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ORIGINAL ARTICLE

Synthesis, spectral characterization and biological evaluation of 1-thiocarbamoyl-3-phenyl-5-hydroxy-5-(-2-pyridyl)-4-pyrazolines via Michael addition

D. Chinnaraja, R. Rajalakshmi *, V. Latha, H. Manikandan

Department of Chemistry, Annamalai University, Annamalai Nagar, 608 002 Tamil Nadu, India

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4-Pyrazoline;
Thiocarbamoyl;
Michael addition;
2-Pyridyl chalcone;
Antibacterial activities

Abstract A series of 1-thiocarbamoyl-3-phenyl-5-hydroxy-5-(2-pyridyl)-4-pyrazolines derivatives have been synthesized using sodium acetate as a catalyst. The spectral characterization and structure of 1-thiocarbamoyl-3-phenyl-5-hydroxy-5-(2-pyridyl)-4-pyrazolines are reported. Spectral techniques employed include ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, D_2O exchange, Mass and IR. Compounds **12–22** exhibited potent antibacterial activity against *Salmonella typhi* and *Pseudomonas aeruginosa* whereas the same set of compounds exerted potent antifungal activity against *Aspergillus niger* and *Aspergillus fumigatus*.

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1. Introduction

Heterocyclic analogues of chalcones were prepared for biological studies (Dhar et al., 1981; Raut, 1960; Ariyan and uschitzky, 1961; Jurasek et al., 1978). N-thiocarbamoyl pyrazolines are considered as important compounds in the organic chemistry because of their application in heterocyclic synthesis and medicine, pyrazolines are compounds with note worthy application and have been reported to show a wide spectrum of biological activities, including antibacterial, antifungal, anti inflammatory, antiamoebic, antidepressant and anticonvulsant (Berghot and Moawad, 2003; Nauduri and Reddy, 1998; Korgaokar et al., 1996; Udupi et al., 1998, Abid and Azam, 2005; Bilgin et al., 1993; Guniz et al., 2000). The pyrazoline function is a quite stable fragment in bioactive moieties to synthesize new compounds possessing biological activities. This prompted us to synthesize various substituted N-thiocarbamoyl pyrazolines. Over the years the synthesis of 2-pyrazoline has received considerable attention. But while attempting to synthesize 2-pyrazoline derivatives using 2-acetyl pyridine based chalcones as precursors, we accidentally got these new 4-pyrazoline derivatives. So, herein we report an efficient approach to synthesize 1-thiocarbamoyl-5-hydroxy-5-(-2-pyridyl)-4-pyrazoline derivatives. Moreover there is no example in the literature for the synthesis of 1-thiocarbamoyl-5-hydroxy-4-pyrazoline.

* Corresponding author. Tel.: +91 4144 238282.
E-mail address: chemrajalaksmi@gmail.com (R. Rajalakshmi).

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2. Experimental section

2.1. Instruments

The IR spectrum was recorded in AVATAR-330 FT-IR spectrophotometer and only noteworthy absorption levels (reciprocal centimetres) were listed. ^{1}H NMR spectra were recorded at 400&500 MHz on Bruker AMX 400&500 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. ^{13}C-NMR spectra were recorded at 100&125 MHz on Bruker AMX 400&500 MHz spectrophotometer using $CDCl_3$. ^{1}H–^{1}H COSY, one-bond ^{1}H–^{13}C correlation spectra, HMBC and D_2O exchange were recorded on bruker AMX 400 NMR spectrometer using standard parameters. 0.05 M solutions of the sample prepared using $CDCl_3$ were used for recording 2D NMR spectra. The tubes used for recording NMR spectra were 5 mm in diameter. The Chemical ionization (CI) Mass spectra were recorded on a VARIAN-SATURN 2200 GC–MS spectrometer. The reactions and the purity of the products were assessed by performing TLC. All the reported melting points were taken in open capillaries and were uncorrected.

3. Synthesis

3.1. Synthesis of 1-phenyl-3-(2-pyridyl) prop-2-en-1-one **(1–10)**

A mixture of 2-acetyl pyridine (0.01 mol) and substituted benzaldehyde (0.01 mol) was stirred in ethanol (50 mL) and then aqueous solution of sodium hydroxide (10% 1 mL) was added at room temperature and again stirred for 15 min. The resulting solid was filtered, washed with water, dried and recrystallized from ethanol. Thus the characterization of 1-phenyl-3-(2-pyridinyl) prop-2-en-1-one (**1**–**6**) is reported (Rajendra prasad et al., 2008).

3.1.1. 3-(4-methylphenyl)-1-(2-pyridyl) prop-2-en-1-one **(7)**

Yield: 78%; mp: 143 °C; pale yellow solid; molecular formula $C_{15}H_{13}ON$; IR υ_{max}(cm^{-1}): 1545 (C═N); 1730 (C═O); 1655 (–CH═CH); ^{1}H NMR ($CDCl_3$) δ(ppm): 2.5 (s, 3H, CH_3); 7.25 (d, 1H, C-3′-H); 7.27 (d, 1H, CO–CH═); 7.94 (s, 1H, C-2-H); 7.65 (d, 1H, C-4′-H); 7.72 (m, 1H, C-5′-H); 7.83 (d, 1H, C-6-H); 8.25 (m, 1H, C-5-H); 8.67 (d, 1H, C-4-H); 8.53 (d, 1H, C-6′-H).

3.1.2. 3-(4-N, N-dimethylaminophenyl)-1-(2-pyridyl) prop-2-en-1-one **(8)**

Yield: 75%; mp: 135 °C; pale yellow powder; molecular formula $C_{16}H_{16}ON_2$; IR υ_{max}(cm^{-1}): 1515 (C═N); 1725 (C═O); 1643 (–CH═CH); ^{1}H NMR ($CDCl_3$) δ(ppm): 2.9 (s, 6H, $(CH_3)_2$); 7.25 (d, 1H, C-3′-H); 7.45 (d, 1H, CO–CH═); 7.53 (d, 1H, C-6-H); 7.85 (s, 1H, C–3–H); 7.95 (m, 2H, C-4′-H, C-5’-H); 8.25 (d, 1H, C-5-H); 8.33 (d, 1H, ═CH–Ar); 8.77 (d, 1H, C-6′-H).

3.1.3. 3-(3-nitrophenyl)-1-(2-pyridyl)prop-2-en-1-one **(9)**

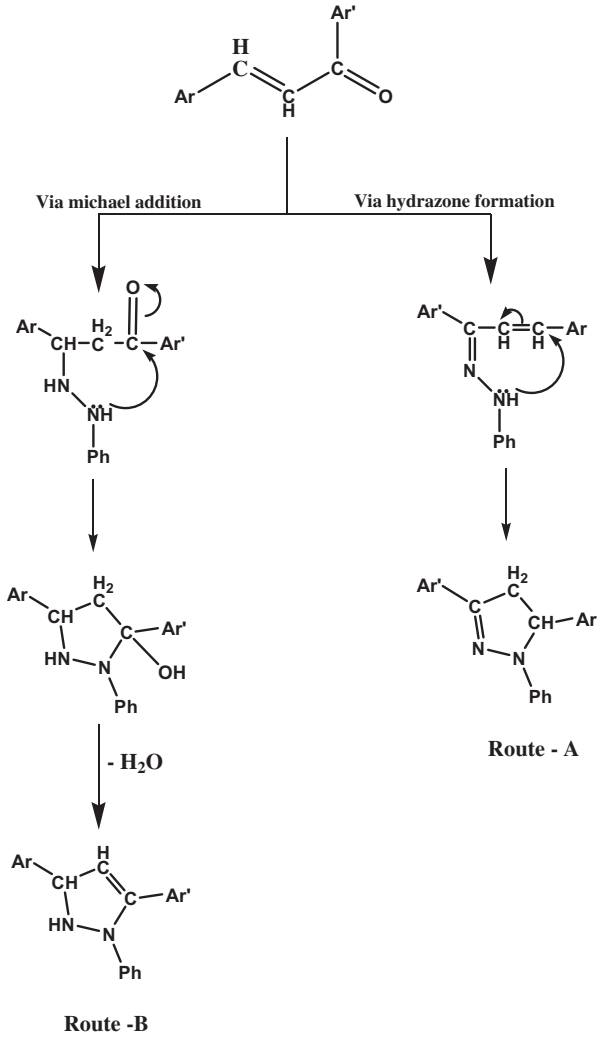
Yield: 82%; mp: 110 °C; yellow solid; molecular formula $C_{14}H_{10}O_3N_2$; IR υ_{max}(cm^{-1}): 1545 (C═N); 1733 (C═O); 1659 (–CH═CH); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.29 (d, 1H, C-3′-H); 7.25 (d, 1H, CO–CH═); 7.11 (s, 1H, C-2-H); 7.54 (d, 1H, C-4′-H); 7.65 (m, 1H, C-5′-H); 7.85 (d, 1H, C-6-H); 8.14 (m, 1H, C-5-H); 8.29 (d, 1H, ═CH–Ar); 8.67 (d, 1H, C-4-H); 8.79 (d, 1H, C-6′-H).

3.1.4. 3-(4-nitrophenyl)-1-(2-pyridyl)prop-2-en-1-one **(10)**

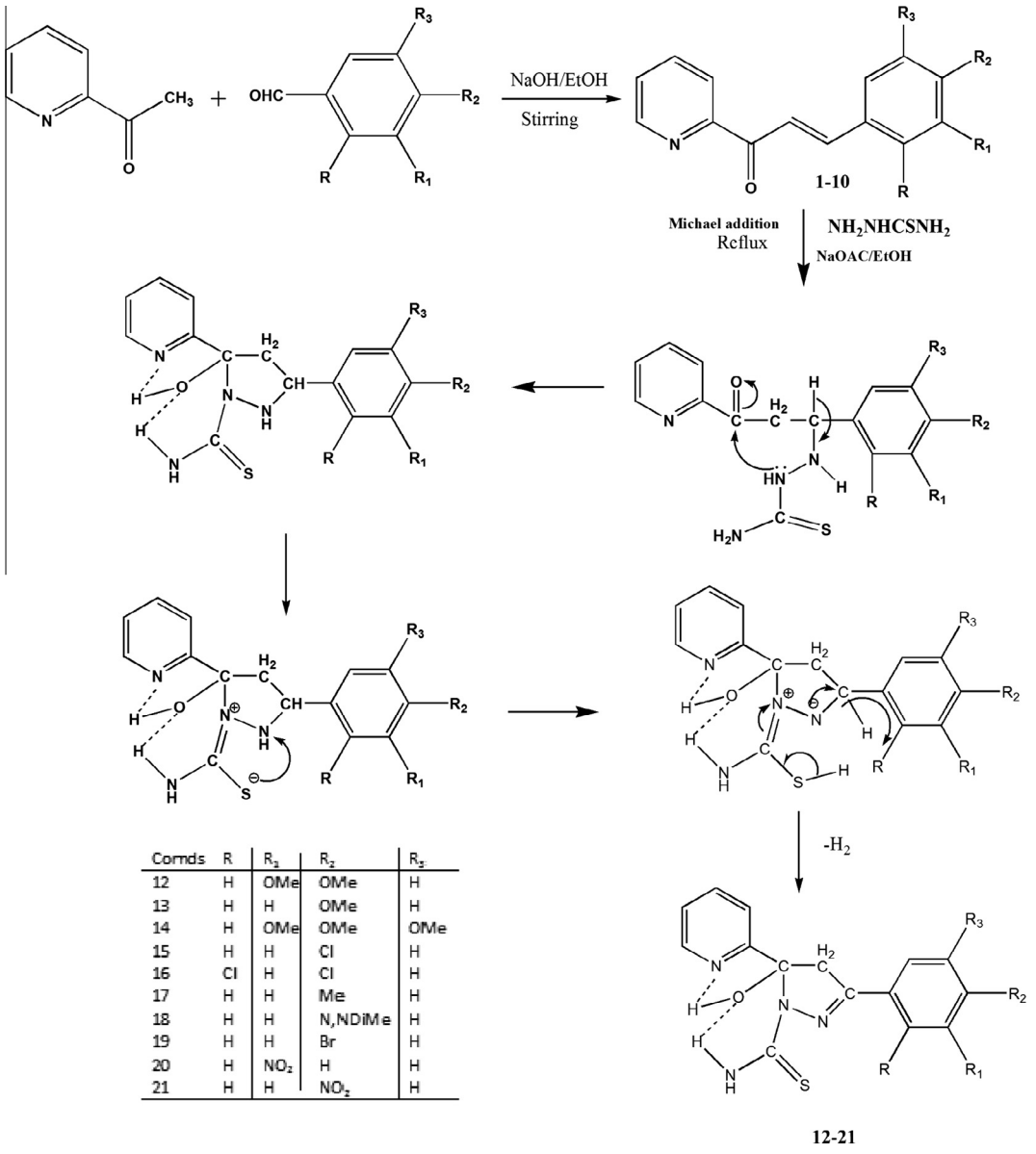
Yield: 80%; mp: 115 °C; yellow solid; molecular formula $C_{14}H_{10}O_3N_2$; IR υ_{max}(cm^{-1}): 1548 (C═N); 1735 (C═O); 1661 (–CH═CH); ^{1}H NMR ($CDCl_3$) δ(ppm): 7.33 (d, 1H, C-3′-H); 7.21 (d, 1H, CO–CH═); 7.13 (s, 1H, C-2-H); 7.55 (d, 1H, C-4′-H); 7.63 (m, 1H, C-5′-H); 7.83 (d, 1H, C-6-H); 8.17 (m, 1H, C-5-H); 8.27 (d, 1H, ═CH–Ar); 8.65 (d, 1H, C-4-H); 8.76 (d, 1H, C-6′-H).

3.2. Synthesis of 3-(3, 4-dimethoxy phenyl)-1-(2-naphthyl) prop-2-en-1-one **(11)**

A mixture of 2-acetyl naphthalene (0.1 mm) and 3, 4-dimethoxy benzaldehyde (0.1 mm) in ethanol was stirred for 10 min in the presence of 10% sodium hydroxide (5 mL). The solution is cooled and the product was filtered and recrystallized from ethanol.



Scheme 1



Comds	R	R_1	R_2	R_3
12	H	OMe	OMe	H
13	H	H	OMe	H
14	H	OMe	OMe	OMe
15	H	H	Cl	H
16	Cl	H	Cl	H
17	H	H	Me	H
18	H	H	N,NDiMe	H
19	H	H	Br	H
20	H	NO_2	H	H
21	H	H	NO_2	H

Scheme 2

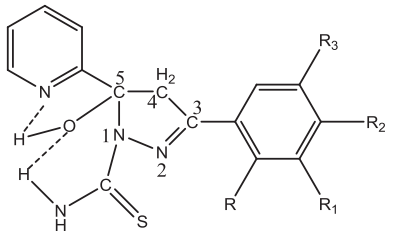


Figure 1 Numbering of the 4-pyrazoline **(12)**.

Compound as yellow crystal; mp 93 °C; yield 85%; mf $C_{21}H_{18}O_3$; υ_{max} cm^{-1} 1729 (C═O), 1655 (HC═CH), 3067(HC═); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 6.77 (d,1H, J = 9.1, HC═CH), 7.49–7.66(m, 7H, aromatic), 7.85–8.11 (m, 3H, aromatic) 8.19 (d, 1H, J = 9.0 CO–CH═CH), 3.8 (s, 6H, $(OCH_3)_2$; ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 189.07 (C═O), 111.8 and 117.77(CH═CH), 55.19,55.25$(OCH_3)_2$, 122.7, 124.8, 126.6, 127.8, 128.11, 128.56, 129.7, 129.9, 130.6, 132.7, 135.4, 136.7, 139.4, and 140.3 (aromatic carbons);GC–MS (m/z): 318.

3.3. General procedure for the synthesis ***(12–21)***

Synthesis of pyrazoline derivatives was performed in a manner as outlined in Scheme 2. The cyclization of chalcones (**1–10**) with thiosemicarbazide under basic condition (saturated CH3COONa) in 50 mL of ethanol led to the formation of pyrazoline derivatives and all the compounds were stable in solid state. The structure of pyrazoline derivatives (**12–21**) is shown in Fig. 1.

3.4. Synthesis of 1-thiocarbamoyl 3-(3, 4-dimethoxyphenyl)-5-hydroxyl (-2-pyridyl)-4-pyrazoline ***(12)***

Compound as yellow powder; mp 204 °C; yield 80%; mf $C_{17}H_{18}N_4O_3S$; υ_{max} cm^{-1} 1617 (C═N), 3170 (HC═Ar),

3273,3347 (NH_2); ^1H NMR (400 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 3.9 (s, 6H, $(OCH_3)_2$), 6.7 (s, 1H, –OH); 6.0–7.1 (broad signals, 2H, NH_2), 6.8–8.5 (m, 7H, Ar–H); ^{13}C NMR (100 MH_Z $CDCl_3$) δ(ppm): 161.61 (C═N), 175.19, (C═S), 50.13(C-4), 95.22 (C-5), 56.14, 56.16 ($(OCH_3)_2$), 151.89 153.22 (ipso carbons), 110.81–149.37.(aromatic carbons); Anal. Calcd. (%) for: C, 56.97; H, 5.06; N, 15.63. Found (%): C, 56.71; H, 5.01; N, 15.42; GC/MS (*m*/*z*): 341.1. and loss of –OH group.

3.5. Synthesis of 1-thiocarbamoyl-3-(4-methoxyphenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(13)***

Compound as yellow powder; mp 184 °C; yield 70%; mf $C_{16}H_{16}N_4O_2S$; υ_{max} cm^{-1} 1620 (C═N), 3167 (HC═Ar), 3336–3419 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 3.8 (s, 3H, (OCH_3)), 6.7 (s, 1H, –OH); 5.9–7.1 (broad signals, 2H, NH_2), 6.9–8.5 (m, 8H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 161.88 (C═N), 175.01, (C═S), 50.00(C-4), 94.98 (C-5), 55.43 (OCH_3), 136.48, 149.21, 152.91 (ipso carbons), 114.22–128.71 (aromatic carbons).

3.6. Synthesis of 1-thiocarbamoyl-3-(3, 4, 5-trimethoxyphenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(14)***

Compound as yellow powder; mp 209 °C; yield 65%; mf $C_{17}H_{20}N_4O_4S$; υ_{max} cm^{-1} 1620 (C═N), 3173 (HC═Ar), 3271–3343 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 3.8 (s, 9H, $(OCH_3)_3$), 6.7 (s, 1H, –OH); 6.0–7.2 (broad signals, 2H, NH_2), 6.8–8.5 (m, 7H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 161.50 (C═N), 175.11(C═S), 51.15(C-4), 95.33(C-5), 56, 11, 56.19, 56.25$(OCH_3)_3$, 149.21, 152.91 (ipso carbons), 114.22–128.71(aromatic carbons).

3.7. Synthesis of 1-thiocarbamoyl-3-(4-chlorophenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(15)***

Compound as white powder; mp 168 °C; yield 65%; mf $C_{15}H_{13}N_4OSCl$; υ_{max} cm^{-1} 1627 (C═N), 2923 (HC═), 3315–3405 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18.3 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 6.6 (s, 1H, –OH); 6.0–7.1 (broad signals, 2H, NH_2), 7.1–8.5 (m, 8H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 161.22(C═N), 175.56(C═S), 49.76(C-4), 95.27 (C-5), 148.87, 149.22, 151.90 (ipso carbons), 120.15–137.06 (aromatic carbons); GC–MS (*m*/*z*): 317.9, and loss of –OH group.

3.8. Synthesis of 1-thiocarbamoyl-3-(2, 4-dichlorophenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(16)***

Compound as white powder; mp 115 °C; yield 65%; mf $C_{15}H_{12}N_4OSCl_2$; υ_{max} cm^{-1} 1625 (C═N), 2929 (HC═Ar), 3319–3409(NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18.5 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 6.6 (s, 1H, –OH); 6.1–7.2 (broad signals, 2H, NH_2), 7.1–8.5 (m, 7H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 162.27(C═N), 175.23(C═S), 49.13(C-4), 95.09 (C-5), 148.86, 149.66, 152.80 (ipso carbons), 120.17–137.76 (aromatic carbons).

3.9. Synthesis of 1-thiocarbamoyl 3-(4-methylphenyl)-5-hydroxyl (-2-pyridyl)-4-pyrazoline ***(17)***

Compound as yellow powder; mp 127 °C; yield 80%; mf $C_{16}H_{16}N_4OS$; υ_{max} cm^{-1} 1613 (C═N), 3175 (HC═Ar), 3273,3347 (NH_2); ^1H NMR (400 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 2.5 (s, 3H, (CH_3)), 6.6 (s, 1H, –OH); 6.0–7.1 (broad signals, 2H, NH_2), 6.8–8.5 (m, 7H, Ar–H); ^{13}C NMR (100 MH_Z $CDCl_3$) δ(ppm): 162.61 (C═N), 174.19, (C═S), 51.13(C-4), 95.13 (C-5), 56.14, 15.16 (CH_3), 151.89 153.22 (ipso carbons), 112.81–149.77 (aromatic carbons).

3.10. Synthesis of 1-thiocarbamoyl-3-(4-N,N-dimethylphenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(18)***

Compound as yellowish orange powder; mp 104 °C; yield 70%; mf $C_{17}H_{19}N_5OS$; υ_{max} cm^{-1} 1621 (C═N), 3187 (HC═Ar), 3332,3415 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 2.9 (s, 6H, $(CH_3)_2$), 6.6 (s, 1H, –OH); 6.0–7.2 (broad signals, 2H, NH_2), 6.9–8.6 (m, 9H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 160.88 (C═N), 177.01, (C═S), 51.00(C-4), 95.98 (C-5), 43.13 (CH_3), 136.48, 149.21, 152.91 (ipso carbons), 113.22–129.71 (aromatic carbons).

3.11. Synthesis of 1-thiocarbamoyl-3-(4-Bromophenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(19)***

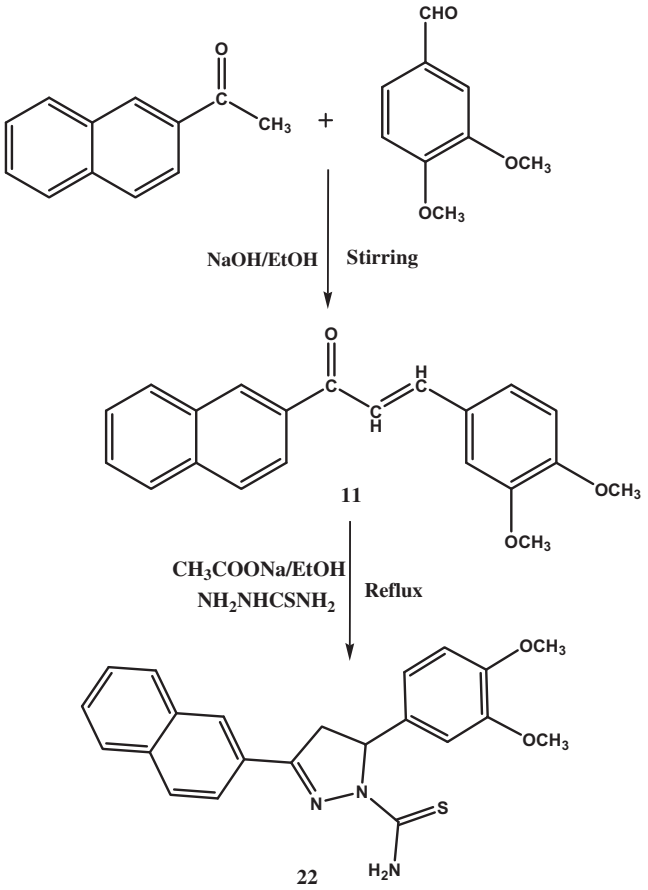
Compound as white powder; mp 133 °C; yield 75%; mf $C_{15}H_{13}N_4OSBr$; υ_{max} cm^{-1} 1626 (C═N), 2921 (HC═), 3315,3405 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.7 (d, 1H, J = 18.3 H_Z, H_A), 3.8 (d, 1H, J = 18 H_Z, H_B), 6.7 (s, 1H, –OH); 6.0–7.1 (broad signals, 2H, NH_2), 7.1–8.5 (m, 8H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 162.22(C═N), 176.56(C = S), 50.76(C-4), 96.27 (C-5), 148.87, 149.22, 151.90 (ipso carbons), 120.15–137.06 (aromatic carbons).

3.12. Synthesis of 1-thiocarbamoyl-3-(3-nitrophenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(20)***

Compound as yellow powder; mp 117 °C; yield 65%; mf $C_{15}H_{13}N_5O_3S$; υ_{max} cm^{-1} 1627 (C═N), 2921 (HC═), 3317,3406 (NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18.3 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 6.8 (s, 1H, –OH); 6.0–7.1 (broad signals, 2H, NH_2), 7.0–8.6 (m, 8H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 160.22(C═N), 174.56(C═S), 51.76(C-4), 96.27 (C-5), 149.87, 151.22, 152.90 (ipso carbons), 120.15–137.06 (aromatic carbons).

3.13. Synthesis of 1-thiocarbamoyl-3-(4-Nitrophenyl)-5-hydroxy-5-(2-pyridyl)-4-pyrazoline ***(21)***

Compound as yellow powder; mp 177 °C; yield 60%; mf $C_{15}H_{13}N_5O_3S$; υ_{max} cm^{-1} 1626 (C═N), 2926 (HC═Ar), 3329, 3993(NH_2); ^1H NMR (500 MH_Z $CDCl_3$) δ(ppm): 3.6 (d, 1H, J = 18.5 H_Z, H_A), 3.7 (d, 1H, J = 18 H_Z, H_B), 6.6 (s, 1H, –OH); 6.1–7.2 (broad signals, 2H, NH_2), 7.1–8.5 (m,



Scheme 3

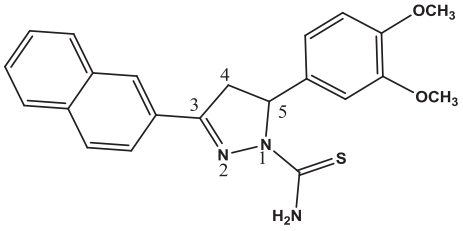


Figure 2 Numbering of 2-pyrazoline **(22)**.

7H, Ar–H); ^{13}C NMR (125 MH_Z $CDCl_3$) δ(ppm): 161.27(C═N), 176.23(C═S), 50.13(C-4), 95.09 (C-5), 148.86, 149.66, 152.80 (ipso carbons), 120.17–137.76 (aromatic carbons).

3.14. Synthesis of 1-thiocarbamoyl-3-naphthyl-5-(3, 4-dimethoxyphenyl) -2-pyrazoline ***(22)***

Synthesis of pyrazoline derivative was performed in a manner as outlined in (Scheme 3). The cyclization of chalcones (**11**) with thiosemicarbazide under basic condition (saturated CH_3COONa) in 50 mL of ethanol led to the formation of pyrazoline compound which was stable in solid state. The structure of pyrazoline compound **22** is shown in Fig. 2.

Compound as yellow wish brown solid; mp 74 °C; yield 65%; mf $C_{22}H_{21}N_3O_2S$; υ_{max} cm^{-1} 1589 (C═N), 3057 (HC═Ar), 3440–3369 (NH_2); ^{1}H NMR (400 MH_Z $CDCl_3$) δ(ppm): 3.4 (dd, 1H, J_1 = 3.6H_Z, J_2 = 17.6H_Z, H_A), 4.1 (dd, 1H, J_1 = 3.3H_Z, J_2 = 17.3H_Z H_B), 6.0 (dd, 1H, J_1 = 3.2H_Z, J_2 = 11.2H_Z H_c), 3.84, 3.85 (s, 6H, $(OCH_3)_2$), 8.9 (d, 2H, J = 8.4, NH_2), 6.9–7.9 (m, 9H, Ar–H); ^{13}C NMR (100 MH_Z $CDCl_3$) δ(ppm): 155.90 (C═N), 175.91, (C═S), 61.30(C-4), 108.10(C-5), 54.87, 54.91 ($(OCH_3)_2$), 148.30 (ipso carbon), 116.57–133.28 (aromatic carbons); GC/MS (m/z): 390.

3.2. Biological activity

The *in vitro* activities of the compounds were tested in Sabouraud's dextrose broth (SDB) (Hi-media, Mumbai) for fungi and Sabouraud's dextrose agar (SDA) for bacteria by the two fold serial dilution method (Dhar et al., 1968). The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in SDA from 24 objective old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores of 1–7 days old were suspended in SDA. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^3–10^7 cfu/mL. The final inoculum six was 10^7 cfu/mL for antifungal assay. Testing was performed at pH 8 of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One millilitre of this was diluted with a further 1 mL of the seeded broth to give the second dilution and so on till dilutions of desired volume were obtained. A set of assay tubes containing only incubated broth was kept as control and likewise solvent controls were also used simultaneously. The tubes were incubated in BOD incubators at 37 °C for bacteria and 28 °C for fungi. Amikacin and amphotericin were used as standards.

4. Results and discussion

4.1. Spectral analysis

Generally chalcones show differential reactivity towards thiosemicarbazide (Shivarama Holla et al., 2000), they can lead to two different pyrazolines as shown in Scheme 1 (Rezessy et al., 2000). The synthesis of 2-pyrazoline derivatives from 2-acetyl pyridine based chalcones using KOH as catalyst has also been reported (Wang et al., 2011). (Scheme 1, route-A).

In the present investigation the condensation of 2-acetyl pyridine based chalcones with thiosemicarbazide in the presence of saturated sodium acetate yields 5-hydroxy-4-pyrazoline (**12**). But the chalcones derived from 2-acetyl naphthalene with thiosemicarbazide under the same reaction conditions give 2-pyrazoline as expected. But in the present study the reaction of 2-acetyl pyridine with thiosemicarbazide follows route-B.

But the reaction does not proceed up to completion and stops before (**12**) is transformed into 2-pyrazoline derivative this may be due to the intramolecular hydrogen bonding between the nitrogen atom of the pyridine ring and the hydroxyl group at C-5 carbon. The hydroxyl group may also be in a hydrogen bonding with the hydrogen atom of the thiocarbamoyl group. The hydroxyl is not in a free state to be eliminated as water so the reaction stopped at this stage and gives 5-hydroxyl-4-pyrazoline derivatives. Compound (**12**) formed is not the excepted product and the formation of (**12**) can be rationalized as shown in Scheme 2.

Table 1 Antibacterial activity of thiocarbamoyl compounds (**12–22**).

Compounds	Zone of inhibition (mm)				
	S. typhi	*S. aureus*	*E. coli*	*K. pneumoniae*	*Pseudomonas*
12	1.5	2.5	1.0	1.0	1.5
13	1.0	2.0	2.0	2.0	1.2
14	3.1	2.5	2.5	2.0	1.2
15	2.5	2.5	2.5	2.5	3.1
16	2.5	2.0	2.5	1.0	1.5
17	2.5	2.5	2.0	1.5	2.5
18	2.5	2.5	1.5	2.5	2.5
19	1.5	1.0	1.0	2.0	1.2
20	1.2	1.2	1.2	1.0	1.2
21	1.2	1.2	1.5	1.2	1.2
22	2.3	1.5	2.0	1.5	1.2
Amikacin	1.00	2.5	2.5	1.00	1.00

Table 2 Antifungal activity of thiocarbamoyl compounds (**12–22**).

Compounds	Zone of inhibition (mm)			
	A. flavus	*C. albicans*	*A. fumigatus*	*A. niger*
12	1.0	2.0	2.5	2.5
13	2.0	2.5	1.0	1.5
14	2.5	2.5	2.0	1.5
15	2.5	2.5	2.5	3.1
16	1.0	1.0	2.0	2.5
17	2.0	1.0	1.0	2.5
18	2.5	2.5	2.	2.5
19	1.0	2.5	2.5	2.5
20	2.0	2.5	2.5	2.5
21	2.5	2.0	1.0	2.5
22	2.5	2.5	2.5	1.0
Amphotericin	2.5	2.5	2.5	2.5

For a safe differentiation the ^{1}H NMR spectrum of both **12&22** is compared. The methine protons of the pyrazoline ring (**22**) exhibits vicinal coupling with both the protons in the methylene carbon which are in different environment therefore it gives three doublets of doublet as excepted. Whereas in the ^{1}H NMR spectrum of hydroxy pyrazoline (**12**) only two doublets are observed. From the HOMOCOSY spectrum of compound **12** it is clear that the closely spaced doublet at 3.6 and 3.7 ppm has cross peak mutually and it should be due to the methylene protons. But in the HOMOCOSY spectrum of compound **22**, the double doublet at 6.0 ppm correlates both with the double doublet at 3.4 and 4.1 ppm, whereas there is only a doublet, instead of doublet doublet in this region, in compound **12**, proving the absence of benzylic proton.

In the ^{13}C NMR spectrum of compound **12** the signal at 50.13 ppm is assigned to methylene carbon (C-4) and the methoxy carbon signals are observed at 56.14 and 56.16 ppm. The signal observed at 161.1 ppm is due to (C=N) carbon. The signal at 95.22 ppm is assigned to C-5 carbon and the signal at 175.19 ppm is due to the thiocarbamoyl carbon.

In the HSQC spectrum of compound **12** it can be noted that the signal at 50.13 ppm has a cross peak with signals at 3.6 ppm and 3.7 ppm. Therefore the signal found at 50.13 ppm is due to methylene carbon (C-4). Another observation is that the signal at 95.22 ppm does not show any correlation with proton signal indicating the C-5 carbon. Further evidence for this structure was obtained from the HMBC spectrum, allows the presence of carbon–proton coupling over two or three bonds.

The peak at 95.22 ppm has a cross peak with signal at 6.71 ppm and the peak at 50.13 ppm also has cross peak with signal at 6.71 ppm. But in the HSQC spectrum there is no correlation between the signal at 95.22 and 6.71 ppm. It indicates that the proton is not attached directly to carbon. This is due to the proton attached to hetero atom. Besides, the signal at 6.71 ppm in ^{1}H NMR spectrum is due to –OH proton and it is attached to C-5 carbon appearing at 95.22 ppm. It is also very clear that the C-5 carbon is attached to C-4 carbon which is appearing at 50.13 ppm. This is because the signal at 95.22 ppm also has a cross peak with signals at 3.6 and 3.7 ppm which inturn are the signals of protons attached to C-4 carbon appearing at 50.13 ppm.

In the mass spectrum of compound **22** the peak observed at $m/z = 390$ is due to the molecular ion. In the mass spectrum of compound **12** there is no discernible molecular ion peak. But the base peak observed at $m/z = 341.1$ is due to loss of –OH group [(M^{+}-17) (358–17)]. This also proves that the thiocarbamoyl group of **12** is not in a free state to be eliminated during ionization as it is involved in hydrogen bonding.

4.2. Biological activity

All synthesized compounds **(12–21** and **22)** are tested for their antibacterial activity against *Salmonella typhi, Stapylococcus aureus, Escheriachia coli, Klebsiella pneomoniae and Pseudomonas aeruginosa* as well as antifungal activities against *Aspergillus flavus, Candida albicans, Aspergillus niger and Aspergillus fumigatus*. The zone of inhibition (mm) of compound **12–21** and **22** against all tested in microorganism is given in Table 1.

The compounds **12–21** and **22** were tested for their antibacterial activity against *S. aureus, E. coli, K. pneumoniae, S. typhi* and *P. aeruginosa*. It is clear that the compounds **12–18** showed very good activity against *S. typhi* and *S. aureus* when compared to the standard. But the compounds **19–22** have a very less activity against all tested organisms.

From the diameter of zone of inhibition (Table 2) of tested compounds **12–21** and **22** it is inferred that they showed very good activity against *A. niger* compared to the standard, but

their activity against all other fungal strains was found to be very poor when compared to the standard amphotericin.

5. Conclusion

It can be concluded that starting with chalcones derived from 2-acetyl pyridine 5-hydroxy-4-pyrazoline derivatives (**12**) can be obtained via Michael addition whereas the 2-pyrazoline derivatives (**22**) were not formed. The same 5-hydroxy-4-pyrazoline derivatives (**12**) can be isolated though this can be transformed into corresponding pyrazoline derivatives. On the other hand cyclization of 2-acetyl naphthalene based chalcones under the same set of reaction conditions proceeds via hydrozone (route-A) and yields the excepted 2-pyrazoline derivative. So it is clear that the intramolecular hydrogen bonding between the nitrogen atom of the pyridine ring and the hydroxyl group at the C-5 carbon prevents the reaction to proceed further and to give the dehydrated product 2-pyrazoline derivative. Antibacterial and antifungal studies show that the synthesized compounds **12–21** and **22** exhibit maximum activity of inhibition against the reported bacterial and fungal strains.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2013.04.006.

References

Abid, M., Azam, A., 2005. Bioorg. Med. Chem. 15, 2213–2220.
Ariyan, Z.S., Suschitzky, H.J., 1961. J. Chem. Soc., 2242–2243.
Berghot, M.A., Moawad, E.B., 2003. Eur. J. Pharm. Sci. 20, 173–179.
Bilgin, A., Palaska, E., Sunal, R., 1993. Arzneimittelforschung 43, 1041–1044.
Dhar, D.N., 1981. The Chemistry of Chalcones and Related Compounds. JohnWiley, New York.
Dhar, M.L., Dhar, M.M., Dhanan, B.N., Mehrotra, B.N., Ray, C., 1968. Indian J. Exp. Biol. 6, 232–247.
Guniz, K.S., Rollas, S., Erdeniz, H., Kiraz, M., Cevdet, E.A., Vidin, A., 2000. Eur. J. Med. Chem. 35, 761–771.
Jurasek, A., Knoppava, V., Danderova, M., Kovar, J., Reinprecht, 1978. Tetrahedron 34, 1833–1836.
Korgaokar, S.S., Patil, P.H., Shah, M.T., Parekh, H.H., 1996. Indian J. Pharm. Sci. 58, 222–225.
Nauduri, D., Reddy, G.B., 1998. Chem. Pharm. Bull. 46, 1254–1260.
Rajendra prasad, Y., Praveen kumar, P., Ravikumar, P., Srinivasan rao, A., 2008. E-J. Chem. 5, 144–148.
Raut, K.B., Wender, 1960. J. Org. Chem. 25, 50–52.
Rezessy, B., Zubovics, Z., Kovacs, J., Toth, G., 2000. Tetrahedron 55, 5909–5922.
Shivarama Holla, B., Akberali, P.M., Shivananda, M.K., 2000. IL Farmaco 55, 256–263.
Udupi, R.H., Kushnoor, A.R., Bhat, A.R., 1998. Indian J. Heterocycl. Chem. 8, 63–66.
Wang, S., Shao, W., Li, H., Liu, C., Wang, K., Zhang, J., 2011. Eur. J. Med. Chem. 46, 1914–1918.