cells treated with the compounds **3h**, **3t,** and **4j** at IC_{50} concentrations, promoted changes in the shape, flagella, and surface of the parasite body similar to those observed in benznidazole-treated cells. The compounds with the highest antimalarials activity were the phthalimido-thiazoles **4l** (IC_{50}= 1.2μM), **4m** (IC_{50}= 1.7μM), and **4n** (IC_{50}= 2.4μM). Together, these data revealed, phthalimido derivatives possess a dual antiparasitic profile with potential effects against *T. cruzi* and lead-like characteristics.

Introduction

Neglected tropical diseases (NTDs) are a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries. NTDs affect more than one billion people and impose a heavy economic burden on developing economies. Drugs available to treat NTDs present some issues: limited number, low efficacy, toxic side effects, and appearance of resistant strains.[1,2] Malaria (caused by *Plasmodium spp*.) and Chagas disease (caused by *Trypanosoma cruzi*) are two vector-borne diseases, with a heavy impact throughout the tropical and subtropical regions where they remain a life-threatening public health problem. Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi* (*T. cruzi*) and new chemotherapies are highly needed.[3] About 6 to 7 million people worldwide, mostly in Latin America, are estimated to be infected with *T. cruzi.*[4]

In the last years, the epidemiology of this infection is changing due to the unprecedented immigration of asymptomatic infected individuals from Latin America to Europe, the United States, and Australia acquired by vertical or through organs or blood products obtained from infected donors.[5] Chagas disease can be treated with benznidazole (BDZ) or nifurtimox.[6] Although highly efficacious, if timely administered, adverse reactions can occur in up to 40% of treated patients.[4,6] BDZ and nifurtimox contraindications include pregnancy and kidney or liver dysfunction.

Malaria is one of the most devastating infectious diseases of our time, in 2017, there were an estimated 219 million cases of malaria (more than 99% due to *P. falciparum*) and 435.000 deaths in 87 countries.[4] There are relatively few effective treatments for *P. falciparum*, apart from artemisinin (ART) combination therapies (ACTs). Nowadays, extensive *P. falciparum* malaria treatment relies on ACT, although resistance is now evident in several Southeast Asian countries.[7–10] Another relevant issue related to the widespread application of ART is the difficulty in maintaining the drug supply; ART is extracted from *Artemisia annua* in low yield, and ART-derivatives are obtained by a long and expensive semi-synthesis process.[11]

There is a recognized need to identify new chemical entities (NCEs) as drug candidates in order to both improve patient care and meet targets for elimination, particularly of neglected parasitic diseases.[12] Phthalimides, thiosemicarbazones and 1,3-thiazoles nucleus are found in several natural products and have also been utilized to develop synthetic drugs and drug-like molecules with a variety of pharmacological effects, binding to a plethora of different targets across target families.[13,14]

Phthalimides, thiosemicarbazones, and 1,3-thiazoles nucleus have been used as platform types to obtains antiplasmodial and anti-*T. cruzi* agents. [15–22] Our research group has explored the pharmacological properties of phthalimide derivatives, and as a result, phthalimido-thiazole derivatives were identified as building blocks to promising anti-*T. cruzi* candidates.[23]

Here we performed the synthesis of 24 phthalimido-thiosemicarbazones, (**3a-x**), and 14 phthalimido-thiazoles (**4a-n**) (**Figure 1**). In this synthetic strategy, a substructure-based compound library was used to choose different substituents around the phenyl ring attached in the thiosemicarbazone moiety, obtaining **3a-x** compounds. For **4a-n** compounds, substituents around the phenyl ring attached at C4 in the thiazole ring were explored. In both series, structural modifications were performed by insertion of substituents on the N3 position of thiosemicarbazone (**3a-x**) or thiazole ring (**4a-n**). With a view to investigates the parasitical profile of new phthalimido-thiosemicarbazones and new phthalimido-thiazoles derivatives, the compounds were tested *in vitro* against the *T. cruzi* parasite trypomastigote form and *P. falciparum*.

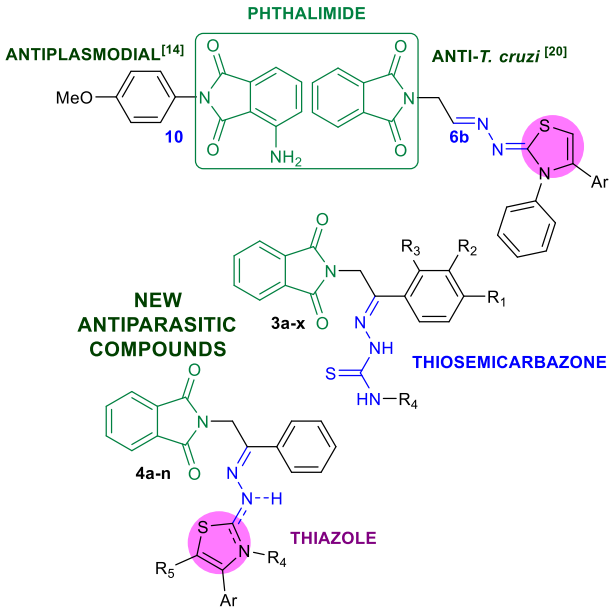


Figure 1. Structural planning of the proposed compounds.

Results and Discussion

Chemistry

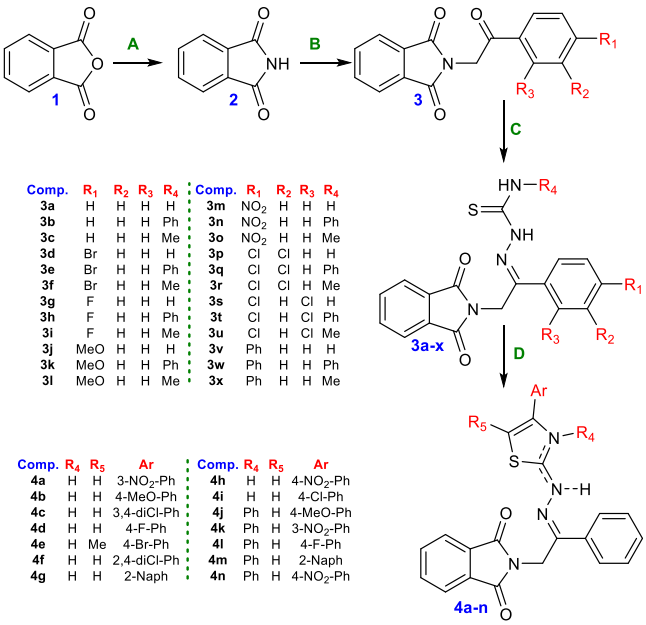
The details of the synthesis are depicted in **Scheme 1**. The composition of newly prepared molecules was confirmed by NMR (^{1}H and ^{13}C), FT-IR, and HR-MS techniques. In NMR spectra, protons and carbons were assigned (see supporting information). All the data were in agreement with our proposed structure.

The 24 phthalimido-thiosemicarbazones (**3a-x**), as shown in **Scheme 1**, were synthesized in a two-step process from phthalimide (**2**) and respective acetophenone. The reaction was carried out in acetone under room temperature (r.t) or reflux for 4h, which led us to intermediate **3**. These intermediates were them reacted with thiosemicarbazides (thiosemicarbazide, 4-phenylthiosemicarbazide, and 4-methyl-3-thiosemicarbazide) and catalytic H_2SO_4 in an ultrasound bath (4-12h) or reflux (24-48h).[24] After 4-12h, thiosemicarbazones **3a-x** precipitated in the reaction mixture and were collected by filtration. The synthesis of the series **4a-n** was performed via Hantzsch cyclization between thiosemicarbazones (**3a** and **3b**) and substituted 2-bromoacetophenones, using isobutanol under ultrasound irradiation, at room temperature for 3h.[25] This reaction condition led to average yields from 27 to 94%. All the synthesized compounds were characterized by infrared (IR), nuclear magnetic resonance (^{1}H, ^{13}C NMR), and mass spectroscopy (ESI-TOF).

NMR data are compatible with the proposed compounds. In theory, two geometrical isomers (E and Z) about the imine (CN) double bond are possible for the thiosemicarbazones and the respective thiazoles. However, analysis of the ^{1}H NMR spectra of the compounds indicated one predominant isomer; the E isomer by comparison with known analogues.[20] Intramolecular H-bonding involving the proton attached to N4 (in DMSO) with the imine N-atom leads to a distinctive singlet around 10.2 ppm,[20] and this is also seen here. Both thiosemicarbazones and respective 1,3-thiazoles were characterized by usual spectroscopy.

The ^{1}H NMR spectra of some compounds showed that phthalimido-thiazoles **4a-n** are composed by diastereomers (supp. Material, **Figures S1** and **S2**). Based on previous crystallized compounds by our group, we suggest that the major isomer formed present the *E-Z* configuration. Indeed, hydrazine double-bond C2=N2 is commonly assigned as *E* configuration.[20,23,25–28] Concerning the exocyclic double-bond N3=C3, we suggest that the predominant configuration is in *Z* configuration.[20,23,28–30]

Besides, a representative ^{1}H-NMR spectrum of compound **4n** is presented in Supplementary Material (**Figure S1**).



Comp.	R_1	R_2	R_3	R_4
3a	H	H	H	H
3b	H	H	H	Ph
3c	H	H	H	Me
3d	Br	H	H	H
3e	Br	H	H	Ph
3f	Br	H	H	Me
3g	F	H	H	H
3h	F	H	H	Ph
3i	F	H	H	Me
3j	MeO	H	H	H
3k	MeO	H	H	Ph
3l	MeO	H	H	Me

Comp.	R_1	R_2	R_3	R_4
3m	NO_2	H	H	H
3n	NO_2	H	H	Ph
3o	NO_2	H	H	Me
3p	Cl	Cl	H	H
3q	Cl	Cl	H	Ph
3r	Cl	Cl	H	Me
3s	Cl	H	Cl	H
3t	Cl	H	Cl	Ph
3u	Cl	H	Cl	Me
3v	Ph	H	H	H
3w	Ph	H	H	Ph
3x	Ph	H	H	Me

Comp.	R_4	R_5	Ar
4a	H	H	3-NO_2-Ph
4b	H	H	4-MeO-Ph
4c	H	H	3,4-diCl-Ph
4d	H	H	4-F-Ph
4e	H	Me	4-Br-Ph
4f	H	H	2,4-diCl-Ph
4g	H	H	2-Naph

Comp.	R_4	R_5	Ar
4h	H	H	4-NO_2-Ph
4i	H	H	4-Cl-Ph
4j	Ph	H	4-MeO-Ph
4k	Ph	H	3-NO_2-Ph
4l	Ph	H	4-F-Ph
4m	Ph	H	2-Naph
4n	Ph	H	4-NO_2-Ph

Scheme 1. Global synthesis of compounds **3a-x** and **4a-n.** Reagents and conditions: (**A**) Urea, 150°C, 3h; (**B**) acetone, phthalimide (**2**), K_2CO_3, KI, respective acetophenone, r.t. or reflux, 4h; (**C**) ethyl alcohol, sulfuric acid, ultrasound or reflux, 30 min, respective thiosemicarbazide, ultrasound (4-12h) or reflux (24-48h). (**D**) compound **3a** (for **4a-l**; or **3b** for **4j-n**), isobutanol, respective acetophenone, ultrasound, 3h.

Cytotoxicity activity against mammalian cells

Initially, we verified the influence of the cyclization of phthalimido-thiosemicarbazone to the phthalimido-thiazole ring on the cytotoxic effect against RAW 264.7 (macrophage-like) cells. Phthalimido-thiosemicarbazones (**3a-x**), showed a range of IC_{50} = 42 to 338 μM, with 8 compounds presenting IC_{50} below 100 μM. Compound **3b** (IC_{50}= 42.03 μM) was the most cytotoxic (**Table 1**). In general, compounds **3a-x** presented IC_{50} values higher than 100 μM (**Table 1**). On the other hand, of the 14 phthalimido-thiazole derivatives (**4a-n**), only compound **4d** showed an IC_{50} value lower than 100μM being the most cytotoxic (IC_{50}= 59.7μM) (**Table 2**).

Antiparasitic activity against extracellular forms of T. cruzi

Observing the trypanocidal activity for trypomastigote form, we observed that 13 of 38 compounds present higher *anti-T. cruzi* activity than the control drug, BDZ (**Table 1**) witch exhibit an IC_{50} value of 14.6μM. Compounds that exhibited IC_{50} lower than BDZ were considered active *anti-T. cruzi* agents. Among series **3a-x** and **4a-n**, compounds **3t** (IC_{50}= 3.60μM), **3h** (IC_{50}= 3.75μM) (**Table 1**) and **4j** (IC_{50}= 4.48μM) (**Table 2**) were the most active.

Based on the results of anti-*T. cruzi* activity of the tested compounds, we first analyzed the effect of substitutions on the phthalimido-thiosemicarbazones, in which the structural variations were made in the phenyl ring from 2-haloacetophenone. No clear correlation was observed between the substituents, once compound **3a** (IC_{50}= 42.88μM) which has no substitution in the phenyl ring, compounds **3g** and **3i** (fluorine), as well compounds **3j-l** (OCH_3), **3m** (NO_2), **3u** (Cl) and **3v** (Ph), presented low activity profile. However, compound **3h** presents fluorine in *para* position, and a phenyl ring in N4 of thiosemicarbazone was the most potent of all series, being twice as potent than reference drug BZD.

Table 1. Cytotoxicity and antiparasitic activity of phthalimido-thiosemicarbazone series (**3a-x**).

Compd.	R1	R2	R3	R4	Cytotoxicity (RAW264.7) CC_{50} μM[a]	IC_{50} Trypo. μM (strain Y)	SI (CC_{50} RAW264.7/ IC_{50} Tryp.)	*P. falciparum* (D7-GFP) % of inhibition at 10μM	IC_{50} *P. falciparum* (3D7-GFP) μM ±SD	SI (CC_{50} RAW264.7/ IC_{50} *P. falciparum*)
3[a]	H	H	H	H	338.97	42.88	7.90	19.00	-	-
3b	H	H	H	Ph	42.03	7.55	5.57	8.97	-	-
3c	H	H	H	Me	331.72	91.80	3.61	21.82	-	-
3d	Br	H	H	H	227.55	8.65	26.31	45.66	-	-
3e	Br	H	H	Ph	50.69	4.90	10.34	29.96	-	-
3f	Br	H	H	Me	84.13	18.82	4.47	24.32	-	-
3g	F	H	H	H	296.04	73.66	4.01	42.66	-	-
3h	F	H	H	Ph	140.47	3.75	37.46	60.36	-	-
3i	F	H	H	Me	246.84	94.41	2.61	88.25	2.90	85.12
3j	MeO	H	H	H	311.61	76.06	4.10	48.76	-	-
3k	MeO	H	H	Ph	48.80	64.52	0.76	38.43	-	-
3l	MeO	H	H	Me	204.55	27.30	7.49	29.81	-	-
3m	NO_2	H	H	H	213.13	52.74	4.04	0.00	-	-
3n	NO_2	H	H	Ph	251.81	11.51	21.88	22.99	-	-
3o	NO_2	H	H	Me	112.08	17.19	6.52	30.56	-	-
3p	Cl	Cl	H	H	157.19	16.62	9.46	59.14	-	-
3q	Cl	Cl	H	Ph	92.87	14.23	6.53	0.00	-	-
3r	Cl	Cl	H	Me	266.08	20.39	13.05	16.10	-	-
3s	Cl	H	Cl	H	243.82	22.44	10.87	36.44	-	-
3t	Cl	H	Cl	Ph	245.57	3.60	68.21	59.82	-	-
3u	Cl	H	Cl	Me	73.75	54.40	1.36	68.89	3.82	19.31
3v	Ph	H	H	H	48.54	32.06	1.51	8.53	-	-
3w	Ph	H	H	Ph	49.19	ND	-	49.05	-	-
3x	Ph	H	H	Me	184.73	12.09	15.28	0.00	-	-
BDZ	-	-	-	-	123.54	14.60	8.46	-	-	-
CQ	-	-	-	-	77.00	-	-	99.23*	0.01	7,700.00

BDZ = benznidazole. IC_{50} = inhibitory concentration for 50%. CC_{50} = cytotoxic concentration for 50%. CC_{50} and IC_{50} values were calculated using concentrations in triplicate, and experiments were repeated, only values with a standard deviation < 10% mean were considered. SI= Selectivity index. *challenged with 1μM due to the correspondent IC_{50} values situated at nM range. CQ: chloroquine

In the same way, compounds with withdrawer substituents, **3p**, **3q**, **3r** (2,3-*di*Cl), **3s,** and **3u** (2,4*di*Cl) did not present good trypanocidal activity. In contrast, compound **3t** (2,4-*di*Cl) and a phenyl ring in *N*4 of thiosemicarbazone moiety, displayed a better activity profile than benznidazole-treated parasites. Comparing *N*4 substitution of thiosemicarbazone moiety, it was observed that phenyl ring in *N*4 of thiosemicarbazone was generally beneficial to the activity against trypomastigote form as can be seen in compounds **3h** and **3t** (**Table 1**).

Concerning compounds **4a-n**, they present no substitution in the phenyl ring from 2-haloacetophenone and a thiazole nucleus, which was attached a phenyl ring at 4 position. In this sub-series, the majority of compounds exhibited a low antiparasitic activity profile. Compounds **4b** (OCH_3) and **4c** (Cl) present in *para* position displayed trypanocidal effects compared with BDZ. Only compound **4j**, which possess fluorine in *para* position of phenyl ring and another phenyl ring in *N*4, exhibited the most potent activity of this series (**Table 2**). Phthalimido-thiosemicarbazones derivatives, namely **3t** (IC_{50}= 3.60μM), and **3h** (IC_{50}= 3.75μM) were more potent than phthalimido-thiazoles derivatives, of which only compound **4j** (IC_{50}= 4.48μM) showed potent trypanocidal profile (**Figure 2**).

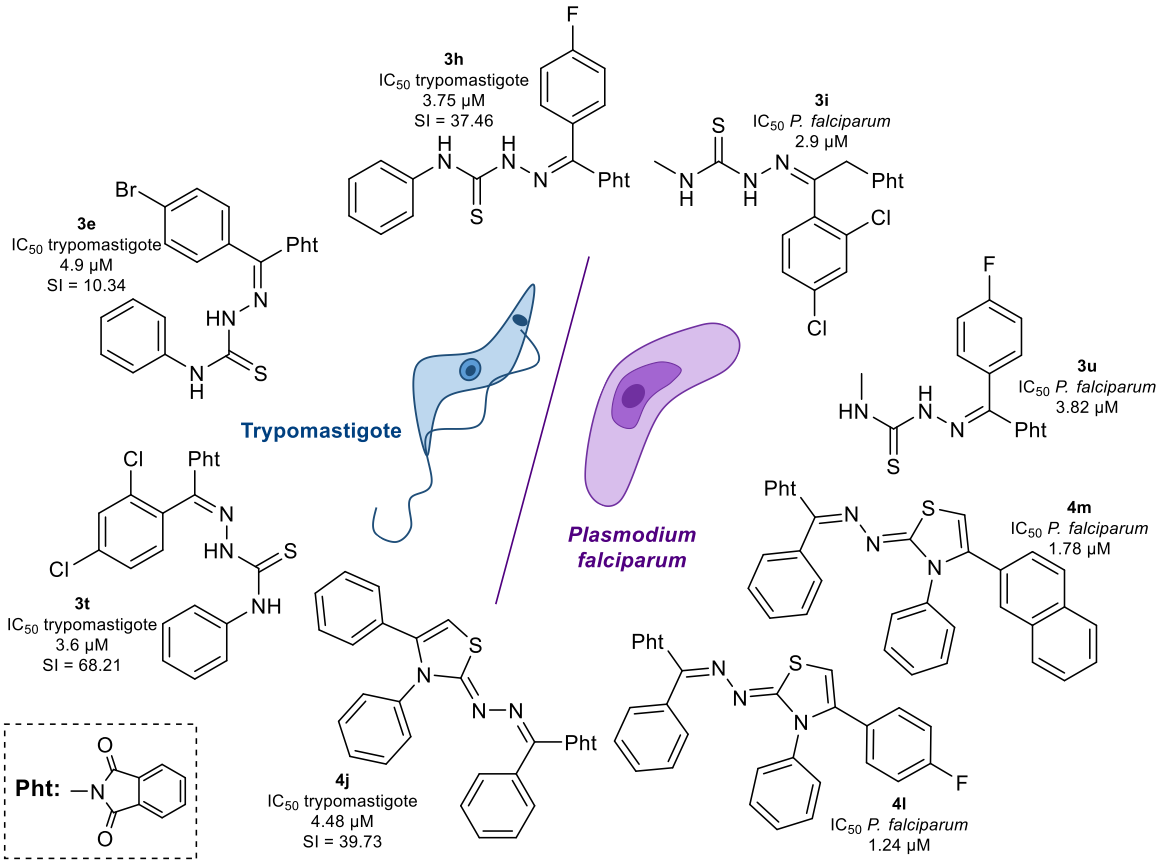


Figure 2. The most active compounds against *T. cruzi* and *P. Falciparum*.

In vitro activity of compounds 3a-v and 4a-n against P. falciparum (3D7-GFP)

The synthesized derivatives were first evaluated at 10 µM fixed concentration for their growth inhibitory activity against the erythrocytic stage of the GFP-expressing *P. falciparum* (3D7-GFP). As the parasite develops inside the red blood cell, the production of GFP-protein increases, hence increasing green fluorescence (GFP fluoresces in the FITC channel). The number of fluorescent events (parasitized RBCs) detected by the flow cytometer, represents the number (or %) of the surviving parasites.[31] Cytotoxicity (CC_{50}) of selected compounds for RAW 264.7 cells and selectivity index (SI) for *P. falciparum* strain 3D7-GFP are reported in **Tables 1** and **2**.

Table 2. Cytotoxicity and antiparasitic activity of phthalimido-thiazoles series (**4a-n**).

Cpd.	R4	R5	Ar	Cytotoxicity (RAW264.7) CC_{50} µM[a]	IC_{50} Trypo. µM (strain Y)	SI (CC_{50} RAW264.7/ IC_{50} Tryp.)	*P. falciparum* (3D7-GFP) % of inhibition at 10µM	IC_{50} *P. falciparum* (3D7-GFP) µM ±SD	SI (CC_{50} RAW264.7/ IC_{50} *P. falciparum*)
4a	-	H	3-NO_2-Ph	>413	51.62	>8,00	14.8	-	-
4b	-	H	4-MeO-Ph	385	8.94	43.06	61.5	-	-
4c	-	H	3,4-*di*Cl-Ph	194.44	6.07	32.03	44.1	-	-
4d	-	H	4-F-Ph	>438	14.85	>29.49	50.7	-	-
4e	-	Me	4-Br-Ph	59.7	50.09	1.19	10.5	-	-
4f	-	H	2,4-*di*Cl-Ph	>394	28.12	>14.01	14.5	-	-
4g	-	H	2-Naph	123	54.02	2.28	17.8	-	-
4h	-	H	4-NO_2-Ph	>413	12.26	>33.69	23.8	-	-
4i	-	H	4-Cl-Ph	111.9	14.06	3.93	28.5	-	-
4j	Ph	H	4-MeO-Ph	178	4.48	39.73	70.2	3.54	50.28
4k	Ph	H	3-NO_2-Ph	>357	10.13	>35.24	81.9	3.69	>96.75
4l	Ph	H	4-F-Ph	>375	187.76	2.00	89.0	1.24	>302.42
4m	Ph	H	2-Naph	>354.2	177.10	2.00	82.5	1.78	>198.99
4n	Ph	H	4-NO_2-Ph	192.3	39.60	4.86	86.8	2.41	79.79
BDZ	-	-	-	123.54	14.60	8.46	-	-	-
CQ	-	-	-	77.00	-	-	99.23*	0.01	7,700.00

BDZ = benznidazole. IC_{50} = inhibitory concentration for 50%. CC_{50} = cytotoxic concentration for 50%. CC_{50} and IC_{50} values were calculated using concentrations in triplicate and experiment were repeated, only values with a standard deviation < 10% mean were considered. SI= Selectivity index. *challenged with 1µM due to the correspondent IC_{50} values situated at nM range. **CQ**: chloroquine

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Growth inhibition assays (as the estimation of IC_{50}) using flow cytometry-based methods have been shown to be highly reproducible, scalable for efficient use in screening antimalarial activity of new compounds and comparable in terms of sensitivity to alternative methods such as hypoxanthine uptake.[32] The use of *Plasmodium* lines expressing green fluorescent proteins has enhanced the sensitivity and reproducibility of flow cytometry methods used to screen the antimalarial activity of new compounds.[33] Scatter plots data from one representative assay are shown in **Figure 3**. The percentage of survival parasites determined as in **Figure 3** was used to determine the % of inhibition at 10μM, presented in **Tables 1** and **2** and to generate a dose-response curve, from which the correspondent IC_{50} was determined (**Tables 1** and **2**).

The reference antimalarial chloroquine (CQ) was included as control and resulted in IC_{50} value in agreement with previous results.[34,35]

All compounds were first screened for antimalarial activity against *P. falciparum* at a fixed dose of 10μM, and those exhibiting over around 70% growth inhibition were selected as active and confirmed by IC_{50} estimation. For the phthalimido-thiosemicarbazones (**3a-x**), only compounds **3i** (88%) and **3u** (68.82%) were considered for refinement of activity.

In the phthalimido-thiazoles series (**4a-n**), compounds **4j** (70.2%), **4k** (81.9%), **4l** (89.0%), **4m** (82.5%), and **4n** (86.8%) demonstrated considerable antimalarial activity, so were also further evaluated. Dose-response curves to determine half-maximal inhibitory concentrations (IC_{50}) against *P. falciparum* strain 3D7-GFP were determined based on cytometry evaluation of parasite growth.

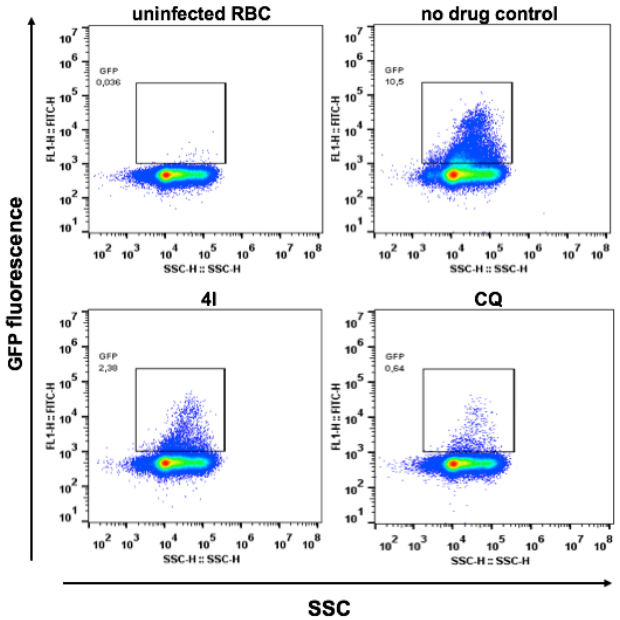

Figure 3. Evaluation of *Plasmodium falciparum* survival using flow cytometry. Scatter plots showing the gating signals of: uninfected red blood cells as negative control (top left panel); parasite culture after 72h of incubation without drug, as positive control (top right panel); parasite culture after 72h of incubation with 10μM of tested compound 4l (bottom left panel); parasite culture after 72h of incubation with 1μM of CQ as a control drug (bottom right panel). The ordinate shows green fluorescence emission from GFP protein (FL1-H::FITC-H), and the abscissa displays the side scatter (SSC-H::SSC-H) signal intensity. Inside each panel is represented the percentage (GFP) of the total events (parasitized red blood cells) recorded in that culture. RBC, red blood cells; GFP, green fluorescent protein; CQ, chloroquine; **4l** tested compound.

For phthalimido-thiosemicarbazone series, compounds **3i** and **3u** presented IC_{50} values of 2.9μM and 3.82μM, respectively. The best performing compounds (with lower IC_{50}) belong to the phthalimido-thiazoles series, being compounds **4l** (IC_{50}= 1.2μM), **4m** (IC_{50}= 1.7μM), and **4n** (IC_{50}= 2.4μM) (**Table 2**). Phthalimido-thiazoles derivatives, compounds **4j-m**, present a phenyl ring at N of R_5 of thiazole nucleus; hence, they present higher lipophilicity, consistent with a higher antimalarial activity. Regarding the selective index against the parasite, compound **4l** presented an SI value higher than 300, a desirable characteristic (SI>10) for an antimalarial candidate molecule (**Table 2**). Compound **4j** was active for both parasites tested (IC_{50}= 4.48μM for *T. cruzi* and 3.54μM for *P. falciparum*) (**Table 2**).

Flow cytometry analysis for trypomastigote form of T. cruzi

It was possible to evaluate the effects of the most active compounds using flow cytometry as a tool (**3h**, **3t** and **4j**), as well as BDZ, on the induction of cell death by apoptosis or necrosis. It was observed that compound **4j** (IC_{50} and 2x IC_{50}) was able to induce significantly labeling compatible with necrosis and apoptosis in trypomastigotes. Similar results were found, in higher proportions, in parasites treated with BDZ, the reference drug and positive control used in this assay. (supp. material, **Figure S2**). Compounds **3h** and **3t**, despite having demonstrated trypanocidal potential in the initial evaluation tests, they do not demonstrate the induction of cell death due to apoptosis or necrosis, with the methodology used (data not show).

Ultrastructural studies for T. cruzi

In view to investigate the effects of phthalimido-thiosemicarbazones and phthalimido-thiazoles on parasite morphology, the most active compounds of this work (**3h**, **3t**, and **4j**) were selected. Analysis by scanning electron microscopy (SEM) showed that *T. cruzi* trypomastigote cells treated with the compounds **3h**, **3t** and **4j** at IC_{50} and 2x IC_{50} concentrations (3.75, 3.60, and 4.48μM respectively), promoted changes in the shape, flagella and surface of the parasite body, compared to the untreated control group.

Compounds **3h** and **3t** showing body writhing, appearance swollen and rounded, flagellum shortening, besides the presence of filamentous structures on the cell surface, while **4j** caused changes similar to those observed in BDZ-treated cells including extravasation of the cytoplasmic content. It was also observed that at IC_{50} and 2x IC_{50} values, the compounds promoted the same morphological changes in the parasites. (supp. material, **Figure S3**).

In the present study, the activity of these compounds (**3h**, **3t** and **4j**) can be seen through ultrastructural alterations that can lead to cell unavailability, reinforcing the findings by Menna-Barreto et al.[36] who analyzing different cell death pathways induced by drugs in *T. cruzi*, suggest that a significant number of morphological alterations is always related to loss of cell viability and parasite death. While the changes caused by **3h** and **3t** are described in the literature as typical events of death due to apoptosis even when, despite the changes in the plasma membrane, it can remain intact,[37] changes by treatment **4j** as leakage of cytoplasmic content suggest death by necrosis or apoptosis. Thus, all SEM and FACS data are in agreement.

Physicochemical and ADME parameters

We evaluated if the synthesized compounds physicochemical properties are within the Lipinski's Rule of Five, which are essential for pharmacokinetics and drug development. For this purpose, physicochemical and ADME properties were calculated using the Swiss ADME (a free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules). Compound obeying at least three of the four criteria is considered to adhere to Lipinski Rule.[38] All 3a-x compounds are compatible with Lipinski Rule. For 4a-n, compounds 4e and 4j-n are not compatible with Lipinski Rule. Another attractive property is the topological polar surface area (TPSA). Compounds with a low TPSA (≤140 Å2) tend to have higher oral bioavailability.[39,40] Must synthesized compounds have appropriate TPSA. Just 4a, 4k, and 4n have TPSA > 140 $Å^2$. Compounds shown variable permeability based on gastrointestinal absorption (GI), according to the BOILED-Egg

predictive model (Brain or Intestine L Estimate D permeation method). Most compounds have shown high gastrointestinal absorption. With respect to oral bioavailability, it is expected 0.55 of the probability of oral bioavailability score > 10% in rat for most compounds. All these data suggest the in silico good drug-likeness profile and excellent chemical stabilities for this most compounds (supp. material, Tables S1 and S2).

Conclusion

Twenty-four phthalimido-thiosemicarbazones (3a-x) and fourteen phthalimido-thiazoles (4a-n) were obtained in reasonable yields using a simple methodology. Compounds with important trypanocidal activity, especially compounds 3t (IC_{50}= 3.60μM), and 3h (IC_{50}= 3.75μM), 4j (IC_{50}= 4.48μM) were identified. Flow cytometry and ultrastructural studies showed that compound 4j was able to induce both necrosis and apoptosis in trypomastigotes. Compound 3t, a phthalimido-thiosemicarbazone derivative, was the most potent trypanocidal agent identified in this work, presented a selective index of 68.60, which was approximately 10 times more selective than BDZ, the standard drug in clinical use. SEM analysis showed that 3h, 3t, and 4j promoted changes in the shape, flagella, and surface of the parasite body. Additionally, compound 4l, a phthalimide-thiazole, exhibited antimalarial activity against sensitive and resistant *P. falciparum* in the low micromolar range (IC_{50} < 10μM), no cytotoxicity against RAW264.7 cells, a considerable selectivity index (SI > 300). Our results indicated phthalimide-based derivatives are promising molecules as potential anti-*Trypanosoma cruzi* and/or antiplasmodial agents.

Experimental Section

***Chemistry*:** All reagents were used as purchased from commercial sources (Sigma Aldrich, Acros Organics, Vetec, or Fluka). Reaction progress was followed by thin-layer chromatography (TLC) analysis (Merck, silica gel 60 F_{254} in aluminum foil). Melting points were determined on a Fisatom 430D electrothermal capillary melting point apparatus and were uncorrected. NMR spectra were measured on either a Varian Unity Plus 400 MHz (400 MHz for ^{1}H and 100 MHz for ^{13}C) or a Bruker AMX-300 MHz (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) instrument. DMSO-d_6 and D_2O were purchased from CIL or Sigma Aldrich. Chemical shifts were reported in ppm, and multiplicities were given as s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet), and coupling constants (J) in hertz. Mass spectrometry experiments were performed on an LC-IT-TOF (Shimadzu). Unless otherwise specified, ESI was conducted in positive ion mode. Typical conditions were as follows: capillary voltage of 3kV, cone voltage of 30V, and peak scan between 50 and 1000 m/z.

General procedures for the synthesis of compounds 3a-x:

Synthesis of Intermediate 2: 4.05 g (67.43 mmol) of urea were blended together with 10.0 g (67.52 mmol) of phthalic anhydride. The powder mixture was transferred to a round-bottomed flask and left in an oil bath with heating to reach the temperature of about 156 ° C for approximately 3h with the half-opened flask. During this time, the compounds were melted, and the new solid formed (a white powder), in addition to gas evolution. A TLC was carried out with the phthalic anhydride, and the product obtained, using an eluent system the mixture of Hexane / Ethyl Acetate in a ratio of 7: 3. For purification, the solid obtained was washed with water in a vacuum filtration system. The sintered funnel containing the product was packed in a desiccator for 48h, and then the phthalimide (Int. **1**) was weighed to obtain the reaction yield data.

***Synthesis of Intermediate 3*:** Into a round bottom flask, 15 ml of acetone P.A., 3.4 mmol of phthalimide (0.50 g), 4.6 mmol K_2CO_3 (0.47 g), and a solid KI spatula tip were added. The mixture was allowed to stir vigorously for 30 minutes at room temperature. 3.4 mmol of acetophenone (2-Bromoacetophenone, 2-Bromo-4'-Fluoroacetophenone, 2-Bromo-4'-Phenylacetophenone, 2,4'-Dibromoacetophenone, 2-Bromo-4'-Methoxyacetophenone, 2-Bromo-3'-Nitroacetophenone, 2-Bromo-3 ', 4'-Dichloroacetophenone and 2,2', 4'-Trichloroacetophenone) and then the system remained under constant stirring for 2-24 h. In all cases, it was necessary to add excess acetophenone in order to reach the end of the reaction. To verify the formation of the products, TLCs were performed with the reagents and the reaction mixture, using as an eluting solvent the mixture of Ethyl Acetate and Hexane in a ratio of 2: 8. The mixture was vacuum filtered, washed with hot acetone and the supernatant dried on a rotary evaporator. The dry solid product was subjected to isopropyl alcohol washing in a vacuum system. The sintered funnel containing the product was packed in a desiccator for 24 hours, and then the acetophenone was weighed to obtain the reaction yield data.

Synthesis of compounds 3a-x: In a round-bottomed flask containing 15 ml of ethyl alcohol, 300 mg of intermediate **3** was added along with 14 drops of P.A. Sulfuric Acid. The mixture was kept under ultrasound or reflux for about 30 min. After that time, the equivalent molar amount of the respective thiosemicarbazide (thiosemicarbazide,4-phenylthiosemicarbazide, 4-methyl-3-thiosemicarbazide) was added to the system. The system was left on ultrasound (4-12h) or reflux (24-48h). In all cases, it was necessary to add excess thiosemicarbazide in order to reach the end of the reaction. Thin Layer Chromatography (TLC) with the reactants and the reaction mixture were prepared using the 3: 7 ethyl acetate and hexane mixture as eluent solvent. The solid obtained was subjected to vacuum filtration and washed with Ethyl Alcohol. The sintered funnel containing the filtered solid was packed in a desiccator for 24 h and then weighed the final product to obtain the reaction yield data.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-phenylethylidene)Hydrazinecarbothioamide (3a): Yield 87.99%; m.p.(°C) 178-180; Rf: 0.48 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 300MHz), δ ppm: 4.9 (s, 2H, CH 2), 7.34 (m, 3H, CH- Ar), 7.79 (m, 6H), 8.45 (s, 1H, NH_2), 10.67 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75MHz) δ ppm: 34.42 (CH_2), 123.76 (C-Ar), 127.39 (C-Ar), 128.57 (C-Ar), 129.87 (C C-Ar), 131.56 (C-Ar), 135.21 (C-Ar), 135.74 (C-Ar), 145.19 (C = N), 167.90 (C = O), 179.79 (C = S). HRMS:339.0788 $[M - H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-phenylethylidene)-N-phenylhydrazinecarbothioamide (3b): Yield 89.62%; m.p. (°C) 223-224; Rf: 0.76 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 300MHz), δ ppm: 4.81 (s, 1H, NH), 5.01 (s, 2H, CH_2), 7.26 (m, 1H), 7.36 (m, 6H, CH-Ar), 7.58 (m, 1H, CH-Ar), 7.83 (m, 6H, CH-Ar), 10.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75MHz) δppm: 34.21 (CH_2), 123.30 (C-Ar), 123.87 (C-Ar), 125.85 (C-Ar), 127.32 (C C-Ar), 128.09 (C-Ar), 129.30 (C-Ar), 131.10 (C-Ar), 134.75 (C-Ar), 135.12 (C- 139.00 (C-Ar), 145.57 (C = N) 147.50 (C-Ar), 167.48 (C = O), 177.42 (C = S). HRMS: 415.0841$[M - H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-phenylethylidene)-N-methylhydrazinecarbothioamide (3c): Yield 50.75%; m.p. (°C) 148-149; Rf: 0.70 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 300MHz), δ ppm: 3.06 (d, 3H, CH_3), 5.24 (s, 2H, CH_2), 7.31 (d, *J* = 6Hz, 2H, Ar), 7.56 (m, 4H, CH-Ar), 7.94 (m, 4H, CH-Ar), 8.1 (d, *J* = 6 Hz, 2H, CH-Ar), 8.54 (d, *J* = 3 Hz, 1H, NH), 10.70 (s, 1H, NH);^{13}C NMR (DMSO-d_6, 75MHz) δ ppm: 31.17 (CH 3), 44.46 (CH 2), 123.25 (C-Ar), 126.90 (C- Ar), 129.34 (C-Ar), 131.51 (C-Ar), 134.52 (C-Ar), 134.76 (C-Ar), 144.50, (C = O), 178.92 (C = S). HRMS: 353.0956 $[M+H]^+$.

(E)-2-(1-(4-bromophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene) hydrazinecarbothioamide (3d): Yield 93.89%; m.p. (°C) 206-208; Rf: 0.48 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 4.90 (s, 2H, CH_2), 7.49 (d, *J* = 8Hz, CH-Ar), 7.77 (m, 4H, CH-Ar), 8.49 (s, 2H, NH_2), 10.69 (s, 1H, NH);^{13} C NMR (DMSO-d_6, 100MHz) δ ppm: 33.65 (CH_2), 123.32 (C-Ar), 128.99 (C-Ar), 131.02 (C- C-Ar), 134.62 (C-Ar), 134.76 (C-Ar), 143.21 (C = N), 167.45 (C = O), 179.33 (C = S). HRMS: 418.9856 $[M+H]^+$.

(E)-2-(1-(4-bromophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-phenylhydrazinecarbothioamide (3e). Yield 94.58%; m.p. (°C) 197-199; Rf: 0.66 (hexane/ethyl acetate 7:3). NMR ^{1}H (DMSO-d_6, 400MHz), δ ppm: 4.99 (s, 2H, CH_2), 7.23 (m, 1H, CH- Ar), 7.34 (m, 4H, 7.38 (m, 2H, CH-Ar), 7.54 (m, 2H, CH- Ar), 7.84 (m, 4H, CH- Ar), 10.20 (s, 1H, NH), 11.05 (s, 1H, NH);^{13}C NMR (DMSO-d_6, 100MHz) δ ppm: 33.97 (CH_2), 122.95 (C-Ar), 123.39 (C-Ar), 125.63 (C-Ar), 126.12 (C-Ar), 128.14 (C-Ar), 129.27 (C-Ar), 131.07 (C-Ar), 131.13 (C-Ar), 134.47 134.84 (C-Ar), 139.02 (C-Ar), 144.13 (C = N), 167.55 (C = O), 177.47 (C = S). HRMS: 493.0031 $[M+H]^+$.

(E)-2-(1-(4-bromophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-methylhydrazinecarbothioamide (3f): Yield 75.96%; m.p. (°C) 191-193; Rf: 0.70 (hexane/ethyl acetate 7:3). NMR ^{1}H (DMSO-d_6, 400MHz), δ ppm: 3.05 (d, J = 4.8 Hz, 3H, CH_3), 4.91 (s, 2H, CH_2), 7.52 (d, J = 8). (D, J = 8.4 Hz, 2H, CH-Ar), 7.81 (m, 4H, CH-Ar), 8.60 (d, J = 4.4 Hz, 1H, NH), 10.72 (s, 1H, NH);^{13}C NMR (DMSO-d_6, 100MHz) δ ppm: 31.18 (CH_3), 33.70 (CH_2), 122.65 (C-Ar), 123.30 (C- Ar), 131.02 (C-Ar), 131.09 (C-Ar), 134.69 (C-Ar), 134.74 (C-Ar), 143.00 (C = O), 178.86 (C = S). HRMS: 430.9623 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-fluorophenyl)ethylidene)hydrazinecarbothioamide (3g): Yield 63.33%; m.p. (°C) 198-200; Rf: 0.28 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 4.92 (s, 2H, CH_2), 7.15 (t, J = 8.8 Hz, 2H, 6H, CH-Ar), 8.47 (s, 1H, NH), 10.67 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6, 100MHz) δ ppm: 34.31 (CH_2), 115.34 (C-Ar), 123.61 (C-Ar), 129.66 (C-Ar), 132.27 (C C-Ar), 134.98 (C-Ar), 143.97 (C = N), 146.58 (C-Ar), 161.85 (CF), 167.90 (C = O) 179.74 (C = S). HRMS: 357.0707 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-fluorophenyl)ethylidene)-N-phenylhydrazinecarbothioamide (3h): Yield 67.62%; m.p. (°C) 205-207; Rf: 0.88 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 5.00 (s, 2H, CH_2), 7.21 (m, 2H, CH- Ar), 7.39 (m, 2H, 7.5 (m, 2H, CH-Ar), 7.58 (d, J = 8Hz, 2H, CH- Ar), 7.83 (m, 4H, , CH-Ar), 10.18 (s, 1H, NH), 11.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100MHz) δppm: 34.63 (CH_2), 115.39 (C-Ar), 123.80 (C-Ar), 125.99 (C-Ar), 126.45 C-Ar), 128.55 (C-Ar), 129.96 (C-Ar), 132.10 (C-Ar), 132.12 (C-Ar), 135.24 139.48 (C-Ar), 144.82 (C = N), 164.45 (CF), 167.98 (C = O), 178.88 (C = S). HRMS: 433.1117 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-fluorophenyl)ethylidene)-N-methylhydrazinecarbothioamide (3i): Yield 55.42%; m.p. (°C) 188-190; Rf: 55.42 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 3.06 (d, J = 4.8 Hz, 3H, CH_3), 4.92 (s, 2H, CH_2), 7.17 (t, J = 9 Hz, 2H, CH-Ar), 7.84 (m, 6H, CH-Ar), 8.58 (d, J = 4 Hz, 1H, NH), 10.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100MHz) δ ppm: 31.12 (CH_3), 33.86 (CH 2), 114.86 (C-Ar), 123.13 (C- Ar), 131.04 (C-Ar), 131.82 (C-Ar), 134.51 (C-Ar), 143.32 (C = N), 161.34 (CF), 167.43 (C C = O), 178.83 (C = S). HRMS: 371.0997 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-methoxyphenyl)ethylidene)hydrazinecarbothioamide (3j): Yield 94.10%; m.p. (°C) 179-181; Rf: 0.34 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 3.71 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.85 (d, J = 8.8 Hz, CH-Ar), 7.80 (m, 6H, CH-Ar), 8.41 (s, 2H, NH2), 10.60 (s, 1H, NH); ^{13} C NMR (DMSO-d_6, 100MHz) δ ppm: 33,69 (CH_2), 55,13 (CH_3-O), 113,53 (C-Ar), 123,32 (C-Ar), 127,57 (C-Ar), 128,46(C-Ar), 131,11 (C-Ar), 134,76 (C-Ar), 144,48 (C=N), 160,14 (C-O), 167,48 (C=O), 179,05 (C=S). HRMS: 369.1122 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-methoxyphenyl)ethylidene)-N-phenylhydrazinecarbothioamide (3k): Yield 96.67%; m.p. (°C) 219-221; Rf: 0.80 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400MHz), δ ppm: 3.73 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 6.88 (d, J = 8.8 Hz, 2H, CH-Ar), 7.22 (m, 1H, CH-Ar), 7.39 (m, 2H, CH- Ar), 7.59 (d, J = 7.6 Hz, , 7.85 (m, 6H, CH-Ar), 10.09 (s, 1H, NH), 10.92 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100MHz) δ ppm: 33.90 (CH_2), 55.24 (CH_3 -O), 113.54 (C-Ar), 123.35 (C-Ar), 125.73 (C-Ar), 127.39 (C-Ar), 128.05 (C-Ar), 128.22 (C-Ar), 128.71 (C-Ar), 129.04 (C-Ar), 131.12 (C-Ar), 134.78 (C-Ar), 139.03 (C-Ar), 147.38 (C = N), 160.31 (C-Ar), 167.51 (C = O), 177.12 (C = S). HRMS: 445.1262 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-methoxyphenyl)ethylidene)-N-methylhydrazinecarbothioamide (3l): Yield 77.77%; m.p. (°C) 189-191; Rf: 0.76 (hexane/ethyl acetate 7:3). NMR ^{1}H (DMSO-d_6, 400MHz), δ ppm: 3.05 (d, J = 4.4 Hz, 3H, CH_3), 3.31 (s, 3H, CH_3), 4.88 (s, 2H (M, 2H, CH-Ar), 10.54 (m, 6H, CH-Ar), 8.4 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 100MHz) δ ppm: 31.13 (CH 3), 30.70 (CH_2), 55.11 (CH 3 -O) 113.51 (C-Ar), 123.16 (C-Ar)), 127.64 (C-Ar), 128.42 (C-Ar), 131.10 (C-Ar), 138.30 (C-Ar), 144.28 (C = (CO), 167.32 (C = O), 178.78 (C = S). HRMS: 383.1166 $[M+H]^+$.

2-(2-(4-nitrophenyl)-2-oxoethyl)isoindoline-1,3-dione (3m): Yield 91.29%; m.p. (°C) 217-218; Rf: 0.38 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6, 300MHz), δ $_{ppm}$: 5.00 (s, 2H, CH_2), 7.61 (m, 2H, CH-Ar), 7.89 (m, 2H, CH-Ar), 8,26 (m, 2H, CH-Ar), 8.58 (m, 2H, CH-Ar), 8.76 (s, 2H, NH_2), 10.79 (s,1H, NH). ^{13}C NMR (DMSO-$_{d6}$, 75MHz) δ $_{ppm}$: 44.71 (CH_2), 123.31 (C-Ar), 128.32 (C-Ar), 130.76 (C-Ar), 131.44(C-Ar), 134.47 (C-Ar), 137.18 (C-Ar), 141.87 (C=N), 147.88 (C-Ar), 167.53 (C=O), 179.45 (C=S). HRMS: 384.0742 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-nitrophenyl)ethylidene)-N-phenylhydrazinecarbothioamide (3n): Yield 94.81%; %; m.p. (°C) 211-213; Rf: 0.44 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-$_{d6}$, 400MHz), δ $_{ppm}$: 5.08 (s, 2H, CH_2), 7.24 (m, 1H, CH-Ar), 7.41 (m, 2H, CH-Ar), 7.54 (d, J=7,2 Hz, 2H, CH-Ar), 7.85 (m, 4H, CH-Ar), 8.17 (d, J=8,4 Hz, 2H, CH-Ar), 8.39 (d, J=8 Hz, 2H, CH-Ar), 10.33 (s, 1H, NH), 11,12 (s, 1H, NH). ^{13}C NMR (DMSO-$_{d6}$, 100MHz) δ $_{ppm}$: 33.96 (CH_2), 121.95 (C-Ar), 123.38 (C-Ar), 125.74 (C-Ar), 126.21 (C-Ar), 128.17 (C-Ar), 129.61 (C-Ar), 131.16 (C-Ar), 133.65 (C-Ar), 134.80 (C-Ar), 137.05 (C-Ar), 139.00 (C-Ar), 142.78 (C=N), 147.87 (C-N), 167.63 (C=O), 177.65 (C=S). HRMS: 460.0442 $[M+H]^+$.

(E)-2-(2-(1,3-dioxoisoindolin-2-yl)-1-(4-nitrophenyl)ethylidene)-N-methylhydrazinecarbothioamide (3o): Yield 86.97%; m.p. (°C) 148-149; Rf: 0.4 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-$_{d6}$, 400MHz), δ $_{ppm}$: 3.07 (d, J=3.6 Hz, 3H, CH_3), 5.00 (s, 2H, CH_2), 7.81 (m, 4H, CH-Ar), 8.16 (d, J=8.4Hz, 2H, CH-Ar), 8.32 (d, J=7.6 Hz, 2H, CH-Ar), 8.74 (d, J=3.6 Hz, 1H, NH_2), 10.81 (s, 1H, NH). ^{13}C NMR (DMSO-$_{d6}$, 100MHz) δ $_{ppm}$: 31.26 (CH_3), 33.76 (CH_2), 123.34 (C-Ar), 129.58 (C-Ar), 131.45 (C-Ar), 133.35 (C-Ar), 134.74 (C-Ar), 137.29 (C-Ar), 141.89 (C=N), 147.89 (C-Ar), 167.57 (C=O), 178.88 (C=S). HRMS: 398.0510 $[M+H]^+$.

(E)-2-(1-(3,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)hydrazinecarbothioamide (3p). Yield 94.5%; m.p. (°C) 233-234; Rf: 0,56 (ethyl acetate/hexane 3:7) ^{1}H NMR (DMSO-d_6 400MHz),, δ $_{ppm}$: 4.91 (s, 2H, CH_2), 7,57 (d, J=8.4 Hz, 1H, CH-Ar), 7.84 (m, 4H, CH-Ar), 8.18 (s, 1H, CH-Ar), 8.27 (s, 1H, CH-Ar), 8.54 (s, 2H, NH_2), 10.71 (s,1H, NH). ^{13}C NMR (DMSO-d_6. 100MHz) δ $_{ppm}$: 33.44 (CH_2), 123.32 (C-Ar), 126.90 (C-Ar), 128.83 (C-Ar), 130.09 (C-Ar), 131.10 (C-Ar), 131.21 (C-Ar), 131.70 (C-Ar), 134.74 (C-Cl), 136.03 (C-Cl), 141.53 (C=N), 167.55 (C=O), 179.33 (C=S). HRMS: 406.9683 $[M+H]^+$.

(E)-2-(1-(3,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-phenylhydrazinecarbothioamide (3q). Yield 90.99; m.p. (°C) 214-216; Rf: 0.82. (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 400MHz), δ $_{ppm}$: 4.99 (s, 2H, CH_2), 7.30 (m, 1H, CH-Ar), 7.40 (m, 2H, CH-Ar), 7.56 (m, 3H, CH-Ar), 7.86 (m, 6H, CH-Ar); 10.29 (s, 1H, NH), 11.05 (s, 1H, NH). ^{13}C NMR (DMSO-d_6 100MHz) δ $_{ppm}$: 33.76 (CH_2), 123.38 (C-Ar), 125.76 (C-Ar), 126.40 (C-Ar), 127.21 (C-Ar), 128.14 (C-Ar), 129.98 (C-Ar), 130.15 (C-Ar), 131.16 (C-Ar), 131.88 (C-Ar), 134.81 (C-Ar), 135.92 (C-Ar), 139.03 (C-Ar), 142.47 (C=N), 167.63 (C=O), 177.60 (C=S). HRMS: 483.0058 $[M+H]^+$.

(E)-2-(1-(3,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-methylhydrazinecarbothioamide (3r): Yield 59,52; m.p. (°C) 209-210; Rf: 0,78. (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6. 400MHz), δ $_{ppm}$: 3.07 (d, J=4.8Hz, 3H, CH_3), 4.91 (s, 2H, CH_2), 7.59 (d, J=8,4 Hz, 1H, CH-Ar), 7.82 (m, 4H, CH-Ar), 8.15 (d, J=2 Hz, 2H, CH-Ar), 8.71 (d, J=4.4 Hz, 1H, NH), 10.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6. 100MHz) δ $_{ppm}$: 31.27 (CH_3),

33.52 (CH_2), 123.35 (C-Ar), 126.96 (C-Ar), 128.78 (C-Ar), 130.18 (C-Ar), 131.13 (C-Ar), 131.19 (C-Ar), 131.67 (C-Cl), 134.78 (C-Cl), 141.43 (C=N), 167.60 (C=O), 178.79 (C=S). HRMS: 421.0617 $[M+H]^+$.

(E)-2-(1-(2,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)hydrazinecarbothioamide (3s): Yield 64.10%; m.p. (°C) 194-195; Rf: 0.52 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 400MHz), δ_{ppm}: 3.32 (s, 2H, CH_2), 7.30 (d, *J*=8,4 Hz, 1H, CH-Ar), 7.47 (d, *J*=8 Hz, 1H, CH-Ar), 7.69 (s, 1H, CH-Ar), 7.84 (m, 4H, CH-Ar), 8.32 (s, 1H, NH), 10.04 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6 100MHz) δ_{ppm}: 42.52 (CH_2), 123.21 (C-Ar), 128.02 (C-Ar), 129.42 (C-Ar), 129.84 (C-Ar), 131.35 (C-Ar), 132.70 (C-Ar), 134.58 (C-Ar), 135.23 (C-Ar), 142.00 (C=N), 167.19 (C=O), 179.10 (C=S). HRMS: 406.9683 $[M+H]^+$.

(E)-2-(1-(2,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-phenylhydrazinecarbothioamide (3t): Yield 81.47%; m.p. (°C) 177-179; Rf: 0.88 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 400MHz), δ_{ppm}: 4.75 (s, 2H, CH_2), 7.16 (1H, CH-Ar), 7.32 (m, 2H, CH-Ar), 7.44 (m, 2H, CH-Ar), 7.56 (m, 2H, CH-Ar), 7.71 (s, 1H, C-Ar), 7.85(m, 4H, C-Ar). ^{13}C NMR (DMSO-d_6 100MHz) δ_{ppm}: 42.54 (CH_2), 123.31 (C-Ar), 125.35 (C-Ar), 127.00 (C-Ar), 127.98 (C-Ar), 128.07 (C-Ar), 128.64 (C-Ar), 129.82 (C-Ar), 131.04 (C-Ar), 131.34 (C-Ar), 132.81 (C-Ar), 133.90 (C-Ar), 134.70 (C-Ar), 135.31 (C-Ar), 138.71 (C-Ar), 142.25 (C=N), 167.26 (C=O), 177.61 (C=S). HRMS: 483.0327 $[M+H]^+$.

(E)-2-(1-(2,4-dichlorophenyl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-methylhydrazinecarbothioamide (3u): Yield 62.69; m.p. (°C) 204-205; Rf: 0,72 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 . 400MHz), δ_{ppm}: : 2,95 (d, *J*=4.4Hz, 3H, CH_3), 4.89 (s, 2H, CH_2), 7.27 (s, 1H, CH-Ar), 7.43 (d, *J*= 2Hz, 1H, CH-Ar), 7.65 (d, *J*=2 Hz, 1H, CH-Ar), 7.83 (m, 4H, CH-Ar), 8,45 (d, *J*=4.8 Hz, 1H, NH), 9,95 (s, 1H, NH). ^{13}C NMR (DMSO-d_6. 100MHz) δ_{ppm}: 31.07 (CH_3), 42.79 (CH_2), 123.22 (C-Ar), 127.01 (C-Ar), 128,58 (C-Ar), 129,36 (C-Ar), 131,32 (C-Ar), 13,53 (C-Ar), 133,13 (C-Ar), 134. 06 (C-Ar), 134.59 (C-Ar), 141.,37 (C=N), 167.19 (C=O), 179.15 (C=S). HRMS: 421.0082 $[M+H]^+$.

(E)-2-(1-([1,1'-biphenyl]-4-yl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)hydrazinecarbothioamide (3v). Yield 78.02; m.p. (°C) 232-234; Rf: 0,46 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 . 400MHz), δ_{ppm}: 4.96 (s, 2H, CH_2), 7.45 (m, 1H, CH-Ar), 7.63 (m, 4H, CH-Ar), 7.70 (d, *J*=7.6 Hz, 4H, CH-Ar), 7.81 (m, 2H, CH-Ar) 7.92 (d, *J*=78.4 Hz, 2H, CH-Ar), 9,21 (s, 2H, NH_2), 10.69 (s, 1H, NH). ^{13}C NMR (DMSO-d6. 100MHz) δ_{ppm}: 42.57 (CH_2), 123.35 (C-Ar), 126.28 (C-Ar), 127.52 (C-Ar), 128.92(C-Ar), 129.82 (C-Ar), 131.46 (C-Ar), 134.53 (C-Ar), 140.70 (C-Ar), 141.50 (C=N), 143.93 (C-Ar), 146.81 (C-Ar), 167.54 (C=O), 179.30 (C=S). HRMS: 415.1059 $[M+H]^+$.

(E)-2-(1-([1,1'-biphenyl]-4-yl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-phenylhydrazinecarbothioamide (3w): Yield 69.83; m.p. (°C) 203-205; Rf: 0.82 (ethyl acetate/hexane 3:7). ^{1}H NMR (DMSO-d_6 400MHz), δ_{ppm}: 5.04 (s, 2H, CH_2), 7.23 (m, 2H, CH-Ar), 7.41 (m, 4H, CH-Ar), 7.66 (m, 6 H, CH-Ar), 7.86 (m, 4H, CH-Ar), 8,00 (d, *J*= 8Hz, 2H, CH-Ar), 10.20 (s, 1H, CH-Ar), 11.03 (s, 1H, CH-Ar). ^{13}C NMR (DMSO-d_6 100MHz) δ_{ppm}: 33.96 (CH_2), 123.35 (C-Ar), 125.50 (C-Ar), 125.87 (C-Ar), 126.28 (C-Ar), 126.71 (C-Ar), 127.76 (C-Ar), 128.10 (C-Ar), 128.25 (C-Ar), 128.92 (C-Ar), 129,03(C-Ar), 131,51(C-Ar), 134.21 (C-Ar), 134.78 (C-Ar), 139,13 (C-Ar), 140,85 (C-Ar), 144.78 (C=N), 167.57 (C=O), 177.34 (C=S). HRMS: 491,1315 $[M+H]^+$.

(E)-2-(1-([1,1'-biphenyl]-4-yl)-2-(1,3-dioxoisoindolin-2-yl)ethylidene)-N-methylhydrazinecarbothioamide (3x): Yield 85.37; m.p. (°C) 241-243; Rf: 0.72 (ethyl acetate/hexane 3:7). ^{1}H NMR H (DMSO-d_6. 300MHz), δ_{ppm}: 3.08 (d, *J*=4.4Hz, 3H, CH_3), 4.95 (s, 2H, CH_2), 7.36 (m, 1H, CH-Ar), 7.46 (m, 2H, CH-Ar), 7.53 (m, 2H, CH-Ar), 7.65 (m, 2H, CH-Ar), 7.82 (m, 2H, CH-Ar), 7.91 (m, 2H, CH-Ar), 7.97 (m, 2H, CH-Ar), 8.60 (d, *J*=4.4 Hz, 1H, NH), 10,70 (s, 1H, NH). ^{13}C NMR (DMSO-d6. 100MHz) δ_{ppm}: 31.19(CH_3), 33.19 (CH_2), 123.30 (C-Ar), 126.26 (C-Ar), 126.53 (C-Ar), 127.48 (C-Ar), 127.69 (C-Ar), 128.65 (C-Ar), 131.13 (C-Ar), 134.44 (C-Ar), 139.14 (C=N), 140.63 (C-Ar), 143.70 (C-Ar), 167.51 (C=O), 178.87 (C=S). HRMS: 429.1289 $[M+H]^+$.

Synthesis of compounds 4a-i: 500mg (14.8mmol) of compound **3a** was solubilized in 20mL of isobutanol at reflux, and different acetophenones were added as shown in **table 2**, after 6h a plate was made, and the reaction was confirmed to have come to an end as soon as possible. Then the product was filtered and washed with ethanol and taken to the desiccator for subsequent reaction yield.

(E)-2-(2-(2-(4-(3-nitrophenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4a): Yield 81.25%; m.p.(°C) 198-202; Rf: 0.45 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 4.70 (s, 2H, CH_2), 5.02 (s, 1H, N-H); 7.43 (s, 1H, C_5-$H_{thiazole}$); 7.53 (s, 1H, CH_{ar}); 8.74-7.72 (m, 12H, CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 34.24 (-CH_2); 107.16 (C5-thiazole); 143.94 (C=N), 167.56 (C=O), 167.28 (C=O), 169,93 (C2-thiazole), 148,28 (C4-thiazole), 136.10 (CH-Ar), 135.42 (CH-Ar), 134.7 (CH-Ar), 123.25 (CH-Ar), 122.04 (CH-Ar), 121.90 (CH-Ar), 119.94 (CH-Ar), 119.80 (CH-Ar), 131.07-126.0 (CH-Ar). HRMS: 483.5244 $[M-H]^+$.

(E)-2-(2-(2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4b): Yield 94.80%; m.p. (°C) 174-178; Rf: 0.40 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 5.01 (s, 2H, CH_2); 3.79 (t, 3H, CH_3); 7.17ppm (C_5-H-thiazole); 5.01ppm (N-H); 7.80 -7.6 (m, 12H, C-H Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 169.82 31 (C2-thiazole), 167.63 (C=O), 167.40 (C=O), 159,03 (C4-thiazole); C=N (144.28); 101.95 (C5-thiazole); 55.62 (CH_2); 34.06 (CH_3); 135.54 (C-OCH_3); 134.69 – 12.318 (CH-Ar). HRMS: 466.5547 $[M-H]^+$.

(E)-2-(2-(2-(4-(3,4-dichlorophenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4c): Yield 54.32%; m.p. (°C) 189-193; Rf: 0.66 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 4.69 (s, 2H, CH_2); 5.00 (s, 1H, N-H); 7.33 (s, 1H, C_5-H-thiazole); 7.51 (s, 1H, CH-Ar); 7.30 – 7.58 (m, 12H, CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 167.56; 167.30] (2C=O); 169,03 (C2-thiazole); 106.72 (C5-thiazole); 144.01 (C4-thiazole); 42,35 (CH_2); 153.96 (C=N); 135.65 – 123.25 (CH-Ar). HRMS: 507.6055 $[M-H]^+$.

(E)-2-(2-(2-(4-(4-fluorophenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4d): Yield 50.88%; m.p. (°C) 180-184; Rf: 0.59 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 4.70 (s, 2H, CH_2); 5.01 (s, 1H, N-H); 7.07 (s, 1H, C5-thiazole); 7.84 - 7.13 (CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 169,61 (C2-thiazole); 167.57; 157.31 (2C=O); 163.10 (C4-thiazole); 159.88 (C2-thiazole); 144.70 (C=N); 103.35 (C6-thiazole); 42.34 (CH_2); 134.62 – 115.19 (CH-Ar). HRMS: 457.0678 $[M-H]^+$.

(E)-2-(2-(2-(5-methyl-4-phenylthiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4e): Yield 53.81%; m.p. (°C) 226-230; Rf: 0,66 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 3,59 (s, 3H, CH_3); 4.97 (s, 2H, CH_2); 7.80 (s, 1H, N-H); 7.90 – 7.27 (m, 14H, CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 175.22 (C2-thiazole); 169.35; 167.83 (2C=O); 144.44 (C=N); 163.,38; (C4-thiazole); 110,27 (C5-thiazole); 62.75 (CH_2); 34.46 (CH_3), 136.22 – 123.17 (CH-Ar). HRMS: 452.0856 $[M-H]^+$.

(E)-2-(2-(2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4f): Yield 27.73%; m.p. (°C) 165-270; Rf: 0.79 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 5.04 (s, 2H, CH_2); 7.28 (s, 1H, C5-thiazole); 8,43 (s, 1H, NH); 8,05 – 7.34 (m, 12H, CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 169.76 (C2-thiazole); 167.33 and 167.30 (2C=O); 149.72 (C4-thiazole); 144.05 (C=N); 106.03 (C5-thiazole); 61.90 (CH_2); 135.54 – 123.25 (CH-Ar). HRMS: 506.6013 $[M-H]^+$.

(E)-2-(2-(2-(4-(naphthalen-2-yl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4g): Yield 52.33%; m.p. (°C) 226-228; Rf: 0,72 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6. 400 MHz), δ_{ppm}: 4.70 (s, 2H, CH_2); 5.03 (s, 1H, N-H); 7.36 (s, 1H, C5-thiazole); 7.53 (s, 1H, CH-Ar); 8.10 – 7.23 (m, 15H, CH-Ar). ^{13}C NMR (DMSO-d_6. 100 MHz), δ_{ppm}: 169.77 (C2-thiazole); 167.56 e 167.33 (2C=O); 144.01 (C4-thiazole);

144.75 (C=N); 106.11 (C5-thiazole); 42.19 (CH_2); 133.09 – 123.00 (CH-Ar). HRMS: 488.6852 $[M-H]^+$.

2-((E)-2-((Z)-(4-(4-nitrophenyl)thiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4h). Yeld 83.29%; m.p. (°C): 218-221; Rf: 0.72(hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 4.7 (s, 2H, CH_2); 5.01 (s, 1H, N-H); 7.6 (s,1H, C5-thiazole); 7.3 – 7.5 (m, 5H, CH-Ar); 7.8 - 8.3 (m, 9H, CH-Ar). ^{13}C NMR (DMSO-d_6, 100 MHz), δ_{ppm}: 169,94 (C2-thiazole); 167.56 e 167.31 (2C=O); 146.16 (C4-thiazole); 144.74 (C=N); 108,96 (C5-thiazole); 25.45 (CH_2); 134.70 – 123.21 (CH-Ar). HRMS: 483.0395 $[M-H]^+$.

(E)-2-(2-(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4i): Yield 72.85%; m.p. (°C): 220 - 224; Rf: 0.72(hexane/ethyl acetate 7:3). ^{1}H NMR (*DMSO*-d_6, 400 MHz), δ_{ppm}: 4.69 (s, 2H, CH_2); 5.00 (s, 1H, N-H); 7.16 (s,1H, C5-thiazole); 7.27 – 7.95 (m, 13H, CH-Ar). ^{13}C NMR (DMSO-d_6, 100 MHz), δ_{ppm}: 169,71 (C2-thiazole), 167.60 (C=O), 167.30 (C=O), 135.51 (C4-thiazole), 134.71 (C=N), 104.83 (C5-thiazole), 34.09 (CH_2), 134.64 – 123.22 (CH-Ar). HRMS: 473.0412 $[M-H]^+$.

Synthesis of compounds 4j-n: 500mg (14.8mol) of compound 3b was solubilized in 20mL of isobutanol at reflux, and different acetophenones were added as shown in table 2, after 6h a plate was made and it was confirmed that the reaction had come to an end shortly after the product was filtered and washed with ethanol and taken to the desiccator for subsequent reaction yield.

2-((E)-2-((Z)-(4-(4-methoxyphenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4j): Yield 34.55% ; m.p. (°C) 214-216; Rf: 0.70 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 3.69 (s, 3H, CH_3); 4.82 (s, 2H, CH_2); 6.55 (s, 1H, C5-thiazole) 6.55, 7.74 – 6.78 (m, 14H, CH-Ar). ^{13}C NMR (DMSO-d_6, 100 MHz), δ_{ppm}: 167.64 (C2-thiazole), 159.50 (C=O), 159.40 (C=O), 153.69 (C4-thiazole), 139.99 (C=N), 101.33 (C5-thiazole), 55.54 (CH_2), 35.93 (CH_3), 133.64 – 123.44 (CH-Ar). HRMS: 544.9669 $[M-H]^+$.

2-((E)-2-((Z)-(4-(3-nitrophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4k): Yield 59.70%; m.p. (°C): 179-181; Rf: 0.75 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 4.84 (s, 2H, CH_2), 6.72 (s,1H,C5-thiazole), 6.96 (s, CH-Ar), 8,10 – 7.23 (m, 17H, CH-Ar). ^{13}C NMR DMSO-d_6, 100 MHz), δ_{ppm}: 170.13 (C2-thiazole), 167.75 (C=O), 167.00 (C=O), 153.84 (C4-thiazole), 148.59 (C=N), 104.67 (C5-thiazole), 41.45 (CH_2), 137.32 – 122.54 (CH-Ar). HRMS: 559.9842 $[M-H]^+$.

2-((E)-2-((Z)-(4-(4-fluorophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4l): Yield 59,65%; m.p. (°C): 213-216); Rf: 0.86 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 4.73 (s, 2H, CH_2); 6. (s,1H, C5-thiazole); 7.74 – 7.04 (m, 19H, CH-Ar). ^{13}C NMR (DMSO-d_6, 100 MHz), δ_{ppm}: 170.42 (C2-thiazole); 167.40 (C=O), 167.00 (C=O), 160.24 (C2-thiazole), 153.58 (C=N), 138.58 (C4-thiazole), 102.23 (C5-thiazole), 35.51 (CH_2), 137.12 – 115.01 (C-Ar). HRMS: 532.8695$[M-H]^+$.

2-((E)-2-((Z)-(4-(naphthalen-2-yl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4m). Yield 81.64%; m.p. (°C): 225 - 228; Rf: 0.84 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 4.85 (s, 2H, CH_2); 6.80 (s,1H, C5-thiazole), 7.12 – 7.83 (m, 21H, CH-Ar). ^{13}C NMR (DMSO-d$_6$, 100 MHz), δ_{ppm}: 170.45 (C2-thiazole), 167.05 (C=O), 167.40 (C=O), 153.49 (C4-thiazole), 139.65 (C=N), 102.75 (C5-thiazole), 35.5 (CH_2), 137.33 – 122.96 (CH-Ar). HRMS: 565.0973 $[M-H]^+$.

2-((E)-2-((Z)-(4-(4-nitrophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)-2-phenylethyl)isoindoline-1,3-dione (4n). Yield 96.74%; m.p. (°C): 250 - 252; Rf: 0.81 (hexane/ethyl acetate 7:3). ^{1}H NMR (DMSO-d_6, 400 MHz), δ_{ppm}: 4.82 (s, 2H, CH_2), 6.75 (s,1H, C5-thiazole), 7.18 – 8,08 (m, 18H, CH-Ar). ^{13}C NMR (DMSO-d_6, 100 MHz), δ_{ppm}: 167.76 (C2-thiazole), 167.04 (C=O), 167.40 (C=O), 148.92 (C4-thiazole), 146.69 (C=N), 105.32 (C5-thiazole), 41.45 (CH_2), 137.04 – 122.97 (CH-Ar). HRMS: 560.0671 $[M-H]^+$.

Sample preparation: Each compound was solubilized in DMSO (Sigma-Aldrich) to obtain a final concentration of 5 μM. Intermediate dilutions were then prepared in RPMI-1640 (Invitrogen ™) supplemented with AlbuMAXII (Invitrogen ™).

Biological assays of toxicity to macrophage RAW264.7 lineage cells: *RAW264.7* macrophages were seeded in 96-well plates containing complete DMEM medium in a 5% CO2 atmosphere at 37ºC for 24h. Subsequently, the compounds were added at different concentrations (6.25μg/ml; 12.5μg/ml; 25μg/ml; 50μg/ml; 100μg/ml, 200μg/ml) and incubated again for 48 h. Each compound was tested in duplicate.

After this period, MTT (5mg / mL in PBS) was added, followed by further incubation for 2h. DMSO was added to dissolve the formazan crystals, and the absorbance was read at 570nm. Negative reaction control was obtained in wells containing only culture medium and cells (untreated). The activity of the benznidazole reference drug was also evaluated. From the culture inhibition values, the CC_{50} was obtained by simple linear regression using GraphPad Prism 5.0 software.

Toxicity to trypomastigotes: Strain Y trypomastigotes were collected from L929 cell supernatants and distributed in a 96 well plate to a final density of 4 x 10^5 cells per well. Each chemical inhibitor was added to the wells in triplicate. Benznidazole was used as a positive control in this assay. The plate was then cultivated for 24 h at 37 ºC and 5% CO_2. After this time, aliquots from each well were collected, and the number of viable parasites (i.e., with apparent motility) was counted in a Neubauer chamber. The wells that did not receive the chemical inhibitors were assumed as 100% the number of viable parasites. The dose-response curves were determined, and the IC_{50} values were calculated by nonlinear regression (Prism, version 4.0) using at least seven concentrations (data points).

Plasmodium falciparum in vitro culture: Laboratory-adapted *P. falciparum* line 3D7-GFP (MRA-1029, MR4. ATCC® Manassas Virginia), a chloroquine-sensitive strain, was continuously cultured using the method of Trager and Jensen, with previously described modifications [29]. Parasites were cultivated at 5% hematocrit, 37°C and atmosphere with 5% of CO_2, human serum was replaced with 0.5% AlbuMAXII (Invitrogen™) in the culture medium. Synchronized cultures were obtained by treatments with a 5% (m/v) solution of D-sorbitol (Sigma-Aldrich) [30].

Antimalarial activity primary screening using flow cytometry: All compounds were screened for their *in vitro* antimalarial activity against chloroquine-susceptible 3D7 strain GFP expressing *P. falciparum* (3D7-GFP). Unsynchronized culture with 2% hematocrit and 1% parasitaemia, was incubated in a 96-well flat-bottom plate with 1uM and 10uM of each compound for 72h (37 °C and 5% CO2). Each plate also included growth controls wells: no drug added and 5nM and 500 nM of chloroquine. Parasite growth was assessed by flow cytometry (Beckman Coulter, Cytoflex) with a 96-well plate reader, using Fl-1 (green fluorescent protein [GFP]; excitation wavelength, 488 nm). Typically, 20,000 to 40,000 RBCs were counted for each well. Samples were analyzed using FlowJo software (Tree Star Inc.).

Dose-response assay: IC_{50} estimation: Compounds exhibiting over 70% growth inhibition in the primary screening assay, were selected as active and confirmed in the dose-response assay to estimate the correspondent IC_{50}. Unsynchronized culture with 2% hematocrit and 1% parasitaemia, was incubated with the tested compounds in 3-fold serial dilutions ranging from 10 to 0.014 μM. After 72h at 37 °C and 5% CO_2, parasite growth was assessed by flow cytometry as described for the antimalarial activity primary screening assay. Half-maximal inhibitory concentrations (IC_{50}) were determined with GraphPad Prism 5 (trial version). At least three experiments, each in duplicate, were performed to obtain the mean IC_{50} presented.

Cell death assessment for T. cruzi: After confirmation of trypanocidal activity, Annexin-FITC/Propidium Iodide labelling was used to characterize cell death modalities induced by incubation with compounds. Metacyclic trypomastigotes were collected from the supernatant of infected L929 cells and then seeded at $4x10^5$ cells/well in RPMI-1640 medium. All compounds were dissolved in DMSO and added to wells at IC_{50} and $2xIC_{50}$ concentrations. Benznidazole (IC_{50} and $2x\ IC_{50}$) and culture medium were used as the positive and negative control, respectively. Plates were incubated at the same conditions used for anti-trypomastigote activity (37ºC, 24h, and 5% CO_2). Briefly, after treatment, parasites were washed with PBS and resuspended in binding buffer (Annexin V Binding Buffer-BD Pharmingen™, USA). For labelling, 10 μL of propidium iodide (50 μg/mL) and 5 μL of Annexin-FITC (BD Pharmingen™, USA) were added for 15 min, at room temperature, in the dark. Flow cytometry was conducted on FACSCalibur (Becton & Dickinson, USA). For each sample, we acquire 20,000 events, and the data were analyzed using Cell Quest software (Becton & Dickinson, USA). Assays were conducted in triplicate. For significance analysis was used ANOVA and Dunnett's test, considering $p < 0.05$.

Ultrastructural studies: The parasites were cultured for 24 h in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) buffered to pH 7.5 and supplemented with HEPES (20 mM), 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL) containing the compound at the IC_{50} concentration and twice the value of IC_{50}. The parasites were collected, washed in PBS, and fixed with 2.5% glutaraldehyde, 4% formaldehyde, and 0.1 M cacodylate buffer at pH 6.8. They were then postfixed in 2% osmium tetroxide (OsO4) in a 0.1 M cacodylate buffer at pH 6.8 and processed for routine scanning electron microscopy (SEM). The parasites were dehydrated in graded ethanol and dried by the critical point method with CO_2. The samples were mounted on aluminium stubs, coated with gold, and examined under a JEOL-5600LV microscope.

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GRAPHICAL ABSTRACT