Med Chem Res (2012) 21:415–420
DOI 10.1007/s00044-010-9547-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Effect of the lipophilic parameter (log *P*) on the anti-parasitic activity of imidazo[1,2-*a*]pyridine derivatives

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Received: 2 July 2010 / Accepted: 15 December 2010 / Published online: 7 January 2011

Abstract A number of imidazo[1,2-*a*]pyridine derivatives were selected and investigated in relation to anti-parasitic (*Trichomonas vaginalis*) activity. After treatment with derivatives, biological activity was assessed by determination of the in vitro viability of cell cultures, using alamar blue as a metabolic indicator. A good correlation was found between the anti-parasitic activity and the partition coefficient log *P* determined experimentally on the tested compounds, which explained up to 84% of the measured activity. A favorable interval (0.9 ± 0.3 log *P*) was found for optimum biological response.

Keywords Anti-parasitic activity · Log *P* · Imidazo[1,2-*a*]pyridine derivatives

Introduction

Parasitic infections exist worldwide and affect large populations, especially in underdeveloped countries where poor hygiene conditions prevail. The appearance of anti-parasitic drug resistant strains, in spite of prophylactic policies, creates a more complex scenario. Therefore, considerable efforts are ongoing to find alternative drugs for the treatment of parasitic diseases.

The fused heterocyclic system imidazo[1,2-*a*]pyridine is recognized as an important pharmacophore, which has been investigated in connection to a great number of pharmacological responses. The structural resemblance of imidazo[1,2-*a*]pyridine with the benzimidazole nucleus is one of the reasons why derivatives of the former were tested as antimicrobial (Teulade *et al*., 1978) as well as anti-parasitic (*Trichomonas fetus*) (Winkelmann *et al*., 1977) agents. On the other hand, the hydrophobic character of a molecule often seems to be a very important physicochemical parameter for explaining the variations of biological activity (Golovanov *et al*., 2002; Ačanski and Đaković-Sekulić, 2004; Eros *et al*., 2002; Gensmantel, 1994). However, a correlation of the hydrophobic character of imidazo[1,2-*a*]pyridines with anti-microbial or -parasitic activity has not been investigated. Therefore, an investigation was carried out to explore the influence of the lipophilic factor of a number of selected imidazo[1,2-*a*]pyridine derivatives on anti-parasitic activity in relation to a highly pathogenic strain (*Trichomonas vaginalis*).

Materials and methods

Chemistry

Most of the chemical compounds selected for this investigation are known. Some of them are commercial products, others have been previously described in the literature, and a few are new compounds. Products **3**–**12** were prepared in house according to synthetic Scheme 1, by applying well-established protocols. Compounds were

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identified through their physical constants as well as spectroscopic data. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, using a Varian Mercury Vx 300 MHz spectrometer. New products were further characterized by combustion analysis.

*Ethyl 3-nitro imidazo[1,2-a]pyridine-2-carboxylate (**3**)*

The title compound was prepared following the protocol described by Lombardino (1965) and isolated as greenish needles, 64.7%, mp: 105–107 and 105°C (Teulade *et al*., 1978).

*Ethyl 5-methyl-3-nitro imidazo[1,2-a]pyridine-2-carboxylate (**4**)*

The title compound was obtained following the same protocol (Lombardino, 1965) as yellow crystals, yield 55%, mp: 96–98 and 101°C (Winkelmann *et al*., 1977).

*3-Nitro imidazo[1,2-a]pyridine-2-carboxylic acid (**5**)*

The title compound was isolated as whitish crystals, yield 83%, mp: 208–209 and 206°C (Winkelmann *et al*., 1977).

*3-Nitroimidazo[1,2-a]pyridine (**6**)*

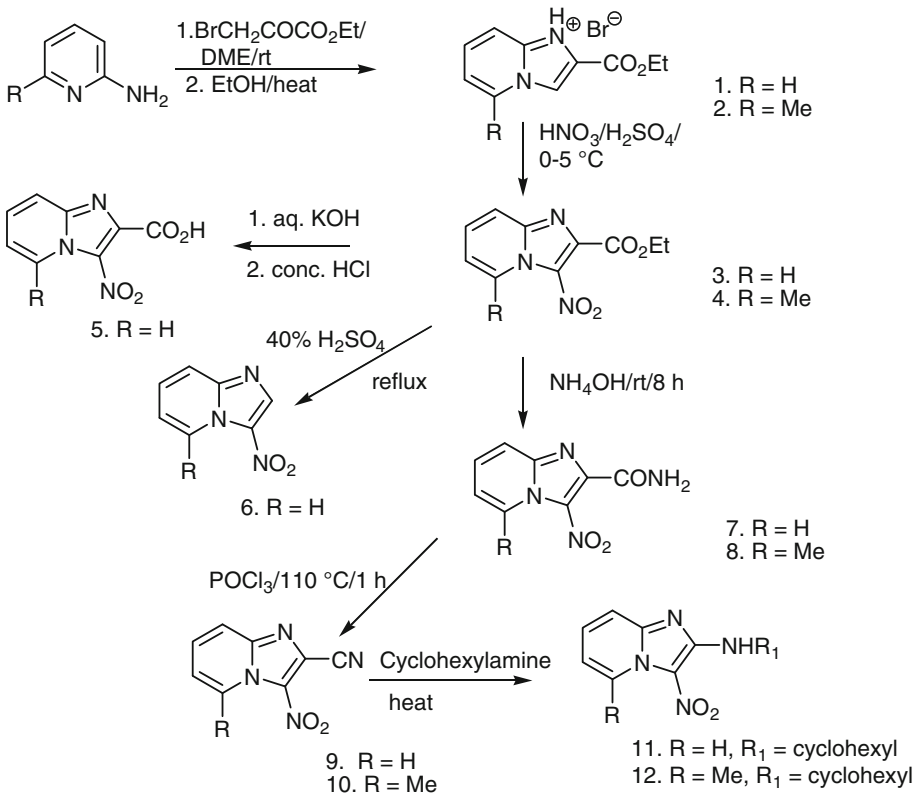
The title compound was isolated as a light yellow solid, yield 86%, mp: 210–211 and 203–204°C (Winkelmann *et al*., 1977).

*3-Nitroimidazo[1,2-a]pyridine-2-carboxamide (**7**)*

The title compound was prepared following a procedure in the literature and isolated as colorless needles in 70% yield, mp: 274–276, 265°C (Teulade *et al*., 1978) and 275–276°C (Rizo *et al*., 2004).

*5-Methyl-3-nitroimidazo[1,2-a]pyridine-2-carboxamide (**8**)*

Following the same protocol (Rizo *et al*., 2004), the title compound was obtained as mustard colored crystals, in 80% yield, mp: 212–214°C. IR νcm^{-1} 1683, 1528. ^{1}H NMR ($DMSO_{d6}$) δ 7.76 (d, $J_{8,7}$ 9.2 Hz, 1H), 7.66 (dd, $J_{7,6}$ 7.0, $J_{7,8}$ 7.0 1H), 7.23 (d, $J_{6,7}$ 7.0, 1H), 2.5 (s, 3H), 7.6 (bs, 1H), 8.2 (bs, 1H). ^{13}C NMR δ 19.0, 114.8, 115.85, 117.75, 130.47, 137.51, 137.56, 143.35, 162.39. $C_9H_8N_4O_3$. Anal. calcd. for: C, 49.09; H, 3.66; N, 25.45; found: C, 49.31; H, 3.83; N, 25.14.



Scheme 1 Synthetic route used to obtain imidazo[1,2-*a*] pyridine derivatives

*3-Nitroimidazo[1,2-a]pyridine-2-carbonitrile (**9**)*

The title compound was prepared following a procedure in the literature (Arias *et al*., 2006) and isolated as reddish crystals in 80% yield, mp: 167–168 and 162°C (Winkelmann *et al*., 1977).

*5-Methyl-3-nitroimidazo[1,2-a]pyridine-2-carbonitrile (**10**)*

The title compound was obtained from 2-carboxamido-5-methyl-3-nitroimidazo[1,2-*a*]pyridine, following the same procedure (Arias *et al*., 2006) and isolated as light brown crystals in 90% yield, mp: 144–146°C. IR ν cm $^{-1}$ 2250. ^{1}H NMR ($DMSO_{d6}$) δ 7.9 (m, 2H), 7.45 (d, $J_{6,7}$ 6.05, 1H) 2.7(s, 3H). ^{13}C NMR δ 21.15, 112.78, 116.42, 119.01, 120.77, 121.4, 133.36, 140.03. 146.99. $C_9H_6N_4O_2$. Anal. calcd. for: C, 53.47; H, 2.99; N, 27.71; found: C, 53.79; H, 3.32; N, 27.58.

*2-(N-cyclohexyl)amino-3-nitroimidazo[1,2-a]pyridine (**11**)*

The title compound was prepared according to the same procedure and isolated as a yellow solid in 62% yield, mp: 156–158 and 157–159°C (Arias *et al*., 2006).

*2-(N-cyclohexyl)amino-5-methyl-3-nitroimidazo[1,2-a]pyridine (**12**)*

5-Methyl-3-nitroimidazo[1,2-a]pyridine-2-carbonitrile was heated with an excess of cyclohexylamine, following the same procedure (Arias *et al*., 2006) at 60–70°C to give the title compound as dark brown crystals in 70% yield, mp: 122–124°C. IR (KBr) ν cm^{-1} 3369, 1601, 1475. ^{1}H NMR ($DMSO_{d6}$) δ 7.54 (dd, $J_{7,8}$ 8.74, $J_{7,6}$ 7.23, 1H), 7.44 (m, 1H), 7.27 (d, $J_{8,7}$ 8.74, 1H), 6.8 (d, $J_{6,7}$ 6.46, 1H), 2.6 (s, 3H), 1.2–2.2 (m, 11H). ^{13}CNMR δ 22.5, 24.6, 25.4, 33.2, 51.3, 112.1, 115.5, 134.5, 143.0, 150.6, 155.9. $C_{14}H_{18}N_4O_2$. Anal. calcd. for: C, 61.30; H, 6.61; N, 20.42; found: C, 61.01; H, 6.69; N, 20.1.

Anti-parasitic activity: in vitro evaluation

Strain treatment

The anti-parasitic activity of the imidazo[1,2-*a*]pyridine derivatives **3–12** was evaluated using *Trichomonas vaginalis GT3* (GT3 is a highly pathogenic strain isolated in the City of Guanajuato, Mexico) micro-cultures in multipanel plates. The culture medium (TYIS-33) was prepared as described by Diamond (1995) and used for both proliferation and biological assays. Previously, fetal bovine serum (FBS, lot A 7502 Microlab, Mexico) and the Diamond vitamin mix (in vitro Laboratories, Mexico) were tested on the coupling and proliferation of the culture until a steady growth was reached. Benzyl penicillium (2%) was incorporated into the medium to ensure axenic conditions.

Experimental conditions

Treatment was carried out at 24 and 48 h. Controls used were metronidazole (Rhone-Poulenk, Mexico) and a series of seven untreated populations (400,000–150,000 trophozoites), and fresh culture broth was included. In order to show that a maximum solvent concentration in the drug sensitivity test had no effect on the viability of the parasites, dimethyl sulphoxide (DMSO, biological grade, Sigma-Aldrich, <0.03%, v/v) was included.

Procedure

The working solutions were prepared by dissolving 10 mg of each compound or of metronidazole, in DMSO and diluting with the culture medium (TYI-S-33), while the multipanel plate (96 wells bottom plane), was prepared by addition of 100 µl of the culture medium to each well. The operation was successively repeated so that the serial twofold dilutions allowed seven concentrations ranging from 0.5 to 32 µg/ml throughout the plate. Then, a suspension (100 µl) of trophozoites (50,000) was incorporated into each well and incubated during 24 h at 37°C. Controls without drug application were included in each plate and contained different amounts of parasites, ranging from 100 (50, 000 parasites) to 1.5% (750 parasites). Alamar blue (20 µl) was added to each well and the plate was monitored during 3 h.

Colorimetric evaluation of trichomonicidal activity was carried out based on the properties of the metabolic indicator alamar blue. The evident end point (blue to pink) and the intermediate color tones of the cultures allowed for assessment of the minimal inhibitory concentration (MIC) required to inhibit cell growth (Yajko *et al*., 1995). Tests were carried out in triplicate with independent runs, under a randomly double blind designed bioassay.

Spectrophotometric (UVWINLAB Perkin Elmer) readings at $\lambda = 630$ for the oxidized form (blue) of alamar blue and at $\lambda = 570$ for the reduced form (pink) were obtained with the aid of the Beckman coulter lector software, thus enabling the calculation of viable and non-viable trophozoites in each culture. Then, the absorbance data obtained from the micro culture readings were interpolated in the corresponding trophozoite population calibration curve to calculate the percentage of dead parasites. This latter value was plotted against the concentration of the respective compound to obtain the concentration-activity profile. The

compound concentration required to cause the death of 50% (EC_{50}) of the microorganisms was estimated as well.

Log *P* calculation

The partition coefficient log *P* was experimentally determined for each compound in a 1-octanol/water (1-octanol, Sigma-Aldrich Analytical grade) system by applying the shake flask methodology (Sangster, 1997). The maximum absorbance (λ_{max}) of each compound, metronidazole included, was obtained by UV spectroscopy. Then, a conveniently stable concentration (0.85 mg/ml) of compound/1-octanol (pre-saturated with water) was prepared as a stock solution. From this, series of dilutions in ethyl alcohol were obtained and read on the spectrophotometer. The concentration of each compound was adjusted through absorbance sweeps so that the λ_{max} values fell within a range of 0.2–0.8 absorbance units (concentrations fell in the interval 0.001–0.01 mg/ml).

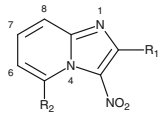
Triplicate test vessels containing the required, accurately measured amounts of the two solvents together [1-octanol/buffer solution (0.25 M, pH 7.4, JT Baker)] with the necessary quantity of the stock solution were prepared for each of the test conditions. In addition, a tube containing an ethyl alcohol/buffer solution (blank) and a tube containing only a solution of 1-octanol/compound (concentrated solution) were included.

The extraction systems of a two-phase 1-octanol-buffer solution were prepared in a calculated volume ratio of 1:1, 1:2 and 2:1 and then shaken for 10 min at 25 ± 1°C in a shaker lab-Line 3D rotator, and allowed to stand for 48 h. Once the partition equilibrium was reached between phases, the concentration of each compound in the organic phase was evaluated by taking an aliquot from each tube for each test condition, and then further analyzed by UV spectroscopy. The total quantity of substance present in both phases was calculated by comparison with the quantity of the substance originally introduced (concentrated solution). Log *P* was calculated as log (ratio of the concentration in the 1-octanol phase to the concentration in the aqueous phase). Metronidazole was included for comparison (Leo *et al*., 1971).

Results and discussion

The trichomonicidal activity profile was obtained as a function of compound concentration, and the EC_{50} values were determined. Molar MICs were calculated from the minimum concentrations that inhibited proliferation of the culture, as indicated by alamar blue. Table 1 shows the trichomonicidal activity exhibited by the tested compounds in terms of both parameters.

Table 1 Trichomonicidal activity of nitro imidazo[1,2-*a*]pyridine derivatives



Compounds	R_1	R_2	Trichomonicidal activity		
			EC_{50} (μM)	MIC (μM)	
			48 h	24 h	48 h
3	COOEt	H	3.96	2.13	2.13
5	COOH	H	21.10	19.30	19.30
6	H	H	2.45	3.01	3.07
7	$CONH_2$	H	41.30	9.71	9.71
9	CN	H	9.68	5.32	2.66
11	NHC_6H_{10}	H	61.53	61.50	30.80
4	COOEt	CH_3	9.11	4.02	4.02
8	$CONH_2$	CH_3	14.63	9.09	9.09
10	CN	CH_3	12.12	4.95	2.48
12	NHC_6H_{10}	CH_3	64.27	58.40	29.20
Metronidazole			1.20	2.92	2.92

Mean values of log *P* are shown in Table 2. Standard deviations from the average readings of the ratios of three volumes were not statistically significant and therefore the method was considered reliable.

Correlation of log *P* and anti-parasitic activity

Average experimental values of log *P* were used to establish the correlation with anti-parasitic activity data. Different mathematical descriptors were tried to establish such correlation. It was found that the mathematical expression shown in Eq. 1 is statistically significant with

Table 2 Log *P* values of imidazo[1,2-*a*]pyridine derivatives

Compounds	Log *P*				
	1-Octanol/water volume ratio				
	1:1	1:2	2:1	Log *P* average	λ_{max}
3	0.983	1.045	0.963	0.997 ± 0.035	265.30
5	−0.041	−0.042	−0.038	−0.040 ± 0.002	252.50
6	0.938	0.960	0.922	0.940 ± 0.016	252.45
7	−0.515	−0.569	−0.540	−0.541 ± 0.022	258.24
9	0.691	0.641	0.692	0.675 ± 0.024	257.80
11	1.541	1.500	1.503	1.514 ± 0.019	230.22
4	1.268	1.204	1.303	1.258 ± 0.041	269.80
8	0.670	0.690	0.695	0.690 ± 0.011	265.50
10	1.193	1.125	1.083	1.135 ± 0.045	263.10
12	1.556	1.500	1.527	1.528 ± 0.023	235.19
Metronidazole	0.120	0.110	0.115	0.119 ± 0.004	310.25

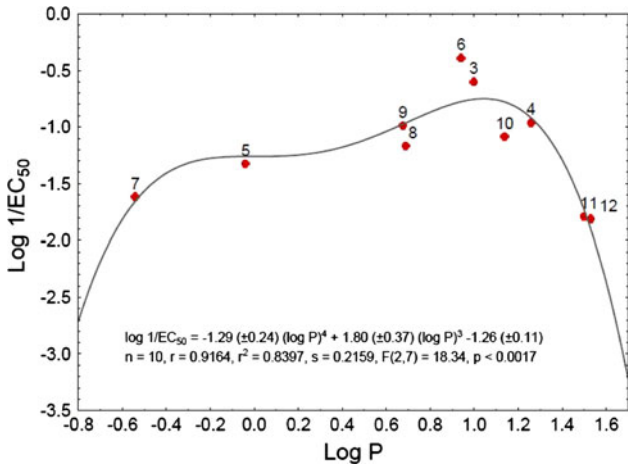


Fig. 1 Correlation of anti-parasitic activity with the hydrophobic parameter log *P*. The STATISTICA program was used to obtain the correlation

each of its coefficients other than zero, and therefore was chosen as a model. The correlation coefficient, $r = 0.916$, was obtained. A polynomial expression of order 4 was examined as well. However, its coefficients were not statistically significant and therefore it was ruled out.

$$\log\ 1/\mathrm{EC}_{50} = -1.29(\pm 0.24)\,(\log P)^4 + 1.80\,(\pm 0.37) \times (\log P)^3 - 1.26(\pm 0.11) \quad (1)$$

$$n = 10$$

$$(r = 0.9164),\ \left(r^2 = 0.8397\right),\ s = 0.2159,\ F(2.7) = 18.34,\ P < 0.0017.$$

Equation 1 shows that the two variables describe up to 84% of the total biological activity in accordance with the determination of the coefficient, $r^2 = 0.8397$. Compound **6** may be excluded from the correlation plot on the basis that it falls 95% of the confidence limits (Moridani *et al*., 2004). Furthermore, if eliminated a better coefficient would result (an increase >0.05 units is actually obtained). However, it was included.

Figure 1 shows the graph obtained by applying the mathematical model described in Eq. 1. In agreement with these results, an optimum interval of lipophilic character does exist where the tested compounds show good biological response.

Most of the compounds showed anti-parasitic activity within 24 h of treatment, compounds **9**, **10**, **11** and **12** showed a maximum effect after 48 h according to the MIC values, suggesting that these molecules should be biotransformed to more active species. After 24 h of treatment, nitro derivative **6** had a trichomonicidal activity (MIC) comparable to that given by metronidazole. Likewise, nitro-ester **3** and the nitro-cyano derivatives **9** and **10** exhibited anti-parasitic activity similar to that shown by metronidazole itself.

As anticipated, the introduction of the methyl group in the pyridine moiety increased the lipophilic character of the imidazo[1,2-*a*]pyridine derivatives, evidenced by greater log *P* values and higher hydrophobicity (Table 2). Log *P* of metronidazole was 0.11, while compound **12** was the most lipophilic (15.28) and the least active.

With regard to the quantitative anti-parasitic activity and its correlation with the partition coefficient, the following observations were made. Nitro-amide **7** was the most hydrophilic of the series (−0.541), with a rather poor activity. In general, the presence of the methyl group (compounds **4**, **10** and **12**) diminished the anti-parasitic activity. Remarkably, with derivative **8**, in which the nitrocarboxamide carried a methyl group, both hydrophobicity as well as the anti-parasitic activity was increased compared to the non-methylated analog **7**. The most active compounds, **3** and **6**, had lipophilic values of 0.997 and 0.94, respectively. In accordance with these results, an optimum range of lipophilic character must exist for the compounds to show an efficient biological response.

If the partition coefficient (log *P*) were the determining physical property influencing the in vitro activity of these compounds, a bell-shaped distribution curve would have been obtained, and all the points in Fig. 1 would fit into one plot, indicating a standard biological effect (Hansch *et al*., 1968). However, the fact that a plateau is present in the curve shape clearly indicates that additional factors (primarily electronic) should be operating as well (Butler *et al*., 1967).

Conclusion

The results indicate that ethyl 3-nitroimidazo[1,2-*a*]pyridine-2-carboxylate **3** and the 3-nitroimidazo[1,2-*a*]pyridine **6** had good anti-parasitic activity, requiring 2.45–3.96 μM to reach the desired effect.

An experimental partition coefficient (log *P*) determination for all the tested compounds indicated an increase in the hydrophobicity of the methyl-substituted imidazo[1,2-*a*]pyridine derivatives. A mathematical descriptor correlating the exhibited pharmacological activity and log *P* was found (0.9 ± 0.3 log *P*), which suggests that an optimum balance between hydrophilic and lipophilic properties is most convenient. Calculations based on Eq. 1 show that 84% of the total activity variation found may be described by these two variables.

Acknowledgments Financial support through grant No. 49937, Consejo Nacional de Ciencia y Tecnología (CONACYT) México is gratefully acknowledgedSpecial thanks to Dr. Marco Meráz,

Departamento Biología Molecular, CINVESTAV México for kindly providing the strains of *Trichomonas vaginalis*

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