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Original article

Synthesis and antiamoebic activity of new 1-*N*-substituted thiocarbamoyl-3,5-diphenyl-2-pyrazoline derivatives and their Pd(II) complexes

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Abstract

Some 1-N-substituted thiocarbamoyl-3,5-diphenyl-2-pyrazoline derivatives (L), **1**–**8** were synthesized by a base-catalyzed Claisen–Schmidt condensation of benzaldehyde with acetophenone followed by cyclization with various N-4 substituted thiosemicarbazides. The palladium(II) complexes [$PdLCl_2$], **1a**–**8a** of these ligands were obtained by reacting them with [Pd(DMSO)2Cl2]. All compounds have been characterized by means of elemental analyses, electronic, IR, 1H NMR and mass spectroscopic data, while the complexes have additionally been characterized by thermogravimetric patterns. The in vitro antiamoebic activity was evaluated against the *HM1:IMSS* strain of *Entamoeba histolytica* and the results were compared with the standard drug, metronidazole. The preliminary test results showed that the complexes had better antiamoebic activity than their respective ligands. Moreover, the complexes showed better inhibition of the test organism. The results suggest that the ligands 4, 7 and the complexes **2a**–**4a**, **6a**–**8a** were found with IC_{50} lower than that of the standard drug metronidazole and thus are better inhibitor of growth of *E. histolytica*.

Keywords: Chalcone; Pyrazolines; Thiocarbamoyl; Palladium(II) complexes; Anti-amoebic activity

1. Introduction

Among the parasitic infections, amoebiasis ranks third world wide after malaria and schistosomiasis [1,2]. More than 50 million people are estimated to suffer from the symptoms of amoebiasis such as hemorrhagic colitis and amoebic liver abscess resulting in 100,000 deaths annually [3,4]. The drug of choice to treat amoebic dysentery is metronidazole, which is associated with serious side effects [5]. However, critical lateral effects have been described, i.e. neurological alterations produced by interaction of the drug with the central nervous system, the impairment of cardiac rhythm due to the chelation of MNZ with calcium ions [6–10]. It is carcinogenic to humans and animals [11]. However, differences in drug sensitivity between strains of *Entamoeba histolytica* have been reported, indicating that there may be a small percentage of amoebae which are either resistant to the drug or may even eventually become resistant due to abuse of antiamoebic agents [12] There are occasional reports of failure with metronidazole suggesting that this could probably be heralding the development of drug resistance clinically [13]. Recurrence of amoebic liver abscess even after treatment with metronidazole has been reported and parasites may survive in spite of adequate treatment [14]. Therefore, it is necessary to search for new antiamoebic agents.

Compounds with a pyrazole structure are known to possess monoamine oxidase inhibitor [15], antibacterial [16], antidepressant [17], hypotensive [18], antipyretic and anti-inflammatory [19] activities. Metal complexes derived from pyrazole have attracted considerable interest not only to their extensive coordination chemistry but also to their catalytic and biological properties [20–22]. Very recently we have reported cyclized pyrazoline analogous of thiosemicarbazone and their in vitro screening against *E. histolytica* [23]. Pharmacological importance of pyrazolines and their derivatives prompted us to synthesize new 1-N-substituted thiocarbamoyl-3,5–diphenyl-2-pyrazoline derivatives **1–8** (Fig. 1) and to enhance the effi-

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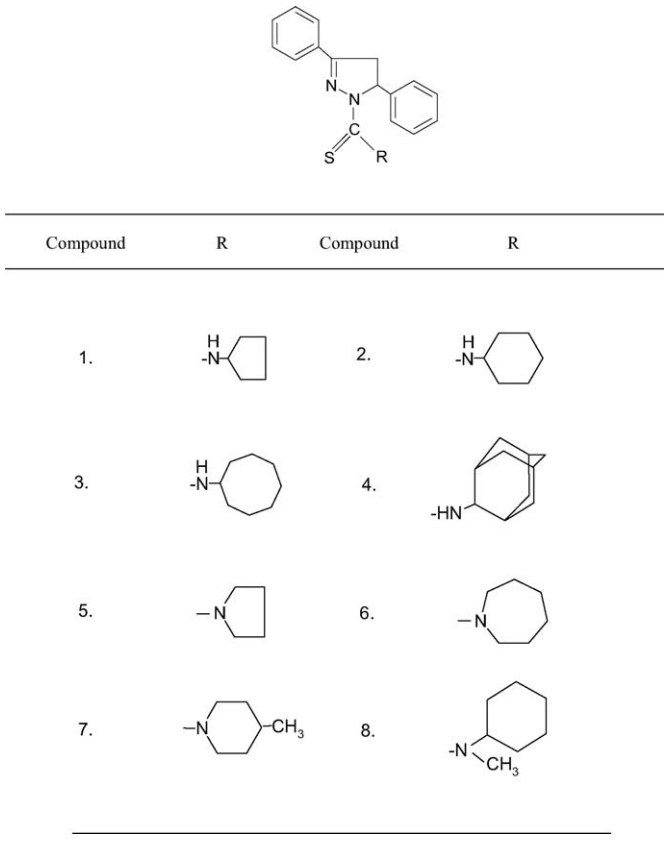


Fig. 1. Structure of 1-N-substituted thiocarbamoyl-3,5-diphenyl-2-pyrazolines (**1**–**8**).

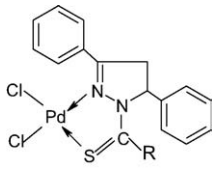


Fig. 2. Structure of Pd(II) complexes of 1-N-substituted thiocarbamoyl–3,5-diphenyl-2-pyrazoline (**1a**–**8a**).

cacy of the ligands by introducing palladium in their molecular structure **1a**–**8a** (Fig. 2). These compounds were subjected to in vitro screening against *HM1:IMSS* strain of *E. histolytica*. To the best of our knowledge this is the first report against *E. histolytica.*

2. Results and discussion

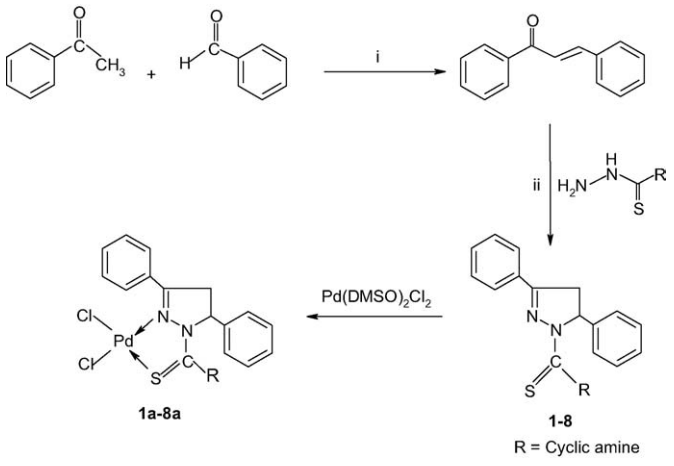
2.1. Chemistry

Chalcone was prepared by reaction of acetophenone with NaOH (11%) (Claisen–Schmidt condensation). The methyl phenyl ketone and benzaldehyde gave high yields above 80%. All the thiosemicarbazide were prepared by reported method [24]. The cyclization of chalcone with N-4 substituted thiosemicarbazides under basic condition leads to the formation of new 1-*N*-substituted-3,5-diphenyl-2-pyrazolines. The yield of the cyclized product of substituted thiosemicarbazide was in the range of 8–25%. The precursor $[Pd(DMSO)_2Cl_2]$ used for the synthesis of palladium complexes was synthesized by the procedure reported in the literature [25]. These compounds were used as ligands to prepare palladium(II) complexes $[PdLCl_2]$ by mixing an equimolar ratio of ligand with $[Pd(DMSO)_2Cl_2]$ in refluxing methanol (Scheme 1). The solution was kept at room temperature overnight. The product was separated by filtration and finally washed with methanol. Crystallization was done in methanol. All the complexes are soluble in DMF and DMSO, sparingly soluble in methanol, ethanol and insoluble in water. The structure of the all ligands and their metal complexes were established by means of their elemental analysis, IR, UV, ^{1}H NMR, electronic spectra, FAB-MS and thermo gravimetric analysis.

2.1.1. IR and electronic spectral studies

Selected diagnostic bands of the IR spectra of the 1-N-substituted thiocarbamoyl-3,5-di phenyl-2-pyrazoline derivatives (**1**–**8**) showed useful information about the structure of the compounds. All the compounds showed intense bands in the region 1302–1377 cm^{-1} due to the ν(C=S) stretch of the thiocarbamoyl group. The IR spectra of all the compounds showed ν(C=N) stretch at 1541–1595 cm^{-1} because of the ring closure [15]. In addition, the absorption bands at 1020–1108 cm^{-1} were attributed to the ν(C–N) stretch vibrations, which also confirm the formation of desired pyrazoline ring in all the compounds. The compounds (**1**–**4**) showed additional sharp bands in the region 3312–3438 cm^{-1} due to the ν(NH) stretch. The IR spectra of the complexes (**1a**–**8a**) showed a strong band appearing in the region 1564–1506 cm^{-1} assignable to the ν(C=N) stretch and shift of this band by 26–64 cm^{-1} to lower frequency indicates the involvement of azomethine nitrogen in coordination. The band due to C=S group is shifted to lower frequency by 21–62 cm^{-1} and this supports the involvement of the thione sulfur in complex formation. This contention is further confirmed by the presence of ν(Pd–N) and ν(Pd–S) band at 532–555 and 430–487 cm^{-1} in the far IR frequency region of the complexes.

The electronic spectra of the cyclized pyrazoline analogues studied in the UV region in methanol, exhibited three absorp-

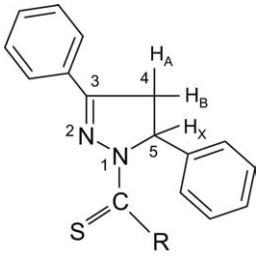


Scheme 1. Reagents and conditions: (i) Methanol, NaOH (11%), 15–28 °C; (ii) Ethanol, reflux, NaOH; (iii) Dry MeOH, 70 °C.

tion bands at 387–290, 288–236 and 232–205 nm assignable to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. The band at 387–290 nm assigned to the transition $n \rightarrow \pi^*$ involving the thione portion (C=S) of thiocarbamoyl group. The two other absorption bands at 288–236 and 232–205 nm were due to $\pi \rightarrow \pi^*$ transition of phenyl ring and $n \rightarrow \sigma^*$ transition of azomethine nitrogen, respectively. The electronic spectra of the Pd(II) complexes were obtained in DMSO in the 211–750 nm region. Several overlapping bands are evident in the spectra of free ligand and its complexes, which complicate the assignation of the transition involved. The comparison of the electronic spectral bands of complexes with their ligands showed that there was a little change in energy of these bands due to extended conjugation of ligand after complex formation. In the spectra of complexes, these bands appeared at ca. 430, 300, and 215 nm, respectively. The bands that appeared at 300 and 215 nm are assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. An intense band at ca. 590 nm in the electronic spectra of the complexes is reasonably assignable to a combination of ligand to metal charge transfer and metal d–d band transitions.

2.1.2. Nuclear Magnetic Resonance spectral studies

The ^{1}H NMR spectra were recorded in $CDCl_3$ support the proposed structure of the compounds. The CH_2 protons of the pyrazoline ring at C-4 carbon appeared as a pair of doublet of doublets at δ 2.5–3.5 and at δ 2.5–3.9 ppm. The CH proton appeared as a doublet of doublets at δ 5.2–6.1 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring. The strong deshielding of the C_5 proton compared with the C-4 protons of the pyrazoline ring can be assumed due to its conformation. (**A**)



The NH proton of thiocarbamoyl group of the ligand **1–4** showed a singlet at δ 7.6–7.7 ppm. The NH proton signal of the complexes **1a–4a** shifted up field as that of free ligand and it appeared at δ 6.1–6.9 ppm.

2.1.3. Thermogravimetric analysis

Thermograms of complexes were recorded under nitrogen with a heating rate of 10 °C min^{-1} between room temperature and 800 °C. All the complexes were stable up to 200 °C. Further increment of temperature cause decomposition of the complexes in two steps. The temperature range for the first step was 209–277 °C. In complexes **1a–4a** and **6a**, the first fragment corresponded to the loss of chlorine and sulfur atoms from the complexes while in the complexes **5a**, **7a**, and **8a**, loss of mixed fragments was observed, which made it difficult to predict the loss of any particular group at any step. The second step starts immediately after the first step and continues until the complete decomposition of the ligand and formation of the end product as palladium sulfide (PdS). The total weight loss of the complexes corresponds to the loss of respective ligands after considering the transfer of one sulfur atom to the metal ion and the residues correspond to the palladium sulfide.

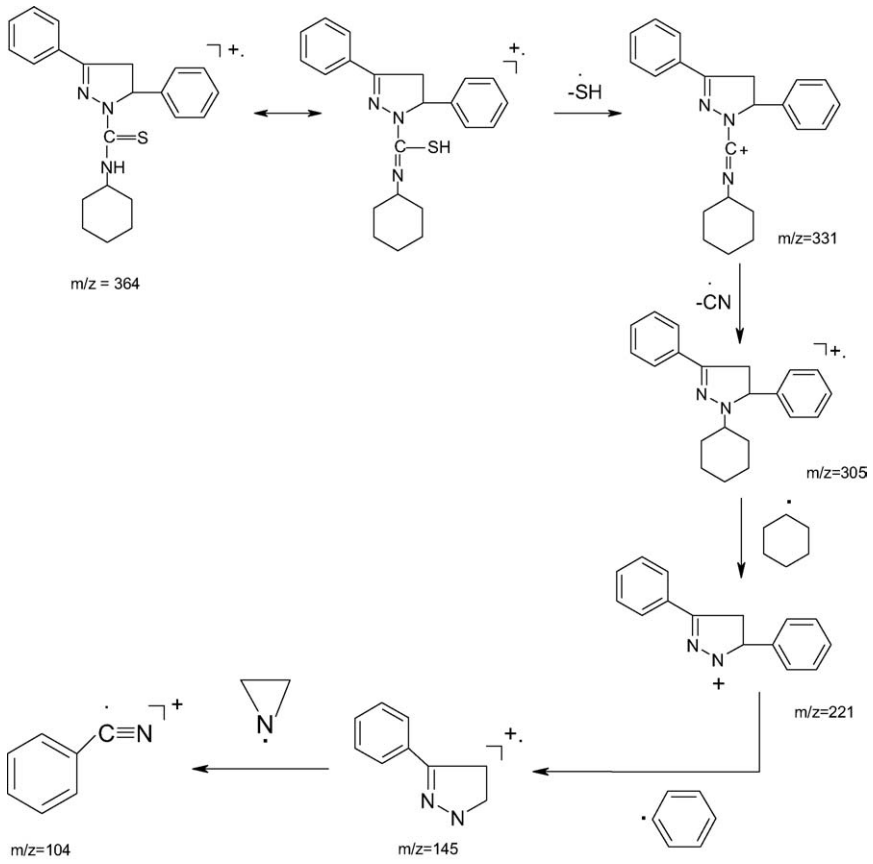
2.1.4. FAB-MS analysis

The characteristic peaks observed within the mass spectra of pyrazoline derivatives (compounds **2**, **2a**) are summarized in Schemes 2 and 3. This mass spectra exhibit molecular ion peaks and contains fragments that confirm the pyrazoline structure in all the compounds [26,27]. The spectra of these compounds suggest that they exhibit thione–thiol tautomerization. The fragmentation of compound **2** showed an M–SH ion at *m*/*z* 331. The pyrazoline derivatives **1–3** showed the molecular ion peak (M + 1) of the ligands. The fragmentation of the ligand occurs via cleavage of [SH] moiety giving desired peaks in all the compounds which correspond to the $[M–SH]^+$ ion. This is followed by elimination of CN radical to produce a tri-substituted pyrazoline ion at *m*/*z* 305. Further elimination of cyclohexyl radical gives rise to the formation of di-substituted pyrazoline ion at *m*/*z* 221, which was observed as a base peak. The removal of phenyl radical produced phenyl pyrazoline ion at *m*/*z* 145. At *m*/*z* 104, phenyl cyanide ion was also detected after the removal of azirinium radical from phenyl pyrazoline ion.

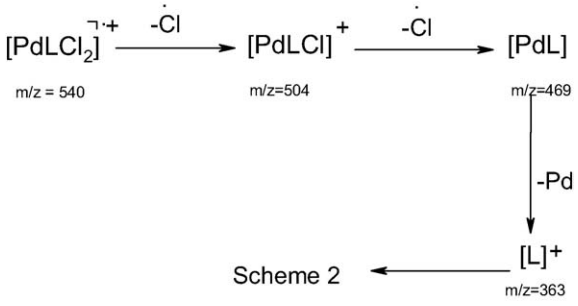
The mass spectra of metal complexes also shows molecular ion peaks (M + 1) confirming their molecular weights and their fragmentation pathways can be initiated by loss of the two chlorine ions giving (M – Cl_2) at *m*/*z* 469. Elimination of Pd metal from thiocarbamoyl pyrazoline was observed at *m*/*z* 363 and its further fragmentation follows the similar pathway in all the complexes.

2.2. Antiamoebic activity

The in vitro antiamoebic activities of 1-N-substituted-3, 5-diphenyl-2-pyrazolines **1–8** and their Pd(II) complexes **1a–8a** were carried out using the HM1:IMSS strain of *E. histolytica* to ascertain the effectiveness of metal complexes in comparison to their pyrarazoline derivatives. Metronidazole was used as the reference drug with IC_{50} 1.8 μM are given in Table 1. The results were estimated as the percentage of growth inhibition compared with the inhibited controls and plotted as probit values as a function of the drug concentration. IC_{50} and 95% confidence limits were interpolated in the corresponding dose response curve. The pyrazoline **1–8** showed IC_{50} values in the range 5.34–1.1 μM. Among all the pyrazoline derivatives, the most active compounds in this class were the compounds which have adamantyl amine (**4**, IC_{50} = 1.39 μM) and 4-methyl piperidine (**7**, IC_{50} = 1.1 μM) as the 1-N-substitution. The complexation of pyrazoline derivative with Pd (II) results in complexes **1a–8a**, which showed IC_{50} in the range 0.42–2.24 μM. All the complexes were found more active than ligands. More-



Scheme 2. Proposed mass fragmentation pattern for compound **2**.



Scheme 3. Proposed mass fragmentation pattern for compound **2a**.

over, the complexes **2a**–**4a** and **6a**–**8a** were more active than metronidazole with less IC_{50} values. The complexation enhances the activity of the ligand, it may be due to chelation, which reduces the polarity of the central metal atom because of partial sharing of its positive charge with the ligand. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *t*-test. The significativity of the difference between the IC_{50} values of metronidazole and the compounds **4, 7, 2a**–**4a** and **6a**–**8a** was evaluated by *t*-test. The values of the calculated *t* were found higher than table value of *t* at 5% level, thus concluding that the character under study is significantly influenced by the treatment. It is concluded that the presence of these bulky groups at position 1-N of thiocarbamoyl group greatly enhanced the antiamoebic activities. Besides, the complex **4a** show the most promising antiamoebic activity with IC_{50} = 0.42 μM. The Pd-complex precursor $[Pd(DMSO)_2Cl_2]$ was also evaluated for antiamoebic activity and compared with Pd(II) complexes and metronida-

Table 1
In vitro antiamoebic activities of 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazolines and Pd(ll) complex against (*HM1:IMSS*) strain of *E. histolytica*

Compound	IC_{50} (μM)	S.D.[a]
1	3.90	0.14
1a	2.24	0.05
2	2.23	0.05
2a	1.44	0.06
3	2.03	0.16
3a	0.70	0.15
4	1.39	0.06
4a	0.42	0.05
5	5.34	0.08
5a	2.08	0.06
6	2.23	0.16
6a	0.77	0.06
7	1.10	0.16
7a	0.83	0.09
8	2.70	0.09
8a	0.90	0.05
$[Pd(DMSO)_2Cl_2]$	8.15	1.73
Metronidazole	1.8	0.1

[a] Standard deviation.

zole, which showed no activity against *E. histolytica*. Detailed studies of the toxicity of these compounds, mechanism of action as well as in vivo studies are in progress.

3. Experimental

Reactions were monitored by TLC analysis using Merck pre-coated aluminum plate silica gel $60F_{254}$ thin layer plates. All the chemicals were purchased from Aldrich Chemical Company (USA). Elemental analysis (C, H, N) was carried out by Central Drug Research Institute, Lucknow, India. Chlorine was estimated by decomposing the complexes with Na_2O_2/NaOH and precipitating as AgCl with $AgNO_3$ after dissolving in dil. HNO_3. Melting points were recorded on KSW melting point apparatus and are uncorrected. Electronic spectra were recorded in methanol on a Shimadzu UV-1601 PC UV-Visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at ambient temperature using a Brucker spectroscopin DPX-300 MHZ spectrophotometer in $CDCl_3$ and DMSO using tetramethylsilane as an internal standard. Splitting patterns are designated as follows; s, singlet, d, doublet, m, multiplet coupling constant *J* is given in Hz. The FAB mass spectra of all the complexes were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/Data System using argon/xenon 6 kV, 10 mA) as the FAB gas and *m*-nitrobenzyl alcohol (NBA) was used as the matrix. Thermograms of the complexes were recorded under nitrogen on a TG 51 thermogravimetric analyzer with increasing the temperature at 10 °C min^{-1}.

3.1. Synthesis of chalcone: a general procedure

A solution of acetophenones (50 mmol) and benzaldehyde (50 mmol) in methanolic NaOH (11%) was stirred for 18 h at 28 °C. The yellow solid obtained, washed with ice-cold water and then rectified spirit, dried and recrystallized by ethanol. Pale yellow crystals (chloroform); Yield: 93%; m.p. 55 °C; Anal. calc. for $C_{15}H_{12}O$; C, 86.21, H, 4.86; found; C, 86.5, H, 5.16; UV/VIS: λ_{max}; nm; 371, 291, 236, 217; IR ν_{max} cm^{-1} 1664 (C=O), 1620 (CH=CH), 1580 (C=C); ^{1}H NMR ($CDCl_3$)/ppm; 7.8 (m, 10H, Ar), 7.74 (d, Hβ, J = 15 Hz, 1H), 7.63 (d, Hα, J = 15 Hz, 1H).

3.2. Synthesis of 1-N-sustituted thiocarbamoyl-3,5-diphenyl-2-pyrazoline: a general method

A mixture of chalcone (10 mmol), thiosemicarbazide (10 mmol) and NaOH (25 mmol) was refluxed in ethanol (25 ml) for 8 h. The solution was poured in to ice water. The precipitate was filtered and recrystallized from methanol [18].

3.2.1. 1-(N-cyclopentyl)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline (1)

Pale yellow crystal (chloroform); Yield: 18%; m.p. 130 °C; Anal. calc. ($C_{21}H_{23}N_3S$): C, 72.21; H, 6.59; N, 12.03; found: C, 72.15; H, 6.61; N, 12.02; UV/VIS: λ_{max}; nm; 371, 291, 245, 223, 211; IR/ν_{max}; cm^{-1}; 3349 (NH), 2945 (C–H), 1595 (C=N), 1370 (C=S), 1076 (C–N); 1H NMR: ($CDCl_3$)/ppm: 7.4 (m, 10H, Ar), 3.1 (dd, HA, JAB = 18.75 Hz, JAX = 12.5 Hz, 1H), 3.8 (dd, HB; JAB = 17.5 Hz, JBX = 6.77 Hz, 1H), 6.1 (dd, Hx, JAX = 10.96 Hz, JBX = 6.77 Hz, 1H), 1.5–1.7 (m, 8H, –CH_2), 7.6 (s, 1H, NH). FAB MS; *m*/*z*; 350 (M + 1), 316, 289, 221, 145, 104.

3.2.2. 1-(N-cyclohexyl)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline (2)

Bright yellow crystal (chloroform); Yield: 13%; m.p. 149 °C; Anal. calc. for ($C_{22}H_{25}N_3S$) C, 72.73, H, 6.89, N, 11.57; found; C, 72.80, H, 6.04, N, 11.57%; UV/VIS; λ_{max} nm 371, 290, 232; IR; ν_{max} cm^{-1} 3315 (NH), 2915 (C–H), 1589 (C=N), 1377 (C=S), 1074 (C–N); ^{1}H NMR ($CDCl_3$)/ppm: 7.4 (m, 10H, Ar), 3.1 (dd, H_A, J_{AB} = 16.7 Hz, J_{Ax} = 11.6 Hz, 1H), 3.8 (dd, H_B, J_{AB} = 16.7; J_{BX} = 10.2 Hz, 1H,), 6.1 (dd, Hx, J_{AX} = 11.6 Hz, J_{BX} = 10.2 Hz, 1H), 7.7 (s, 1H, NH), 1.2-2.1 (m, 10H, CH_2) . FAB MS; *m*/*z* 364 (M + 1), 330, 304, 221, 145, 104.

3.2.3. 1-(N-cyclooctyl)-thiocarbamoyl–3,5–diphenyl–2-pyrazoline (3)

Cream solid (chloroform) Yield: 12%; m.p. 137 °C; Anal. calc. for ($C_{24}H_{29}N_3S$); C, 73.66; H, 7.42; N, 10.74; found: C, 73.68, H, 7.62; N, 10.62%; UV/VIS λ_{max} nm; 366, 301, 223, 205, 246; IR: ν_{max} (cm^{-1}) 3312 (NH), 2920 (C–H), 1590 (C=N),1375 (C=S), 1076 (C–N); ^{1}H NMR ($CDCl_3$)/ppm: 7.1–7.7 (m, 10H, Ar), 3.11 (dd, CH_A; J_{AB} = 4.5 Hz, J_{AX} = 2.11) 3.83 (dd, H_B, J_{AB} = 16.6 Hz, J_{AX} = 9.52 Hz, 1H), 6.11 (dd, Hx, J_{AX} = 2.58, J_{BX} = 7.75, 1H), 7.7 (s, 1H, NH), 1.2–2.1 (m, 14 H, CH_2). FAB MS; 392 (M + 1), 391 (M^+), 358, 332, 221, 145, 106.

3.2.4. 1-(N-adamantylamine)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline (4)

Mud yellow powder (chloroform); Yield: 10%; m.p. 165 °C; Anal. calc. for ($C_{26}H_{29}N_3S$): C, 75.18; 6.99; N, 10.12, found: C, 75.38; H, 6.16; N, 10.7; UV/VIS λ_{max} nm; 380, 288, 236; IR; ν_{max} cm^{-1} 3438 (NH), 2907 (C–H), 1595 (C=N), 1302 (C=S), 1258 (C–N), 1098 (C=S); ^{1} H NMR ($CDCl_3$)/ppm; 7.3 (m, 10H, Ar), 3.3 (dd, H_A J_{AB} = 17.2 Hz, J_{AX} = 9.5 Hz, 1H), 3.3 (dd, H_B, J_{AB} = 17.2 Hz, J_{BX} = 9.4 Hz, 1H), 3.5 (dd, H_x, J_{AX} = 10.8 Hz, J_{BX} = 9.25, 1H), 1.2 –2.2 (m, 18H, CH_2), 7.8 (s, 1H, NH).

3.2.5. 1–(N-pyrrolidine)thiocarbamoyl 3,5-diphenyl-2-pyrazoline (5)

Bright orange crystal (chloroform); Yield: 12%; m.p. 162 °C, Anal. calc. for ($C_{20}H_{21}N_3S$); C, 71.64, H, 6.27, N, 12.54; found; C, 71.54,H, 6.34, N, 12.46; UV/VIS; λ_{max}; nm; 371, 291, 245, IR; ν_{max}; 2925 (CH), 1578 (C=N), 1333 (C=S), 1066 (C–N); 1H NMR(CDCl3) 7.7–7.8 (m, 10H, Ar), 3.10 (dd, HA, JAB = 17.2 Hz, JAX = 10.5 Hz, 1H); 3.7 (dd, HB, JAB = 17.2 Hz, JBX = 8.5 Hz, 1H), 5.3 (dd, Hx, JAX, = 10.5 Hz, JBX = 8.5 Hz, 1H), 2.2 -1.2 (m, 1H, CH2).

*3.2.6. 1-(N-4-methy piperidine)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline (**6**)*

Bright yellow crystal (chloroform); Yield: 15%; m.p. 180 °C; Anal. calc. for ($C_{22}H_{25}N_3S$): C, 72.73; H, 6.89; N, 11.57; found C, 72.76; H, 6.68; N, 11.59; UV/VIS; λ_{max}; nm: 271, 245, 370: IR/V_{max};cm^{-1}; 2918 (C–H), 1595 (C=N), 1349 (C=S), 1066 (C–N); ^{1}H NMR ($CDCl_3$);7.1 (m, 10H, Ar), 2.2 (dd, H_A, J_{AB} = 17.8 Hz, J_{AX}= 10.7 Hz, 1H), 2.5 (dd, H_B J_{AB} = 17.8 Hz, J_{BX} = 7.14 Hz,1H), 4.5 (d, H_x J_{AX} = 12.1 Hz, J_{BX} = 9.3 Hz, 1H), 1.01 (d, 1H, CH_3), 1.8 (m, 8H, CH_2).

3.2.7. 1-(N-methyl cyclohexyl)-1-thiocarbamoyl 3,5-diphenyl-2-pyrazoline (7)

Bright yellow crystal (chloroform); Yield: 8% m.p. 155 °C, Anal. calc. for (C_{23} $H_{27}N_3S$); C 73.21, H,7.16,N,11.14, found; C, 3.19, H, 7.12, N,11.11; UV/VIS: λ_{max};nm; 387, 371, 292, 243, 233, 222 IR.v_{max} cm^{-1}; 2925(CH), 1541(C=N), 1108 (CN), 1303(C=S); ^{1}HNMR $(CDC1)_3$/ppm; 7.7 (m, 10H, Ar), 3.1 (dd, H_A, J_{AB} = 18.2 Hz, J_{AX} = 10.9 Hz, 1H), 3.8 (dd, H_B J_{AB} = 18.2 Hz, J_{BX} = 7.3 Hz, 1H), 6.1 (dd, H_X J_{AX} = 10.9 Hz, J_{BX} = 10.9 Hz, 1H), 1.5–17 (m, 8H, CH_2), 7.6 (d, 1H, NH), 2.5 (s, 3H, CH_3).

*3.2.8. 1-(N-hexamethylineimine)-1-thiocarbamoyl-3,5-diphenyl-2-pyrazoline (**8**)*

Orange crystal (chloroform); Yield: 11%; m.p. 143 °C; Anal. calc. for ($C_{22}H_{25}N_3S$); C,72.73, H, 6.59, N, 11.57, found; C, 72.67, H,6.62, N,11.39, UV/VIS λ_{max} nm; 371, 294, 242, 217; I.R. υ_{max} cm^{-1} 2918 (C–H), 1595 (C=N), 1066 (C–N), 1307 (C=S); ^{1}H NMR ($CDCl_3$)/ppm; 7.4 (m, 10H, Ar), 3.3 (dd, H_A, J_{AB} = 17.5 Hz, J_{AX} = 11.6 Hz, 1H), 3.5 (dd, H_B, $J_{AB;}$ = 17.5 Hz, J_{BX} = 9.25 Hz, 1H), 3.8 (dd, Hx, J_{AX} = 10.9 Hz, J_{BX} = 9.25 Hz, 1H), 2.1 (m, 8H, CH_2).

3.3. Palladium(II) complexes of N-sustituted thiocarbamoyl-3-5-diphenyl-2-pyrazoline; a general method

All Pd (II) complexes were prepared by mixing the equimolar ratio of ligand and $[Pd(DMSO)_2Cl_2]$ in hot dry methanol with continues stirring. The reaction mixture was refluxed for 4 h.The solution was kept at 0 °C overnight, the product was separated by filtration and finally washed with methanol and dried in vacuo.

*3.3.1. [Palladium{(1-N-cyclopentyl)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline}chloride] (**1a**)*

Yellow yield (DMSO); 87.28%; m.p. 242 °C; Anal. calc. for [($C_{21}H_{23}N_3SPdCl_2$] C, 47.89, H, 4.37, N 7.98, Cl, 13.49 found; C, 47.50, H, 4.69, N, 7.61, Cl, 13.42; UV/VIS λ_{max} nm; 746, 371, 292, 243, 214; IR υ_{max}; cm^{-1}; 3205 (N–H), 2955 (C–H), 1535 (C=N), 1349 (C=S), 1030 (C–N), 540 (M–N), 483 (M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm; 7.4 (m, 10H, Ar), 3.4 (dd, 1H, H_A), 2.4 (dd,1H, H_B), 5.8 (dd, 1H, H_X), 1.2–1.9 (m, 8H, CH_2), 7.9 (d, 1H, NH). FAB MS; *m/z* 526 (M + 2), 490, 454, 348, 315, 289, 221, 144, 107.

*3.3.2. [Palladium{(1-N-cyclohexyl)-thiocarbamoyl-3,5-diphenyl-2-pyrazoline}chloride] (**2a**)*

Brown amorphous solid (DMSO); Yield: 87%; m.p. 242 °C; Anal. calc. for [($C_{22}H_{25}N_3SPdCl_2$]; C, 48.89, H, 4.63, N, 7.78, Cl, 13.14; found; C, 48.05, H, 4.69, N, 7.61, Cl, 13.12; UV/VIS λ_{max}; nm; 744, 430, 371, 290, 242, 215; IR. υ_{max} cm^{-1} 3434 (N–H), 2931 (C–H), 1552 (C=N), 1330 (C=S), 540 (M–N), 483 (M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm; 7.4 (m, 10 H, Ar), 3.4 (dd, 1H, H_A), 3.9 (dd, 1H, H_B), 4.3 (dd 1H, H_X), 7.8 (d, 1H, NH), 2.1 2.7 (m, 10 H, CH_2) FAB MS; *m/z* 542 (M + 2), 505, 468, 362, 330, 305, 221, 146, 107.

*3.3.3. [Palladium(1-N-cyclooctyl)–thiocarbamoyl-3,5-diphenyl-2-pyrazoline)chloride] (**3a**)*

Mud brown amorphous solid (DMSO); Yield: 73%; m.p. 235 °C; Anal. calc. for ($C_{24}H_{29}N_3SPdCl_2$]; C, 50.70, H, 5.10, N, 7.39, Cl, 12.49; found C, 50.49, H, 5.31, N, 7.40, Cl, 12.45 UV/VIS λ_{max}; nm; 748, 371, 294, 224, 211; IR υ_{max} 3434 (N–H), 2924 (C–H), 1350 (C=S), 1029 (C–N), 547 (M–N), 480 (M–S); ^{1}H NMR; ($(CD_3)_2SO$)/ppm; 7.4 (m,10 H, Ar), 2.4 (dd, 1H, H_A) 3.4 (dd, 1H, H_B), 5.9 (dd, 1H, H_X), 6.1 (d, 1H, NH), 1.5–1.6 (m, 14 H, CH_2). FAB-MS; *m/z* 568 (M + 1), 532, 496, 390, 356, 328, 220, 143, 107.

*3.3.4. [Palladium{(1-N-adamantylamine)thiocarbamoyl-3-5-diphenyl-2-pyrazoline} chloride] (**4a**)*

Brown (DMSO); Yield: 80%; m.p. 272 °C; Anal. calc. [($C_{26}H_{29}N_3SPdCl_2$]; C, 52.70, H, 4.89, N, 7.09, Cl, 11.96; found; C, 52.52, H, 4.89, N, 7.05, Cl, 11.90; UV/VIS; λ_{max} nm; 734, 370, 295, 236, 215; IR; υ_{max}; cm^{-1} 3441 (N–H), 2923 (C–H), 1261 (C=S), 1541 (C=N), 1024 (C–N), 550 (M–N), 487 (M–S); ^{1}H NMR; ($(CD_3)_2$ SO)/ppm; 7.3 (m, 10H, Ar), 2.6 (dd, 1H, H_A), 3.3 (dd, 1H, H_B), 5.8 (dd, 1H, H_x), 6.9 (d, 1H, NH), 1.5 (m, 14 H, CH_2).

*3.3.5. [Palladium{(1-N-pyrrolidine)thiocarbamoyl–3,5-diphenyi–2-pyrazoline}chloride] (**5a**)*

Brown powder (DMSO); Yield: 92%; m.p. 223 °C; Anal. calc. [$C_{20}H_{21}N_3SPdCl_2$]; C,46.88, H, 4.10, N, 8.20 Cl, 13.85; found; C, 46.61, H,4.10 N, 8.19, Cl, 13.80; UV/VIS λ_{max}; nm; 744, 371, 290, 242, 215; IR υ_{max}; cm^{-1} 2925 (C–H), 1550 (C=N), 1312 (C=S), 1032 (C–N), 555 (M–N), 482 (M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm; 7.5 (m, 10 H, Ar), 3.6 (dd, 1H, H_A), 3.2 (dd, 1H, H_B), 3.1 (dd, 1H, H_X), 1.9–1.7 (m, 4H, CH_2).

*3.3.6. [Palladium[(1-N-4-methylpiperidine)thiocarbamoyl-3-5-diphenyl-2-pyrazoline} chloride] (**6a**)*

Red brown solid (DMSO); Yield: 73%; m.p. 256 °C; Anal. calc. [$C_{22}H_{25}N_3SPdCl_2$]; C, 48.89, H, 4.37, N, 7.98, Cl, 13.14 found; C, 48.73, H, 4.22, N, 7.9I, Cl, 13.10. UV/VIS; λ_{max}; nm: 750, 371, 292, 243, 214; IR υ_{max}; cm^{-1}; 2925 (C–H), 1550 (C=N), 1261 (C=S), 1096 (C–H), 542 (M–N), 430 (M–S); ^{1}H NMR; ($(CD_3)_2SO$); 7.1 (m, 10H, Ar), 2.2 (dd, 1H, H_A), 2.5 (dd, 1H, H_B), 4.5 (d, 1H, H_X),1.01 (d, 3H, CH_3), 1.8 (m, 8H, CH_2), 1.5 (m, 4H, CH_2).

*3.3.7. [Palladium{(1-N-methylcyclohexylamine) thiocarbamoyl-3,5-diphenyl–pyrazoline} chloride] (**7a**)*

Mud brown solid (DMSO); Yield: 84% m.p. 260 °C; Anal. Calc. for $[C_{23}H_{27}N_3SPdCl_2]$ C, 49.82, H, 4.87, N, 7.58, Cl 12.8, found; C, 49.61, H, 4.65, N 7.84, Cl, 12.4; UV/VIS λ_{max} nm; 745, 443, 373, 288, 205, 243; IR υ_{max} cm^{-1} 2923 (C–H), 1506 (C=N), 1303 (C=S), 1043(C=N), 548 (M–N), 432 (M–S); ^1H NMR ($(CD_3)_2SO$)/ppm; 7.4 (m, 10 H, Ar), 3.4 (dd, 1H, H_A), 2.5 (dd, 1H, H_B), 5.8 (dd, 1H, H_X), 1.2–1.9 (m, 10H, CH_2).

*3.3.8. [Palladium{(1-N-hexamethylenimine)thiocarbamoyl-3,5-diphenyl-2-pyrazoline} chloride] (**8a**)*

Red brown solid (DMSO); Yield: 88% m.p. 256 °C; Anal. calc. $[C_{22}H_{25}N_3SPdCl_2]$ C, 48.89, H, 4.63, N, 7.78, Cl, 13.15; found; C, 48.43, H, 4.22, N, 3.9, Cl, 13.45; UV/VIS λ_{max}; nm; 741, 371, 292, 243, 214 IR υ_{max} cm^{-1}; 2925 (C–H), 1550 (C=N), 1261 (C=S), 1096 (C–N), 532 (M–N), 475 (M–S); ^1H NMR; ($(CD_3)_2SO$)/ppm; 7.5 (m, 10H, Ar), 2.8 (dd, 1H, H_A), 3.5 (dd, 1H, H_B), 6.1 (dd, 1H, H_X), 2.1(m, 10H,CH_2).

3.4. Organism culture and in vitro screening against E. histolytica

Preliminary experiments were carried out to determine the antiamoebic activities of the in vitro culture against *HM1:IMSS* strain of *E. histolytica* as previously described in [28]. The *E. histolytica* strain *HM1:IMSS* was cultured in tubes (15 cm^3) by using Diamond TYIS-33 medium [29]. All the compounds were dissolved in DMSO (40 μl). The maximum concentration of DMSO did not exceed 0.1% at which level no inhibition of amoebal growth occurred [30,31] and is further confirmed by the experiments carried out in our laboratory, followed by adding enough culture medium to obtain concentration of 1 mg ml^{-1}. Stock solutions of the compounds were prepared freshly before use at a concentration of 0.1 mg ml^{-1}. Twofold serial dilutions were made in the wells of 96-well microtiter plate (Costar). Each test includes metronidazole as a standard amoebicidal drug, with control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoebae suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 ml of fresh medium, chilling the culture tube on ice to detach the organisms from the side of the flask. The number of amoeba per milliliter was estimated with a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organisms per milliliter by adding fresh medium and 170 μl of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μl). An inoculum of 1.7×10^4 organisms per well was chosen so that confluent, but not excessive growth, took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoebae in the plate was checked with a low power microscope. By inverting the plate, the culture media was removed with gentle shaking and then immediately washed with sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoebae. It was allowed to dry at room temperature. After drying, the amoeba were fixed with chilled methanol by keeping it in ice bath for 15 min. drying and staining with (0.5%) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and allowed to dry. A 200 μl portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which the IC_{50} value was found.

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