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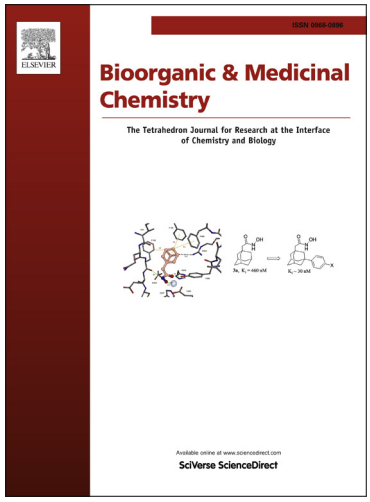

Synthesis of 3-tetrazolylmethyl-4*H*-chromen-4-ones via Ugi-azide and biological evaluation against *Entamoeba histolytica*, *Giardia lamblia* and *Trichomona vaginalis*

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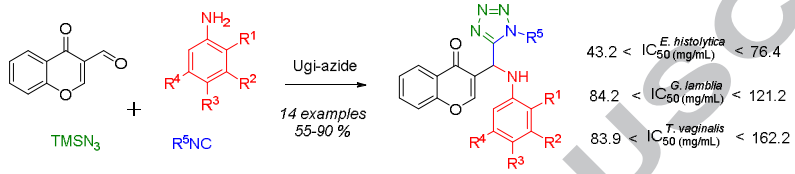

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ABSTRACT

The synthesis of novel 3-tetrazolylmethyl-4*H*-chromen-4-ones via an Ugi-azide multicomponent reaction and their biological evaluation against *Entamoeba histolytica*, *Giardia lamblia* and *Trichomona vaginalis* are described. Reported yields are moderate to good and biological results show that these compounds could be considered as candidates to anti-parasitic drugs, especially against *Giardia lamblia*.

1. Introduction

Parasitic infections are some of the most common human health problems and are often caused by contaminated food or water. Infections caused by intestinal protozoa are responsible for high morbidity and mortality worldwide. The human intestine is a major target for these pathogenic microorganisms resulting in severe infections such as dysentery and diarrhea.[1]

The parasitic disease known as amebiasis, which is caused by the *Entamoeba histolytica* (*a*, Fig. 1) is actually the second leading cause of death by parasites.[2] The *Giardia lamblia* (*b*, Fig. 1) causes the giardiasis, a highly contagious disease which can lead to death.[3] The *Trichomona vaginalis* (*c*, Fig. 1) causes the trichomoniasis, which together with the candidiasis are the most common women's sexually transmitted infections.[4] These three anaerobic protozoa, despite the significant differences in their life-cycle and pathogenic properties are customarily grouped together based on their carbohydrate metabolism and lack of mitochondria.[5]

Metronidazole (*d*, Fig. 1) is a 5-nitroimidazole that shows activity against anaerobic microorganisms, which has been considered as the drug of choice against protozoan infections including giardiasis and amoebic dysentery. For decades, the use of metronidazole has been noteworthy for its efficiency. However, potential carcinogenic, teratogenic, embryogenic effects and clinical and laboratory-generated drug-resistance have been described.[6] Prolonged treatment or high doses of metronidazole often cause side effects such as headache, dry mouth, metallic taste, glossitis and urticaria.[1, 3] Because of these undesired side effects and considering the possible development of new metronidazole resistant strains, there is a clear need for new, effective and safer anti-protozoa agents.[1] In an effort to improve a theraphy for giardiasis and amoebic dysentery, the creation of chemical libraries is necessary to obtain novel drugs with high activity combined with low toxicity. In this context, multicomponent reactions (MCR) provide products with the needed diversity for the discovery of new leading compounds. This diversity and easy accessibility to a large number of compounds, combined with throughput screening techniques make MCR a very important synthetic tool in modern drug discovery processes.[7]

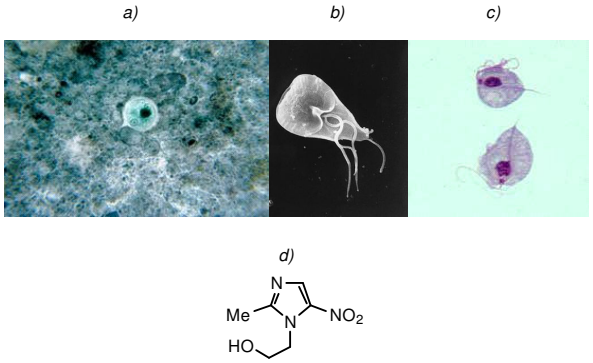


Figure 1.[8] a) *Entamoeba histolytica*, b) *Giardia lamblia*, c) *Trichomona vaginalis*, d) Metronidazole.

Chromen-4-ones are heterocyclic systems present in numerous natural and synthetic products showing interesting biological activities such as anti-asthmatic,[9a] anti-Parkinson's disease,[9b] mild anti-ulcer,[9c] anti-Alzheimer,[9d] anti-viral,[9e] anti-fungic,[9f] anti-tumoral[9g] and anti-HIV.[9h]

Among the most important chromen-4-ones, are the 2- and 3-aryl-chromen-4-ones known as flavones and isoflavones respectively, which often are excellent naturally ocurring antioxidants.[10]

Several 3-substituted-chromen-4-ones are of great relevance in medicinal chemistry because they present a variety of privileged biological activities such as anti-microbial,[11a-c] anti-bacterial,[11b] anti-allergenic,[11d] anti-anaphylactic,[11e,f] anti-tumoral[9g] and anti-cancer,[11g,h] together in some cases with low toxicity.

In this context, the 3-substituted-chromen-4-one formononetin (**2**), which was isolated from the *Dalbergia frutescens*, is a stronger antiparasitic drug than metronidazole against *Giardia intestinalis*[12] (Figure 2).

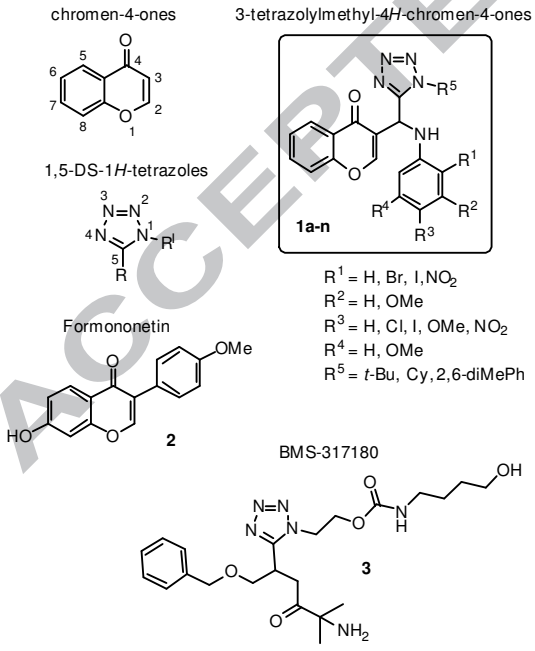


Figure 2. Objective compounds **1a-n**, formononetin (**2**) and the BMS-317180 (**3**).

Some synthetic methodologies have been described to prepare 3-substituted-chromen-4-ones from the chromen-4-one, mainly based on direct C-C couplings such as the Heck reaction,[13a] Pd-catalized cross coupling reaction,[13b] Suzuki-Miyaura,[13c-e] organolithium mediated alkylation[13f-h] and xanthate based alkylation.[13i]

Some 3-substituted-chromen-4-ones and particularly the 3-formyl-chromen-4-one can be efficiently prepared using a Vilsmeier-Haack reaction.[14] This compound is an excellent 1,2 and 1,4 acceptor.[15] There are only three reports using it as starting material in multicomponent reactions.[16]

On the other hand, 1,5-disubstituted-1*H*-tetrazoles (1,5-DS-1*H*-T) are bioisosteres of the *cis*-amide bonds of peptides as a result of the similarities in their physicochemical properties.[17] This kind of heterocyclic systems are the core of several synthetic compounds such as the *in clinical* phase drug BMS-317180 (**3**) (Fig. 2), which was reported by Bristol-Myers Squibb Company as a potent orally available ligand of the growth hormone secretagogue (GHS).[18]

Several reports describe the synthesis of compounds having the 1,5-DS-1*H*-T ring system among the most important are those based on both, [2+3]-dipolar cycloadditions of azides with cyanides[19] and in the Ugi-azide multicomponent reaction.[20]

The synthesized compounds **1a-n** have the 1,5-DS-1*H*-T and the 3-substituted-chromen-4-one moieties. In this context, the Ugi-azide reaction combined with suitably post-condensation processes have been used to prepare various tetrazole containing heterocyclic scaffolds such as ketopiperazine,[21a] azepine,[21b] benzodiazepine,[21c] *bis*-pyrrolidinone,[21d] quinoxaline,[21e] indazoline,[21f] isoindolinone,[21g] amodiaquine,[21h] pyrrolidine,[21i] azepinoindolone[21j] and tetrahydrocarboline-tetrazoles.[21k] In the other hand, several methods have been described for the synthesis of 3-substituted-chromen-4-one containing scaffolds such as phenyl,[22a] *bis*-chromen,[22b] pyrimidin,[22c] indolinone,[22c] pyrazol,[22c] imidazol[22d] and spiropyrolidinone-chromen-4-ones.[16c] It is noteworthy that as far as we known, only three reports describe the synthesis of compounds having the tetrazol-chromen-4-one heterocyclic system based on stepwise synthetic methods.[11b, e, f.]

In this work, we describe the first synthesis of 3-tetrazolylmethyl-4*H*-chromen-4-ones by an Ugi-azide reaction in moderate to good yields (55-90%) under mild conditions (room temperature). Our hypothesis was "compounds having both scaffolds, tetrazole and chromone in the same molecule, will show antiprotozoal activity". As we discussed below, tetrazoles and chromones have been described to be independently active against some parasites such as *E. histolytica* and *Leishmania*. Thus, based on this idea and in the side effects caused by the metronidazole, we are convinced it is really necessary increasing the amount of available antiparasitic compounds that can be prepared in a minimum of steps. The compounds **1a-n** were prepared in only one synthetic procedure with good yields.

2. Results and discussion

*2.1. Synthesis of the 3-tetrazolylmethyl-4*H*-chromen-4-ones*

The synthetic methodology involved a sequential combination of the 3-formylchromen-4-one (**4**) with the anilines **5**, the trimethylsilylazide (**6**) and the isocyanides (**7**) to prepare the series of 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n** (Table 1).

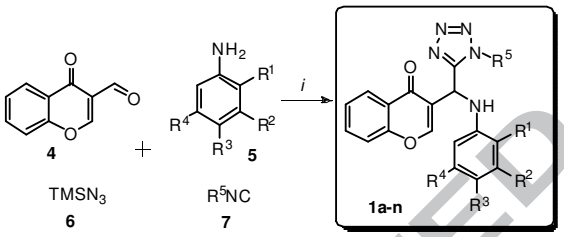
After an exhaustive exploration of the reaction conditions varying the temperature, solvents and acidic catalysts, optimal

parameters to prepare the 3-tetrazolylmethyl-4*H*-chromen-4-one **1a** were found (Table 1).

It is noteworthy that anilines, which commonly are not good nucleophiles, were used as amino component in the Ugi-azide reaction. It has been reported that Lewis acids favor the condensation reactions between anilines with aldehydes or ketones.[23] Thus, a catalytic amount of $InCl_3$ (5% mol) was added to increase the electrophilicity of the 3-formyl-chromone (**4**) promoting its condensation with the substituted anilines **5**. The catalyst-free classic Ugi-azide method usually works very well when good nucleophilic amines were used.[20b-c, 21f,k]

Following with the results and discussion, cyclohexyl isocyanide was used instead of *t*-butyl isocyanide to prepare the compound **1b** in 78% yield. Aniline was used instead of *o*-iodoaniline to prepare the compounds **1c-d** in 86 and 82% yields respectively. *p*-chloro and *p*-iodoanilines were used to prepare the compounds **1e-h** obtaining 72 to 85% yields. Surprisingly, when the poorly nucleophilic *p*-nitroaniline was used to prepare the compound **1i**, 70% yield was observed. When 2,6-dimethylisocyanide was used, product **1j** was obtained in 55% yield. The latter experiment gave us the lowest yield probably due to the high steric hindrance generated by the substituent of the isocyanide (R^5 = 2,5-diMePh). The amine having more ERG, the 2,3,4-trimethoxyaniline was used to prepare the compound **1k** with the highest yield (90%). To complete the series, *o*-bromo and *o*-nitroanilines were used in the Ugi-azide reaction to prepare the products **1l-n** with yields close to 70% (Table 1).

Table 1. Synthesis of the 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n**.



Prod.	R^1	R^2	R^3	R^4	R^5	yield (%)[a]
1a	I	H	H	H	*t*-Bu	85
1b	I	H	H	H	Cy	78
1c	H	H	H	H	*t*-Bu	86
1d	H	H	H	H	Cy	82
1e	H	H	Cl	H	*t*-Bu	76
1f	H	H	Cl	H	Cy	72
1g	H	H	I	H	*t*-Bu	85
1h	H	H	I	H	Cy	79
1i	H	H	NO_2	H	Cy	70
1j	H	OMe	OMe	H	2,6-diMePh	55
1k	H	OMe	OMe	OMe	Cy	90
1l	Br	H	H	H	*t*-Bu	72
1m	Br	H	H	H	Cy	70
1n	NO_2	H	H	H	Cy	71

i) $InCl_3$ (5% mol); *i*-PrOH; R. T.; 2 h

[a] Measured after recrystallization

The 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n** were characterized by spectroscopic analysis: ^{1}H and ^{13}C-NMR, IR and HRMS. Additionally, an adequate crystal of compound **1a** was obtained for X-ray analysis [24] (Figure 3).

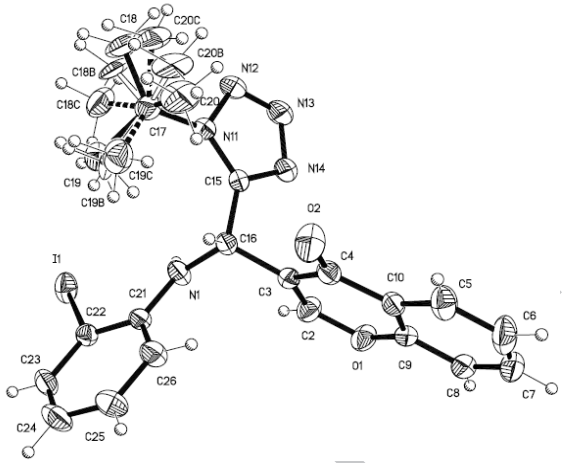


Figure 3. ORTEP diagram of compound **1a**.

2.2. Biological evaluation

In 2008, Diwakar and co-workers designed and synthesized a series of 3-(Z)-tetrazolylvinylchromen-4-one, which showed *in vitro* both, antibacterial (*E. faecalis, S. pneumonia and E. coli*) and antimicrobial (*S. aureus*) activity.[11b]

Diwakar's work[11b] prompted us to synthesize our novel 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n**, which were biologically tested against *E. histolytica*, *G. lamblia* and *T. vaginalis* (Table 2). These unicellular eukaryotes have become in a serious worldwide health problem because they have been able to resist the majority of the currently known drugs such as metronidazole.[25]

Antiprotozoal activity of chromen-4-ones[26a-c] and tetrazoles[26d] against parasites such as *Leishmania* has been practically unexplored. In this context, the antiprotozoal activity of tetrazoles[27] particularly against *E. histolytica* has been described only in one report, but the stepwise prepared compounds were obtained in low overall yields. However, as far as we know, this is the first report in which antiprotozoal activity of compounds having both, tetrazole and chromen-4-one heterocyclic scaffolds against three kinds of parasites, *E. histolytica, G. lamblia* and *T. vaginalis* is described.

Biological activity results are depicted in Table 2. The data is presented in terms of the half-maximal inhibitory concentration (IC_{50}). The antiprotozoal activity of the synthesized compounds **1a-n** was compared with the activity of metronidazole (positive control), which is the most widely commercially available antiamoebic drug.[28] A total of 14 compounds were studied. Among these compounds, **1k** which has three methoxy groups in R^2, R^3 and R^4 positions of the amine moiety, showed the best activity against *E. histolytica* (IC_{50} = 43.2 μg/mL) and *G. lamblia* (IC_{50} = 84.2 μg/mL), while **1n** was the most active against *T. vaginalis* (IC_{50} = 83.9 μg/mL). All compounds showed selectivity on *G. lamblia*. However, these values are considerable higher than those presented by metronidazole (IC_{50} = 0.23 μg/mL against *E. histolytica*, IC_{50} = 1.22 μg/mL against *G. lamblia* and IC_{50} = 0.037 μg/mL against *T. vaginalis*) (Table 2).

Table 2. Half Maximal inhibitory concentration of the 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n** against *E. histolytica, G. lamblia and T. vaginalis.*

	IC_{50}[a]					
Product	*E. histolytica*		*G. lamblia*		*T. vaginalis*	
	μg/mL	μM	μg/mL	μM	μg/mL	μM
1a	67.3	134.3	103.8	207.2	140.2	279.8
1b	64.3	122.0	104.1	197.5	148.4	281.6
1c	70.4	187.6	118.7	316.4	162.2	432.3
1d	73.3	182.7	114.7	285.9	150.2	374.4
1e	65.9	161.1	110.0	268.9	151.7	370.8
1f	76.4	175.6	94.9	218.1	123.7	284.3
1g	71.1	141.9	98.7	197.0	123.4	246.3
1h	61.9	117.4	119.4	226.5	116.9	221.8
1i	51.8	116.1	110.2	247.0	91.4	204.9
1j	53.8	111.3	116.2	240.5	92.6	191.6
1k	43.2	87.9	84.2	171.4	86.2	175.5
1l	56.7	125.1	109.8	242.3	106.2	234.4
1m	54.6	114.0	121.2	253.0	93.3	194.7
1n	55.5	124.4	86.5	193.9	83.9	188.0
Met.[b]	0.23	—	1.22	—	0.037	—
For.[c]	—	—	0.03	—	—	—

[a] *In vitro* antiprotozoal activity of compounds **1a-n**. *n = 3*
[b] **Met.** = metronidazole
[c] **For.** = formononetin

2.3. Structure Activity Relationship

The SAR study revealed that the isocyanide substituent (R^5) does not significantly affect to the antiprotozoal activity against *E. histolytica* as can be seen comparing the value for **1a** (R^5 = *t*-Bu, IC_{50} = 67.3 μg/mL) with the value of **1b** (R^5 = Cy, IC_{50} = 64.3 μg/mL) or the value of **1c** (R^5 = *t*-Bu, IC_{50} = 70.4 μg/mL) with the value of **1d** (R^5 = Cy, IC_{50} = 73.3 μg/mL). The same behavior was observed for these compounds against the other two studied protozoa. Another brief variation in the activity against *E. histolytica* could be observed analyzing the effect of the iodine atom position as can be seen comparing the value of **1a** (R^1 = I, IC_{50} = 67.3 μg/mL) with the value of **1g** (R^3 = I, IC_{50} = 71.1 μg/mL) or **1b** (R^1 = I, IC_{50} = 64.3 μg/mL) with **1h** (R^3 = I, IC_{50} = 61.9 μg/mL). Respect to *G. lamblia*, comparing the value of **1a** (R^1 = I, IC_{50} = 103.8 μg/mL) with the value of **1g** (R^3 = I, IC_{50} = 98.7 μg/mL), the difference of the activities was kept to a minimum, but, if a comparison is made between the activity of **1b** (R^1 = I, IC_{50} = 104.3 μg/mL) with the activity of **1h** (R^3 = I, IC_{50} = 119.4 μg/mL), a considerable difference can be observed, which signifies the position of iodine atom really affect to *G. lamblia* when R^5 = Cy. A comparison of values for both, **1a** (R^1 = I, IC_{50} = 140.2 μg/mL) with **1g** (R^3 = I, IC_{50} = 123.4 μg/mL) and **1b** (R^1 = I, IC_{50} = 148.4 μg/mL) with **1h** (R^3 = I, IC_{50} = 116.9 μg/mL) allows to observe a considerable difference in the activity against *T. vaginalis*. Another revelation based on the SAR study can be seen by analyzing the effect of the substitution of chlorine by iodine in position R^3. The value for the activity against *E. histolytica* of compound **1e** (R^3 = Cl, IC_{50} = 65.9 μg/mL) respect to the value of **1g** (R^3 = I, IC_{50} = 71.1 μg/mL) gave a small advantage for the compound having chlorine. This behavior is reversed making a comparison between the values of compound **1e** (R^3 = Cl, IC_{50} = 110.0 μg/mL) with the value of compound **1g** (R^3 = I, IC_{50} = 98.7 μg/mL) against *G. lamblia* and also between the values of compound **1e** (R^3 = Cl, IC_{50} = 151.7 μg/mL) with the value of compound **1g** (R^3 = I, IC_{50} = 123.7 μg/mL) against *T. vaginalis*, in which an advantage for the iodine-containing compounds is observed. Another interesting SAR study was made analyzing the effect of the high electronegative nitro group in R^3 and in R^1 positions. Thus, the activity of **1i** (R^3 = NO_2, IC_{50} = 110.2 μg/mL) is lower than the activity of **1n** (R^1 = NO_2, IC_{50} = 86.5.4 μg/mL). Based on the SAR study, one of the conclusions is that the *G. lamblia* is the most susceptible parasite to the structural diversity. Other interesting observations derived from the SAR study are described below: *i)* compounds having *o*-bromide atom in the aniline system (**1l** and **1m)** show a considerable better activity than those having *o*-iodine atom in the aniline system (**1a** and **1b**); *ii)* compound **1k**, which has three methoxy groups in R^2, R^3 and R^4 positions of the amine moiety show better activity than compound **1j**, which has only two methoxy groups, in R^2 and R^3 positions of the amine moiety; *iii)* compound **1k**, which is the most active among them against *E. histolytica* and *G. lamblia*, clearly shows a lower activity than metronidazole and a worse activity against *G. lamblia* than the formononetin, which actually is one of the most promising antiparasitic drug, especially against *G. lamblia*; *iv)* compound **1n**, which is the most active of all compounds against *T. vaginalis*, shows also no better antiprotozoal activity (Table 2).

3. Conclusions

This work describes the first one pot synthesis of novel 3-tetrazolylmethyl-4*H*-chromen-4-ones and their evaluation against *E. histolytica*, *G. lamblia* and *T. vaginalis*. Compound **1k** shows the best antiprotozoal activity against *E. histolytica* and *G. lamblia*, while the compound **1n** shows the best activity against *T. vaginalis*. Compounds **1a-n** showed the best activity against *G. lamblia* among the three parasites, but none of them was better than metronidazole. These compounds could represent a suitable alternative when the studied parasites become resistant to the majority of the current antiparasitic drugs.[29] As main conclusion, it can be considered that our hypothesis was true because the 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n**, which have both scaffols, chromone and tetrazole, showed a moderate antiprotozoal activity.

4. Experimental section

4.1 General Information, instrumentation and chemicals

Commercially available starting materials were purchased from Sigma-Aldrich and were used without further purification. The solvents were distilled and dried using common procedures. IR spectra were recorded on a Perkin Elmer 100 FT-IR spectrometer (in cm^{-1}). ^1H and ^{13}C NMR spectra were acquired in Bruker (500, 400, 300 MHz) spectrometers. $CDCl_3$ was used as solvent and chemical shifts were reported in ppm. Coupling constants were reported in Hz. Internal reference for ^1H-NMR spectra is respect to TMS at 0.0 ppm. Internal reference for ^{13}C-NMR spectra is respect to $CDCl_3$ at 77.23 ppm. HRMS were recorded on a JEOL GC-mate spectrometer by EI+ ionization mode. Reaction progress was monitored by TLC on precoated silica gel Kieselgel 60 F254 plates. The spots were visualized under UV light (254 and 365 nm). Cold anhydrous diethyl ether was used as recrystallization solvent. Melting points were determined on a Fisher-Johns apparatus and were uncorrected. The Purity of the synthesized compounds was supposed nearly to 100% due to the fact that perfect crystals of them were used for characterization and to prepare the samples for biological tests.

4.2. General procedure for the synthesis of the 3-tetrazolylmethyl-4H-chromen-4-ones **1a-n** (GP).

To a stirred solution of 3-formylchromone (**4**) (1.0 mmol, 1.0 equiv.) in anhydrous isopropanol with $InCl_3$ (5% mol) at room temperature, anilines **5** (1.0 mmol, 1.0 equiv.), azidotrimethylsilane (**6**) (1.0 mmol, 1.0 equiv.) and isocyanides **7** (1.0 mmol, 1.0 equiv) were sequentially added. After 2 h at room temperature, the solvent was removed until dryness. Then, the crude was dissolved in DCM (20 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL), followed by a treatment with an excess of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Finally a recrystallization using cold anhydrous diethyl ether was made to afford the corresponding 3-tetrazolylmethyl-4*H*-chromen-4-ones **1a-n**.

4.3. Characterization of the 3-tetrazolylmethyl-4H-chromen-4-ones **1a-n**.

3-((1-(tert-butyl)-1H-tetrazol-5-yl)((2-iodophenyl)amino)methyl)-4H-chromen-4-one (**1a**)

Yield: 85%; white solid; mp = 150-152 °C; R_f = 0.39 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3334 (N-H), 1640 (C=O), 1283 (N-N=N), 1230 (C–O–C); ^{1}H-NMR (500 MHz, $CDCl_3$): 8.27 (s, 1 H, CH), 8.17 (dd, *J* = 8.0, 1.5 Hz, 1 H, ArH), 7.72-7.68 (m, 2 H, ArH), 7.49 (d, *J* = 8.4 Hz, 1 H, ArH), 7.45-7.42 (m, 1 H, ArH), 7.22-7.18 (m, 1 H, ArH), 6.63 (d, *J* = 8.1 Hz, 1 H, ArH), 6.57-6.53 (m, 2 H, ArH and CH), 4.80 (d, *J* = 8.1 Hz, 1 H, NH), 1.85 (d, *J* = 10.7 Hz, 9 H, 3 CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): 176.8, 156.5, 155.7, 153.6, 144.0, 139.5, 134.2, 129.9, 125.6, 123.4, 120.9, 118.4, 111.4, 85.7, 62.3, 45.4, 30.0; HRMS (EI+) calcd. for $C_{21}H_{20}N_5O_2I$: 501.0662, found: 501.0662.

3-((1-cyclohexyl-1*H*-tetrazol-5-yl)((2-iodophenyl)amino) methyl)-4*H*-chromen-4-one (**1b**)

Yield: 78%; white solid; mp = 156-158 °C; R_f = 0.42 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3355 (N-H), 1637 (C=O), 1290 (N-N=N), 1227 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$): 8.25 (d, *J* = 0.6 Hz, 1 H, CH), 8.21 (ddd, *J* = 8.0, 1.7, 0.4 Hz, 1 H, ArH), 7.73-7.67 (m, 2 H, ArH), 7.49-7.41 (m, 2 H, ArH), 7.21-7.15 (m, 1 H, ArH), 6.63-6.51 (m, 1 H, ArH), 6.27 (d, *J* = 6.9 Hz, 1 H, CH), 5.45 (d, *J* = 7.2 Hz, 1 H, NH), 4.77-4.66 (m, 1 H, CH), 2.06-1.86 (m, 8 H, 4 CH_2), 1.54-1.41 (m, 2 H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.6, 156.4, 155.7, 153.7, 144.3, 139.6, 134.3, 129.7, 125.8, 125.7, 123.4, 121.0, 120.8, 118.4, 111.7, 85.8, 58.5, 44.9, 33.3, 32.9, 25.28, 25.23, 24.9; HRMS (EI+) calcd. for $C_{23}H_{22}N_5O_2I$: 527.0819, found: 527.0834.

3-((1-(tert-butyl)-1H-tetrazol-5-yl)(phenylamino)methyl)-4H-chromen-4-one (**1c**)

Yield: 86%; white solid; mp = 220-222 °C; R_f = 0.32 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3345 (N-H), 1640 (C=O), 1285 (N-N=N), 1227 (C–O–C); ^{1}H-NMR (500 MHz, $CDCl_3$): 8.31 (s, 1 H, CH), 8.16 (d, *J* = 8.0 Hz, 1 H, ArH), 7.70-7.66 (m, 1 H, ArH), 7.47 (d, *J* = 8.4 Hz, 1 H, ArH), 7.43-7.39 (m, 1 H, ArH), 7.19-7.15 (m, 2 H, ArH), 6.80-6.75 (m, 1 H, ArH), 6.68 (d, *J* = 7.9 Hz, 2 H, ArH), 6.46 (d, *J* = 7.6 Hz, 1 H, CH), 4.79-4.76 (m, 1 H, NH), 1.85 (s, 9 H, 3 CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): 176.9, 156.5, 155.6, 134.1, 129.6, 125.6, 125.5, 123.5, 118.4, 113.3, 62.3, 45.2, 30.0; HRMS (EI+) calcd. for $C_{21}H_{21}N_5O_2$: 375.1695, found: 375.1679.

3-((1-cyclohexyl-1H-tetrazol-5-yl)(phenylamino)methyl)-4H-chromen-4-one (**1d**)

Yield = 82%; white solid; mp = 218-220 °C; R_f = 0.37 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1} 3373 (N-H), 1640 (C=O), 1290 (N-N=N), 1226 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.30 (s, 1 H, CH), 8.19 (dd, *J* = 8.0, 1.4 Hz, 1 H, ArH), 7.69 (ddd, *J* = 8.6, 7.1, 1.7 Hz, 1 H, ArH), 7.48-7.40 (m, 2 H, ArH), 7.22-7.15 (m, 2 H, ArH), 6.83-6.76 (m, 1 H, ArH), 6.73-6.68 (m, 2 H, ArH), 6.18 (d, *J* = 7.0 Hz, 1 H, CH), 4.98 (d, *J* = 7.1 Hz, 1 H, NH), 4.75-4.66 (m, 1 H, CH), 2.09-1.88 (m, 8 H, 4 CH_2), 1.51-1.38 (m, 2 H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.7, 156.4, 155.4, 154.2, 145.0, 134.2, 129.6, 125.6, 123.5, 121.4, 119.4, 118.4, 113.8, 58.4, 44.6, 33.3, 32.7, 29.7, 25.2, 24.9; HRMS (EI+) calcd. for $C_{23}H_{23}N_5O_2$, 401.1852, found: 401.1850.

3-((1-(tert-butyl)-1H-tetrazol-5-yl)((4-chlorophenyl)amino)methyl)-4H-chromen-4-one (**1e**)

Yield = 76%; white solid; mp = 200-202 °C; R_f = 0.34 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3316 (N-H), 1646 (C=O), 1287 (N-N=N), 1225 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.29 (d, *J* = 0.9 Hz, 1 H, CH), 8.18-8.15 (m, 1 H, ArH), 7.69 (ddd, *J* = 8.6, 7.0, 1.6 Hz, 1 H, ArH), 7.49-7.46 (m, 1 H, ArH), 7.42 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1 H, ArH), 7.10 (d, *J* = 8.9 Hz, 2 H, ArH), 6.62 (d, *J* = 9.9 Hz, 2 H, ArH), 6.41 (d, *J* = 6.1 Hz, 1 H, CH), 4.94 (d, *J* = 6.5 Hz, 1 H, NH), 1.83 (s, 9 H, 3 CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.8, 156.5, 155.7, 153.9, 143.6, 134.2, 129.5, 125.6, 124.0, 123.5, 121.4, 118.3, 114.6, 62.4, 45.3, 30.0; HRMS (EI+) calcd. for $C_{21}H_{20}N_5O_2Cl$: 409.1306, found: 409.1319.

3-((4-chlorophenyl)amino)((1-cyclohexyl-1H-tetrazol-5-yl)methyl)-4H-chromen-4-one (**1f**)

Yield = 72%; yellow solid; mp = 210-212 °C; R_f = 0.37 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3344 (N-H), 1640 (C=O), 1285 (N-N=N), 1227 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.27 (d, *J* = 0.7 Hz, 1 H, CH), 8.19 (ddd, *J* = 8.0, 1.9, 0.5 Hz, 1 H, ArH), 7.70 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1 H, ArH), 7.48-7.41 (m, 2 H, ArH), 7.15-7.09 (m, 2 H, ArH), 6.66-6.61 (m, 2 H, ArH), 6.15 (d, *J* = 7.7 Hz, 1 H, CH), 5.15 (d, *J* = 7.8 Hz, NH), 4.73-4.61 (m, 1 H, CH), 2.02-1.78 (m, 8 H, 4 CH_2), 1.53-1.38 (m, 2 H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.6, 156.4, 155.6, 154.0, 143.5, 134.3, 129.5, 125.8, 125.6, 124.2, 123.4, 121.1, 118.4, 114.9, 58.4, 44.5, 33.3, 32.8, 25.2, 24.8; HRMS (EI+) calcd. for $C_{23}H_{22}N_5O_2Cl$: 435.1462, found: 435.1462.

3-((1-(tert-butyl)-1H-tetrazol-5-yl)((4-iodophenyl)amino)methyl)-4H-chromen-4-one (**1g**)

Yield = 85%; white solid; mp = 166-168 °C; R_f = 0.26 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3316 (N-H), 1641 (C=O), 1286 (N-N=N), 1224 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.27 (d, *J* = 0.8 Hz, 1 H, CH), 8.17 (dd, *J* = 8.0, 1.3 Hz, 1 H, ArH), 7.73-7.67 (m, 1 H, ArH), 7.50-7.40 (m, 4 H, ArH), 6.51-6.46 (m, 2 H, ArH), 6.44 (d, *J* = 8.3 Hz, 1 H, CH), 4.78 (d, *J* = 8.3 Hz, 1 H, NH), 1.84 (s, 9 H, 3 CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.7, 156.5, 155.7, 153.9, 144.5, 138.3, 134.2, 125.6, 121.4, 118.4, 115.6, 80.5, 62.3, 44.9, 30.0; HRMS (EI+) calcd. for $C_{21}H_{20}N_5O_2I$: 501.0662, found: 501.0662.

3-((1-cyclohexyl-1H-tetrazol-5-yl)((4-iodophenyl)amino)methyl)-4H-chromen-4-one (**1h**)

Yield = 79%; white solid; mp = 162-164 °C; R_f = 0.29 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3318 (N-H), 1645 (C=O), 1286 (N-N=N), 1230 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.26 (s, 1 H, CH), 8.18 (dd, *J* = 8.0, 2.0 Hz, 1 H, ArH), 7.73-7.67 (m, 1 H, ArH), 7.47-7.40 (m, 4 H, ArH), 6.53-6.47 (m, 2 H, ArH), 6.15 (d, *J* = 7.6 Hz, 1 H, CH), 5.31 (d, *J* = 7.8 Hz, 1 H, NH), 4.72-4.60 (m, 1 H, CH), 1.99-1.74 (m, 8 H, 4 CH_2), 1.51-1.38 (m, 2 H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.6, 156.4, 155.6, 153.9, 144.7, 138.2, 134.3, 125.7, 125.6, 123.3, 121.0, 118.4, 115.8, 80.5, 58.5, 44.1, 33.2, 32.8, 25.25, 25.19,

24.9; HRMS (EI+) calcd. for $C_{23}H_{22}N_5O_2I$: 527.0819, found: 527.0854.

3-((1-cyclohexyl-1H-tetrazol-5-yl)((4-nitrophenyl)amino)methyl)-4H-chromen-4-one (**1i**)

Yield = 70%; yellow solid; mp = 210-212 °C; R_f = 0.14 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3330 (N-H), 1650 (C=O), 1281 (N-N=N), 1235 (C–O–C); ^{1}H-NMR (500 MHz, $CDCl_3$): 8.26 (s, 1 H, CH), 8.23 (d, *J* = 8.0 Hz, 1 H, ArH), 8.06 (d, *J* = 9.1 Hz, 2 H, ArH), 7.75-7.71 (m, 1 H, ArH), 7.50-7.46 (m, 2 H, ArH), 6.72 (d, *J* = 9.1 Hz, 2 H, ArH), 6.35 (d, *J* = 7.2 Hz, 1 H, CH), 6.32 (d, *J* = 7.2 Hz, 1 H, NH), 4.70-4.63 (m, 1 H, CH), 2.07-2.01 (m, 2 H, CH_2), 2.00-1.88 (m, 4 H, 2 CH_2), 1.79-1.73 (m, 1 H, H of CH_2), 1.52-1.41 (m, 2 H, CH_2), 1.36-1.25 (m, 1 H, H of CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$): 175.7, 155.7, 155.2, 152.7, 149.5, 139.0, 133.8, 125.5, 125.2, 124.8, 122.4, 119.8, 117.5, 111.3, 57.4, 42.1, 31.8, 31.4, 23.7, 23.6, 23.3; HRMS (EI+) calcd. for $C_{23}H_{22}N_6O_4$: 446.1703, found: 446.1710.

3-((3,4-dimethoxyphenyl)amino)((1-(2,6-dimethylphenyl)-1H-tetrazol-5-yl)methyl)-4H-chromen-4-one (**1j**)

Yield = 55%; brown solid; mp = 171-173 °C; R_f = 0.11 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3330 (N-H), 1648 (C=O), 1289 (N-N=N), 1222 (C–O–C); ^{1}H-NMR (500 MHz, $CDCl_3$): 8.26 (s, 1 H, CH), 8.00 (d, *J* = 8.0 Hz, 1 H, ArH), 7.71-7.66 (m, 1 H, ArH), 7.44 (d, *J* = 8.5 Hz, 1 H, ArH), 7.43-7.38 (m, 1 H, ArH), 7.36-7.32 (m, 1 H, ArH), 7.17 (d, *J* = 7.6 Hz, 1 H, ArH), 7.13 (d, *J* = 7.6 Hz, 1 H, ArH), 6.61 (d, *J* = 8.5 Hz, 1 H, ArH), 6.23-6.21 (m, 1 H, ArH), 6.08 (d, *J* = 8.5 Hz, 1 H, CH), 5.83 (d, *J* = 8.9 Hz, 1 H, ArH), 4.56 (d, *J* = 9.0 Hz, 1 H, NH), 3.75 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 1.90-1.86 (m, 6 H, 2 CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): 175.9, 156.2, 156.1, 155.3, 150.0, 143.1, 139.4, 136.2, 136.1, 134.1, 131.5, 131.1, 128.9, 128.8, 125.8, 125.6, 123.5, 121.3, 118.2, 112.8, 105.7, 100.3, 56.4, 55.8, 45.2, 17.4, 17.2; HRMS (EI+) calcd. for $C_{27}H_{25}N_5O_4$: 483.1907, found: 483.1922.

3-((1-cyclohexyl-1H-tetrazol-5-yl)((3,4,5-trimethoxyphenyl)amino)methyl)-4H-chromen-4-one (**1k**)

Yield = 90%; yellow solid; mp = 160-162 °C; R_f = 0.14 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR) ν_{max}/cm^{-1}: 3332 (N-H), 1650 (C=O), 1281 (N-N=N), 1235 (C–O–C); ^{1}H-NMR (400 MHz, $CDCl_3$): 8.21 (s, 1 H, CH), 8.04 (d, *J* = 7.9 Hz, 1 H, ArH), 7.59-7.54 (m, 1 H, ArH), 7.35-7.28 (m, 2 H, ArH), 6.06 (d, *J* = 7.9 Hz, 1 H, CH), 5.86 (s, 2 H, ArH), 5.01 (d, *J* = 8.1 Hz, 1 H, NH), 4.53-4.45 (m, 1 H, CH), 3.61 (s, 6 H, 2 OCH_3), 3.59 (s, 3 H, OCH_3), 1.87-1.72 (m, 8 H, 4 CH_2), 1.36-1.24 (m, 2 H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): 175.3, 155.0, 154.6, 153.0, 152.7, 140.4, 133.0, 129.9, 124.5, 124.2, 122.0, 120.5, 117.1, 90.4, 63.0, 59.7, 57.0, 54.7, 43.2, 31.8, 31.5, 24.0, 23.9, 23.5; HRMS (EI+) calcd. for $C_{26}H_{29}N_5O_5$: 491.2169, found: 491.2164.

3-((2-bromophenyl)amino)((1-(tert-butyl)-1H-tetrazol-5-yl)methyl)-4H-chromen-4-one (**1l**)

Yield = 72%; yellow solid; mp = 180-182 °C; R_f = 0.37 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR)ν_{max}/cm^{-1}: 3300 (N-H), 1650 (C=O), 1281 (N-N=N), 1235 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.28 (d, *J* = 0.8 Hz, 1 H, CH), 8.17 (dd, *J* = 8.0, 1.6 Hz, 1 H, ArH), 7.72-7.65 (ddd, *J* = 8.6, 7.1, 1.7 Hz, 1 H, ArH), 7.49-7.39 (m, 3 H, ArH), 7.20-7.14 (m, 1 H, ArH), 6.74-6.65 (m, 2 H, ArH), 6.56 (d, *J* = 8.2 Hz, 1 H, CH), 4.96 (d, *J* = 8.2 Hz, 1 H, NH), 1.85 (s, 9 H, 3 CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.7, 156.5, 155.7, 153.7, 141.7, 134.2, 132.9, 129.0, 125.6, 123.4, 121.3, 120.1, 118.4, 112.1, 110.2, 62.3, 45.0, 30.0; HRMS (EI+) calcd. for $C_{21}H_{20}N_5O_2Br$: 453.0800, found: 453.0804.

3-((2-bromophenyl)amino)(1-cyclohexyl-1H-tetrazol-5-yl)methyl)-4H-chromen-4-one (**1m**)

Yield = 70%; yellow solid; mp = 184-186 °C; R_f = 0.47 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR) ν_{max}/cm^{-1}: 3300 (N-H), 1640 (C=O), 1283 (N-N=N), 1232 (C–O–C); ^{1}H-NMR (300 MHz, $CDCl_3$): 8.26 (d, *J* = 0.7 Hz, 1 H, CH), 8.23-8.19 (m, 1H), 7.70 (ddd, *J* = 8.6, 7.1, 1.7 Hz, 1 H, ArH), 7.50-7.41 (m, 3 H, ArH), 7.18-7.12 (m, 1 H, ArH), 6.71-6.64 (m, 2 H, ArH), 6.28 (d, *J* = 7.6 Hz, 1 H, CH), 5.57 (d, *J* = 7.6 Hz, 1 H, NH), 4.77-4.66 (m, 1 H, CH), 2.06-1.86 (m, 8 H, 4 CH_2), 1.52-1.41 (m, 2 H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$): 176.6, 156.4, 155.6, 153.7, 141.9, 134.3, 132.9, 128.8, 125.8, 125.7, 123.4, 121.0, 120.0, 118.4, 112.3, 110.4, 58.5, 44.5, 33.3, 32.9, 25.3, 25.2, 24.9; HRMS (EI+) calcd. for $C_{23}H_{22}N_5O_2Br$: 479.0957, found: 479.0956.

3-((1-cyclohexyl-1H-tetrazol-5-yl)((2-nitrophenyl)amino)methyl)-4H-chromen-4-one (**1n**)

Yield = 71%; Yellow solid; mp = 180-182 °C; R_f = 0.28 (Hexanes-AcOEt, 7:3 V/V); FT-IR (ATR) ν_{max}/cm^{-1}: 3338 (N-H), 1640 (C=O), 1263 (N-N=N), 1227 (C–O–C); ^{1}H-NMR (500 MHz, $CDCl_3$): 9.0 (d, *J* = 6.5 Hz, 1 H, NH), 8.31 (s, 1 H, CH), 8.25 (d, *J* = 7.7 Hz, 2 H, ArH), 7.76-7.71 (m, 1 H, ArH), 7.51-7.44 (m, 3 H, ArH), 6.85 (d, *J* = 8.51 Hz, 1 H, ArH), 6.83-6.79 (m, 1 H, ArH), 6.48 (d, *J* = 6.7 Hz, 1 H, CH), 4.73-4.65 (m, 1 H, CH), 2.08-2.01 (m, 2 H, CH_2), 1.99-1.85 (m, 4 H, 2 CH_2), 1.79-1.73 (m, 1 H, 1 H of CH_2), 1.54-1.39 (m, 2 H, CH_2), 1.36-1.25 (m, 1 H, CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$): 176.1, 156.4, 156.3, 153.2, 142.0, 136.6, 134.6, 133.6, 127.2, 126.0, 125.8, 123.3, 121.1, 118.5, 117.6, 113.9, 58.5, 43.0, 33.2, 33.0, 25.3, 25.2, 24.8; HRMS (EI+) calcd. for $C_{23}H_{21}N_6O_4$: 445.0397, found: 445.0399.

4.4. Evaluation of antiprotozoal activity. Antiprotozoal assays: Entamoeba histolytica strain HM1-IMSS used in all experiments was grown axenically at 37 ºC in TYI-S-33 medium supplemented with 10% heat inactivated bovine serum. In the case of *Giardia lamblia*, strain IMSS: 8909:1 was grown in TYI-S-33 modified medium supplemented with 10% calf serum and bovine bile. Trophozoites of *Trichomona vaginalis* strain GT3 were maintained in TYI-S-33 medium supplemented with10% bovine serum. The trophozoites were axenically maintained and were employed for assays in the log phase of growth. In vitro susceptibility tests were performed using the subculture method previously described.[30] Briefly, *E. histolytica* (6×10^3) or *G. lamblia* (5×10^4) trophozoites were incubated for 48 h at 37 ºC in the presence of different concentrations (2.5-200 μg/mL) of pure compounds **1a-n** in dimethyl sulfoxide (DMSO). Each test included standard amoebicidal and giardicidal drugs, a control (culture medium plus trophozoites and DMSO) and a blank (culture medium). After incubation, the trophozoites were detached by chilling and 50 μL samples of each tube were subcultured in fresh medium for another 48 h, without antiprotozoal samples. The final number of parasites was determined with a hemocytometer and the percentages of trophozoites growth inhibition were calculated by comparison with the control culture. The results were confirmed by a colorimetric method: the trophozoites were washed and incubated for 45 min at 37 ºC in a saline phosphate buffer with MTT (3-[4,5-dimethylhiazol-2-il]-2,5-diphenyl tetrazolium bromide) and phenazine methosulfate. The dye produced (formazan) was extracted and the absorbance was determined at

570 nm. The experiments were performed in duplicate for each protozoan and repeated three times.

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8. The images depicted in Fig. 1 are of public domain and were taken from the Center for Disease Control and Prevention's Public Health Image Library. For the *E. histolytica* image, ID number: #1474, author: Dr. George Healy; for the *G. lamblia* image, ID number: #8698, author: Janice Carr.
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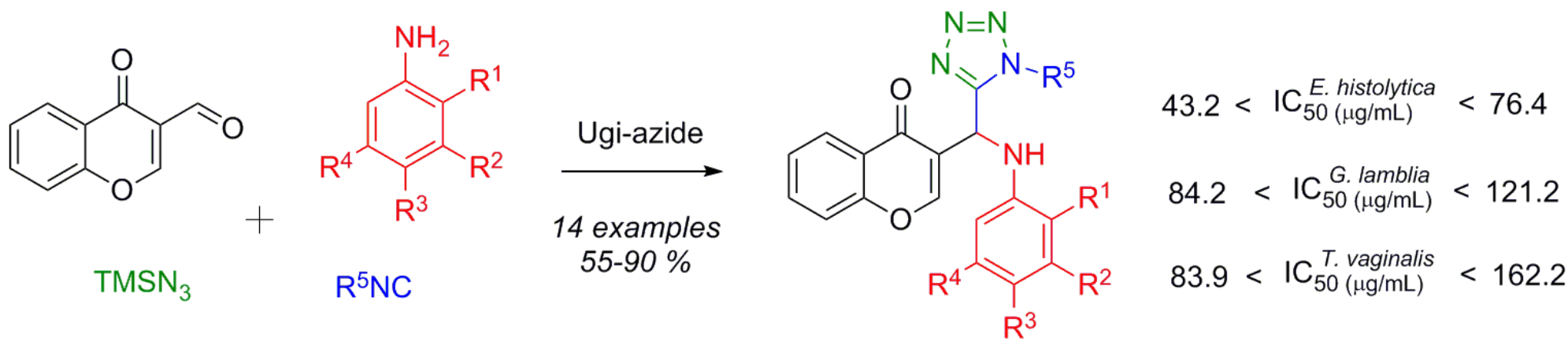
Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.


TMSN3
R5NC
Ugi-azide
14 examples
55-90 %
43.2 < IC50 (μg/mL) E. histolytica < 76.4
84.2 < IC50 (μg/mL) G. lamblia < 121.2
83.9 < IC50 (μg/mL) T. vaginalis < 162.2