Leishmanicidal Activity of New Megazol Derivatives

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Abstract: A series of thirteen new megazol derivatives, designed exploring the molecular hybridization approach between megazol (**3**) and heterocombretastatins (**2**), was synthesized. These new compounds were tested for *in vitro* antiparasitic activity upon axenic amastigotes of *Leishmania donovani*. Biological results led us to identify a new potent megazol derivative (**4g**), which presents an IC_{50} = 0.081μg/mL, more active tham the reference drug miltefosine (IC_{50} = 0.131μg/mL).

Key Words: Leishmanicidal, megazol derivatives, molecular hybridization, heterocombretastatin, cytotoxic, sulfones.

1. INTRODUCTION

Visceral leishmaniasis, caused by *Leishmania donovani*, is an endemic disease in many parts of the world and affects an estimated 15 million people world-wide [1]. Drugs currently in use, such as pentavalent antimony compounds, pentamidine, miltefosine or amphotericin B, are inadequate due to their toxicity, lack of efficacy, availability and affordability, and the inability to eliminate all parasite life cycles stages from the host [2]. Consequently, the search for new drugs for the treatment of the leishmaniases is of highest priority.

Combretastatins are an important group of anticancer drugs [3, 4], isolated from the bark of the South African tree *Combretum caffrum*. In particular, cis-combretastatins A-4 (**1**) disrupt tubulin aggregation, thereby preventing metastasis and angiogenesis, which places among the most active of antineoplastic agents. Del Rey and co-workers [5] described the *in vitro* antileishmanial activity of heterocombretastatins (**2**) against three different species of *Leishmania*.

Megazol (**3**), a 5-nitroimidazole derivative synthesized by American Cyanamid Co. in 1968 [6], was identified as being active against *Trichomonas* species. Investigators from FIOCRUZ have shown that megazol is also highly active against *T. cruzi* [7, 8]. Against *Leishmania infantum* megazol reduces 95% of the total parasites at 6.2μM [9]. Because of its high anti-protozoal activity, megazol's core structure has been used as a template for the design of new compounds against Chagas disease [10, 11], leishmaniose [12] and, recently, some sulfanylated megazol derivatives exhibited antituberculosis activity [13, 14].

In continuation of our studies based on the synthesis and evaluation of the antiparasitic activity of megazol derivatives, we describe the leishmanicidal and cytotoxicity profile of new 5-benzyl-sulfanyl/sulfonyl megazol derivatives (**4** and **5**). The design of the inhibitors explored the modulation of the methoxy substituted phenylacethyl moiety of the previously described active heterocombretastatin (**2**) pharmacophore (A, Fig. **1**) combined with the megazol (**3**) core. Thus, the incorporation of the sulfone moiety (B) was used as a nonclassical bioisoster of the carbonyl group [15, 16]. In this work, we present the preliminary results of the biological evaluation of these new 5-benzyl-sulfanyl/sulfonyl megazol derivatives.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Megazol (**3**) was employed as starting material [17, 18]. The corresponding chloride (**6**) was prepared exploiting the diazotation and Sandmeyer reaction with CuCl generated *in situ* [19]. The key intermediate, 2-mercapto-5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazole (**7**), was prepared reacting **6** with thiourea in refluxing ethanol [20, 21]. The condensation of this intermediate with the appropriate benzyl chloride afforded the sulfides derivatives (**4a-h**). Sulfones derivatives series (**5**) were prepared by usual procedures from sulfides (**4**), using an excess of oxone, as described in Scheme **1** [22].

2.2. Biological Evaluation

The antileishmanial and cytotoxic properties of megazol derivatives are shown in Table **1**, and their inhibitory activities at 4.85μg/mL were determined. With these results of the leishmanicidal profile, we elected the most active compounds (**4e**, **4g**, **4h**, **5a**, **5b**, **5d**, **5e** and **5h**) to determine their IC_{50}. The sulfones derivatives (**5**) shown higher percentage of inhibition *in vitro* activity when compared with the corresponding sulfides (**4**), but most of them was much more cytotoxic them the sulfides, which considerable decrease the selectivity index (SI) (IC_{50} for cytotoxicity divided by IC_{50} for Leishmanicidal activity). The sulfide (**4g**) was the most active compound (IC_{50} = 0.081μg/mL) and presents the best SI (240).

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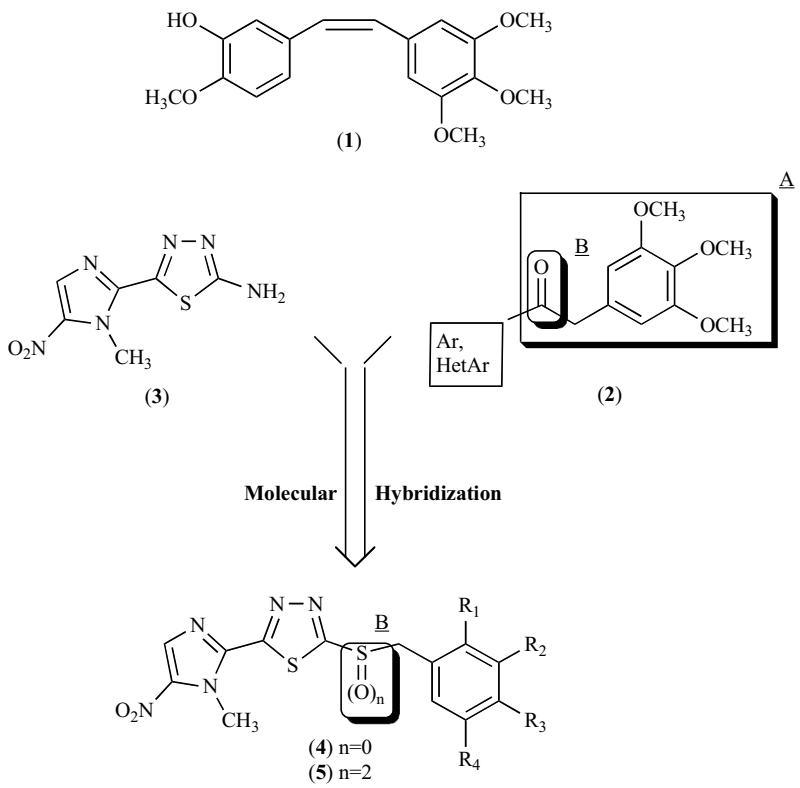


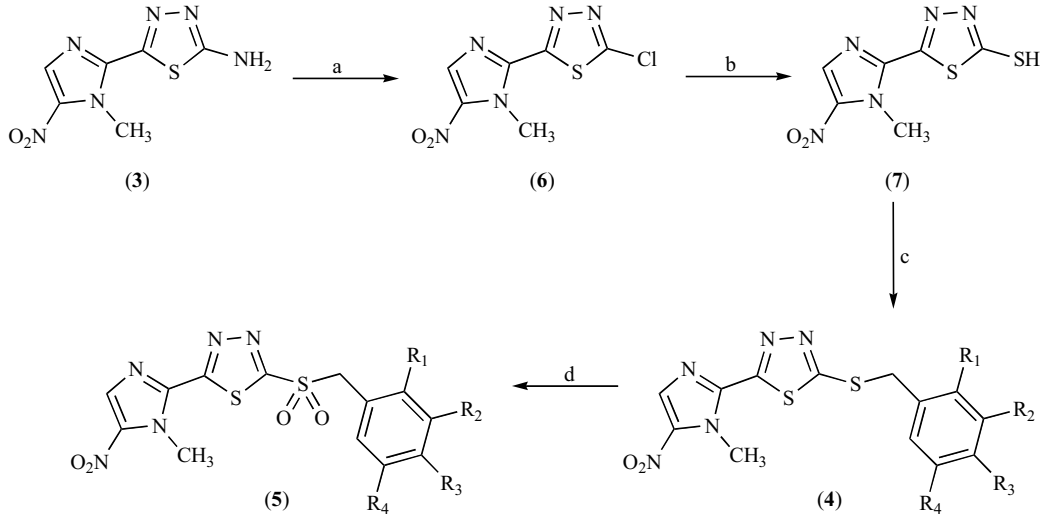
Fig. (1). Design concept of the megazol derivatives (**4**) and (**5**).

2.3. Prediction of *In Vivo* Absorption: Lipinski's Rule of Five

Lipinski's Rule of Five [23, 24] has been widely used as a filter for substances that would likely to be further developed in drug design programs. This simple rule states that oral bio-availability is likely to occur if at least three of the following rules are obeyed: a) hydrogen bond donors ≤ 5 (OH and NH groups); b) hydrogen bond acceptors ≤ 10 (N and O atoms); molecular weight < 500; d) calculated log P (ClogP) < 5.

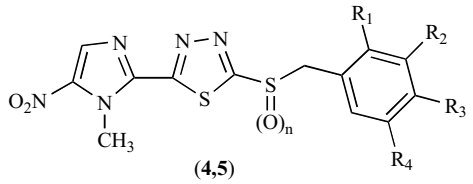
In addition to leishmanicidal activity, *in vivo* absorption capabilities of two promising megazol derivatives from this series were tentatively assessed by means of theoretical calculations following this approach [25].

The results of the calculations for the sulfide (**4g**) and the sulfone (**5h**) showed that both presented a potential for good



Scheme 1. Reagents and conditions: (a) $NaNO_2$, HCl, Cu^0; (b) Thiourea, Ethanol, Reflux; (c) KOH (85% aq.); (d) $PhCH_2Cl$, 23-88%; (e) Oxone, Al_2O_3, $CHCl_3$, Reflux

Table 1. *In Vitro* Leishmanicidal Activity and Cytotoxicity of Megazol Derivatives (4, 5)



(**4,5**)

Cpd	R_1	R_2	R_3	R_4	n	*L. donovani* *Axenic Amastigotes*			Cytotoxicity L-6 Cells	SI
						conc[a]	% inhib.	IC_{50}[a]	IC_{50}[a]	
4a	H	H	H	H	0	4.85	38.7	nd	38.64	-
5a	H	H	H	H	2	4.85	100	0.494	1.36	2.8
4b	H	H	OMe	H	0	4.85	32.3	nd	38.24	-
5b	H	H	OMe	H	2	4.85	100	0.326	2.75	8.4
4c	H	H	Cl	H	0	4.85	45.6	nd	>90	-
4d	H	H	F	H	0	4.85	24.9	nd	49.00	-
5d	H	H	F	H	2	4.85	100	0.598	14.77	24.7
4e	Cl	Cl	H	H	0	4.85	73.2	5.32	>90	>16
5e	Cl	Cl	H	H	2	4.85	100	0.404	3.71	9.2
4f	H	OMe	OMe	H	0	4.85	72.1	nd	65.95	-
4g	H	OMe	OMe	OMe	0	4.85	78.5	0.081	19.44	240
4h	H	$-OCH_2O-$		H	0	4.85	76.3	6.83	>90	>13
5h	H	$-OCH_2O-$		H	2	4.85	100	0.268	2.91	10.9
Miltefosine	-	-	-	-	-	-	-	0.131		

[a] μg/mL.

nd = not done.

in vivo absorption, since they satisfied Lipinski's rule of five without violations (Table **2**).

3. CONCLUSION

In conclusion, thirteen new megazol derivatives, based on a combination of the heterocombretastatin pharmacophore and megazol (**3**) have been prepared, and their inhibitory activities were determined. We discovered a new potent thioeter megazol derivative (**4g**) which present an IC_{50} 1.6 more potent them the reference drug miltefosine, which could be a good start of point to further studies in the search to find new lead compounds with different framework.

4. EXPERIMENTAL

4.1. Chemistry

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer in potassium bromide pellets and frequencies are expressed in cm^{-1}. NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400.00 MHz (^1H) and 100 MHz (^{13}C). Chemical shifts are reported in ppm (δ). Proton and carbon spectra were typically obtained at room temperature. GC/MS analyses were carried on GC (6890M)/MS (5973) Agilent Technologies system. Thin-layer chromatography (TLC) on silica

Table 2. Molecular Descriptors for Lipinski's Rule of Five of 4g and 5h Derivatives

Compound.	MW	CLOGP	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Satisfies the Rule of Five?
4g	423.5	2.86	0	10	yes
5h	409.4	2.26	0	9	yes

gel was run in ethyl acetate/methanol mixture and spots were observed by Ultraviolet light. The progress of all reactions was monitored by TLC, which was performed on 2.0 X 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light (254–265 nm).

*4.1.1. General Procedure for Preparing 2-Substituted benzylsulfanyl-5-(1-methyl-5-nitro-1***H***-imidazol-2-yl)-1,3,4-thiadiazole)(4a-4h)*

The mercapto derivative (**7**) (0.003 mol.) was suspended in water (9 mL), and 0.3 mol. of KOH (85% solution) was added under stirring at room temperature. After a few minutes (5-10 min), the solution was brought to 0°C in an ice bath, and the appropriate benzyl chloride (0.003 mol) was dropped in with vigorous stirring. The reaction mixture was monitored by TLC. After 4 hours, the reaction was completed, water (30 mL) was added and the precipitated solid was filtered, washed with water and crystallized from acetone. The following compounds were prepared according to the general procedure.

4.1.1.1. 2-Benzylsulfanyl-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4a)

Yield 88%, m p. 152.8 °C. IR (KBr, ν cm^{-1}) 3117, 1531, 1331, 1085. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.53 (s, 3H, CH_3); 4.72 (s, 2H, CH_2); 7.31-7.58 (m, 5H, Ph); 8.13 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 34.52, 37.08, 127.30, 128.10, 128.64, 132.06, 135.58, 139.94, 140.55, 159.47, 167.36. CG/MS: (*m/z*) [M$^+$] 333.

*4.1.1.2. 2-(4-Methoxy-benzylsulfanyl)-5-(1-methyl-5-nitro-1***H***-imidazol-2-yl)-[1,3,4]thiadiazole (4b)*

Yield 82%, m.p. 155.5°C. IR (KBr, ν cm^{-1}):3131, 1519, 1351, 1121. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 3.73 (s, 3H, OCH_3); 4.36 (s, 3H, CH_3); 4.61 (s, 2H, CH_2); 6.92 (d, *J*= 8.00 Hz, 2H, Ph); 7.43 (d, *J* = 8.00 Hz, 2H, Ph); 8.27 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 35.27, 37.06, 55.01, 113.54, 113.97, 127.50, 129.92, 130.39, 133.08, 139.95, 140.73, 158.81, 159.49, 168.12. CG/MS: (*m/z*) [M$^+$] 363.

4.1.1.3. 2-(4-Chloro-benzylsulfanyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4c)

Yield 39%, m.p. 134.4°C. IR (KBr, ν cm^{-1}): 3116, 1531, 1482, 1085. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.51 (s, 3H, CH_3); 4.71 (s, 2H, CH_2); 7.40 (d, *J* = 8.00 Hz, 2H, Ph); 7.59 (d, *J* = 8.00 Hz, 2H, Ph); 8.11 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 34.53, 36.13, 128.09, 130.39, 132.07, 132.60, 134.88, 139.90, 159.64, 166.95. CG/MS: (*m/z*) [M$^+$] 367.

4.1.1.4. 2-(4-Fluoro-benzylsulfanyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4d)

Yield 23%, m.p. 116.2°C. IR (KBr, ν cm^{-1}): 3139, 1525, 1351, 1074. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.36 (s, 3H, CH_3); 4.66 (s, 2H, CH_2); 7.54 (d, *J* = 8.50 Hz, 2H, Ph); 7.56 (d, *J* = 8.50 Hz, 2H, Ph); 8.26 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 35.25, 36.51, 115.27, 115.44, 131.12, 131.19, 132.33, 133.05, 139.30, 140.73, 159.67, 160.58, 167.73. CG/MS: (*m/z*) [M$^+$] 351.

4.1.1.5. 2-(2,3-Dichloro-benzylsulfanyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4e)

Yield 55%, m.p. 139.1°C. IR (KBr, ν cm^{-1}):3123, 1529, 1353, 1081. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.37 (s, 3H, CH_3); 4.81 (s, 2H, CH_2); 7.37 (t, *J* = 8.00 Hz, 1H, Ph); 7.62 (dd, *J* = 8.00 Hz, 1H, Ph); 8.28 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, Acetone-d_6): 36.11, 36.98, 129.02, 131.10, 131.24, 133.11, 133.64, 133.85, 137.64, 141.37, 161.50, 168.00. CG/MS: (*m/z*) [M$^+$] 402.

4.1.1.6. 2-(3,4-Dimethoxy-benzylsulfanyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4f)

Yield 76%, m.p. 145.8°C. IR (KBr, ν cm^{-1}): 3116, 1519, 1357, 1084. ^{1}H-NMR (400 MHz; δ ppm, Acetone-d_6): 3.79 (s, 3H, OCH_3); 3.81 (s, 3H, OCH_3); 4.52 (s, 3H, CH_3); 4.64 (s, 2H, CH_2); 6.92 (d, *J* = 8.40 Hz, 1H, Ph); 7.07 (dd, *J* = 2.0 Hz, *J* = 8.40 Hz, 1H, Ph); 7.15 (d, *J* = 2.0 Hz, 1H, Ph); 8.11 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, Acetone-d_6): 35.27, 37.53, 55.34, 111.50, 112.35, 121.50, 127.64, 133.10, 139.94, 140.71, 148.40, 159.48, 168.16. CG/MS: (*m/z*) [M$^+$] 393.

4.1.1.7. 2-(1-Methyl-5-nitro-1H-imidazol-2-yl)-5-(3,4,5-trimethoxy-benzylsulfanyl)-[1,3,4]thiadiazole (4g)

Yield 52%, m.p. 185°C. IR (KBr, ν cm^{-1}): 3138, 1570, 1347, 1124. ^{1}H-NMR (400 MHz; δ ppm, $CDCl_3$): 3.85 (s, 3H, OCH_3); 3.87 (s, 6H, OCH_3); 4.56 (s, 3H, CH_3); 4.60 (s, 2H, CH_2); 6.68 (s, 2H, Ph); 8.08 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, $CDCl_3$): 35.63, 38.66, 56.17, 60.81, 106.29, 130.50, 133.15, 137.89, 140.53, 153.43, 159.66, 168.42. CG/MS: (*m/z*) [M$^+$] 423.

4.1.1.8. 2-(Benzo[1,3]dioxol-5-ylmethylsulfanyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (4h)

Yield 72%, m.p. 197.4°C. IR (KBr, ν cm^{-1}): 3149, 1530, 1350, 1075. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.36 (s, 3H, CH_3); 4.59 (s, 2H, CH_2); 6.01 (s, 2H, O-CH_2-O); 6.88 (d, *J* = 8.00 Hz, 1H, Ph); 6.99 (dd, *J* = 2.0 Hz, *J* = 8.00 Hz, 1H, Ph); 7.07 (d, *J* = 2.0 Hz, 1H, Ph); 8.27 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 36.09, 38.65, 102.36, 109.08, 110.31, 123.87, 130.66, 133.66, 141.49, 148.49, 148.96, 161.00, 168.66. CG/MS: (*m/z*) [M$^+$] 377.

4.1.2. General Procedure for Preparing 2-Substituted benzylsulfonyl-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole) (5a, 5b, 5d, 5e and 5h)

The 2-substituted benzylsulfonyl derivatives (2.0 mmol) was added to vigorously stirred suspension of wet alumina (2.0g) and Oxone® (3.70g, 6.0 mmol) in chloroform (10 mL). The mixture was heated at reflux. After 48 hours, the mixture was cooled and the solids washed with chloroform. Removal of solvent afforded sulfone derivatives. The following compounds were prepared according to the general procedure.

4.1.2.1. 2-(1-Methyl-5-nitro-1H-imidazol-2-yl)-5-benzylsulfinyl-[1,3,4]thiadiazole (5a)

Yield 54%. m.p. 162.2 °C. IR (KBr, ν cm^{-1}): 3123, 1525, 1331, 1367, 1162. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.45 (s, 3H, CH_3); 4.95 (s, 2H, CH_2); 7.21-7.29 (m, 5H, Ph);

8.03 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 34.81, 60.73, 126.18, 128.21, 128.64, 130.88, 132.15, 138.87, 141.09, 164.83, 168.20. CG/MS: (*m/z*) [M^+] 365.

4.1.2.2. 2-(4-Methoxy-benzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (5b)

Yield 56% (the reaction time was 48 hours). m.p. 160.2 °C. IR (KBr, ν cm^{-1}): 3140, 1525, 1346, 1426, 1146. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 3.74 (s, 3H, OCH_3); 4.14 (s, 3H, CH_3); 5.15 (s, 2H, CH_2); 6.92 (d, J = 8.00 Hz, 2H, Ph); 7.26 (d, J = 8.00 Hz, 2H, Ph); 8.34 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 36.41, 55.77, 61.84, 115.27, 119.24, 133.74, 140.52, 161.65, 166.36, 170.03. CG/MS: (*m/z*) [M^+] 395.

4.1.2.3. 2-(4-Fluoro-benzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (5d)

Yield 15% (the reaction time was 48 hours). m.p. 226.4 °C. IR (KBr, ν cm^{-1}): 3131, 1519, 1460, 1352, 1170. ^{1}H-NMR (400 MHz; δ ppm, Acetone-$_{d6}$): 4.60 (s, 3H, CH_3); 5.12 (s, 2H, CH_2); 7.18(m, 2H, Ph); 7.50 (m, 2H, Ph); 8.17 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 35.47, 60.05, 115.40, 124.17, 132.71, 133.58, 139.41, 141.08, 161.24, 163.14, 179.40.

4.1.2.4. 2-(2,3-Dichloro-benzylsulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (5e)

Yield 53% (the reaction time was 48 hours). m.p. 192.1 °C. IR (KBr, ν cm^{-1}): 3129, 1520, 1461, 1336, 1154. ^{1}H-NMR (400 MHz; δ ppm, DMSO-$_{d6}$): 4.42 (s, 3H, CH_3); 5.44 (s, 2H, CH_2); 7.45 (d, 1H, Ph); 7.51 (d, J = 7.5 Hz, 1H, Ph); 7.74 (d, J = 8.0 Hz, 1H, Ph); 8.36 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 35.52, 55.33, 60.82, 111.50, 114.20, 119.43, 123.82, 133.14, 138.88, 139.40, 149.01, 163.07, 168.11. CG/MS: (*m/z*) [M^+] 434 .

4.1.2.5. 2-(Benzo[1,3]dioxol-5-ylmethanesulfinyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-[1,3,4]thiadiazole (5h)

30% (the reaction time was 48 hours). m.p. 194.3 °C. IR (KBr, ν cm^{-1}): 3144, 1532, 1362, 1406, 1206. ^{1}H-NMR (400 MHz; δ ppm, DMSO-d_6): 4.42 (s, 3H, CH_3); 5.14 (s, 2H, CH_2); 6.04 (s, 2H, O-CH_2-O); 6.80-6.87 (m, 2H, Ph); 6.95 (d, 1H, Ph); 8.35 (s, 1H, Im). ^{13}C-NMR (100 MHz, δ ppm, DMSO-d_6): 36.40, 62.14, 101.01, 102.48, 109.36, 112.22, 120.88, 126.55, 133.74, 140.52, 149.12, 149.73, 169.34. CG/MS: (*m/z*) [M^+] 409.

4.2. Leishmanicidal Activity

Amastigotes of Leishmania donovani strain MHOM/ET/67/L82 were grown in axenic culture at 37°C in SM medium [25] at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% $C0_2$ in air. 100 µl of culture medium with 10^5 amastigotes from axenic culture with or without the compound to test were seeded in 96-well microtiter plates. Two formats were used: A medium throughput assay using two set compound concentration (4.85 µg/ml) and determining the % inhibition of growth *vs*. the untreated controls, and a serial drug dilution assay using seven 3-fold dilutions covering a range from 30 µg/ml to 0.041 µg/ml and determining the 50% inhibition concentration (IC_{50}). If the inhibition at 0.81 µg/ml was below 50%, then the IC_{50} value was not determined. For compounds **4e** and **4h** the IC_{50} value was directly determined without a prior determination at the two fixed concentrations. Each drug was tested in duplicate and each assay was repeated at least once. After 72 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10µl of Alamar Blue (12.5 mg resazurin dissolved in 100 ml phosphate buffered saline) were then added to each well and the plates incubated for another 2 hours. Then the plates were read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. Data were analysed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (=inhibition) was expressed as percentage of the fluorescence of control cultures. For the serial drug dilution assay inhibition values were plotted against the drug concentrations and IC_{50} values were calculated from the sigmoidal inhibition curves.

4.3. Cytotoxicity

100 µl RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum containing 4x104 L-6 cells (rat skeletal myoblasts) were added to each well of a 96-well microtiter plate. After 24 hours, the medium was removed from all wells and replaced by 100 µl of fresh medium containing a 3-fold serial drug dilution covering a range from 200 µg/ml to 0.274 µg/ml, except for the control wells. After 72 hours of incubation 10 µl of Alamar Blue (12.5 mg resazurin dissolved in 100 ml phosphate buffered saline) were added to each well and the plates were incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer as described for the leishmanicidal assay.

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REFERENCES

[1] Ashford, R.W.; Desjour, P.; DeRaadt, P. Estimation of population at risk of infection and number of cases of leishmaniasis. *Parasitol. Today*, **1992**, *8*, 104-105.

[2] Alvar, J.; Croft, S.; Olliaro, P. Chemotherapy in the treatment and control of leishmaniasis. *Adv. Parasitol.*, **2006**, *61*, 223-274.

[3] Pettit, G.R.; Cragg, G.M.; Herald, D.L.; Schmidt, J.M.; Lohavanijaya, P. Anti-neoplastic agents .84. Isolation and structure of combretastatin. *Can. J. Chem.*, **1982**, *60*, 1374-1376.

[4] Pettit, G.R.; Singh, S.B.; Hamel, E.; Lin, C.M.; Alberts, D.S.; Garcia-Kendall, D. Antineoplastic agents .145. Isolation and structure of the strong cell-growth and tubulin inhibitor combretastatin-a-4. *Experientia,* **1989**, *45*, 209-211.

[5] del Rey, B.; Ramos, A.C.; Caballero, E.; Inchaustti, A.; Yaluff, G.; Medarde, M.; Arias, A.R.; San Feliciano, A. Leishmanicidal activity of combretastatin analogues and heteroanalogues. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2711-2714.

[6] Berkelhammer, G.; Asato, G. 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole - a new antimicrobial agent. *Science*, **1968**, *162*, 1146.

[7] Lages-Silva, E.; Filardi, L.S.; Brener, Z. Effect of the host specific treatment in the phagocytosis of trypanosoma-cruzi blood forms by mouse peritoneal-macrophages. *Mem. Inst. Oswaldo Cruz*, **1990**, *85*, 401-405.

[8] Filardi, L.S.; Brener, Z. A nitroimidazole-thiadiazole derivative with curative action in experimental trypanosoma-cruzi infections. *Ann. Trop. Med. Parasitol.*, **1982**, *76*, 293-297.

[9] Chauviere, G.; Bouteille, B.; Enanga, B.; de Albuquerque, C.; Croft, S.L.; Dumas, M.; Perie, J. Synthesis and biological activity of nitro heterocycles analogous to megazol, a trypanocidal lead. *J. Med. Chem.*, **2003**, *46*, 427-440.

[10] Carvalho, S.A.; da Silva, E.F.; Santa-Rita, R.M.; de Castro, S.L.; Fraga, C.A.M. Synthesis and antitrypanosomal profile of new functionalized 1,3,4-thiadiazole-2-arylhydrazone derivatives, designed as non-mutagenic megazol analogues. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 5967-5970.

[11] Carvalho, S.A.; Lopes, F.A.S.; Salomão, K.; Romeiro, N.C.; Wardell, S.M.S.V.; de Castro, S.L.; da Silva, E.F.; Fraga, C.A.M. Studies toward the structural optimization of new brazilizone-related trypanocidal 1,3,4-thiadiazole-2-arylhydrazone derivatives. *Bioorg. Med. Chem.*, **2008**, *16*, 413-421.

[12] Foroumadi, A.; Emami, S.; Pournourmohammadi, S.; Kharazmi, A.; Shafiee, A. Synthesis and *in vitro* leishmanicidal activity of 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-substituted-1,3,4-thiadiazole derivatives. *Eur. J. Med. Chem.*, **2005**, *40*, 1346-1350.

[13] Foruomadi, A.; Soltani, F.; Moallemzadeh-Haghighi, H.; Shafiee, A. Synthesis, *in vitro*-antimycobacterial activity and cytotoxicity of some alkyl alpha-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetates. *Arch. Pharm. Life Sci.*, **2005**, *338*, 112-116.

[14] Foruomadi A.; Kargar, Z.; Sakhteman, A.; Sharifzadeh, Z.; Feyzmohammadi, R.; Kazemi, M.; Shafiee, A. Synthesis and antimycobacterial activity of some alkyl [5-(nitroaryl)-1,3,4-thiadiazol-2-ylthio]propionates. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 1164-1167.

[15] Chihiro, M.; Nagamoto, H.; Kitano, K.; Komatsu, H.; Sekiguchi, K.; Tabusa, F.; Mori, T.; Tominaga, M.; Yabuuchi, Y. Novel thiazole derivatives as inhibitors of superoxide production by human neutrophils - synthesis and structure-activity- relationships. *J. Med. Chem.*, **1995**, *38*, 353-358.

[16] Hanna, N.B.; Bhattacharya, B.K.; Robins, R.K.; Avery, T.L.; Revankar, G.R. Sulfinosine congeners - synthesis and antitumor-activity in mice of certain n9-alkylpurines and purine ribonucleosides. *J. Med. Chem.*, **1994**, *37*, 177-183.

[17] Albright, J.D.; Shepherd, R. G. Megazol was prepared by reproducing the experimental conditions described In: Reactions of 1,2-dimethyl-5-nitroimidazole, novel methods of conversion of 2-methyl group to a nitrile. *J. Heterocycl. Chem.*, **1973**, *10*, 899-907.

[18] Papaioannou, C.G. U.S. Patent 3,682,942. 1972 [Chem. Abstr. **1972**, *77*, 152193].

[19] Foroumadi, A.; Daneshtalab, M.; Shafiee, A. Synthesis and *in vitro* antifungal activity of 2-aryl-5-phenylsulfonyl-1,3,4-thiadiazole derivatives. *Arzneim. Forsch.,* **1999**, *49*, 1035-1038.

[20] Foroumadi, A.; Asadipour, A.; Mirzaei, M.; Karimi, J.; Emani, S. Antituberculosis agents. V. Synthesis, evaluation of *in vitro* antituberculosis activity and cytotoxicity of some 2-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives. *Il Farmaco,* **2002**, *57*, 765-769.

[21] Clerici, F.; Pocar, D.; Guido, M.; Loche, A.; Perlini, V.J.; Brufani, M. Synthesis of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. *J. Med. Chem.,* **2001**, *44*, 931-936.

[22] Greenhalgh, R.P. Selective oxidation of phenyl sulphides to sulphoxides or sulphones using oxone® and wet alumina. *Synlett,* **1992**, *3*, 235-236.

[23] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.,* **1997**, *23*, 3-25.

[24] Lipinski, C.A. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov. Today: Technol.*, **2004**, *1*, 337-341.

[25] www.molinspiration.com

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