Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 2811–2819

TETRAHEDRON

One-pot syntheses of 2-pyrazoline derivatives

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Received 6 November 2002; revised 6 February 2003; accepted 4 March 2003

Abstract—Hydrazinediium dithiocyanate and α,β-unsaturated ketones give in one-pot reactions 1-thiocarbamoyl-2-pyrazolines and 1-formyl-2-pyrazolines. The syntheses of pyridine-2-thiones, pyrimidine-2-thiones and bicyclo[2.2.2]octan-2-ones from ammonium thiocyanates and ketones by analogous procedures are reviewed. The mechanisms of the ring formations are discussed. Crystal structure analyses of a 1-thiocarbamoyl- and a 1-formyl-2-pyrazoline are given. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Ammonium thiocyanates and α,β-unsaturated ketones have already been cyclized to products having pyridine-2-thione, pyrimidine-2-thione or bicyclo[2.2.2]octan-2-one structure. This paper reports the reaction of α,β-unsaturated ketones with hydrazinediium dithiocyanate giving 1-thiocarbamoyl- or 1-formyl-2-pyrazolines depending on the structure of the ketone.

2. Results and discussion

The reaction of the β-alkyl ketones **1a**,**b** with dialkylammonium thiocyanates gives the 4-dialkylamino-5,6-dihydropyridinethiones **2a**–**c**.[1,2] However, from the corresponding β-phenylketone **1c** the bicyclo[2.2.2]octan-2-ones **3a**–**d** have been formed under the same reaction conditions.[3,4] The dihydropyrimidine-2-thiones **4a**–**d**[5–8] were yielded upon heating of alkylammonium or ammonium thiocyanates with α,β-unsaturated ketones **1a**,**c**,**d** (Scheme 1).

The following mechanisms (Scheme 2) have been proposed for the above-mentioned reactions. When the thiocyanate ion is added in β-position of the β-alkyl ketones **1a**,**b**,**d** isothiocyanates **8** are formed. The latter react with secondary amines giving enamines **9** which cyclize to the dihydropyridine-2(1*H*)-thiones **2** in good yields.[1,2] However, ammonia or methylamine are added preferably to the isothiocyanato group of **8** giving thioureas **12** which cyclize to the pyrimidine-2(1*H*)-thiones **4**.[9]

The reaction between benzylidene acetone (**1c**) and dialkylammonium rhodanides **7** proceeds in a completely different way. Most likely the formation of enamines **10** becomes the first step followed by a Diels-Alder addition of unchanged **1c** giving 4-acetylcyclohex-1-en-1-ylammoniumsalts **11**. Those cyclize to the bicyclo[2.2.2]octan-2-ones **3** (Scheme 2). In ^{1}H NMR spectra, the discrimination between the two similar chains (C-5/C-6 and C-7/C-8) was achieved by the correlation of NOE spectra and w-couplings as reported in Ref. 3.

But no formation of bicyclo-octanone was observed, when **1c** was treated with diethylammonium thiocyanate **7e**. Surprisingly we were able to isolate a small amount of the dihydropyridine-2(1*H*)-thione **2d** from the reaction mixture by means of column chromatography. This indicates that the above cyclizations are competitive reactions. Accordingly the structure of the ketone only favours the formation of a certain cyclic product but does not rule out the generation of another.

We refluxed α,β-unsaturated ketones with hydrazinediium dithiocyanate. Recently, we reported the formation of 1,2,4-triazepine-3-thiones from α,β-unsaturated ketones **1c**,**e**–**h** and hydrazinediium dithiocyanate **13**.[10] Their structural elucidation was based on the comparison of the chemical shifts in their ^{13}C NMR spectra with reported data of formerly prepared 1,2,4-triazepine-3-thiones.[11] With the aid of a single crystal structure analysis we found out recently

Keywords: α,β-unsaturated ketones; cyclization; hydrazinediium dithiocyanate; pyrazolines.
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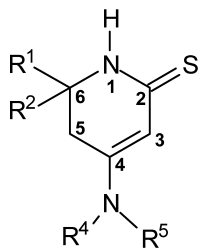
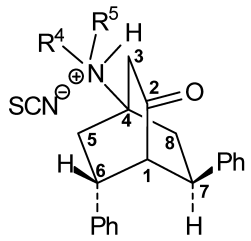
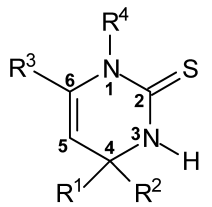
doi:10.1016/S0040-4020(03)00338-7



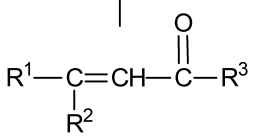
2a: $R^1 = R^2 = R^4 = R^5 = Me$
2b: $R^1 = R^2 = Me$, $R^4 + R^5 = -(CH_2)_2-O-(CH_2)_2-$
2c: $R^1 = iPr$, $R^2 = H$, $R^4 = R^5 = Me$
2d: $R^1 = Ph$, $R^2 = H$, $R^4 = R^5 = Et$



3a: $R^4 = R^5 = Me$
3b: $R^4 + R^5 = -(CH_2)_2-O-(CH_2)_2-$
3c: $R^4 + R^5 = -(CH_2)_4-$
3d: $R^4 + R^5 = -(CH_2)_5-$



4a: $R^1 = R^2 = R^3 = R^4 = Me$
4b: $R^1 = Ph$, $R^2 = H$, $R^3 = Me$, $R^4 = H$
4c: $R^1 = R^2 = R^3 = Me$, $R^4 = H$
4d: $R^1 = Me$, $R^2 = H$, $R^3 = Ph$, $R^4 = H$



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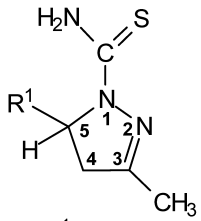
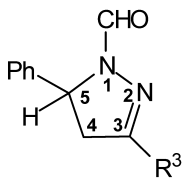


5a: $R^1 = Ph$
5b: $R^1 = 4\text{-}OMe\text{-}Ph$
5c: $R^1 = 4\text{-}Me_2N\text{-}Ph$
5d: $R^1 = 2\text{-}furyl$
5e: $R^1 = 2\text{-}thienyl$



6a: $R^3 = Ph$
6b: $R^3 = 4\text{-}Cl\text{-}Ph$
6c: $R^3 = 4\text{-}Me\text{-}Ph$
6d: $R^3 = 4\text{-}OMe\text{-}Ph$
6e: $R^3 = 2\text{-}OH\text{-}Ph$

1	R^1	R^2	R^3	1	R^1	R^2	R^3
a	Me	Me	Me	**g**	2-furyl	H	Me
b	iPr	H	Me	**h**	2-thienyl	H	Me
c	Ph	H	Me	**i**	Ph	H	Ph
d	Me	H	Ph	**j**	Ph	H	4-Cl-Ph
e	4-OMe-Ph	H	Me	**k**	Ph	H	4-Me-Ph
f	$4\text{-}Me_2N\text{-}Ph$	H	Me	**l**	Ph	H	4-OMe-Ph

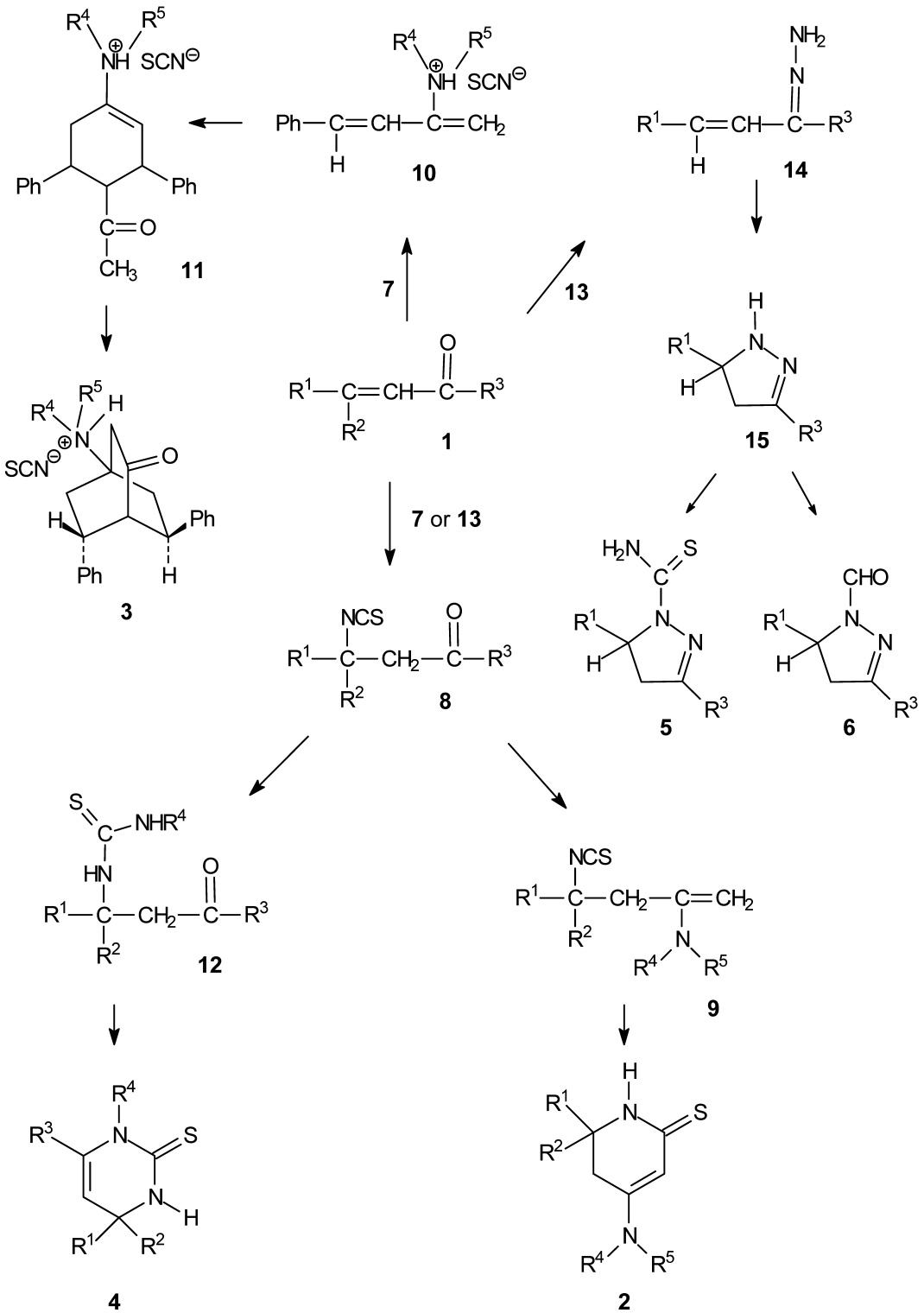
Scheme 1.

that these products are not 1,2,4-triazepine-3-thiones but 1-thiocarbamoyl-2-pyrazolines **5a**–**e** which have the same elemental composition. We assume the following reaction mechanism for the pyrazoline formation: first the imines **14** are formed which cyclize to the pyrazolines **15**. The latter are added to the thiocyanato group giving 1-thiocarbamoyl-2-pyrazolines **5a**–**e** (Scheme 2).

2.1. X-Ray crystal structure of 5b

The crystal structure analysis of **5b** established the compound as a 2-pyrazoline derivative. A search in the Cambridge Structural Database[12] resulted in no structural data for 1-thiocarbamoyl-2-pyrazolines. As shown in Figure 1, two molecules of **5b** are held together by two N–H···S hydrogen bonds [N···S 3.355(3) Å, N–H···S 169(4)°]. The 2-pyrazoline ring adopts an envelope conformation [C5 has a deviation of 0.231(16) Å from the least-squares plane through the atoms N1 to C4]. The phenyl ring bonded to C5 encloses an angle of 86.68(15)° with the least-squares plane of the 2-pyrazoline ring. The thiocarbamoyl group is almost co-planar to the bond N1–N2 [N7–C7–N1–N2=−2.1(4)°].

Because substances with thiosemicarbazide partial structure have been reported to exhibit antimalarial[14] as well as antimycobacterial[15] activities the 1-thiocarbamoyl-2-pyrazoline **5a** has been screened for its activity against causative


10
11
14
7
13
1
15
7 or 13
3
8
5
6
12
9
4
2
7a: R4 = R5 = Me
7b: R4 + R5 = –(CH2)2–O–(CH2)2–
7c: R4 + R5 = –(CH2)4–
7d: R4 + R5 = –(CH2)5–
7e: R4 = R5 = Et
7f : R4 = R5 = H
7g: R4 = Me, R5 = H
7
13

Scheme 2.

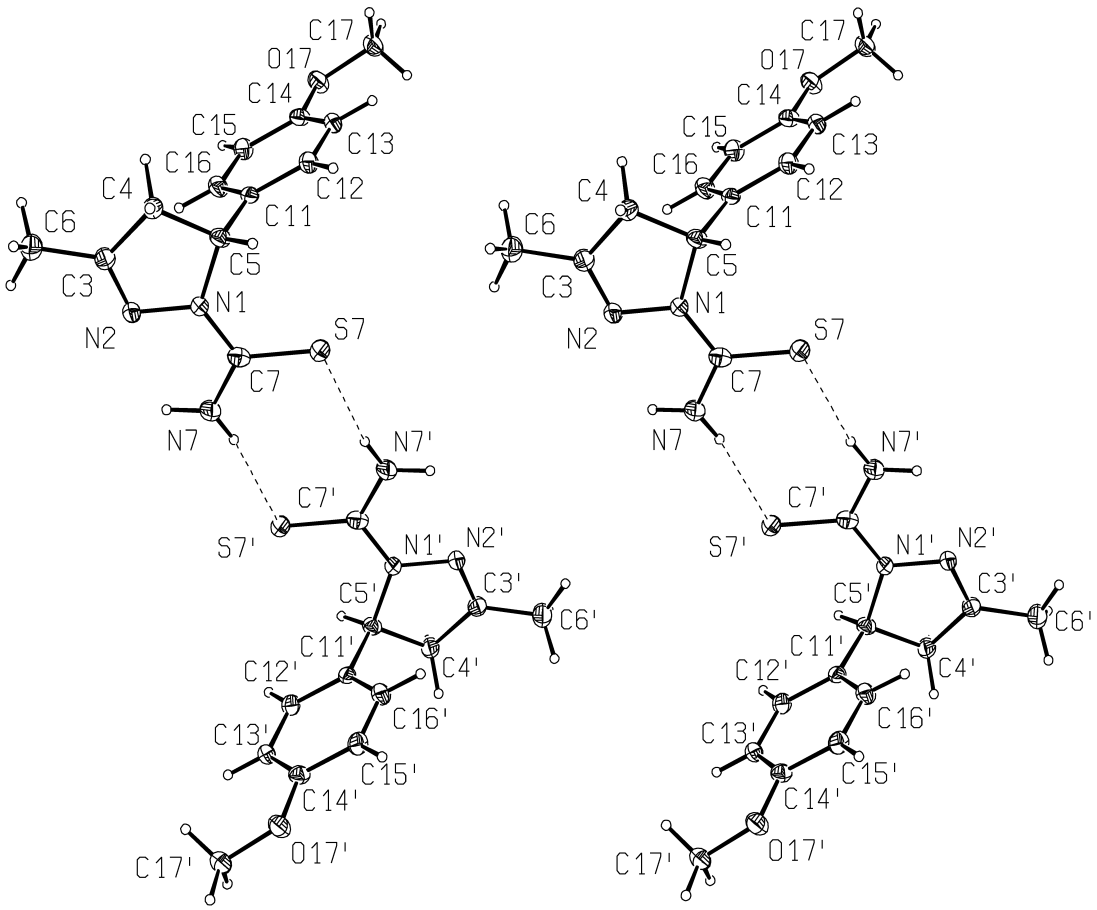


Figure 1. Stereoscopic ORTEP[13] plot of two molecules of **5b** showing the atomic numbering scheme. The probability ellipsoids are drawn at the 50% probability level.

organisms of tropical diseases including *Plasmodium falciparum* K_1, *Trypanosoma cruzi* and *Trypanosoma b. rhodesiense*. However, **5a** which has formerly been prepared in a two-step procedure[16] showed no antiprotozoal activity (Table 1).

When the chalcone derivatives **1i–l** were heated with hydrazinediium dithiocyanate **13** high amounts of the 1-formyl-2-pyrazolines **6a–d** were formed. The formylation of the pyrazoline intermediates **15** proceeds via a transamidation reaction of DMF which was used as solvent.

Both pyrazoline derivatives **5a** and **6a** were formed by the reaction of the 1-unsubstituted pyrazolines **15** with hydrazinediium dithiocyanate **13** under the same conditions verifying the reaction mechanisms.

With the aid of ^{1}H NMR spectroscopy 1-formyl and 1-thiocarbamoyl pyrazolines were detected in all mother liquors revealing the formation of products **5** and **6** as competitive reactions.

The substitution of N-1 of the 2-pyrazolines **5** and **6** was established by long-range couplings. Crosspeaks from 5-H to C=S were observed in the HMBC spectra of compounds **5**. The formyl protons of compounds **6** resonating at 9 ppm in their ^{1}H NMR spectra show correlations to C-5. In the ^{13}C NMR spectra of **5** the signals for the formyl carbons typically appear at 160 ppm, whereas the C=S carbons of compounds **5** resonate at 176 ppm. The signals for the C-3 carbons of the 2-pyrazolines **5** and **6** were observed at ca. 157 ppm. The structures of compounds **6a–d** were established by a single crystal structure analysis.

2.2. X-Ray crystal structure of 6a

The molecular structure and labelling scheme of **6a** are shown in Figure 2. The 2-pyrazoline ring is almost planar

Table 1. Antiprotozoal activities of compound **5a**, expressed as IC_{50} (μg/ml)

Compound	*P. falciparum* K_1	*T.b.rhodesiense*	*T. cruci*	Cytotoxicity. L6
5a	>5000	9.36	49.0	>90.0
Standard[a]	0.0018	0.000849	1.25	4.3

Values represent the average of four determinations (two determinations of two independent experiments).
[a] Substances used as standard are mentioned in Section 4.

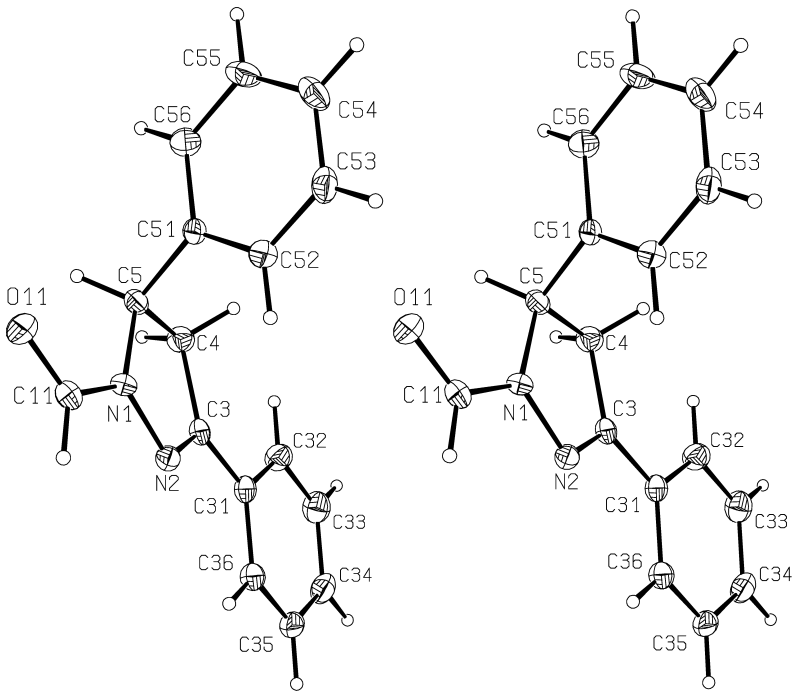


Figure 2. Stereoscopic ORTEP[13] plot of **6a** showing the atomic numbering scheme. The probability ellipsoids are drawn at the 50% probability level.

[the max. deviation from the least-squares plane is 0.055(9) Å] and the phenyl ring bonded to C3 is almost co-planar to it [5.06(13)°] whereas the phenyl ring bonded to C5 encloses an angle of 77.67(13)° with the least-squares plane of the 2-pyrazoline ring. The carboxaldehyde group is *trans* oriented to N2 [O11–C11–N1–N2=−178.5(2)°] as observed in all the other crystal structure determinations of 1-carbonyl-2-pyrazolines found in the Cambridge Structural Database.[12]

Substances **6a** and **6d** have already been prepared from the corresponding pyrazoline with formic acid.[17,18] The 2-hydroxyphenyl analogue **6e** of compound **6a** showed the highest antifungal activity in an assay against *Alternaria alternata*, *Macrophomina phaseoli*, *Colletotrichum falcatum* and *Fusarium oxysporum*.[19]

3. Conclusion

When α,β-unsaturated ketones are cyclized with ammonium thiocyanates or hydrazinediium dithiocyanates the mainly formed product is predictable from the structures of both reactants. Reactions with alkylammonium or ammonium thiocyanates give dihydropyrimidine-2-thiones. Dialkylammonium thiocyanates and β-alkyl substituted α,β-unsaturated ketones afford dihydropyridine-2-thiones whereas bicyclo[2.2.2]octan-2-ones are obtained from their β-phenyl analogues.

Hydrazinediium dithiocyanates and β-aryl substituted α,β-unsaturated ketones give 2-pyrazoline derivatives. From methylketones 1-thiocarbamoyl-2-pyrazolines are formed in moderate yields. However, phenylketones give 1-formyl-2-pyrazolines in good yields. The 1-thiocarbamoyl-2-pyrazoline **5a** was investigated for its activity against causative organisms of tropical deseases. **5a** was not active against *Plasmodium falciparum* K_1, *Trypanosoma cruzi* and *Trypanosoma b. rhodesiense*.

4. Experimental

4.1. General

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200 and are uncorrected. IR spectra: infrared spectrometer system 2000 FT (Perkin–Elmer). UV/VIS: Lambda 17 UV/VIS-spectrometer (Perkin–Elmer). NMR spectra: Varian Inova 400 (300 K) 5 mm tubes, TMS resonance as internal standard. ^{1}H- and ^{13}C-resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation spectra. HMBC spectra were optimized for 8 Hz. For NOE measurements oxygen was carefully removed by bubbling Ar through the solutions. ^{1}H- and ^{13}C-resonances are numbered as given in the formulae. Assignments marked with an asterisk are interchangeable. MS: Varian MAT 711 spectrometer 70 eV electron impact, Kratos profile spectrometer 70 eV electron impact, Micromass Tofspec (MALDI). Microanalyses: EA 1108 CHNS-O apparatus (Carlo Erba), Microanalytical Laboratory at the Institute of Physical Chemistry, Vienna. Materials: Column-chromatography (CC): silica gel 60 (Merck, 70–230 mesh), pore-diameter 60 Å, thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254}, 0.2 mm, 200×200 mm^2); the substances were detected in UV light at 254 nm.

*General procedure for the synthesis of thiocyanates **7d,e** and **13**.* Equivalent amounts of the amino component and ammonium thiocyanate were dissolved in the required volume of water and stirred for 1 h at room temperature. The solvent was evaporated and the residue was triturated

with ethanol or benzene, filtered with suction and dried over phosphorus pentaoxide.

4.1.1. (*RS*-(±)-4-Diethylamino-5,6-dihydro-6-phenylpyridine-2(1*H*)-thione (2d). 82 g (0.56 mol) Benzylidene acetone **1c** and 37.1 g (0.28 mol) diethylammonium thiocyanate **7e** were suspended in 110 ml of dimethylformamide and refluxed for 4 h at 150°C at a water separator. After cooling to room temperature, the solvent was removed in vacuo and the residue purified by means of CC using a mixture of toluene/chloroform/ethanol (4:4:1) as eluent. The solvent of the fraction containing the product was removed in vacuo and the residue triturated with ethanol. The solid was filtered off and recrystallized from ethanol, giving 100 mg of **2c** as yellow crystals. Mp: 153°C (ethanol); IR (KBr): $\tilde{\nu}$=3179 (m), 2973 (w), 1563 (s), 1521 (s), 1491 (w), 1473 (m), 1424 (m), 1414 (m), 1362 (m), 1336 (w), 1141 (s), 1079 (m), 1057 (m), 705 (w) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=346 (4.602) nm; ^1H NMR ($CDCl_3$, δ, 400 MHz): 1.67 (t, J=7.0 Hz, 6H, ($CH_3CH_2)_2$), 2.59–2.71 (m, 2H, 5-H), 3.24–3.33 (m, 4H, ($NCH_2)_2$), 4.65 (dd, J=12.3, 6.0 Hz, 1H, 6-H), 5.72 (s, 1H, 3-H), 6.72 (s, 1H, NH), 7.29–7.41 (m, 5H, aromatic H) ppm; ^{13}C NMR ($CDCl_3$, δ, 100 MHz): 12.91 (($CH_3CH_2)_2$), 33.66 (C-5), 44.35 ($N(CH_2)_2$), 57.27 (C-6), 97.64 (C-3), 126.72, 128.64, 128.99 (aromatic C), 139.79 (aromatic C_q), 152.50 (C-4), 191.70 (C-2) ppm; MS (EI$^+$): m/z (%)=260 (100.0) [M^+], 227 (17.8), 188 (14.0), 154 (7.8), 124 (8.5), 100 (8.5), 83 (9.3), 70 (8.9). Anal. calcd for $C_{15}H_{20}N_2S$+0.125H_2O (262.65): C 68.59, H 7.77, N 10.67, S 12.21; found: C 68.52, H 7.78, N 10.77, S 12.03; HRMS (MALDI) calcd ($C_{15}H_{20}N_2S$): 260.1347; found: 260.1348.

4.1.2. (6*RS*,7*RS*)-(±)-6,7-Diphenyl-4-piperidinobicyclo-[2.2.2]octan-2-one×HNCS (3d). 67.4 g (0.46 mol) Benzylidene acetone **1c** and 39.0 g (0.27 mol) piperidinium thiocyanate **7d** were suspended in 250 ml of dimethylformamide and refluxed for 4 h at 220°C at a water separator. After cooling to room temperature, the solvent was removed in vacuo and the residue crystallized from ethanol over night. After recrystallization from ethanol, 15.9 g (16.7%) of **3d** were obtained as beige crystals. Mp: 264°C (ethanol); IR (KBr): $\tilde{\nu}$=2958 (s), 2877 (m), 2601 (w), 2444 (s), 1725 (s), 1497 (s), 1452 (m), 1361 (m), 1330 (m), 755 (s), 698 (s) cm^{-1}; UV (CH_3OH): λ (log ε)=212 (4.005) nm; ^1H NMR (DMSO-d_6, δ, 400 MHz): 1.40–1.54 (m, 1H, CH_2), 1.64–1.80 (m, 3H, CH_2, $(CH_2)_2$), 1.82–1.96 (m, 3H, 8-H, $(CH_2)_2$), 2.35 (dd, J=12.5, 9.3 Hz, 1H, 5-H), 2.53 (s, 1H, 1-H), 2.57 (ddd, J=12.5, 9.3, 2.3 Hz, 1H, 5-H), 2.80 (ddd, J=13.7, 9.4, 3.3 Hz, 1H, 8-H), 2.83 (dd, J=17.4, 2.2 Hz, 1H, 3-H), 3.01 (dd, J=17.7, 3.2 Hz, 1H, 3-H), 2.90–3.34 (m, 2H, $N(CH_2)_2$), 3.40 (t, J=9.4 Hz, 1H, 7-H), 3.54 (t, J=9.3 Hz, 1H, 6-H), 3.64 (br, d, J=10.2 Hz, 1H, $N(CH_2)_2$), 3.77 (br, d, J=10.5 Hz, 1H, $N(CH_2)_2$), 7.08–7.49 (m, 10H, aromatic H), 9.24 (br, s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, δ, 100 MHz): 21.63 (CH_2), 23.62 (($CH_2)_2$), 29.60 (C-5), 34.35 (C-8), 34.58 (C-7), 36.41 (C-6), 43.46 (C-3), 47.50, 48.01 ($N(CH_2)_2$), 53.61 (C-1), 64.40 (C-4), 126.95, 127.18, 127.76, 128.83, 128.94 (aromatic C), 140.39, 142.91 (aromatic C_q), 207.83 (C-2) ppm; MS (base, EI$^+$): m/z (%)=359 (100.0) [M^+], 316 (11.6), 268 (24.8), 255 (66.7), 227 (36.4), 213 (23.3), 178 (12.0), 136 (14.0), 91 (10.9). Anal. calcd for $C_{26}H_{30}N_2OS$ (418.60): C 74.60, H 7.22, N 6.69, S 7.66; found: C 74.45, H 7.43, N 6.77, S 7.44; HRMS (MALDI) calcd ($C_{25}H_{29}NO$): 359.2249; found: 359.2251.

4.1.3. 5-Aryl-3-methyl-1-thiocarbamoyl-2-pyrazolines (5) and 3-aryl-1-formyl-5-phenyl-2-pyrazolines (6). *General procedure*. Hydrazinediium dithiocyanate **13** and the α,β unsaturated ketones **1c**, **1e**–**l** were dissolved in dimethyl formamide and refluxed at a water separator for 4 h. The solvent was evaporated in vacuo and the residue triturated with ethanol. Products **6a**–**d** crystallized over night, were filtered and recrystallized from ethanol. Only a part of compounds **5a**–**e** crystallized. The solid was treated with charcoal in ethanol, filtered and crystallized twice from ethanol. The filtrate has to be purified by CC over silica gel (eluent: CH_2Cl_2 for **5a** and CH_2Cl_2/MeOH (20:1) for **5b**–**e**).

4.1.4. (*RS*)-(±)-3-Methyl-5-phenyl-1-thiocarbamoyl-2-pyrazoline (5a). 12.7 g (84.5 mmol) Hydrazinediium dithiocyanate (**13**) and 14 g (95.8 mmol) benzylidene acetone (**1c**) in 200 ml of dimethylformamide gave yellowish plates. Yield: 5.0 g (27.0%); mp: 237°C (ethanol); IR (KBr): $\tilde{\nu}$=3397 (s), 3252 (s), 3149 (s), 1592 (s), 1498 (s), 1421 (m), 1380 (s), 1364 (s), 1326 (m), 828 (m), 756 (m), 699 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=276 (4.235), 235 (3.890) nm; ^1H NMR (400 MHz, δ, $CDCl_3$): 2.07 (s, 3H, CH_3), 2.73 (dd, J=18.3, 3.4 Hz, 1H, 4-H), 3.46 (dd, J=18.3, 11.3 Hz, 1H, 4-H), 5.87 (dd, J=11.3, 3.4 Hz, 1H, 5-H), 5.95 (br, s, 1H, NH), 6.87 (br, s, 1H, NH), 7.15–7.35 (m, 5H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 16.14 (CH_3), 47.09 (C-4), 63.08 (C-5), 125.21, 127.48, 128.84 (aromatic C), 141.80 (aromatic C_q), 158.11 (C-3), 176.40 ($CSNH_2$) ppm; MS (EI+): m/z (%)=220 (12.9) [M+H$^+$], 219 (93.9) [M^+], 186 (39.3), 178 (41.0), 177 (65.1), 163 (39.3), 159 (33.6), 145 (21.3), 137 (61.0), 119 (19.0), 115 (16.6), 104 (90.2), 103 (100.0), 91 (24.7), 83 (60.3), 77 (43.1), 69 (13.6), 65 (11.5), 60 (34.6), 56 (14.2), 51 (23.7), 42 (29.5). Anal. calcd for $C_{11}H_{13}N_3S$ (219.31): C 60.24, H 5.97, N 19.16, S 14.62; found: C 59.96, H 6.03, N 19.14, S 14.02.

4.1.5. (*RS*)-(±)-5-(4-Methoxyphenyl)-3-methyl-1-thiocarbamoyl-2-pyrazoline (5b). 7.6 g (50 mmol) Hydrazinediium dithiocyanate (**13**) and 14 g (50 mmol) 4-methoxybenzylidene acetone (**1e**) in 80 ml of dimethylformamide gave yellowish prisms. Yield: 4.9 g (40%); mp: 182°C (ethanol); IR (KBr): $\tilde{\nu}$=3419 (s), 3254 (s), 3149 (s), 1596 (s), 1514 (s), 1491 (s), 1455 (m), 1385 (s), 1361 (s), 1324 (m), 1252 (s), 1184 (s), 1034 (s), 823 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=273 (4.257), 235 (4.065) nm; ^1H NMR (400 MHz, δ, DMSO-d_6): 2.02 (s, 3H, CH_3), 2.60 (dd, J=18.3, 3.1 Hz, 1H, 4-H), 3.50 (dd, J=18.4, 11.4 Hz, 1H, 4-H), 3.72 (s, 3H, OCH_3), 5.68 (dd, J=11.3, 3.3 Hz, 1H, 5-H), 6.86 (d, J=8.7 Hz, 2H, *m*-aromatic H), 7.02 (d, J=8.7 Hz, 2H, *o*-aromatic H), 7.37 (br, s, 1H, NH), 7.70 (br, s, 1H, NH) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d_6): 15.96 (CH_3), 46.59 (C-4), 55.22 (OCH_3), 61.93 (C-5), 113.88 (*m*-aromatic C), 126.80 (*o*-aromatic C), 135.33 (aromatic C_q), 158.27 (*p*-aromatic C), 158.55 (C-3), 175.75 ($CSNH_2$) ppm; MS (EI+): m/z (%)=250 (8.8) [M+H$^+$], 249 (54.1) [M^+], 233 (9.1), 216 (32.4), 207 (57.4), 193 (26.4), 189 (13.5), 175 (19.6), 167 (37.2), 166 (16.9), 149 (10.8), 135 (11.5), 134 (100.0), 133 (64.2), 119 (14.9), 91 (25.0), 77 (17.6), 75 (8.8), 65 (15.5), 60 (18.9), 42 (16.2). Anal. calcd

for $C_{12}H_{15}N_3OS$ (249.34): C 57.81, H 6.06, N 16.85, S 12.86; found: C 57.71, H 6.10, N 16.89, S 12.38.

X-Ray diffraction data of ***5b***. All the measurements were performed using graphite-monochromatized Mo K_α radiation at 97 K: $C_{12}H_{15}N_3OS$, M_r 249.33, monoclinic, space group $P2_1/c$, a=6.264(2) Å, b=15.212(3) Å, c=12.990(4) Å, β=95.06(2)°, V=1233.0(6) Å^3, Z=4, d_{calc}=1.343 g cm^{-3}, μ=0.250 mm^{-1}. A total of 3109 reflections were collected (Θ_{max}=26°), from which 2428 were unique (R_{int}=0.0443), with 1685 having $I>2\sigma(I)$. The structure was solved by direct methods (SHELXS-97)[20] and refined by full-matrix least-squares techniques against F^2 (SHELXL-97)[21]. The non-hydrogen atoms were refined with anisotropic displacement parameters without any constraints. The H-atoms of the NH_2 group were refined without any positional constraints but with common isotropic displacement parameters. The H atoms bonded to C atoms were refined with common isotropic displacement parameters for the H atoms bonded to the same C atom and with idealized geometries of approximately tetrahedral angles. The C–H distances were fixed to 0.98, 0.99, 1.00, and 0.95 Å for the methyl, secondary, tertiary, and phenyl H atoms, respectively. For 167 parameters final R indices of R=0.0514 and wR^2=0.1501 (GOF=1.046) were obtained. The largest peak in a difference Fourier map was 0.348 e Å^{-3}. The final atomic parameters, as well as bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre (CCDC202863).

4.1.6. (*RS*)-(±)-5-(4-Dimethylaminophenyl)-3-methyl-1-thiocarbamoyl-2-pyrazoline (5c). 1.6 g (11 mmol) Hydrazinediium dithiocyanate (**13**) and 2.0 g (11 mmol) 4-dimethylaminobenzylidene acetone (**1f**) in 100 ml of dimethylformamide gave white needles. Yield: 1.6 g (57%); mp: 238°C (ethanol); IR (KBr): $\tilde{\nu}$=3427 (m), 3261 (m), 3153 (m), 1621 (m), 1592 (s), 1529 (s), 1488 (s), 1381 (m), 1363 (s), 810 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=266 (4.458) nm; ^{1}H NMR (400 MHz, δ, DMSO-d_6): 2.02 (s, 3H, CH_3), 2.58 (dd, J=18.2, 3.1 Hz, 1H, 4-H), 2.85 (s, 6H, $(NCH_3)_2$), 3.48 (dd, J=18.2, 11.1 Hz, 1H, 4-H), 5.62 (dd, J=11.1, 3.1 Hz, 1H, 5-H), 6.64 (d, J=8.7 Hz, 2H, *m*-aromatic H), 6.92 (d, J=8.5 Hz, 2H, *o*-aromatic H), 7.31 (br, s, 1H, NH), 7.63 (br, s, 1H, NH) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d_6): 16.00 (CH_3), 40.42 ($N(CH_3)_2$), 46.58 (C-4), 62.00 (C-5), 112.50 (*m*-aromatic C), 126.37 (*o*-aromatic C), 130.93 (aromatic C_q), 149.64 (*p*-aromatic C), 158.59 (C-3), 175.65 ($CSNH_2$) ppm. Anal. calcd for $C_{13}H_{18}N_4S$ (262.38): C 59.51, H 6.92, N 21.35, S 12.22; found: C 59.50, H 6.98, N 21.38, S 11.94.

4.1.7. (*RS*)-(±)-5-Furyl-3-methyl-1-thiocarbamoyl-2-pyrazoline (5d). 7.7 g (51 mmol) Hydrazinediium dithiocyanate (**13**) and 7 g (51 mmol) furfurylidene acetone (**1g**) in 85 ml of dimethylformamide gave grey plates. Yield: 2.7 g (25%); mp: 220–222°C (ethanol); IR (KBr): $\tilde{\nu}$=3406 (s), 3263 (s), 3153 (s), 1593 (s), 1489 (s), 1432 (w), 1368 (s), 815 (m), 763 (s) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=269 (4.222), 236 (3.985) nm; ^{1}H NMR (400 MHz, δ, DMSO-d_6): 2.04 (s, 3H, CH_3), 2.82 (dd, J=18.1, 3.4 Hz, 1H, 4-H), 3.42 (dd, J=18.1, 11.4 Hz, 1H, 4-H), 5.81 (dd, J=11.4, 3.4 Hz, 1H, 5-H), 6.23 (d, J=3.4 Hz, 1H, 3′-H), 6.37 (m, 1H, 4′-H), 7.36 (br, s, 1H, NH), 7.53 (d, J=0.8 Hz, 1H, 5′-H), 7.77 (br, s, 1H, NH) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d_6): 15.86 (CH_3), 42.82 (C-4), 56.30 (C-5), 107.22 (C-3′), 110.50 (C-4′), 142.02 (C-5′), 153.46 (C-2′), 158.52 (C-3), 175.80 ($CSNH_2$) ppm; MS (EI+): m/z (%)=210 (13.5) [M+H$^+$], 209 (100.0) [M$^+$], 192 (10.1), 180 (27.0), 176 (10.8), 168 (56.8), 167 (16.9), 153 (35.8), 151 (15.5), 140 (26.4), 139 (12.1), 135 (10.1), 126 (13.5), 121 (28.4), 114 (64.9), 97 (13.5), 94 (82.4), 91 (25.0), 83 (22.3), 81 (65.5), 77 (19.6), 69 (30.4), 65 (48.0), 60 (55.4), 53 (25.0), 51 (18.9), 42 (39.2). Anal. calcd for $C_9H_{11}N_3OS$ (209.27): C 51.66, H 5.30, N 20.08, S 15.32; found: C 51.64, H 5.33, N 20.04, S 15.18.

4.1.8. (*RS*)-(±)-3-Methyl-5-(2-thienyl)-1-thiocarbamoyl-2-pyrazoline (5e). 5.2 g (35 mmol) Hydrazinediium dithiocyanate (**13**) and 7 g (35 mmol) thenylidene acetone (**1h**) in 60 ml of dimethyl formamide gave yellow needles. Yield: 2.3 g (29%); mp: 220°C (ethanol); IR (KBr): $\tilde{\nu}$=3389 (s), 3251 (s), 3150 (s), 1592 (s), 1494 (s), 1420 (m), 1367 (s), 1326 (m), 1305 (m), 1235 (w), 1222 (w), 1197 (w), 1072 (w), 825 (s), 705 (s), 634 (w), 570 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=279 (3.714), 237 (3.576) nm; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 2.11 (s, 3H, CH_3), 2.90 (dd, J=18.2, 2.7 Hz, 1H, 4-H), 3.43 (dd, J=18.2, 10.7 Hz, 1H, 4-H), 6.01 (br, s, 1H, NH), 6.19 (dd, J=10.7, 2.7 Hz, 1H, 5-H), 6.81 (br, s, 1H, NH), 6.93 (dd, J=5.0, 3.7 Hz, 1H, 4′-H), 7.02 (d, J=3.5 Hz, 1H, 5′-H*), 7.19 (d, J=5.0 Hz, 1H, 3′-H*) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 16.15 (CH_3), 46.77 (C-4), 58.82 (C-5), 124.36 (C-3′*), 124.74 (C-5′*), 126.70 (C-4′), 144.23 (C-2′), 158.25 (C-3), 176.30 ($CSNH_2$) ppm; MS (ES+): m/z (%)=226 (11.5) [M+H$^+$], 225 (77.7) [M$^+$], 184 (20.2), 183 (38.5), 171 (10.1), 169 (100.0), 165 (18.9), 151 (36.5), 143 (14.9), 133 (12.2), 111 (11.5), 110 (79.7), 109 (73.6), 97 (21.6), 83 (18.2), 74 (10.1), 69 (23.6), 65 (13.5), 60 (29.7), 45 (21.6). Anal. calcd for $C_9H_{11}N_3S_2$ (225.33): C 47.97, H 4.92, N 18.65, S 28.64; found: C 48.10, H 4.95, N 18.44, S 28.50.

4.1.9. (*RS*)-(±)-1-Formyl-3,5-diphenyl-2-pyrazoline (6a). 7.2 g (48 mmol) Hydrazinediium dithiocyanate (**13**) and 10 g (48 mmol) chalcone (**1i**) in 85 ml of dimethylformamide gave 9.8 g (81%) of **6a**. Mp: 158°C (ethanol), (mp[20] 153°C); IR (KBr): $\tilde{\nu}$=1655 (s), 1594 (m), 1425 (m), 1379 (m), 1327 (m), 1139 (m), 763 (s), 695 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=282 (4.319), 233 (3.824) nm; ^{1}H NMR (400 MHz, δ, DMSO-d_6): 3.20 (dd, J=18.1, 4.8 Hz, 1H, 4-H), 3.92 (dd, J=18.1, 11.6 Hz, 1H, 4-H), 5.54 (dd, J=12.0, 4.8 Hz, 1H, 5-H), 7.23–7.49 (m, 8H, aromatic H), 7.78–7.81 (m, 2H, aromatic H), 8.92 (s, 1H, HC=O) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d_6): 42.50 (C-4), 58.68 (C-5), 125.84, 126.88, 127.65, 128.90, 128.99, 130.71 (aromatic C), 130.90, 141.49 (aromatic C_q), 156.25 (C-3), 159.82 (HC=O) ppm; MS (ES+): m/z (%)=251 (19.6) [M+H$^+$], 250 (100.0) [M$^+$], 222 (16.2), 221 (40.5), 145 (82.4), 119 (35.5), 118 (14.9), 104 (59.1), 103 (14.9), 91 (26.3), 77 (36.5); HRMS (MALDI) calcd ($C_{16}H_{14}N_2O$): 250.1106, found: 250.1127. Anal. calcd for (250.30); calcd: C 76.78, H 5.64, N 11.19; found: C 76.34, H 5.58, N 11.34.

X-Ray diffraction data of ***6a***. All the measurements were performed using graphite-monochromatized Mo Kα radiation at 90 K: $C_{16}H_{14}N_2O$, M_r 250.29, monoclinic, space group $P2_1/c$, a=10.469(2) Å, b=16.227(4) Å, c=

7.506(2) Å, β=97.38(2)°, V=1264.6(5) Å^3, Z=4, d_{calc}=1.315 g cm^{-3}, μ=0.084 mm^{-1}. A total of 2966 reflections were collected (Θ_{max}=25°), from which 2221 were unique (R_{int}=0.0446), with 1638 having $I>2\sigma(I)$. The structure was solved by direct methods (SHELXS-97)[20] and refined by full-matrix least-squares techniques against F^2 (SHELXL-97).[21] The non-hydrogen atoms were refined with anisotropic displacement parameters. The H-atoms were included at calculated positions with their isotropic displacement parameters fixed to 1.2 times U_{eq} of the C atom they are bonded to. The C–H distances were fixed to 0.99, 1.00, and 0.95 Å for the secondary, tertiary, and phenyl hydrogen atoms, respectively. For 174 parameters final R indices of R=0.0584 and wR^2=0.1448 (GOF=1.068) were obtained. The largest peak in a difference Fourier map was 0.258 e Å^{-3}. The final atomic parameters, as well as bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre (CCDC192916).

4.1.10. (*RS*)-(±)-3-(4-Chlorophenyl)-1-formyl-5-phenyl-2-pyrazoline (6b). 5.6 g (37 mmol) Hydraziniediium dithiocyanate (**13**) and 9 g (37 mmol) 4′-chlorochalcone (**1j**) in 100 ml of dimethylformamide gave 7.5 g (71%) of **6b**. Mp: 179°C (ethanol); IR (KBr): $\tilde{\nu}$=1652 (s), 1596 (m), 1426 (s), 1403 (m), 1356 (m), 1321 (m), 762 (m), 701 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=290 (4.402), 233 (3.995) nm; ^{1}H NMR (400 MHz, δ, DMSO-d$_6$): 3.21 (dd, J=18.3, 4.8 Hz, 1H, 4-H), 3.91 (dd, J=18.3, 11.8 Hz, 1H, 4-H), 5.54 (dd, J=12.0, 4.8 Hz, 1H, 5-H), 7.22–7.37 (m, 5H, aromatic H), 7.54 (d, J=8.5 Hz, 2H, aromatic H), 7.80 (d, J=8.6 Hz, 2H, aromatic H), 8.91 (s, 1H, HC=O) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d$_6$): 42.41 (C-4), 58.88 (C-5), 125.88, 127.59, 127.69, 128.63, 128.91, 129.08 (aromatic C), 129.81, 135.31, 141.37 (aromatic C$_q$), 155.31 (C-3), 159.90 (HC=O) ppm. Anal. calcd for $C_{16}H_{13}N_2OCl$ (284.75): C 67.49, H 4.60, N 9.84, Cl 12.45; found: C 67.27, H 4.52, N 9.83, Cl 12.31.

4.1.11. (*RS*)-(±)-1-Formyl-3-(4-methylphenyl)-5-phenyl-2-pyrazoline (6c). 5.6 g (37 mmol) Hydraziniediium dithiocyanate (**13**) and 7.7 g (35 mmol) 4′-methylchalcone (**1k**) in 100 ml of dimethylformamide gave 6.5 g (70%) of **6c**. Mp: 168°C (ethanol); IR (KBr): $\tilde{\nu}$=1655 (s), 1597 (m), 1428 (s), 1360 (m), 1328 (m), 827 (m), 756 (m), 700 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=287 (4.409), 232 (3.967) nm; ^{1}H NMR (400 MHz, δ, DMSO-d$_6$): 2.35 (s, 3H, CH_3), 3.25 (dd, J=18.2, 4.8 Hz, 1H, 4-H), 3.90 (dd, J=18.0, 11.8 Hz, 1H, 4-H), 5.52 (dd, J=11.6, 4.8 Hz, 1H, 5-H), 7.21–7.37 (m, 7H, aromatic H), 7.68 (d, J=8.4 Hz, 2H, aromatic H), 8.90 (s, 1H, HC=O) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d$_6$): 21.19 (CH_3), 42.53 (C-4), 58.57 (C-5), 125.82, 126.86, 127.63, 128.90, 129.57 (aromatic C), 128.17, 140.62, 141.55 (aromatic C$_q$), 156.23 (C-3), 159.70 (HC=O) ppm. Anal. calcd for $C_{17}H_{16}N_2O$ (264.33): C 77.25, H 6.10, N 10.60; found: C 76.98, H 6.15, N 10.72.

4.1.12. (*RS*)-(±)-1-Formyl-3-(4-methoxyphenyl)-5-phenyl-2-pyrazoline (6d). 2.6 g (17 mmol) Hydraziniediium dithiocyanate (**13**) and 4.1 g (17 mmol) 4′-methoxychalcone (**1l**) in 50 ml of dimethylformamide gave 3.2 g (68%) of **6d**. Mp: 145°C (ethanol); (mp[22] 125–126°C); IR (KBr): $\tilde{\nu}$=1680 (s), 1608 (m), 1519 (m), 1426 (s), 1325 (m), 1260 (s), 1174 (m), 828 (m), 773 (m), 700 (m) cm^{-1}; UV (CH_2Cl_2): λ (log ε)=292 (4.308), 234 (3.946) nm; ^{1}H NMR (400 MHz, δ, DMSO-d$_6$): 3.17 (dd, J=18.1, 4.8 Hz, 1H, 4-H), 3.81 (s, 3H, OCH_3), 3.89 (dd, J=17.9, 11.8 Hz, 1H, 4-H), 5.50 (dd, J=11.8, 4.6 Hz, 1H, 5-H), 7.02 (d, J=8.9 Hz, 2H, aromatic H), 7.21–7.37 (m, 5H, aromatic H), 7.74 (d, J=8.9 Hz, 2H, aromatic H), 8.87 (s, 1H, HC=O) ppm; ^{13}C NMR (100 MHz, δ, DMSO-d$_6$): 42.59 (C-4), 55.53 (OCH_3), 58.51 (C-5), 114.43, 125.81, 127.60, 128.60, 128.89 (aromatic C), 123.39, 141.60, 161.27 (aromatic C$_q$), 155.99 (C-3), 159.55 (HC=O) ppm. Anal. calcd for $C_{17}H_{16}N_2O_2$ (280.33): C 72.84, H 5.75, N 9.99; found: C 72.60, H 6.00, N 9.86.

4.2. Biology

IC_{50} values are given in Table 1.

Antimalarial activity of ***5a***. Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay was used.[23] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates for 48 h. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by liquid scintillation counting 24 h after the addition of the radiolabel. The counts were expressed as percentage of the control cultures, sigmoidal inhibition curves were drawn and IC_{50} values calculated. Standard was artemisinine.

Activity of ***5a*** *against Trypanosoma cruzi*. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μl in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 hours 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene) were added in 100 μl per well with 2× of a serial drug dilution. The plates were incubated at 37°C in 5% CO_2 for 4 days. After 96 h the minimum inhibitory concentration (MIC) was determined microscopically. For measurement of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The colour reaction which developed during the following 2–4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated. Benznidazole was used as standard.

Activity of ***5a*** *against Trypanosoma b. rhodesiense and cytotoxicity*. Minimum essential medium (50 μl) supplemented according to Baltz et al.[24] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μl of trypanosome suspension (*T.b.rhodesiense* STIB 900) was added to each well and the plate incubated at 37°C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μl) was then added to each well and incubation continued for a further 2–4 h. The plate was then read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and emission wavelength of 590 nm.[25] Fluorescence development was expressed as percentage of the control, and IC_{50} values determined. Melarsoprol served as standard giving an IC_{50} of 0.000849 μg/ml whereas **5a** exhibits an IC_{50} of 9.36 μg/ml.

Cytotoxicity was assessed using the same assay and L-6 cells using mefloquine as standard.

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