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Synthesis of N1-Substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarboxamide as Novel Small Molecule Inhibitors of Cysteine Protease of *T. cruzi*

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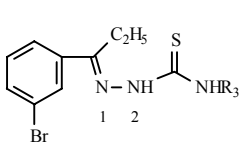
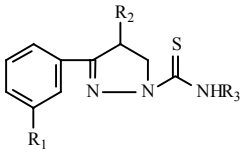
Abstract: A series of N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarboxamide were prepared from the Mannich bases of aryl ketones in good yields. Some derivatives were found to be active against the cysteine protease of *T.cruzi*..

Keywords: N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarboxamide, synthesis, *T.cruzi*. cysteine protease inhibitor.

It has been reported that the pyrazoline scaffold has activity against a broad range of therapeutic targets (*e.g* antibacterial、antiviral、anti-inflammatary)[1,2]. In our previous studies of the small molecule inhibitors of cysteine protease of *T.cruzi* (cruzain), the compounds, 1-(3′-bromopropiophenone)-4-subsituted-thiosemicarbazones**VII**, have been designed and tested both *in vitro* as potential antitrypanosomal agents and in the cell culture assay against *T. cruzi* (the causative agent of Chagas' disease in Latin America). In order to explore the structure-activity relationship we changed the structure of **VII** by attaching the ethyl group to the N-2 atom to form a pyrazoline ring to investigate the influence of restricting the flexibility and the impact on the N-2 atom. A serial N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarboxamide $\mathbf{IV_{1\text{-}27}}$ were designed and synthesized.



VII



$\mathbf{IV_{1\text{-}27}}$

Here, we present an efficient synthetic strategy for generation of N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarboxamide derivatives **IV** from the convenient starting materials in good yields. According to the literature, pyrazoline ring are normally synthesized from hydrazine monohydrate and α, β–unsaturated ketons

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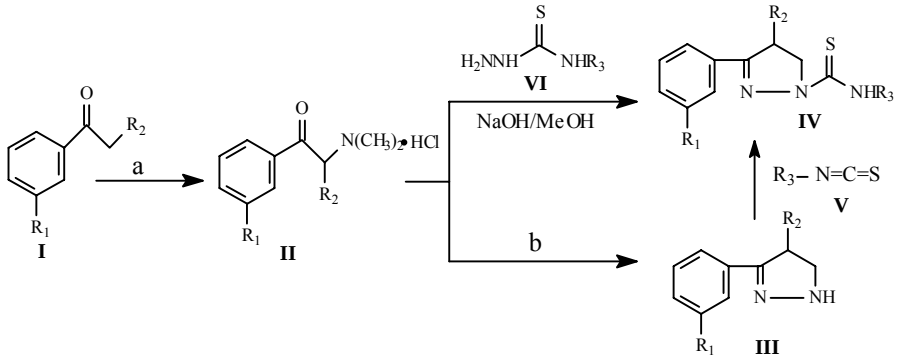
(chalcone) or its potential progenitor , such as β–halo-ketone, Mannich bases[3,4]. Based on these approaches, we explored to prepare the N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyra- zolethiocarboxamide **IV** through the cyclization of Mannich bases of aryl ketones with thiosemicarbazide or 4-alkyl- substituted-thiosemicarbazides. For the preparation of N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethiocarbo-xamide $\mathbf{IV_{19\text{-}27}}$, we synthesize the intermediate 3-aryl-2-pyrazolin $\mathbf{III_{19\text{-}27}}$ first, then it was reacted with the prepared aryl isothiocyanate $\mathbf{V_{19\text{-}27}}$ to give the target compound $\mathbf{VI_{19\text{-}27}}$. The resulting approach is outlined in **Scheme 1**.

Scheme 1



a. HCHO / $HNMe_2$•HCl / C_2H_5OH, 80-86% ; b. H_2NNH_2 / NaOH/ CH_3OH, 78-84%;

The compound **II** were obtained according to Mannich reaction procedures. Ketone **I** (1.0 mol), paraformaldehyde (1.3 mol) and dimethylamine hydrochloride (1.3 mol) in 100 mL absolute alcohol were refluxed for 4-6 hrs to afford **II**. **II** reacted with 1.0 mol of **VI** in 10 mL methanol and 0.3 mL aqueous solution of NaOH (50%) at reflux temperature for 1-4 hrs to give title compounds **IV** in yields 80-86% directly. The end of the reaction was monitored by TLC. If **II** reacted with hydrazine monohydrate in methanol and NaOH by above procedure **III** were obtained. **III** were refluxed in toluene with 1.0 mol aryl isothiocyanate **V** [5] for 1-2 hrs to give $\mathbf{IV_{19\text{-}27}}$ in yields 80-87%. This is another way to get **IV**. Thiocarbamate[6] (1.0 mol) and hydrazine monohydrate (1.2 mol) were stirred at 80-90°C for 2 hours, the intermediate $\mathbf{VI_{4\text{-}18}}$ were obtained in good yield.

Conclusion

Total 27 compounds of N1-substituted-3-aryl-4-alkyl-4, 5-dihydro-1H-1-pyrazolethio-carboxamide ($\mathbf{IV_{1\text{-}27}}$) were designed and synthesized, all compounds have not been reported in literature before. Their structures were confirmed by HRMS (EI) and ^{1}H-NMR spectroscopy[7]. All compounds were tested *in vitro* against cruzain. The structures and IC_{50} values of the synthesized compounds were outlined in **Table 1.**

The result showed that, the most compounds exhibited certain activity against cruzain *in vitro*, in which, compounds $\mathbf{IV_1}$、$\mathbf{IV_2}$ and $\mathbf{IV_3}$ have better activities than the

control compound **tf-175**. The further bioassay of these compounds in cell culture and on animal models against cruzain is underway.

The nonpeptidic nature of these compounds, coupled with their low cost of synthesis, makes this class of inhibitors to be very promising candidates for the development of new antitrypanosomal chemotherapy.

Table 1 The structures and biological data of synthesized compounds

Compounds	R_1	R_2	R_3	mp°C	$IC_{50}(\mu M)$
IV_1	Cl	CH_3	H	131.3-132.6	0.23
IV_2	Br	CH_3	H	107.4-109.9	0.08
IV_3	CF_3	CH_3	H	120.8-121.9	0.04
IV_4	Cl	H	CH_3	172.3-173.1	0.4
IV_5	Br	H	CH_3	189.2-190.3	1
IV_6	CF_3	H	CH_3	192.7-193.4	1
IV_7	Cl	H	C_2H_5	197.3-199.1	1.5
IV_8	Br	H	C_2H_5	219.9-220.3	1.5
IV_9	CF_3	H	C_2H_5	205.1-206.6	1.5
IV_{10}	Cl	H	2-tetrehydrafuranomethylene	195.1-196.7	10
IV_{11}	Br	H	2-tetrehydrafuranomethylene	207.8-108.9	10
IV_{12}	CF_3	H	2-tetrehydrafuranomethylene	194.2-195.9	1.7
IV_{13}	Cl	H	4-morpholinoethylene	189.9-191.2	10
IV_{14}	Br	H	4-morpholinoethylene	197.3-198.8	10
IV_{15}	CF_3	H	4-morpholinoethylene	208.9-209.7	10
IV_{16}	Cl	H	1-piperidinoethylene	216.8-217.6	10
IV_{17}	Br	H	1-piperidinoethylene	207.8-208.9	10
IV_{18}	CF_3	H	1-piperidinoethylene	199.6-201.3	10
IV_{19}	Cl	H	m-ClC_6H_4	211.9-213.1	1.4
IV_{20}	Br	H	m-ClC_6H_4	213.6-214.9	1.4
IV_{21}	CF_3	H	m-ClC_6H_4	222.1-223.5	1.6
IV_{22}	Cl	H	m-$CF_3C_6H_4$	241.8-242.9	1
IV_{23}	Br	H	m-$CF_3C_6H_4$	235.0-236.4	1
IV_{24}	CF_3	H	m-$CF_3C_6H_4$	218.1-219.7	1
IV_{25}	Cl	H	p-$CF_3C_6H_4$	198.9-199.5	1.4
IV_{26}	Br	H	p-$CF_3C_6H_4$	221.1-222.6	1.4
IV_{27}	CF_3	H	p-$CF_3C_6H_4$	194.3-195.2	1.4
tf-175*					0.45

* tf-175 is one of the most potent peptidyl inhibitors of cruzain in our previous work.

Acknowledgment

All bioassay data were performed by Elizabeth Hansell and Patricia Doyle, Department of Pathology, University of California, San Francisco, CA 94143, U.S.A

ReferenceS and Notes

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5. Arylamines (1.0 mol) was stirred with thiophosgene (1.0 mol) in CH_2Cl_2/H_2O at < 10℃ for 0.5 hrs, the organic layer was washed with water, dried over anhydrous $MgSO_4$, after removing the solvent the resulting oil was heated in toluene at 100℃ for 1 hour to give corresponding aryl isothiocyanate $\mathbf{V_{19\text{-}27}}$.
6. Treating alkylamines with equivalent phenyl thiochloroformate and triethylamine in CH_2Cl_2 at 0°C, to give corresponding thiocarbamate, which were pure enough to be used to the preparation of $\mathbf{IV_{4\text{-}18}}$ without further purification.
7. ^{1}H-NMR（DMSO, δ ppm）and HRMS (EI) data for partial compounds: $\mathbf{IV_1}$: ^{1}H-NMR 1.17 (d, 3H, J = 7.2), 3.80 (m, 1H), 3.94 (dd, 1H, J = 4.4, 11.6), 4.19 (t, 1H, J = 11.2), 7.50 (m, 2H), 7.75 (d, 1H, J = 6.8), 7.93 (bs, 1H), 8.03 (s,1H), 8.07 (bs, 1H); HRMS (EI) *m/z* (M^+) calcd for $C_{11}H_{12}ClN_3S$ 253.0440, found 253.0447. $\mathbf{IV_2}$: ^{1}H-NMR 1.17 (d, 3H, J = 7.2), 3.80 (m, 1H), 3.92 (dd, 1H, J = 4.2, 11.6), 4.19 (t, 1H, J = 11.6), 7.41 (t, 1H, J = 8.0), 7.63 (m, 1H), 7.78 (d, 1H, 8.0), 7.94 (bs, 1H), 8.07 (bs, 1H), 8.16 (m, 1H); HRMS (EI) *m/z* (M^+) calcd for $C_{11}H_{12}BrN_3S$ 296.9935, found 296.9926. $\mathbf{IV_3}$: ^{1}H-NMR 1.19 (d, 3H, J = 6.8), 3.88 (m, 1H), 3.97 (dd, 1H, J = 4.4, 11.2), 4.22 (t, 1H, J = 11.2), 7.69 (t, 1H, J = 7.6), 7.80 (d, 1H, J = 7.6), 8.02 (bs, 1H), 8.08 (d, 1H, J = 8.4), 8.11 (bs, 1H), 8.30 (s, 1H); HRMS (EI) *m/z* (M^+) calcd for $C_{12}H_{12}F_3N_3S$ 287.0704, found 287.0701. $\mathbf{IV_5}$: ^{1}H-NMR 1.19 (d, 3H, J = 4.4), 3.26 (t, 2H, J = 9.6), 4.15 (t, 2H, J = 10.0), 7.43 (t, 1H, J = 8.0), 7.64 (d, 1H, J = 8.4), 7.74 (d, 1H, J = 7.6), 8.13 (s, 1H), 8.48 (d, 1H, J = 4.4); HRMS (EI) *m/z* (M^+) calcd for $C_{11}H_{12}BrN_3S$ 296.9935, found 296.9939. $\mathbf{IV_8}$: ^{1}H-NMR 1.14 (t, 3H,J=7.2),2.27(m, 2H), 3.61 (q, 2H, J = 7.6), 7.35 (t, 1H, J = 8.0), 7.58 (d, 1H, J = 8.0), 7.88 (d, 1H, J = 8.0), 8.10 (s,1H), 8.60 (bs, 1H); HRMS (EI) *m/z* (M^+) calcd for $C_{12}H_{14}BrN_3S$ 311.0092, found 311.0095. $\mathbf{IV_{11}}$: ^{1}H-NMR 2.09-2.18(m,4H), 2.22(t,2H,J=7.2),2.41-2.48(m,2H), 2.79(d,2H,J=7.6),3.21-3.35(m,2H),3.38-3.41(m,1H), 7.33 (t,1H,J=8.0),7.55(d,1H,J=8.0),7.69(t,1H,J=6.4),7.80(d,1H,J=7.6),8.48(t,1H,J=5.6);HRMS (EI) *m/z* (M^+) calcd for $C_{15}H_{18}BrN_3OS$ 367.0354, found 367.0358. $\mathbf{IV_{20}}$: ^{1}H-NMR 3.36(t, 2H, J = 10.0), 4.25 (t, 2H, J = 10.0), 7.19 (dd, 1H, J = 1.2, 8.0), 7.38 (t, 1H, J = 8.0), 7.45 (t, 1H, J = 8.0), 7.58 (d, 1H,J= 8.0), 7.68 (dd, 1H, J = 1.2, 8.0), 7.73 (t, 1H, J = 1.6), 7.86 (d, 1H, J = 8.0), 8.26 (t, 1H, J = 1.6), 10.19 (s, 1H); HRMS (EI) *m/z* (M^+) calcd for $C_{16}H_{13}BrClN_3S$ 394.9682, found 394.9694. $\mathbf{IV_{26}}$: ^{1}H-NMR 3.37(t, 2H, J = 10.0), 4.26 (t, 2H, J = 10.0), 7.24(d, 1H,J = 8.0), 7.39 (d, 1H, J = 7.2), 7.48 (d, 1H, J = 8.4), 7.58 (d, 1H,J= 8.0), 7.69 (dd, 1H,J =3.2, 8.4),7.78 (t,1H,J=5.6), 7.89(s,1H), 8.27(t,1H,J=7.6), 10.20(s,1H); HRMS (EI) *m/z* (M^+) calcd for $C_{17}H_{13}BrF_3N_3S$ 426.9926, found 426.9920.

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