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Der Pharma Chemica, 2011, 3 (5):102-112
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Novel pyrazolinyl thiocarboxamide derivatives: Design, synthesis and biological evaluation as antibacterial, antifungal and cytotoxic agents

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ABSTRACT

*Novel 1-N-pyrazolinyl thiocarboxamides, 5-(3,4-Dimethoxyphenyl)-3-[2-(3, 4-dimethoxyphenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**1b**); 5-(4-Chloroyphenyl)-3-[2-(4-chlorophenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**2b**) and 5-p-Tolyl-3-(2-p-tolyl-ethenyl)-2-pyrazoline-1-thiocarboxamide (**3b**) were synthesized from their corresponding α, β-unsaturated dienones, 1, 5-bis (3, 4-dimethoxyphenyl)-penta- 1, 4-dien-3-one (**1a),** 1, 5-bis (4-chlorophenyl) penta-1, 4-dien-3-one **(2a),** 1, 5-bis (4-methylphenyl)-penta-1, 4-dien-3- one **(3a)** and thiosemicarbazide in the presence of catalytic amount of conc. hydrochloric acid respectively. The structures of these compounds were assigned by FT-IR, ^{1}H- and ^{13}C-NMR, DCI-MS and elemental analysis. The in vitro cytotoxicity of these compounds were evaluated against human cancers: breast (MCF7), lung (NCI-H460), CNS (SF-268) cell lines; antibacterial in (E. coli, S. viridans, S. epidermatis, B. subtilis) and antifungal in C. albicans, and compared with the standard drugs, chloramphenicol and Fluconozole. These compounds showed moderate to high antibacterial and antifungal activity in different species as compared to standard drugs. However, negligible cytotoxic properties were found against three cancerous cell lines. These compounds hold potential for their use in anti-infective agents also.*

Key Words: α, β-unsaturated dienones; N-pyrazolinyl thiocarboxamides; Antimicrobial; Cytotoxicity; Standard drug.

INTRODUCTION

Pyrazolines are well known and important nitrogen-containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the

research activity in this field. They have several prominent effects, such as antibacterial [1-2] antifungal [3], anti-inflammatory [4-5], analgesic [6], antiallergic [7], antiviral [8], anti-HIV [9], antidepressant [10], cerebroprotective [11], cardiovascular agents [12-13], Parkinson & Alzheimer [14], and antiamoebic properties [15]. More recently, some new class of pyrazoline derivatives have been reported acting as selective inhibitors towards Gram-positive and Gram-negative bacteria [16]. Therefore, pyrazoline derivatives constitute an important class of compounds in drug research. For these reasons, synthetic chemists continued to explore new derivatives using different procedures and substrates [17-18]. Moreover, pyrazoline derivatives from chalcone structural modifications are known pharmacophores of a number of biologically active and medicinally useful molecules [19-20]. It has also been reported that some thiocarboxamide moiety containing systems possess good *in vitro* antibacterial activity against Gram-positive bacteria [21]. Encouraged by the diverse biologically important molecules [22-30], it was decided to prepare new class of pyrazoline derivatives.

In view of the above observations, this study was undertaken to design and synthesize some novel pyrazolinyl thiocarboxamide derivatives (**1b-2b**). These compounds were further screened for cytotoxicity against cancerous cell lines and also used to assess their biopotential as antibacterial and antifungal agents in comparison with standard drugs, chloramphenicol and fluconozole.

MATERIALS AND METHODS

Reagents and solvents were of commercial grade and used without further purification. Reactions were monitored on thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} coated on glass plate. Column chromatography was performed on silica gel 60 (70-230 mesh). Melting points were determined on a Koffler hot-plate apparatus and are uncorrected. FT-IR spectra were recorded as KBr discs on a Perkin Elmer spectrophotometer RX1. ^{1}H- and ^{13}C-NMR spectrum were recorded on Varian unity 400 at 100 MHz spectrometers using TMS as internal standard in acetone-d_6 and DMSO-d^{6} solvent. The splitting patterns of ^{1}H-NMR were designated as follows: s: singlet; d; doublet; t: triplet; dd: double doublet etc.and chemical Shift were written in ppm (δ-value). Desorption Chemical Ionization mass spectroscopy (DCI-MS) were performed in the positive ion mode using ammonia gas in a Ribermag R10-10B quadrupole mass spectrometer. Elemental analysis (C, H, N) was performed by Central Drug Research Institute (CDRI) Chemistry Laboratory Lucknow, UP, INDIA. The products names were written according to IUPAC nomenclature. Alamar Blue TM used from (BioSource International, Inc., USA), nutrient agar medium and Sabouraud dextrose agar medium from [Hi-Media Lab. Pvt. Mumbai, India]. Chloramphenicol and Fluconozole obtained from commercially available source.

Synthesis of 1, 5-Bis (3, 4-dimethoxyphenyl)-penta- 1, 4-dien-3-one (1a), 1, 5-bis (4-chlorophenyl) penta-1, 4-dien-3-one (2a) and 1, 5-bis (4-methylphenyl)-penta-1, 4-dien-3-one (3a)

The dienones; (**1a),** (**2a)** and (**3a)** were prepared following a reported method [31] by condensing acetone with benzaldehydes (in 1:2 molar ratio) in the presence of 2.5 equivalents of sodium hydroxide.

Synthesis of 5-(3,4-Dimethoxyphenyl)-3-[2-(3,4-dimethoxyphenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (1b)

A mixture of 1,5-bis (3,4-dimethoxyphenyl)-penta-1, 4-dien-3-one (**1a**) (950 mg, 2.68 mmol) and thiosemicarbazide (732 mg 8.05 mmol) were dissolved in ethanol, 0.2 ml concentrated HCl was added and refluxed with stirring for 10 h, while reaction progress and completion was monitored by TLC. The reaction mixture was cooled at room temperature and poured onto crushed ice and filtered. The solid obtained was subjected to silica gel column chromatography using benzene-ethyl acetate (8:2 v/v) as eluent. Elution of the column gave orange solid (**1c**) in 5% yield. Rf value: 0.60 (toluene: ethylacetate, 8:2 v/v). Further elution of the column yielded a dark brown solid, which on recrystallzation from benzene-acetone afforded product (**1b**), a brown needle shaped crystalline solid. 5-(3,4-dimethoxyphenyl)-3-[2-(3,4-dimethoxyphenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**1b**): Yield: 749 mg, (65%). mp: 130^{o}C. R_f -value: 0.48 (toluene: ethyl acetate, 8:2 v/v).

Elemental analysis:. Calcd. for ($C_{22}H_{25}N_3O_4S$): C, 61.81, H, 5.89, N, 9.83; found: C, 61.68, H, 5.70, N, 9.85; FT-IR: ν_{max} (cm^{-1}): 3447, 3338 (NH), 2934, 2834 (C-H), 1659 (C=C), 1589 (C=N), 1513, 1465 (phenyl), 1345 (C=S), 1261, 1136 (-C-O-C), 1020 (C-N), 961, 805, 759, 612, 575.; ^{1}H-NMR (The δ_H and δ_C values are given in Tables 1 & 2, respectively).; DCI-MS (NH_3): *m/z* 428 (100.0) $[M+H]^+$.

Synthesis of 5-(4-Chloroyphenyl)-3-[2-(4-chlorophenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (2b) and 1-N-[1, 5-bis (4-chlorophenyl)-penta-1, 4- dien-3-ylidene]-thiosemicarbazide (2c):

A solution of 1,5-bis (4-chlorophenyl)-penta- 1,4-dien-3-one (2a) (680 mg 2.25 mmol) and thiosemicarbazide (614 mg, 6.75 mmol) were dissolved in ethanol, 0.2 ml concentrated HCl was added and refluxed with stirring for 10 h, while reaction progress and completion was monitored by TLC. The reaction mixture was cooled at room temperature and poured onto crushed ice and filtered. The gummy residue obtained was subjected to silica gel column chromatography using benzene-ethyl acetate (8:2 v/v) as eluent. Elution of the column gave orange solid, which on recrystallization from benzene-acetone yielded, 1-N-[1,5-bis(4-chlorophenyl)-penat-1,4-dien-3-ylidene]-thiosemicarbazide (2c) as light orange crystalline globules: Yield 64 mg (10%). m.p.: 130° C; R_f value: 0.62 (toluene-ethyl acetate, 8:2 v/v).

Elemental analysis: Calcd. for ($C_{18}H_{15}Cl_2N_3S$) C, 57.45, H, 4.02, N, 11.17; found: C, 57.62, H, 3.98, N,11.21; FT-IR: ν_{max} (cm^{-1}): 3423, 3247 (NH) 1595 (C=N), 1595, 1497(phenyl), 1197 (C=S), 1086 (C–N), 1008, 957, 812, 708 (C-Cl), 623, 520, 480; ^{1}H-NMR: δ 7.77 (2H, d, J=8.55Hz, H-Ar-2,6); 7.46 (2H, d, J=8.55Hz, H-Ar-3,5), 7.64 (2H, d, J=8.40Hz, H-Ar'-2,6), 7.41 (2H, d, J=8.40Hz, H-Ar'-3,5), 7.46 (1H, d, J=16.48Hz, H-1), 7.32 (1H, d, J=16.48Hz, H-2), 7.18 (1H, d, J=16.17Hz, H-4), 7.38(1H,d,J=16.17Hz, H-5), 7.54, 7.90 (1H, s, NH_2), 9.87 (1H, s, NH); ^{13}C-NMR:δ 138.37 (C-1), 126.54 (C-2), 146.33 (C-3), 118.88 (C-4), 134.46 (C-5), 129.50-136.54 (Ar+Ar'), 180.86 (C=S); DCI-MS (NH_3): m/z 376/378/380 [(M+1)/(M+3)/(M+5), 100/67.1/13.6].

Further elution of the column yielded a dark brown solid, which on recrystallization from benzene-acetone afforded product, 5-(4-Chloroyphenyl)-3-[2-(4-chlorophenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**2b**), a brown needle shaped crystalline solid. Yield 548 mg

(59.96%). mp: 260^{o}C. R_f- value: 0.58 (toluene: ethyl acetate, 8:2 v/v).

Elemental analysis: Calcd. for ($C_{18}H_{15}Cl_2N_3S$) C, 57.45, H, 4.02, N, 11.17; found: C, 57.62, H, 3.98, N, 11.21; FT-IR: ν_{max}(cm^{-1}): 3471. 3355 (NH), 2817 (C-H) 1584 (C=N), 1584, 1475 (phenyl) 1351 (C=S), 1088 (C-N), 1007, 957, 829, 774, 671.; ^{1}H-NMR: (The δ_H and δ_C values are given in Tables 3&4 respectively).; DCI-MS (NH_3): m/z 376/378/380 [(M+1)/(M+3) /(M+5) /100/70.2/14.7].

Synthesis of 1-N-[1, 5-Bis (4-methylphenyl)-penta-1, 4-dien-3-ylidene]-thiosemicarbazide (3c)

A mixture of 1, 5-bis (4-methylphenyl)-penta-1, 4-dien-3- one (600mg, 2.2mmol) and thiosemicarbazide (625mg, 6.87mmol) were dissolved in ethanol, 0.2 ml acetic acid was added and refluxed with stirring 10 h, while reaction progress and completion was monitored by TLC. The reaction mixture was cooled at room temperature and poured onto crushed ice and filtered. Filtrate was crystallized using benzene-acetone solvent to obtain a yellow solid product (3c), a orange crystalline needles, Yield 498 mg, (65%). m.p.: 160 ^{o}C ; $R_{f\text{-value}}$: 0.52 (pet. ether-ethyl acetate, 8:2 v/v).

Elemental analysis:. Calcd. for ($C_{20}H_{21}N_3S$) C, 71.61, H, 6.31, N, 12.53; found: C, 71.53, H, 6.25, N, 12.55; ^{1}H NMR: δ 7.31 (1H, d, J=16.02 Hz, H-1), 7.23 (1H, d, J=16.02 Hz, H-2), 7.08 (1H, d, J=16.0 Hz, H-4), 7.30 (1H, d, J=16.0 Hz, H-5), 2.35(3H, s, H-Ar-CH_3), 2.33 (3H, s, H-Ar'-CH_3), 7.62(2H,d, J=8.08Hz, H-Ar-2,6), 7.24(2H, d, J=8.08Hz, H-Ar-3,5), 7.49 (2H, d, J=8.24Hz, H-Ar'-2,6), 7.20(2H, d, J=8.24Hz, H-Ar'-3,5), 7.53, 7.86(2H, s, NH_2), 9.80(1H, s, NH). ^{13}C-NMR: δ 139.82 (C-1), 125.01(C-2), 147.51(C-3), 117.07(C-4), 135.96 (C-5), 180.64(C=S), 21.32(C-Ar-CH_3), 21.26(C-Ar'-CH_3), 128.54(C-Ar-2,6), 130.25(C-Ar,3,5), 134.90(C-Ar-1), 140.23(C-Ar-4), 134.15 (C-Ar'-1), 127.94(C-Ar'-2, 6), 130.23(C-Ar'-3,5), 139.30(C-Ar'-4). DCI-MS (NH_3): m/z 336(100) $[M+H]^+$.

RESULTS AND DISCUSSION

In present study we report here the synthesis of dienones **(i)** 1, 5-bis (3, 4-dimethoxyphenyl)-penta- 1, 4-dien-3-one (**1a**) and **(ii)** 1, 5-bis (4-chlorophenyl) penta-1, 4-dien-3-one (**2a**) with thiosemicarbazide in presence of hydrochloric acid which yielded compounds, 5-(3, 4-dimethoxyphenyl)-3-[2-(3,4-dimethoxyphenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**1b),** 5-(4-chloroyphenyl)-3-[2-(4-chlorophenyl)-ethenyl]-2-pyrazoline-1-thiocarboxamide (**2b**). In the former, the thiosemicarbazone **(1c)** formed as intermediate being minor could not be isolated while in the latter, the thiosemicarbazone, 1-N-[1, 5-bis (4-chlorophenyl)-penta-1, 4- dien-3-ylidene]-thiosemicarbazide ***(2c)*** was isolated in poor yield .The reaction of the dienone, 1, 5-bis (4-methylphenyl)-penta-1, 4-dien-3- one **(3a)** with thiosemicarbazide has been carried out in presence of acetic acid in which the corresponding thiosemicarbazone, 1-N-[1, 5-Bis (4-methylphenyl)-penta-1, 4-dien-3-ylidene]-thiosemicarbazide ***(3c)*** formed could not cyclize to give desired product, 5-p-Tolyl-3-(2-p-tolyl-ethenyl)-2-pyrazoline- 1-thiocarboxamide (**3b**). Due to the failure of this reaction in acetic acid medium, the above two reactions **(i&ii)** have been carried out in the presence of hydrochloric acid. (**Scheme 1**). It showed that the strong acid is essential for their cyclization. The reaction mechanism for the cyclization is considered either via Michael addition reaction or hydrazone or thiosemicarbazone formation [3]. Products formation indicated that it might be following hydrazone or thiosemicarbazone path, due to more stable

regioisomer formed as 2-pyrazolines instead of 3-pyrazolines during Michael addition.

All compounds were characterized by their elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR, DCI-MS spectra. **FT-IR**: The selected diagnostic bands of 1-N-substituted pyrazolinyl thiocaboxamides showed useful information about the structure of the compounds. Intense bands in the region 1197-1345 cm^{-1} due to (C=S) stretch and 1589-1595 cm^{-1} due to (C=N) ring formation. An additional absorption band around 1020-1086 cm^{-1} was attributed to (C-N) stretching vibration, which also confirmed the formation of pyrazoline ring and bands in 3423-3447 cm^{-1} due to (NH) stretch. **^{1}H-NMR**: The ^{1}H-NMR spectra were recorded using acetone-d_6 with TMS as internal standard. The spectra of (**1b)** and (**2b)** showed three double doublets of ABX splitting pattern due to the presence of geminal and vicinal coupling of the two diastereotopic protons (H_A& H_B) at C-4 with the methine proton Hx at C-5 of the pyrazoline ring [32]. The large coupling constants (*J*=11.29 Hz/11.60 Hz) of H- 4 and H-5 showed that they are anti-periplanar to each other. The doublets with large coupling constants (*J*=16.48 Hz) were attributed for the trans oriented ethylenic protons. **DCI-MS** (NH_3): The DCI-MS (NH_3) showed $[M+H]^+$ peak as the base peak confirming their molecular weights which correspond to the sum of molecular weights of the respective dienones and thiosemicarbazide minus one molecule of water, indicating the formation of thiosemicarbazones or their cyclic isomers, pyrazolines. The significant peak which appeared corresponding to the loss of NH_2-$CSNH_2$ from $[M+H]^+$ suggested that the compounds **(1b)** and **(2b)** contains pyrazoline ring with 1-N attached to –$CSNH_2$ group. Other fragments further supported this structure. **^{13}C-NMR**: The ^{13}C-NMR spectra showed signals, which are in strong agreement with the assigned structure. The peak at 178.08/ 178.37 is due to the presence of C=S. Finally, the elemental analysis of these compounds confirmed the right composition. Based on these spectral evidences and elemental composition, the stereo chemical structures were assigned as represented in **Fig. 1**.

Pharmacology:
The biological properties of compounds (**1b)** and (**2b)** were evaluated for their cytotoxicity against three different cell lines in human cancers as lung, breast and CNS cancers **(Table 5)**. Moreover, in vitro, biological screening of the compounds (**1b)**, (**2b)** and (**2c)** are also summarized as antibacterial in *Escherichia coli*, *Streptococcus viridans*, *Staphylococcus epidermatis*, *Bascillus subtilis* and antifungal in *candida albicans.* (**Table 6**).

Cytotoxicity against malignant human tumour cells: Compounds **(1b, 2b)** were evaluated for cytotoxicity against three-cell lines panel consisting of three types of human cancers; breast (MCF7), lung (NCI-H460) and CNS (SF-268) as one dose primary anticancer assay. Each cell line was inoculated and preincubated for 24-48 h on a micro titer plate. Test agents were then added at a single concentration and the culture incubated for further 48 h. End point determinations were made with alamar Blue TM [30]. Results of each test agent were reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduced the growth of any one of the cell lines to approximately 32% or less were considered to be active. The preliminary screening results are shown in **Table 5**. Thus, the compounds (**1b)** and (**2b)** were found not to be active against any of the cell lines.

Antimicrobial activity: Compounds (**1b), (2b)** and (**2c)** were evaluated in vitro for their antimicrobial activity against bacteria *Escherichia coli*, *Streptococcus viridans*, *Staphylococcus*

epidermatis, *Bacillus subtilis*, and fungus, *Candida albicans* at different concentrations using DMSO as solvent at 200-400 µg/ml concentrations by agar well diffusion method of Perez et al. [33] as adopted earlier by Arena and Ahmad [34]. The antimicrobial activity of the test compounds was assayed on nutrient agar medium [Hi-Media Lab. Pvt. Mumbai, India]. The antifungal activity was tested using Sabouraud dextrose agar medium [Hi-Media, Lab. India]. Briefly 0.1 ml of the diluted inoculum (10^6 CFU/ml) of test organism was spread on NA/SDA (Nutrient Agar/Sabouraud dextrose Agar) plates. Wells of 8 mm diameter were punctured into the agar medium and filled separately with 100 µl of compound, solvent blank and an antibiotic (chloramphenicol, 100 µg/ml) to which the test bacteria were sensitive. Fluconozole at the concentration of 100 µg/ml was used as the control against C. *albicans*. After 24 h of incubation at 37^0C, the zones of inhibitions were measured in mm. Chloramphenicol was used as standard for bacteria and Fluconozole was taken as standard for fungus for comparison at the same concentration. By visualizing the antimicrobial data, it was observed that compounds (**1b)** and (**2b)** is active against *E. coli* and *S. viridans*. The compound (**2b)** showed considerable activity against the fungus used whereas (**1b)** did not show any significant activity. The semicarbazone (**2c)** showed broad-spectrum activity against Gram-negative *E. coli* and all Grams positive bacteria used as well as against the fungus *Candida albicans* .These compounds may be directly explored in the preparation of topical anti-infective agents. However, further exploration requires detailed study on exact MIC values and least toxicity to host cell and system. (**Table 6**).

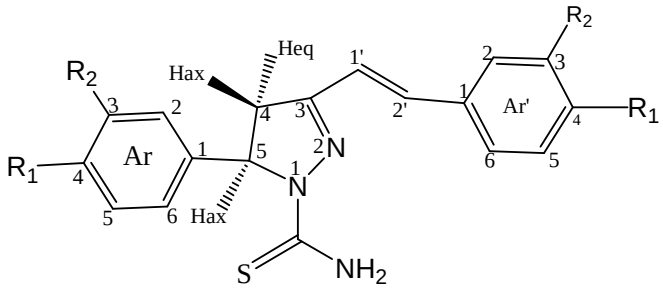


Fig. 1: It represents structure and preferred conformation of N-pyrazolinyl thiocarboxamides based on spectral findings.

Table 1: ^{1}H-NMR spectral data of compound (1b)

Hnumber	δ (ppm)	Integration	Multiplicity	J (Hz)
1’	7.01	1H	d	$J_{1'2'}$ 16.48
2’	7.04	1H	d	$J_{1'2'}$ 16.48
4_{eq}	3.08	1H	dd	$J_{4eq,4ax}$ 17.55, $J_{4eq,5ax}$ 3.21
4_{ax}	3.76	1H	dd	$J_{4eq,4ax}$ 17.55, $J_{4ax,5ax}$ 11.29
5_{ax}	5.91	1H	dd	$J_{4eq,5ax}$ 3.21 , $J_{4ax,5ax}$ 11.29
Ar-2	6.84	1H	d	$J_{Ar\text{-}2,6}$ 2.14
Ar-6	6.69	1H	dd	$J_{Ar\text{-}2,6}$ 2.14,$J_{Ar\text{-}5,6}$ 8.24
Ar-3	-	-	-	-
Ar-5	6.86	1H	d	$J_{Ar\text{-}5,6}$ 8.24
Ar’-2	7.26	1H	d	$J_{Ar'\text{-}2,6}$ 2.14
Ar’-6	7.12	1H	dd	$J_{Ar'\text{-}2,6}$ 2.14 $J_{Ar'\text{-}5,6}$ 8.24
Ar’-3	-	-	-	-
Ar’-5	6.96	1H	d	$J_{Ar'\text{-}5,6}$ 8.24
4_XOMe-$Ar_{-3,4+}$Ar’$_{-3,4}$	3.76-3.88	12H	s	-

Table 2: C-NMR spectral data of compound (1b)

C number	δ (ppm)
1’	122.4
2’	139.72
3	157.66
4	42.42
5	63.78
Ar-1	136.69
Ar-2	111.31[a]
Ar-3	151.79[b]
Ar-4	150.52
Ar-5	113.90[b]
Ar-6	118.92[a]
Ar’-1	129.97
Ar’-2	110.88
Ar’-3	150.77
Ar’-4	149.69
Ar’-5	112.81
Ar’-6	118.49
>C= S	178.08
4xOMe-Ar-$_{3,4}$+Ar’-$_{3,4}$	56.25

[a] *Assignment may be reversed*
[b] *Assignment may be reversed*

Table-3 ^{1}H-NMR spectral data of compound (2b)

Hnumber	δ (ppm)	Integration	Multiplicity	J (Hz)
1’	7.09	1H	d	$J_{1'2'}$ 16.48
2’	7.15	1H	d	$J_{1'2'}$ 16.48
4_{eq}	3.10	1H	dd	$J_{4eq,4ax}$ 17.55, $J_{4eq,5ax}$ 3.81
4_{ax}	3.84	1H	dd	$J_{4eq,4ax}$ 17.55, $J_{4ax,5ax}$ 11.60
5_{ax}	5.98	1H	dd	$J_{4eq,5ax}$ 3.81, $J_{4ax,5ax}$ 11.60
Ar-2,6	7.34	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5,}$ 8.55
Ar-3,5	7.21	2H	d	$J_{Ar\text{-}2,6,\ Ar\text{-}3,5,}$ 8.55
Ar’-2,6	7.63	2H	d	$J_{Ar'\text{-}2,6,\ Ar'\text{-}3,5,}$ 8.55
Ar’3’5	7.43	2H	d	$J_{Ar'\text{-}2,6,\ Ar'\text{-}3,5,}$ 8.55

Table 4: C-NMR spectral data of compound (2b)

C number	δ (ppm)
1’	121.82
2’	138.22
3	156.87
4	42.24
5	63.67
Ar-1	142.96
Ar-2,6	129.88
Ar-3,5	129.42
Ar-4	135.20
Ar’-1	135.82
Ar’-2,6	129.65
Ar’-3,5	128.30
Ar’-4	113.16
>C= S	178.37

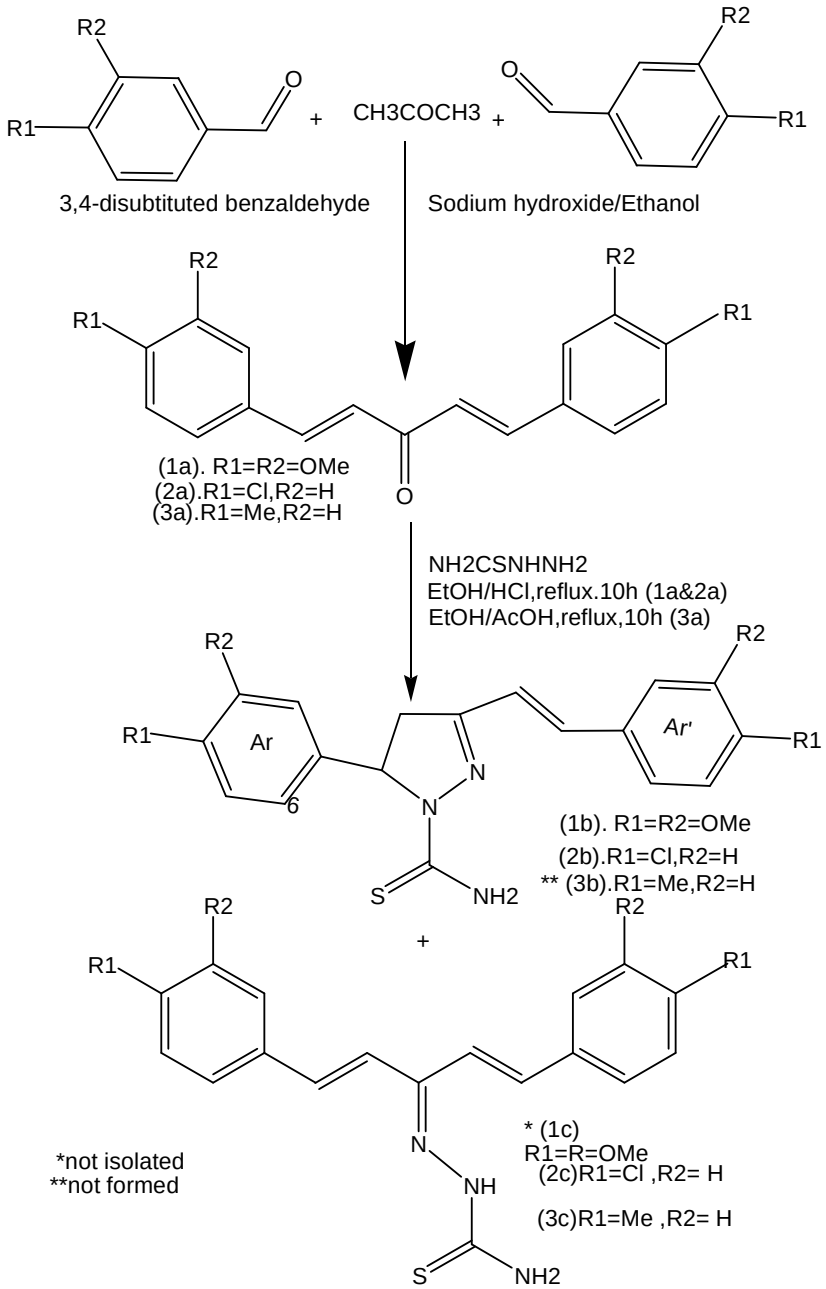
Table 5: Cytotoxic Activity Against 3-cell lines of Human Cancers

Test Compounds	Concentration	Lung (NCI-H460)	Breast(MCF-7)	CNS(SF-268)	Activity
1b	$1x10^{-4}$ M	87	84	91	Inactive
2b	$1x10^{-4}$ M	119	98	123	Inactive

Table 6: Antimicrobial Activity of the Compounds by Agar Well Diffusion Method

Compounds	Antimicrobial activity in terms of zone of inhibition in mm															
	E.C.			S.V.			S.E.			B.S.			C.A.			
	Effective conc.µg/well			Effective conc.µg/well			Effective conc.µg/well			Effective conc.µg/well			Effective conc.µg/well			
	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400	
1b	+++	+++	+++	++++	++++	++++	+	+	+	-	-	+	-	-	-	
2b	+++	+++	+++	++++	++++	++++	++	++	+++	++	+++	++++	+	+	++	
2c	+++	++++	++++	++	+++	+++	+	++	++	+	+++	+++	+	+	++	
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Zone of Inhibition of Standard Drugs																
Chloramphenicol	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++				
Fluconozole													++++	++++	++++	

Scheme 1



Scheme 1. Synthesis of (1b)&(2b):1a/2a,NH2CSNHNH2(molar ratio1:3) EtOH,Conc.HCl (few drop),refulx,10h

CONCLUSION

In conclusion, we synthesized new pyrazoline derivatives and examined their cytotoxicity against human cancer cell lines. It has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. However, antibacterial and antifungal activities is discussed with respect to standard drugs, chloramphenicol, fluconozole and found better than these in some cases.

Acknowledgements
We thank Dr. V.L. Narayanan of National Cancer Institute (NCI), Maryland, USA for anticancer screening and Dr. Athar Habib Siddiqui, Department of Pharmacological and Pharmaceutical Sciences University of Houston, Houston, Texas for antimicrobial evaluation. Authors are grateful to Prof. Guy Lemiere, Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaans, Belgium for recording NMR and Mass Spectra.

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