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Der Pharma Chemica, 2015, 7(4):85-89
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

An efficient route to synthesis of pyrazoline carboxamides bearing thiophene moiety as antimicrobial agents

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ABSTRACT

*A series of new pyrazoline carbothioamides bearing thiophene moiety were synthesized by simple one pot procedure. The cyclocondensation reaction of chalcones **1a-g** and semicarbazine **2** in the presence potassium hydroxide in ethanol yielded 5-(4-aryl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamides **3a-g** in excellent yield. The synthesized new products were characterized by ^{1}H NMR, ^{13}C NMR, Mass spectral studies and elemental analysis. The compounds were tested for their antimicrobial susceptibility against different bacterium and fungi species.*

Key words: Antibacterial, antifungal, carboxamide, cyclocondensation, inhibition.

INTRODUCTION

Nitrogen containing five membered heterocyclic compounds featured prominently in early studies of organic chemistry. The exploitation of a simple molecule with different functionalities for the synthesis of heterocycles is a worthwhile contribution. Among the nitrogen heterocycles, pyrazolines are considered as useful building blocks for the construction of biologically potent molecules. α, β-unsaturated carbonyl compounds forms the central core for the synthesis of biologically important heterocycles such as benzothiazepine [1,2] and pyrazolines [3,4]. Pyrazolines occupies a prime position in medicinal chemistry for their intense biological applications [5]. The usual methods employed for the synthesis of pyrazolines are 1,3-dipolar cycloaddition of nitrile imines to alkene [6,7], cyclocondensation of α, β-unsaturated carbonyl with hydrazine or phenyl hydrazine [8,9].

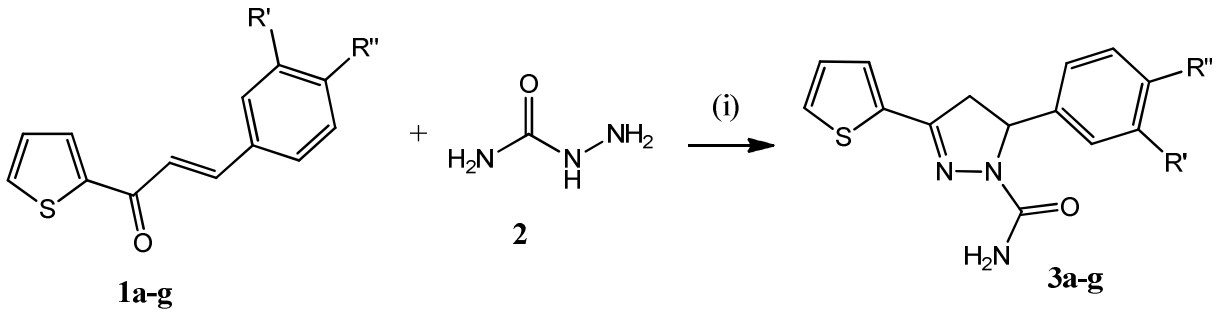
Pyrazolines are known to possess inhibition against kinesin spindle protein (KSP) and neuronal nitric oxide synthase (nNOS) an indicative of potential neuroprotective properties [10], antiproliferative activity against MCF-7 with IC50 of 0.08 lM and potent inhibitory activity in tumor growth inhibition [11]. Pyrazolines also known to exhibit antimicrobial and antioxidant, anti-inflammatory [12], antidepressant [13], anticancer, analgesic, anti-tubercular, anticonvulsant, antipyretic, antihelmintic, herbicidal, antiviral, antitumor and antiangiogenic properties.

In view of the diverse synthetic and biological applications associated with the pyrazolines, we herein report the synthesis of series of novel pyrazoline carboxamides by an accessible procedure and the results of their antimicrobial activities.

MATERIALS AND METHODS

All the reagents and chemicals used are of analytical grade. Melting points were determined by open capillary method and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrophotometer respectively in $CDCl_3$ with TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectrophotomer TOF mode. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (4:1) as eluent.

General procedure for the synthesis of 5-Aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamides, 3a-g: A mixture of substituted chalcones (**1a-g**) (0.01mol) and thiosemicarbazine hydrochloride (**2**) (0.01mol) and potassium hydroxide (0.02mol) in ethyl alcohol (20 mL) was refluxed on a water bath for 6-8 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold water and stirred. The solid separated was filtered, washed with ice cold water and recrystallized from ethyl alcohol to obtain target molecules (**3a-g**) in good yield. The reaction pathway is depicted in scheme-1.



Reagents and condition: (i) 10% alcoholic KOH, Reflux, 4-6 h

3 a) R'=H, R''=H; b) R'=H, R''=Cl; c) R'=H, R''=F; d) R'=H, R''=NO_2
e) R'=H, R''=CH_3; f) R'=H, R''=OCH_3; g) R'=OCH_3, R''=OCH_3

Scheme-1: Reaction pathway for the synthesis of pyrazoline analogues

Antimicrobial activity of the synthesized compounds was done by paper disc diffusion method [14]. The test compounds (**3a-g**) at the concentration of 50 µg/mL in methanol in the nutrient agar media were screened for their antibacterial activity against bacteria species *Escherichia coli, Salmonella typhimurium, Bacillus substilis*, and for their antifungal activity against *Aspergillus niger, Aspergillus flavus, Fusarium oxysporium*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively.

RESULTS AND DISCUSSION

5-Phenyl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3a**:
Obtained from 3-phenyl-1-(thiophen-2-yl)prop-2-en-1-one, **1a** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow crystalline solid in 88% yield. Mp 171-174°C. ^{1}H NMR ($CDCl_3$): δ 3.109-3.165 (dd, 1H, C_4-H_b), 3.739-3.813 (dd, 1H, C_4-H_a), 5.497- 5.527 (dd, 1H, C_5-H_c), 5.540 (s, 2H, -NH_2), 7.020-7.389 (m, 8H, Ar-H and thiophene ring-H). ^{13}C NMR ($CDCl_3$): δ 43.68 (1C, C-4), 60.19 (1C, C-5), 125.43 (2C, Ar-C), 127.54 (1C, 5m ring-C), 127.61 (1C, 5m ring-C), 128.27 (2C, 5m ring-C), 128.88 (2C, Ar-C), 134.96 (1C, Ar-C), 142.24 (1C, Ar-C), 147.43 (1C, C-3), 154.94 (1C, C=O). MS (m/z): 274 (6), 273 (8), 272 (M+1, 100%), 255 (30), 132 (15). Anal. Calcd. for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49%. Found: C, 61.91; H, 4.80; N, 15.40%.

5-(4-Fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3b**:
Obtained from 3-(4-fluorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1b** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 81% yield. Mp 156-159°C. ^{1}H NMR ($CDCl_3$): δ 3.111-3.158 (dd, 1H, C_4-H_b), 3.720-3.834 (dd, 1H, C_4-H_a), 5.462- 5.510 (dd, 1H, C_5-H_c), 5.544 (s, 2H, -NH_2), 7.120-7.588 (m, 7H, Ar-H and thiophene ring-H). ^{13}C NMR ($CDCl_3$): δ 43.62 (1C, C-4), 60.23 (1C, C-5), 125.24 (2C, Ar-C), 126.14 (1C, 5m ring-C), 127.33

(1C, 5m ring-C), 128.45 (2C, 5m ring-C), 128.75 (2C, Ar-C), 134.04 (1C, Ar-C), 142.12 (1C, Ar-C), 147.03 (1C, C-3), 155.04 (1C, C=O). MS (m/z): 290 (M+1, 22), 289 (M+, 100), 273 (45). Anal. Calcd. for $C_{14}H_{12}FN_3OS$: C, 58.12; H, 4.18; N, 14.52%; Found: C, 58.00; H, 4.16; N, 14.50%.

5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3c**:
Obtained from 3-(4-chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1c** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 74% yield. Mp 105-108°C. ^{1}H NMR ($CDCl_3$): δ 3.120-3.172 (dd, 1H, C_4-H_b), 3.744-3.934 (dd, 1H, C_4-H_a), 5.450- 5.567 (dd, 1H, C_5-H_c), 5.580 (s, 2H, -NH_2), 7.020-7.576 (m, 7H, Ar-H and thiophene ring-H). ^{13}C NMR ($CDCl_3$): δ 36.66 (1C, C-4), 76.20 (1C, C-5), 116.18 (1C, Ar-C), 117.20 (1C, Ar-C), 127.05 (1C, Ar-C), 127.91 (1C, Ar-C), 127.99 (1C, 5m ring-C), 128.32 (1C, 5m ring-C), 129.34 (1C, 5m ring-C), 129.46 (1C, 5m ring-C), 142.51 (1C, Ar-C), 155.04 (1C, C=O), 156.03 (1C, C-3). MS (m/z): 307 (M+, ^{37}Cl, 33), 305 (M+, ^{35}Cl, 100), 289 (50). Anal. Calcd. for $C_{14}H_{12}ClN_3OS$: C, 54.99; H, 3.96; N, 13.74%; Found: C, 54.91; H, 3.91; N, 13.70%.

5-(4-Nitrophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3d**:
Obtained from 3-(4-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1d** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 66% yield. mp 168-171°C. ^{1}H NMR ($CDCl_3$): δ 3.140-3.184 (dd, 1H, C_4-H_b), 3.753-3.922 (dd, 1H, C_4-H_a), 5.462- 5.550 (dd, 1H, C_5-H_c), 5.578 (s, 2H, -NH_2), 7.320-7.996 (m, 7H, Ar-H and thiophene ring-H). Anal. Calcd. for $C_{14}H_{12}N_4O_3S$: C, 53.16; H, 3.82; N, 17.71%; Found: C, 53.10; H, 3.78; N, 17.65%.

5-(4-Methylphenyl)-3-(Thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3e**:
Obtained from 3-(4-methylphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1e** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 66% yield. Mp 168-171°C. ^{1}H NMR ($CDCl_3$): δ 2.297 (s, 3H, CH_3), 3.089-3.146 (dd, 1H, C_4-H_b), 3.708-3.781 (dd, 1H, C_4-H_a), 5.460- 5.490 (dd, 1H, C_5-H_c), 5.503 (s, 2H, -NH_2), 7.017-7.382 (m, 7H, Ar-H and thiophene ring-H). ^{13}C NMR ($CDCl_3$): δ 21.08 (1C, CH_3), 43.72 (1C, C-4), 60.01 (1C, C-5), 125.39 (2C, Ar-C), 127.55 (1C, 5m ring-C), 128.24 (1C, Ar-C), 129.54 (2C, Ar-C), 135.07 (1C, 5m ring-C), 137.24 (1C, 5m ring-C), 139.39 (1C, 5m ring-C), 147.39 (1C, C-3), 150.24 (1C, Ar-C), 155.10 (1C, C=O). MS (m/z): 286 (M+1, 39), 285 (M+, 32), 268 (8), 241 (10), 240 (100). Anal. Calcd. for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73%; Found: C, 63.03; H, 5.20; N, 14.66%.

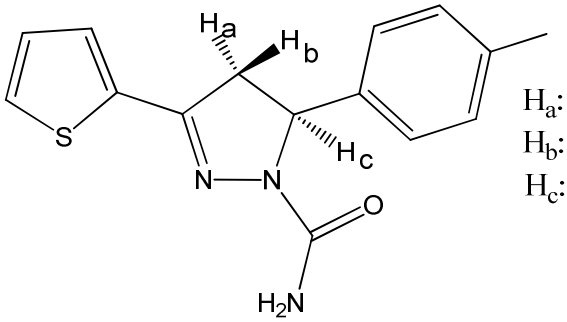
5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3f**:
Obtained from 3-(4-methoxylphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1f** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 89% yield. mp 182-184°C. ^{1}H NMR ($CDCl_3$): δ 3.107-3.164 (dd, 1H, C_4-H_b), 3.712-3.792 (dd, 1H, C_4-H_a), 3.830 (s, 3H, OCH_3), 5.449- 5.479 (dd, 1H, C_5-H_c), 5.492 (s, 2H, -NH_2), 6.834-6.863 (d, 2H, Ar-H), 7.148-7.249 (m, 3H, thiophene ring-H), 7.378-7.393 (d, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 43.54 (1C, C-4), 55.50 (1C, OCH_3), 62.38 (1C, C-5), 125.71 (2C, Ar-C), 127.00 (1C, 5m ring-C), 129.41 (1C, Ar-C), 129.55 (1C, Ar-C), 129.86 (1C, 5m ring-C), 130.33 (1C, 5m ring-C), 134.45, (1C, 5m ring-C), 137.46 (1C, Ar-C), 138.47 (1C, Ar-C), 151.45 (1C, C-3), 156.20 (1C, C=O). MS (m/z): 302 (M+1, 100), 301 (M+, 26), 285 (08), 151 (58). Anal. Calcd. for $C_{15}H_{15}N_3O_2S$: C, 59.78; H, 5.02; N, 13.94%; Found: C, 59.72; H, 5.04; N, 13.87%.

5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide, **3g**:
Obtained from 3-(3,4-dimethoxylphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **1g** (0.001 mole) and semicarbazide, **2** (0.001 mole) as pale yellow solid in 89% yield. Mp 158-161°C. ^{1}H NMR ($CDCl_3$): δ 3.121-3.156 (dd, 1H, C_4-H_b), 3.712-3.784 (dd, 1H, C_4-H_a), 3.856 (s, 6H, OCH_3), 5.449- 5.479 (dd, 1H, C_5-H_c), 5.492 (s, 2H, -NH_2), 6.834-6.863 (d, 2H, Ar-H), 7.148-7.249 (m, 3H, thiophene ring-H), 7.308-7.563 (d, 2H, Ar-H). MS (m/z): 331 (M+, 100), Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68%; Found: C, 57.90; H, 5.12; N, 12.56%.

RESULTS AND DISCUSSION

The general synthetic pathway employed is depicted in the scheme-1. The structure proof of the products (**3a-g**) was provided by ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis. The structural assignments were made by NMR analysis by considering compound (**3e**) as the representative compound. In its ^{1}H NMR spectra, H_a, H_b and H_c protons of the pyrazoline ring appeared as a doublet of doublet. The doublets of H_a appeared at δ 3.708-3.781 ppm; doublets of H_b appeared at δ 3.089-3.146 ppm; and that of H_c at δ 5.460- 5.490 ppm. Doublets of H_a and H_b are due to diastereotopic nature of methylene protons. Among H_a, H_b and H_c protons, H_c is the most deshielded due to its close proximity to benzene ring. H_c couples not only with H_a but also with H_b and appears as doublet of doublet instead of a triplet; exhibited a typical ABX spin system with H_c as a doublet of doublets (Fig-1). The NH_2 protons

deshielded due to adjacent C=O group and appears as singlet at δ 5.503 ppm. Further the compound showed the signals due to aromatic and thiophene ring protons in the expected region. All the synthesised compounds showed the similar ^{1}H NMR signals.

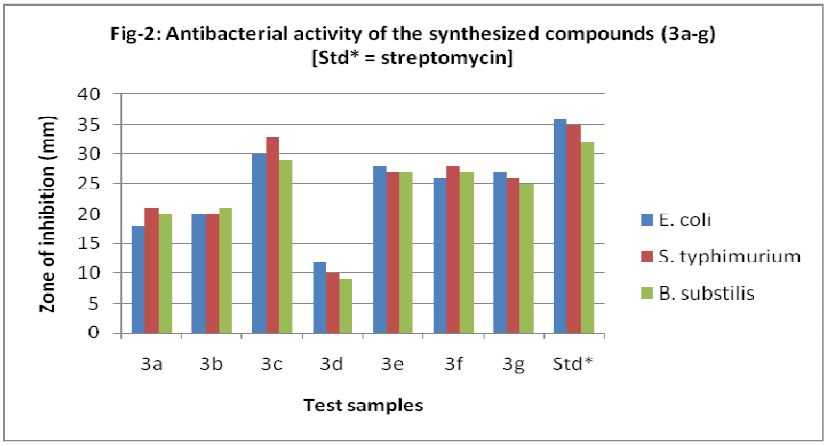


H_a: 3.708-3.781 (dd, 1H, *J*=19.0, 10 Hz)
H_b: 3.089-3.146 (dd, 1H, *J*=18.0, 8.5 Hz)
H_c: 5.460- 5.490 (dd, 1H, *J*=18.5, 9.5 Hz)

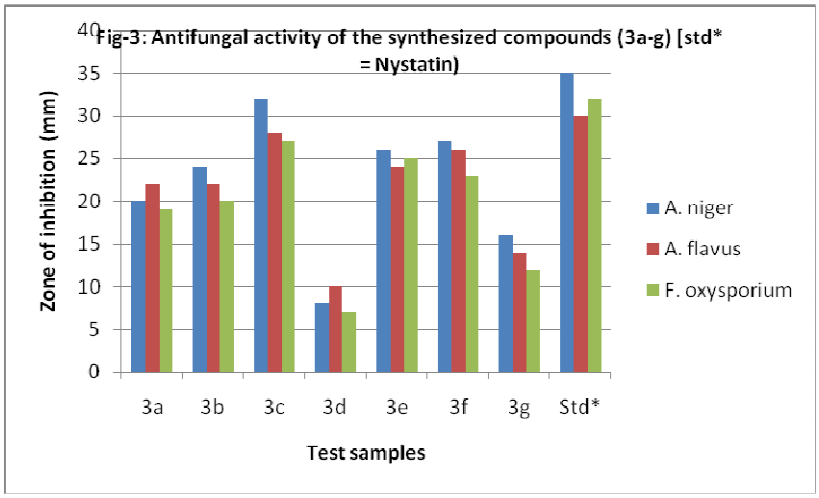
Fig-1: Proton chemical shifts and couplings of **3a**

In ^{13}C NMR, the compound (**3a**) showed signals due to C-3-atom at δ 147.39 ppm, for C-4 atom at δ 43.72 ppm. The C-5 atom signal appeared at δ 60.01 ppm. An intense signal appeared at δ 155.10 ppm was due to C=O carbon atom. Further it showed the signals due to aromatic carbons, thiophene ring carbons and the substituent carbons in the expected region. All the compounds (**3a-g**) showed the similar consistent pattern signals in the spectrum, which strongly favors the formation of the products. All new compounds gave significantly stable molecular ion peaks with a relative abundance ranging from 8-100%. The common possible fragmentation involves with the removal of NH_3, CO, etc. The satisfactorily elemental analysis further supports structure of the products.

Antimicrobial activity: The results of antibacterial and antifungal activity of the synthesized compounds against different bacterium and fungi species were depicted in Fig-2 and Fig-3 respectively.



The results of the study revealed that synthesized new compounds (**3a-g**) exhibited varied antibacterial and antifungal activity against the tested organisms. The compound (**3d**) having strong electron withdrawing $–NO_2$ substitution showed poor inhibition against the organisms tested in comparison with the standards. The compound (**3c**) having –Cl substitution showed promising activity against all the bacterium and fungi species. The compounds (**3e-g**) showed remarkable activity against *E. coli, S. typhimurium, B. substilis, A. niger* and *A. flavus* organism, but poor activity against *F. oxysporium.* The compounds (**3a, 3b**) exhibited moderate activity against all the bacterium and fungi species tested.



CONCLUSION

An accessible procedure for the synthesis of pyrazoline carboxamides, the efficacy of the synthesized compounds as antimicrobial agents validates the significance of this study. Among the series of the compounds reported, 5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide acts as potential antifungal and antibacterial agent.

Acknowledgements
The authors are grateful to IOE Instrumentation facility, University of Mysore, for recording NMR spectra of the compounds reported.

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