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Original Research Article

Synthesis and in vitro antiprotozoal activity of some 2-amino-4-phenyloxazole derivatives

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Abstract

Purpose: *To prepare some 2-amino-4-(p-substituted phenyl)-oxazole derivatives and to evaluate their in vitro antiprotozoal activity against Giardia lamblia and Trichomonas vaginalis.*
Methods: *The 2-amino-4-(p-substituted phenyl)-oxazoles (a-g) were synthesized by microwave (MW) irradiation of mixtures of p-substituted 2-bromoacetophenones and urea in dimethylformamide (DMF). All compounds were identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and low- and high-resolution mass spectrometry (HRMS). NMR assignments were made based on heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments. Each synthesized compound's melting point was determined. Antiprotozoal activity against Giardia intestinalis and Trichomonas vaginalis was quantified using a rigorous and sensitive subculture method. The commercial drug, metronidazole, was used as positive control. The 50 % inhibitory concentration (IC_{50}) of the antiprotozoal agents for each protozoa was determined.*
Results: *Seven 2-amino-4-(p-substituted phenyl)-oxazoles (a-g) were synthesized. The most active compounds against G. lamblia was 2-amino-4-(p-benzoyloxyphenyl)-oxazole (3d) with an IC_{50} of 1.17 µM, while compound 3e (2-amino-4-(p-bromophenyl)-oxazole) showed the highest anti-trichomonal activity (IC_{50}, 1.89 µM).*
Conclusion: *The in vitro antigiardial activity of 2-amino-4-(p-benzoyloxyphenyl) oxazole was higher than that exhibited by metronidazole; however, it is necessary increase the number of synthetic derivatives in order to be able to determine their structure-activity relationship.*

Keywords: *Antiprotozoal, 2-Amino-4-phenyl-oxazoles, Giardia lamblia, Trichomonas vaginalis*

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INTRODUCTION

Giardiasis is an intestinal infection in humans by *Giardia lamblia* (syn. *Giardia intestinalis*, *Giardia duodenalis*), a cosmopolitan anaerobic gastrointestinal protozoan. Approximately one billion people worldwide have giardiasis, and an estimated 280 million cases occur annually, leading to 2.5 million deaths [1]. *Giardia lamblia* exhibits high prevalence in a variety of domestic, livestock and wild animals [2].

The colonization of the human small intestine by *G. lamblia* causes diarrhea, epigastric pain, nausea, vomiting, cramps, and weight loss [3]. Chronic giardiasis leads to malabsorption syndrome associated malnutrition, including

failure-to-thrive in children. Reported long-term clinical sequelae include significant reductions in height and IQ in children aged 7 - 9 years who had suffered multiple episodes of chronic diarrhea, lasting more than two weeks, during the first 24 months of life [4].

The most common sexually-transmitted disease in the world is trichomoniasis, caused by the amitochondriate protozoan parasite *Trichomonas vaginalis.* Disease incidence is estimated at 276 million new cases a year [5]. This protozoan pathogen mainly affects the squamous epithelium of human female and male urogenital tracts. Infection by *T. vaginalis* in women leads to vaginal discharge and edema, vulvar itching, erythema, infertility, and can affect pregnancy course, causing premature birth or low birth weight. In men, it causes dysuria, epididymitis, urethritis, and prostatitis [6,7]. Trichonomiasis has also been associated with a predisposition to cervical and prostate cancers, and increased risks of HIV and HPV acquisition and transmission [8].

Although giardiasis and trichomoniasis are significant worldwide public health issues, research into their pharmaceutical treatment has been slow [9, 10]. The most commonly used antigiardiasic drugs are 5-nitroimidazoles, metronidazole, tinidazole, secnidazole, and ornidazole [11]; while only metronidazole and tinidazole are recommended for treating *T. vaginalis* infections [8]. Most of the drugs currently used to treat these diseases were developed decades ago and produces side effects such headache, vertigo, nausea, allergic reactions, and in some cases neurotoxicity. In addition reports exist of metronidazole-resistant *G. lamblia* and *T. vaginalis* strains [11].

Our research into antiprotozoal compounds has recently focused on the antiprotozoal activity of five-membered heterocyclic systems. Aryloxazole derivatives are reported to have significant antiprotozoal activities (IC_{50} from 0.07 to > 5 µg/mL) [12]. In a previous paper we reported on the synthesis of several 2-amino-4-arylthiazole and evaluation of their *in vitro* anti-giardiasic activity with encouraging results [13]. Based on these findings, we synthesized seven 2-amino-4-(*p*-substituted-phenyl)-1,3-oxazoles, and evaluated their antiprotozoal activity *in vitro* against pathogenic protozoa *Giardia lamblia* and *Trichomonas vaginalis*.

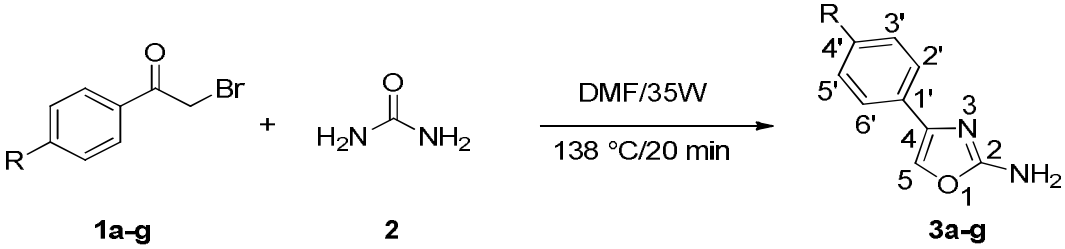
EXPERIMENTAL

General

Starting materials and reagents were purchased from Sigma-Aldrich and used without further purification. All solvents were reagent grade. Microwave irradiations were done in a CEM Discover microwave reactor in standard open vessels. Temperature was monitored during the reaction with the IR pyrometer of the microwave reactor. Analytical thin-layer chromatography (TLC) was done using a 25 µm particle size and a 60 Å pore diameter silica gel matrix (containing a fluorescent indicator at 254 nm) (Sigma-Aldrich). Compounds were viewed using ultraviolet light. Flash chromatography was done using Sigma-Aldrich silica gel (0.002 - 0.025 mm particle size). Melting points were determined on an Electrothermal IA9100 apparatus in open capillaries. Low- and high-resolution mass spectra (MS) were obtained on a Jeol GC-Mate II under electron impact (EI) at 70 eV. ^{1}H and ^{13}C spectra at 400.1 and 100.6 MHz, respectively, as well as 2D homonuclear and heteronuclear experiments were performed on a Bruker DPX400 Avance spectrometer. Chemical shifts were registered in ppm (δ) using the residual solvent signal (DMSO-d6) as internal reference. The synthesis route of the title compounds (**3a-3g**) is shown in Figure 1.

Procedure for synthesis of 2-amino-4-(*p*-phenylsubstituted)oxazoles (3a-g)

A mixture of *p*-substituted 2-bromoacetophenone (**1a-g**) (5 mmol) and urea (**2**) (35 mmol) in DMF (1 mL) was subjected to MW irradiation (35 W) at 138 °C for 20 min (Figure 1).



1a-g **2** **3a-g**

R = H(a), Me(b), OMe (c), OBz (d), Br (e), F(f), NO_2(g)

Figure 1: Synthesis of 2-amino-4-aryloxazoles

After completion of the reaction the solvent was removed at reduced pressure. The final product was purified by flash column chromatography using hexane:ethyl acetate (mixtures of increasing polarity) as eluent to yield 2-amino-4-aryloxazoles **3a-g**.

In vitro giardicidal and trichomonicidal assay

G. lamblia strain IMSS:0696:1 was cultured in TYI-S-33 modified medium, supplemented with 10 % calf serum and bovine bile, and *T. vaginalis* strain GT3 was cultured in TYI-S-33 medium, supplemented with 10 % bovine serum. For the bioassay, the compounds were dissolved in 1 mL of dimethylsulfoxide (DMSO) and added to microtubes containing 1.5 mL of medium to reach concentrations of 1, 2, 10, and 20 µg/mL. The solutions were inoculated with *G. lamblia* or *T. vaginalis* to achieve an inoculum of 4×10^4 trophozoites/mL and then incubated for 24 h (*T. vaginalis*) or 48 h (*G. lamblia*) at 37 ºC. Each test included metronidazole as positive control and trophozoites incubated in culture medium with DMSO used in the experiments as the negative control. After the incubation, trophozoites were washed and sub-cultured for another 48 h in fresh medium alone. At the end of this period, trophozoites were counted using a haemocytometer and the 50 % inhibitory concentration (IC_{50}) was calculated.

Computational studies

All the quantum chemical and descriptor calculations for each synthesized compound (**3a-g**) were performed with the Spartan10 code. Pople's 6-31+G(d,p) basis set was used along the B3LYP functional. Frequency calculations were performed to characterize all the stationary points at the same computational level. The minimum structures were identified from the vibrational analysis with all real frequencies.

Statistical analysis

The compound concentration that inhibited 50 % of trophozoite growth (IC_{50}) was calculated by dose-response (variable slope) technique. All concentrations were evaluated in duplicate and each assay run in triplicate. All statistical analyses were performed using GraphPad Prism ver. 4 statistical software.

RESULTS

All compounds were identified by ^1H and ^{13}C NMR spectroscopy and low and high resolution mass spectrometry. The NMR assignments were based on HSQC and HMBC experiments, and all data were in full agreement with the assigned structures. The spectroscopic data of title compounds (**3a-g**) are presented as follows.

2-Amino-4-phenyloxazole (3a)

White amorphous solid; yield: 29 %; m. p. 137 - 139 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 7.87 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 6.74 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.7, 139.1, 132.1, 128.6, 127.4, 127.3, 124.8.; EIMS (*m/z*): 160 (93), 131 (12), 104 (100), 89 (19), 77 (15), 63 (7), 51 (6); HREIMS: $C_9H_8N_2O$, calcd.: 160.0637, found: 160.0607.

2-Amino-4-(*p*-methylphenyl)-oxazole (3b)

White amorphous solid; yield: 21 %; m. p. 159 - 161 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 7.81 (s, 1H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H), 6.71 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 139.0, 136.4, 129.3, 129.1, 126.8, 124.7, 20.9; EIMS (*m/z*): 174 (82), 145 (10), 118 (100), 103 (11), 91 (8), 77 (8), 65 (4), 51 (4); HREIMS: $C_{10}H_{10}N_2O$, calcd.: 174.0793, found: 174.0735.

2-Amino-4-(*p*-metoxiphenyl)-oxazole (3c)

Light red amorphous solid; yield: 12 %; m. p. 166 - 168 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 7.75 (s, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.68 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 158.5, 138.8, 126.0, 125.9, 124.9, 114.0, 55.1; EIMS (*m/z*): 190 (88), 161 (6), 134 (100), 119 (8), 107 (3), 91 (16), 81 (3), 77 (11), 63 (3), 51 (8); HREIMS: $C_{10}H_{10}N_2O_2$, calcd.: 190.0742, found: 190.0755.

2-Amino-4-(*p*-benzoyloxyphenyl)-oxazole (3d)

Light yellow amorphous solid; yield: 10 %; m. p. 174 - 176 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, *J* = 7.2 Hz, 2H), 7.92 (s, 1H), 7.83-7.68 (m, 3H), 7.62 (t, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.78 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 161.7, 149.6, 138.4, 134.2, 130.0, 129.9, 129.1, 129.0, 127.4, 125.8, 122.1; EIMS (*m/z*): 280 (27), 105 (100), 77 (43), 51 (12); HREIMS: $C_{16}H_{12}N_2O_3$, calcd.: 280.0848, found: 280.0840.

2-Amino-4-(*p*-bromophenyl)-oxazole (3e)

Yellow amorphous solid; yield: 30 %; m. p. 182 - 184 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 7.94 (s, 1H), 7.59-7.53 (m, 4H), 6.79 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.7, 138.1, 131.5, 131.4, 127.9, 126.7, 120.0; EIMS (*m/z*): 240 (84)

238 (85), 212 (4), 211 (4), 210 (4), 209 (4), 184 (99), 182 (100), 157 (6) 131 (9) 129 (3), 104 (9), 103 (26), 102 (27), 89 (47), 76 (20), 63 (18), 50 (14); HREIMS: $C_{10}H_7BrN_2O$, calcd.: 237.9742, found: 237.9731.

2-Amino-4-(*p*-fluorophenyl)-oxazole (3f)

White amorphous solid; yield: 30 %; m. p. 189 - 191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86 (s, 1H), 7.67 (dd, *J* = 8.1, 5.8 Hz, 2H), 7.20 (t, *J* = 8.7 2H), 6.76 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.7, 161.3 (d, *J* = 244.5 Hz, CF), 138.2, 128.6 (d, *J* = 3.2 Hz, CF), 127.1, 126.6 (d, *J* = 8.1 Hz, CF), 115.5 (d, *J* = 21.6 Hz, CF); EIMS (*m/z*): 178 (69) 149 (6), 122 (100), 107 (22), 95 (12), 75 (10), 69 (1), 57 (5), 50 (4); HREIMS: $C_{10}H_7FN_2O$, calcd.: 178.0542, found: 178.0605.

2-Amino-4-(*p*-nitrophenyl)-oxazole (3g)

Light red amorphous solid; yield: 36 %; m. p. 187 - 189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (d, *J* = 8.8 Hz, 2H), 8.18 (s, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 6.95 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.0, 146.0, 138.7, 137.6, 130.5, 125.4, 124.1; EIMS (*m/z*): 205 (100) 175 (4), 149 (53), 119 (5), 103 (22), 89 (13), 76 (8), 63 (8), 50 (4); HREIMS: $C_{10}H_7N_3O_3$, calcd.: 205.0487, found: 205.0423.

***In vitro* giardicidal and trichomonicidal assay**

The results for *in vitro* giardicidal and trichomonicidal activities of synthesized compounds are summarized in Table 1.

Computational results

The physicochemical descriptors obtained for each synthesized 2-amino-4-(*p*-substituted phenyl)oxazol are summarized in Table 1.

DISCUSSION

2-amino-4-(*p*-benzoyloxyphenyl)oxazole (**3d**), was obtained as light yellow amorphous solid. The HREIMS gave an ion peak at *m/z* 280.0847 corresponding to the molecular formula $C_{16}H_{12}N_2O_3$ (calcd 280.0848), which was in good agreement with ^{13}C NMR and DEPT data. The ^{1}H NMR spectrum revealed the presence of two sharp singlets at δ 6.74 and 7.87, attributed to the protons of amino group and H-5, respectively. Moreover, three doublets (δ 7.29, 7.73, 8.15) and two triplets (δ 7.62 and 7.76; *J* = 7.6 Hz each) were present in the aromatic region. The two first doublets showed the same coupling constant value (8.6 Hz); this splitting pattern corresponds to *p*-disubstituted aromatic ring, and according to the literature the signals were assigned to H8,10 and H7,11, respectively [13]. The last one doublet (δ 8.15, *J* = 8.0 Hz) as well as the triplets at δ 7.62 (*J* = 8.0 Hz) and 7.76 (*J* = 8.0 Hz) indicates the presence of the monosubstituted aromatic ring. These signals were assigned to positions H2',6'; H3',5' and H4', respectively. The ^{13}C NMR spectrum and DEPT experiments confirmed the presence of sixteen carbon atoms, corresponding to six quaternary carbons and ten methines. In the same fashion, the structures of the other synthetized compounds were confirmed using NMR and EM data.

Table 1: Antiprotozoal activity and physicochemical descriptors of synthesized 2-amino-4-(*p*-substitutedphenyl) oxazoles

Comp.	[a]IC_{50} (μM ± SD[b])		Polarizability	CLogP	CMR[c]	MW[d]	NA[e]
	G. lamblia	*T. vaginalis*			(cm^3/mol)		
3a	8.84 ± 1.04	6.61 ± 1.27	53.65	1.592	46.25	160	20
3b	9.07 ± 1.04	6.57 ± 1.17	55.13	2.091	52.15	174	23
3c	9.02 ± 1.04	6.35 ± 1.17	54.23	1.616	53.50	190	24
3d	1.17 ± 1.15	4.94 ± 1.05	63.08	3.174	77.58	280	33
3e	5.67 ± 1.13	1.89 ± 1.07	55.16	2.480	53.94	238	21
3f	30.15 ± 1.14	10.33 ± 1.13	54.02	1.760	46.65	178	21
3g	3.55 ± 1.12	6.60 ± 1.20	55.96	1.388	-	205	23
MTZ[d]	1.40 ± 0.07	0.72 ± 0.03					

[a]50% inhibitory concentration, [b]standard deviation, [c]calculated molar refractivity, [d]molecular weigth, [e]number of atoms, [d]metronidazole (positive control)

Considering the fact that thiazole and 1,3-oxazole are molecular scaffolds containing divalent bioisosteres (oxygen and sulfur) [14], and that 2-amino-4-(*p*-phenyl substituted) thiazoles exhibit antigiardiasic activity [13], the synthesized 2-amino-4-(*p*-phenylsubstituted)-oxazoles were tested against the amitochondriate protozoa *G. lamblia* and *T. vaginalis*.

Six of the seven assayed compounds exhibited antiprotozoal activity against both protozoa at IC_{50} below than 10 µM.

Small molecules are normally tested for *in vitro* hit identification at concentrations of 1 - 50 µM [16]; any compound exhibiting activity at a concentration lower than 25µM is considered as having significant activity [17]. Using this criterion, the results show both protozoa to be susceptible to almost all the synthesized oxazoles at concentrations below 10 µM. Versus *T. vaginalis*, 2-amino-4-(*p*-bromophenyl)-oxazole (**3e**) exhibited an IC_{50} of 1.89 µM (0.45 µg/mL). When challenged against *G. lamblia,* 2-amino-4-(*p*-benzoyloxyphenyl)oxazole (**3d**)), a new oxazole, inhibited growth at 1.17 µM (0.33 µg/mL) while 2-amino-4-(*p*-nitrophenyl)-oxazole (**3g**) did so at 3.55 µM (0.73 µg/mL). Due to their IC_{50} <1 µg/mL, these compounds can be considered as hits [18]. Unexpectedly, the compound **3d** exhibited antigiardial activity higher than that of metronidazole, the most common drug used treating giardiasis. In a previous QSAR study, we prepared 2-amino-4-(*p*-phenylsubstituted)thiazoles that had antigiardiasic activity (IC_{50}) ranging from 20.3 - 108.3 µM [13]. In this study, it was determined that EM2 (polarizability) and Hypnotic-80 (lipophilicity) were the two most significant descriptors of a synthesized thiazoles' antigiardial activity [13]. The Hypnotic-80 descriptor correlates to compounds with 162 - 360 g/mol molecular weight, number of atoms from 20 - 45, a calculated Log P ranging from 0.5 to 3.99, and a calculated molar refractivity (CMR) of 43 - 97cm^3/mol [19]. The oxazoles synthesized in the present study exhibited a higher antiprotozoal activity than previously prepared thiazoles, some being up to twelve times more active [13]. Their calculated EM2 and Hypnotic-80 values confirmed this activity (Table 1). And, as expected, all these oxazoles met the criteria for the Hypnotic-80 descriptor for Log P (1.388 - 3.174) and molar refractivity (46.25 - 77.58 cm^3/mol). Of note this that the oxazoles with the highest activity against *G. lamblia* and *T. vaginalis* also had the highest values for polarizability, CLogP, CMR, molecular weight and number of atoms.

To the best of our best knowledge, this is the first time that 2-amino-4-(*p*-benzoyloxyphenyl)oxazole (**3d**), the most active compound against *G. lamblia*, has been synthesized.

CONCLUSION

Bioisosteric replacement of sulfur by oxygen in the heterocyclic moiety is highly significant for antiprotozoal activity since the synthesized oxazoles exhibited antigiardial activity up to 12-fold greater than that of related thiazoles. The *in vitro* antigiardial activity of the novel 2-amino-4-(*p*-benzoyloxyphenyl)oxazole is higher than that exhibited by the commercial drug, metronidazole. Building on these promising preliminary results will require developing a library of 2-amino-4-(p-substituted phenyl) oxazoles for analysis in QSAR, cytotoxicity and *in vivo* toxicological studies.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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