Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(4):118-121
(http://derpharmachemica.com/archive.html)

Synthesis of novel bioactive pyrazole carbothioamides and their antifungal activity studies

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ABSTRACT

*A series of new pyrazoline carbothioamides bearing furan moiety were synthesized by simple one pot procedure. The method involves base catalyzed cyclocondensation reaction of chalcones **1a-e** and thiosemicarbazide **2** in ethanol under optimum temperature. The reaction yielded the title compounds 5-aryl-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamides **3a-e** in excellent yields. The synthesized new compounds were characterized by spectral studies and elemental analysis, and were evaluated in vitro for their antifungal susceptibility.*

Key words: Antimicrobial, chalcone, cyclocondensation, furanoyl, inhibition.

INTRODUCTION

Five membered heterocyclic compounds, in particular pyrazoles and their derivatives were treated as an important class of compounds in synthetic organic and pharmaceutical chemistry. Pyrazoles and their analogues were considered as useful synthons for the construction of bioactive molecules. The exploitation of simple molecules such as α, β-unsaturated carbonyl compounds to biologically potent heterocycles is a worthwhile contribution in organic synthesis [1,2]. α,β-unsaturated ketones forms the central core for the construction of pharmaceutically important molecules such as benzothiazepine [3-5] and pyrazolines [6-8]. The usual methods employed for the synthesis of pyrazolines are 1,3-dipolar cycloaddition of nitrile imines to alkene [9], cyclocondensation of α, β-unsaturated carbonyl with phenyl hydrazine [10].

Pyrazolines are known to possess inhibition against kinesin spindle protein (KSP) and neuronal nitric oxide synthase (nNOS) an indicative of potential neuroprotective properties [11], antiproliferative activity against MCF-7 with IC50 of 0.08 lM and potent inhibitory activity in tumor growth inhibition [12] and antidepressant [13] properties.

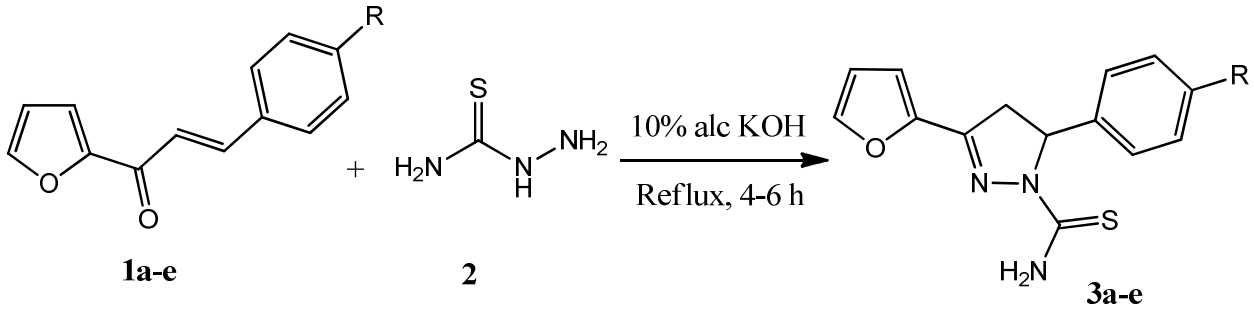
In view of the diverse synthetic and biological applications associated with the pyrazolines, we herein report the synthesis of a series of novel pyrazoline carbothioamides by an easy and accessible procedure and the results of their antimicrobial activities.

METERIALS 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-(furan-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamides, 3a-e: A mixture of substituted chalcones (**1a-e**) (0.001mol) and thiosemicarbazine hydrochloride (**2**) (0.001mol) and potassium hydroxide (0.02 mol) 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-e**) in good yield. The reaction pathway is depicted in scheme-1.



1a-e **2** **3a-e**

3a) R = H; b) R = CH_3; c) R = OCH_3; d) R = NO_2; e) R = Br.

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

The antimicrobial activity of the synthesized compounds was done by a paper disc diffusion method [14]. The test compounds (**3a-e**) at the concentration of 50 µg/mL in methanol in the nutrient agar media were screened for their antifungal activity against *Aspergillus niger, Aspergillus flavus, Fusarium oxysporium*. The antibiotic nystatin was used as standard drug against fungi species.

RESULTS AND DISCUSSION

3-(Furan-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3a**: Obtained from 1-(furan-2-yl)-3-phenylprop-2-en-1-one, **1a** (0.001 mole) and thiosemicarbazide, **2** (0.001 mole) as light brown solid in 78% yield, mp 128-130 °C. ^{1}H NMR ($CDCl_3$): δ 3.079-3.132 (dd, 1H, C_4-H_b), 3.713- 3.763 (dd, 1H, C_4-H_a), 5.940-5.977 (dd, 1H, C_5-H_c), 6.608-7.697 (m, 8H, Ar-H and furan ring-H), 8.240 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 39.86 (1C, C-4), 67.76 (1C, C-5), 108.21 (1C, 5m ring-C), 109.65 (1C, 5m ring-C), 125.56 (1C, Ar-C), 126.92 (2C, Ar-C), 128.54 (2C, Ar-C), 140.54 (1C, 5m ring-C), 141.75 (1C, 5m ring-C), 143.21 (1C, Ar-C), 153.32 (1C, C-3), 176.43 (1C, C=S). MS (m/z): 271 (M+, 100%). Anal. Calcd. for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49%. Found: C, 61.90; H, 4.72; N, 15.38%

3-(Furan-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3b**: Obtained from 1-(furan-2-yl)-3-(p-tolyl)prop-2-en-1-one, **1b** (0.001 mole) and thiosemicarbazide, **2** (0.001 mole) as light greenish solid in 89% yield, mp 120-121 °C. ^{1}H NMR ($CDCl_3$): δ 2.120 (s, 3H, CH_3), 3.067-3.104 (dd, 1H, C_4-H_b), 3.698- 3.716 (dd, 1H, C_4-H_a), 6.134-6.187 (dd, 1H, C_5-H_c), 6.712-6.798 (m, 7H, Ar-H and furan ring-H), 8.144 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 23.43 (1C, CH_3), 38.62 (1C, C-4), 69.05 (1C, C-5), 109.04 (1C, 5m ring-C), 109.85 (1C, 5m ring-C), 125.23 (2C, Ar-C), 129.54 (2C, Ar-C), 136.36 (1C, Ar-C), 138.34 (1C, Ar-C), 140.92 (1C, 5m ring-C), 141.86 (1C, 5m ring-C), 155.43 (1C, C-3), 178.75 (1C, C=S). MS (m/z): 285 (M+, 100). Anal. Calcd. for $C_{15}H_{15}N_3OS$: C, 63.13; H, 5.30; N, 14.73%; Found: C, 63.04; H, 5.16; N, 14.59%.

3-(Furan-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3c**: Obtained from 1-(furan-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one, **1c** (0.001 mole) and thiosemicarbazide, **2** (0.001 mole) as light brown solid in 78% yield, mp 78-80°C. ^{1}H NMR ($CDCl_3$): δ 3.063-3.143 (dd, 1H, C_4-H_b), 3.734- 3.782 (dd, 1H, C_4-H_a), 3,843 (s, 3H, OCH_3), 5.934-5.987 (dd, 1H, C_5-H_c), 6.821-7.732 (m, 7H, Ar-H and furan ring-H), 8.352 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 37.54 (1C, C-4), 55.43 (1C, OCH_3), 71.65 (1C, C-5), 108.54 (1C, 5m ring-C), 109.16 (1C, 5m ring-C), 115.12 (2C, Ar-C), 126.91 (2C, Ar-C), 140.54 (1C, 5m ring-C), 141.76 (1C, 5m ring-C), 134.24 (1C, Ar-C)

,155.51 (1C, C-3), 157.65 (1C, Ar-C), 176.12 (1C, C=S). MS (m/z): 301 (M+, 100). Anal. Calcd. for $C_{15}H_{15}N_3O_2S$: C, 59.78; H, 5.02; N, 13.94%; Found: C, 59.61; H, 4.91; N, 13.80%.

3-(Furan-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3d**: Obtained from 1-(furan-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one, **1c** (0.001 mole) and thiosemicarbazide, **2** (0.001 mole) as light yellow solid in 80% yield, mp 218-220 °C. ^{1}H NMR ($CDCl_3$): δ 3.065-3.143 (dd, 1H, C_4-H_b), 3.723- 3.781 (dd, 1H, C_4-H_a), 5.932-5.964 (dd, 1H, C_5-H_c), 7.143-7.996 (m, 7H, Ar-H and furan ring-H), 8.328 (s, 2H, NH_2). MS (m/z): 316 (M+, 100). Anal. Calcd. for $C_{14}H_{12}N_4O_3S$: C, 53.16; H, 3.82; N, 17.71%; Found: C, 53.12; H, 3.74; N, 17.60%.

5-(4-Bromophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide, **3e**: Obtained from 1-(furan-2-yl)-3-(4-bromophenyl)prop-2-en-1-one, **1c** (0.001 mole) and thiosemicarbazide, **2** (0.001 mole) as light green solid in 77% yield, mp 98-104°C. ^{1}H NMR ($CDCl_3$): δ 3.034-3.123 (dd, 1H, C_4-H_b), 3.802- 3.858 (dd, 1H, C_4-H_a), 5.918-5.976 (dd, 1H, C_5-H_c), 7.017-7.882 (m, 7H, Ar-H and furan ring-H), 8.403 (s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$): δ 38.13 (1C, C-4), 70.54 (1C, C-5), 108.53 (1C, 5m ring-C), 109.23 (1C, 5m ring-C), 121.27 (1C, Ar-C), 127.30 (2C, Ar-C), 131.65 (2C, Ar-C), 140.27 (1C, 5m ring-C), 140.76 (1C, Ar-C), 141.71 (1C, 5m ring-C), 155.39 (1C, C-3), 176.16 (1C, C=S). MS (m/z): 350 (M+, ^{81}Br, 98), 348 (M+, ^{79}Br, 100). Anal. Calcd. for $C_{14}H_{12}BrN_3OS$: C, 48.01; H, 3.45; N, 12.00%; Found: C, 48.12; H, 3.20; N, 11.88%.

RESULTS AND DISCUSSION

The general synthetic pathway employed is depicted in the scheme-1. The structure proof of the products (**3a-e**) 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 (**3c**) 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 3.734- 3.782 ppm; doublets of H_b appeared at δ 3.063-3.143 ppm; and that of H_c at δ 5.934-5.987 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 a benzene ring. H_c couples not only with H_a but also with H_b and appears as a doublet of doublet instead of a triplet; exhibited a typical ABX spin system with H_c as a doublet of doublets. The NH_2 protons deshielded due to adjacent C=S group and appears as singlet at δ 8.352 ppm. Further the compound showed the signals due to methoxy substitution, aromatic and thiophene ring protons in the expected region. All the synthesized compounds showed the similar ^{1}H NMR signals.

In ^{13}C NMR, the compound (**3c**) showed signals due to C-3-atom at δ 155.51 ppm, for C-4 atom at δ 37.54 ppm. The C-5 atom signal appeared at δ 71.65 ppm. An intense signal appeared at δ 176.12 ppm was due to C=S carbon atom. Further, it showed the signals due to methoxy substitution, aromatic and thiophene ring carbons in the expected region. The synthesized compounds (**3a-e**) showed the similar consistent pattern signals in their ^{13}C NMR spectra, which strongly favors the formation of the products. All new compounds showed M+ molecular ion peaks as base peak. The satisfactorily elemental analysis further supports the structure of the products.

The antifungal activity results of the synthesized compounds **3**(**a-e**) revealed that all compounds exerted moderate to excellent activity against all the tested organisms. However, compound **3c** and **3e** having methoxy and bromo substitution showed excellent activity in comparison with the standard. The methyl substitution in the compound **3b** active at *A. niger*. Remaining compounds **3d** and **3a** having nitro and no substitution exhibits very poor inhibition against all the tested organisms.

Table-1: Zone of inhibition of compounds 3(a-e) against fungal species

Compound	Zone of inhibition (measured as diameter in mm)*		
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporium*
3a	24	24	27
3b	29	29	30
3c	29	33	36
3d	19	21	20
3e	33	38	37
Nystatin	28	32	34

**Values are mean of three replicates (n=3)*

CONCLUSION

An accessible procedure for the synthesis of pyrazoline carbothiomides, the efficacy of the synthesized compounds as antifungal agents validates the significance of this study.

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

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