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Synthesis and cytotoxicity study of pyrazoline derivatives of methoxy substituted naphthyl chalcones

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Abstract 2-Acetyl naphthalene reacts with various methoxy substituted benzaldehyde in the presence of 10 % sodium hydroxide solution giving functionalized chalcones. The synthesized chalcones when further reacted with hydrazine hydrate in the presence of acetic acid afforded *N*-acetyl pyrazolines. All the synthesized products were confirmed by various spectral data such as FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS studies. All the synthesized compounds were screened for cytotoxicity against various cell lines.

Keywords Methoxy substituted chalcones · Pyrazolines and Cytotoxicity studies

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Introduction

The chalcones or enones possessing methoxy substitutents have demonstrated their potential as effective pharmaceutical agents and in many applications [1, 2], and it has been observed that methoxy groups present in the chalcones are good acceptors which facilitate hydrogen bonding, while the 2-naphthyl ring is electron rich and can form π-stacking which play vital roles in orientating the inhibitors within the active site, as well as significantly contributing to the biological activity observed for these inhibitors [3]. Similarly, naproxen and nabumetone are effective COX (cyclooxygenase) inhibitors, both the drugs possessing naphthyl and methoxy groups in the core structure. Based on the this importance, the present study envisioned the synthesis and pharmaceutical evaluation of methoxy and naphthyl ring incorporated chalcones. The enones or chalcones provide a bifunctional site for 1-3 dinucleophile affording the synthesis of various heterocycles like pyrazolines [2–6], isoxazole, thiazine and oxazine derivatives [7]. Pyrazolines are important nitrogen-containing heterocyclic compounds, and are reported to have anti-microbial [8–10], anti-inflammatory [11–13] anti-cancer [14–16], anti-oxidant [17], anti-viral [18], anti-depressant [19], anti-diabetic, anti-mycobacterial [20, 21], and anti-malarial properties [22, 23]. Pyrazolines are found in applications such as dyestuff, analytical reagents, and in the agricultural industry. Therefore, the pyrazoline moiety has a predominant role in medicinal chemistry since it is part of many scaffolds which show an impressive array of activity. Moreover, pyrazolines play an important role in heterocyclic chemistry and are used extensively in organic chemistry.

Pyrazoline derivatives exhibit growth inhibitory properties on several cancer cell lines [24] and also act as effective inhibitors of heptosyltransferase [25]. Some of the pyrazolines were effective in inhibiting the accumulation of prion protein [26], the abnormal protease-resistant form. When pyrazolones were part of a tricyclic system, it is expected to possess a cytotoxicity profile by inhibiting tubulin polymerization [27].

The colchicine binding site in tubulin is a better target for many small molecules like combretastatin A-4 [28]. Various structure activity studies proved that chalcones have better binding characteristics in the colchicine binding site in tubulin [29]. Since the tubulin binding activity is more predominant in chalcones than in cis configuration, it is ideal to lock the stereochemistry to cis orientation. This is achieved by fusing the carbon–carbon double bond in the bridge with five-membered or six-membered rings[30–40]. Pyrazolines should possess a twisted geometry for better activity and improved biological solubility [30].

The chemistry and cytotoxicity characteristics of various substituted pyrazolines have been investigated worldwide in recent years to synthesize novel pyrazolines from easily available starting materials and to evaluate their possible cytotoxicity profiles. Therefore, in the view of above facts, we synthesized some 3-(naphthalen-2-yl)-5-phenyl-1H-pyrazolines. All the derivatives were confirmed by spectral study and evaluated for their cytotoxicity profile against various cell lines.

Results and discussion

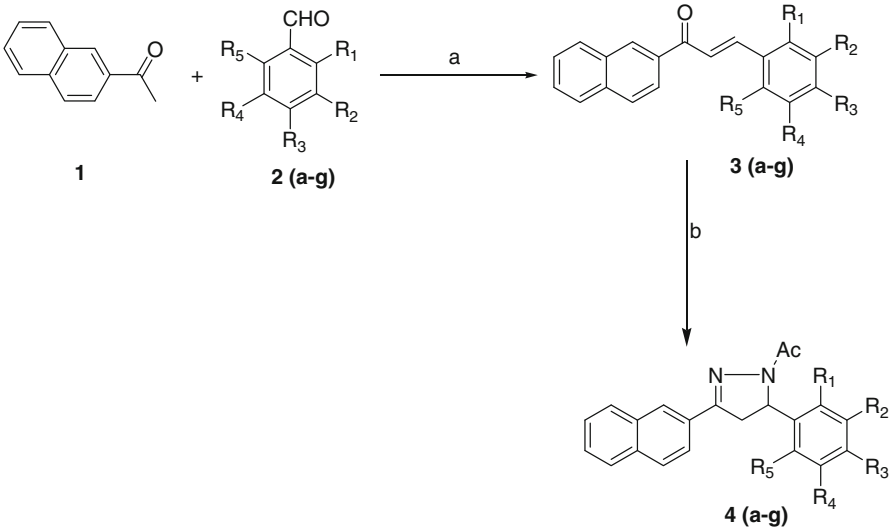
The synthetic route for the pyrazolines are shown in Scheme 1. Compound **1** is 2-acetyl naphthalene on reaction with various methoxy substituted benzaldehydes

2a–**g** in ethanol in the presence of 10 % sodium hydroxide solution for 3 h. Chalcones, 1-(naphthalen-2-yl)-3-phenylprop-2-en-1-one, **3a**–**g** were obtained and purified by recrystallisation from methanol. The acetylated pyrazolines, **4a**–**g**, were obtained from chalcones **3a**–**g** by refluxed with hydrazine hydrate in acetic acid for 3 h. The reaction was monitored by thin layer chromatography and melting points were determined by an open capillary tube method. All the synthesized scaffolds were characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic techniques (Table 1).

The *N*-acetyl pyrazoline, 1-acetyl-5-(3,5-dimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole (**5f**) carbonyl peak appears at 1,664 cm^{-1} in IR spectra. The two methoxy methyl groups appeared in downfield at δ 3.75 ppm and the *N*-acetyl methyl group appeared in at δ 2.48 ppm. The pyrazoline ring methyne (CH) split into quartet δ 5.54 ppm by adjacent methylene of the pyrazoline ring. The two methylene protons appeared at δ 3.25 and 3.82 ppm. The methoxy substituted three aromatic protons appeared as multiplet at δ 6.33–6.40 ppm and all the remaining aromatic protons appeared at δ 7.49–8.05 ppm. The HRMS of compound **5f** showed a molecular ion peak M+ in the positive mode. The molecular ion peak of **5f** was observed at *m*/*z* 374.7421.

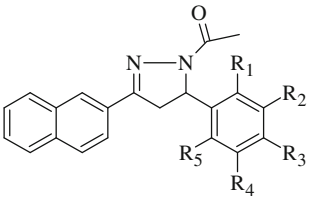
Biological activity

The cytotoxicity of the synthesized pyrazolines towards different cell lines, viz., HCT-15 (human colon carcinoma), HeLa (cervical carcinoma), and A549 (lung adenocarcinoma), were determined by MTT {[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]} assay. The formazan produced during the MTT assay formed by the viable cells was solubilized by addition of 100 mL isopropanol.



Scheme 1 Synthesis of substituted *N*-acetyl pyrazoles. Reagents and conditions: **a** NaOH, ethanol, 3–4 h. **b** $N_2H_4{\cdot}H_2O$, acetic acid, ethanol, reflux, 3 h

Table 1 Pyrazolines synthesized



Compound	R_1	R_2	R_3	R_4	R_5	Time (h)	Yield (%)
4a	OCH_3	H	H	OCH_3	H	3	82
4b	OCH_3	OCH_3	OCH_3	H	H	4	59
4c	H	OCH_3	OCH_3	OCH_3	H	3	81
4d	OCH_3	H	OCH_3	H	H	3.5	81
4e	OCH_3	H	OCH_3	OCH_3	H	4	58
4f	H	OCH_3	H	OCH_3	H	3	89
4g	OCH_3	H	OCH_3	H	OCH_3	3.75	79

The absorbance was recorded at 540 nm in a micro plate reader (Bio-tek, ELx 800). The % cytotoxicity was calculated using the formula

$$\%\ \text{cytotoxicity} = 100 \times \left(\frac{(\text{Abs}_{\text{test}} - \text{Abs}_{\text{Blank}}) - (\text{Abs}_{\text{control}} - \text{Abs}_{\text{Blank}})}{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{Blank}})} \right)$$

Table 2 % cytotoxicity of pyrazolines **4a**–**g** against various cell lines

Conc. tested (μg/mL)	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**
% cytotoxicity in HCT-15 (human colon carcinoma) cells							
12.5	63.98	56.28	64.97	54.53	46.92	53.62	0.64
25	70.60	59.56	99.92	70.83	84.16	67.40	0.69
50	69.84	72.51	101.07	87.43	96.50	72.89	20.79
100	78.45	85.22	100.76	93.53	98.86	74.41	29.40
% cytotoxicity in HeLa (cervical carcinoma) cells							
12.5	40.34	10.21	42.65	14.97	60.02	46.50	0
25	40.95	18.37	66.09	30.27	98.79	51.97	3.17
50	45.54	27.53	100.17	43.57	99.09	53.69	0.11
100	47.26	53.83	100.35	66.95	98.88	60.75	6.68
% cytotoxicity in A549 (lung adenocarcinoma) cells							
12.5	45.27	27.10	42.96	0.87	27.92	40.37	0
25	45.30	33.20	93.93	24.39	74.27	48.73	0
50	46.62	65.98	97.74	54.95	98.88	78.82	4.75
100	63.22	61.77	96.42	78.70	98.93	85.59	15.83

Table 3 IC_{50} values compounds against various cell lines

Compounds	HCT-15 (μM)	HeLa (μM)	A549 (μM)
4a	0.0213	<100.00	0.1736
4b	0.0272	0.2225	0.0865
4c	0.0197	0.0370	0.0346
4d	0.0320	0.2134	0.1200
4e	0.0309	0.0247	0.0432
4f	0.0320	0.0400	0.0667
4g	<100.00	<100.00	<100.00

All pyrazolines, **4a**–**g**, of different concentrations were tested for the cytotoxic potential against all three cell lines and the results are shown in Table 2. The minimum concentration required for 50 % cytotoxicity and inhibitory concentration required for 50 % cytotoxicity (IC_{50}) values are set out in Table 3.

Against HCT15, all the compounds showed significant activity and compounds **4c** and **4a** showed IC_{50} values of 0.0197 and 0.0213 μM, respectively. Compounds **4a**, **4d**, **4e** and **4f** also exhibited better activity. Compounds **4d** and **4a** showed significant cytotoxicity activity against A549 with IC_{50} values of 0.1250 and 0.1736 μM, respectively, and all other compounds except **4g** showed comparable inhibition of cell growth. Pyrazoline, **4e**, had a better IC_{50} value of 0.0247 μM against HeLa, and **4c** and **4f** showed significant effects with IC_{50} values of 0.0370 and 0.0400 μM, respectively. Compound **4g** did not show any activity against any of the cells employed for the study below 100 μg/mL.

Conclusion

In summary, we have described synthesis and biological evaluations of pyrazolines derived from methoxy substituted chalcones, which possess cytotoxicity against various cell lines for cervical cancer (HeLa), colon carcinoma (HCT15), and adenocarcinoma (A549). All the tested compounds showed potential activities against colon carcinoma (HCT15). Among them, compound **4c**, 1-acetyl-5-(2-naphthyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, was found to be most effective with IC_{50} values of 0.197, 0.0370, and 0.0346 μM against HCT15, A549, and HeLa, respectively.

Experimental section

Materials and methods

Melting points were recorded with an open capillary tube and corrected with benzoic acid. Infrared spectra were recorded on a Perkin-Elmer 1720 FT IR. ^1H NMR spectra were recorded on a Bruker AC 400 MHz, spectrometer using tetra methyl silane (TMS) as an internal standard in $CDCl_3$. The HRMS mass spectra were recorded on a JEOLSX 102/DA-6000 mass spectrometer.

Chromatographic separations were done using silica gel (60–120 mesh). Thin layer chromatography (TLC) was performed using glass plates coated with silica gel G, and the spots visualization were done by iodine vapor. Chemicals were purchased from Sigma-Aldrich, India and Sd-Fine chemicals India and were used without further purification. All the solvents were purchased from Sd-Fine chemicals, India, and used without further purification.

Synthesis of 1-acetyl-5-(2,5-dimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole

The synthetic pathway of acetylated pyrazolines from methoxy substituted chalcones is depicted in Scheme 1. The sodium hydroxide catalyzed Claisen–Schmidt condensation of equimolar mixture of 2-acetylnaphthalene, **1**, and various methoxy-substituted benzaldehydes, **2a**–**g** under stirring for 3 h in ethanol and kept in an ice bath overnight afforded the respective chalcones, **3a**–**g**, in excellent yields. The transformation was clean and efficient.

The reactions were carried out by dissolving the equimolar quantity of appropriate ketones and aldehydes in a minimum amount of ethanol and stirred in the presence of 10 % NaOH under suitable temperature for appropriate time periods. The reaction after completion (as monitored by thin layer chromatography, using EtOAc: Pet. Ether solvent system) was kept aside overnight at room temperature for the precipitation of crude chalcones, which were filtered off and recrystallized from methanol.

All the chalcones were treated with hydrazine hydrate in hot glacial acetic acid to afford 1-acetyl-3,5-diaryl-2-pyrazolines in good yields. It was done by refluxing the desired chalcones (5 mmol) with hydrazine hydrate (25 mmol) in hot acetic acid (30 mL) for 3 h, and the completion of reaction was confirmed by TLC using the mobile phase ethyl acetate and hexane in various proportions (1:9, 2:8, 3:7). After completion of the reaction, the reaction mixture was poured over crushed ice (250 mL) and the precipitate was collected by vacuum filtration. The product was washed with water, dried, and recrystallized from methanol.

*1-Acetyl-5-(2, 5-dimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (**4a**)*

Yellow solid, mp 113–115 °C; IR (KBr) 1,661 (CO), cm^{-1} ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.51 (s, 3H), 3.13–3.19 (d, $J = 4.6$ Hz, 1H), 3.71 (s, 3H), 3.79 (s, 3H), 5.85–5.89 (q, $J = 4.6$ Hz, 1H), 6.62–6.62 (d, $J = 2.9$ Hz, 1H), 6.71–6.74 (dd, $J = 5.8$ Hz, $J = 3.0$ Hz, 1H), 6.81–6.83 (d, $J = 9.2$ Hz, 1H), 7.49–7.52 (m, 2H), 7.48–7.8 (m, 3H), 7.906 (s, 1H), 8.04–8.07 (dd, $J = 7.0$ Hz, $J = 1.6$ Hz, 1H) HRMS [EI, M+] calcd for $C_{23}H_{22}N_2O_3$ *m/z* 374.4324, found 374.3861.

*1-Acetyl-5-(2,3,4-trimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (**4b**)*

Pale yellow, mp 136–138 °C; IR (KBr) 1,664 (CO), cm^{-1} ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (s, 3H), 3.21–3.23 (d, $J = 4.6$ Hz, 1H), 3.77 (s, 3H), 3.84 (s, 3H), 3.93 (s, 3H), 5.72–5.76 (q, $J = 4.8$ Hz, 1H), 6.58–6.60 (d, $J = 8.6$ Hz, 1H),

6.81–6.84 (d, $J = 8.8$ Hz, 1H), 7.50–7.53 (m, 2H), 7.82–7.87 (m, 3H), 7.93 (s, 1H), 8.07–8.09 (dd, $J = 6.8$ Hz, $J = 1.6$ Hz, 1H), HRMS [EI, M+] calcd for $C_{24}H_{21}N_2O_4$ *m/z* 404.4584, found 404.3401.

*1-Acetyl-5-(2-naphthyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole, (**4c**)*

Light yellow, mp 129–131 °C; IR (KBr) 1,655 (CO), cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 3.28–3.33 (q, $J = 4.8$ Hz, 1H), 3.80–3.89 (m, 9H), 4.00 (s, 1H), 5.55–5.59 (q, $J = 4.8$ Hz, 1H), 6.54 (s, 2H), 7.50–7.56 (m, 2H), 7.83–7.89 (m, 3H), 7.94 (s, 1H), 8.07–8.09 (dd, $J = 6.8$ Hz, $J = 1.6$ Hz, 1H), HRMS [EI, M+] calcd for $C_{24}H_{24}N_2O_4$ *m/z* 404.4584, found 404.0028.

*1-Acetyl-5-(2,4-dimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (**4d**)*

Yellow solid, mp 109–111 °C; IR (KBr) 1,663 (CO), cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H), 3.62–3.87 (m, 7H), 5.78–5.82 (q, $J = 4.5$ Hz, 1H), 6.39–6.42 (dd, $J = 6.0$ Hz, $J = 2.3$ Hz, 1H), 6.46–6.47 (d, $J = 2.2$ Hz, 1H), 6.96–6.98 (d, $J = 8.4$ Hz, '1H), 7.46–7.58 (m, 2H), 7.79–7.97 (m, 5H), 8.05–8.08 (d, $J = 7.0$ Hz, 1H), HRMS [EI, M+] calcd for $C_{23}H_{22}N_2O_3$ *m/z* 374.4324, found 374.7421.

*1-Acetyl-5-(2,4,5-trimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (**4e**)*

Light brown, mp 152–155 °C; IR (KBr) 1,660 (CO), cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 3.18–3.19 (q, $J = 4.8$ Hz, 1H), 3.76–4.00 (m, 10H), 5.77–5.81 (q, $J = 4.8$ Hz, 1H), 7.48–7.55 (m, 2H), 7.81–7.90 (m, 3H), 7.93 (s, '1H), 8.06–8.09 (dd, $J = 6.8$ Hz, $J = 1.6$ Hz, 1H), HRMS [EI, M+] calcd for $C_{24}H_{24}N_2O_4$ *m/z* 404.4584, found 404.2773.

*1-Acetyl-5-(3,5-dimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (**4f**)*

Off white, mp 113–115 °C; IR (KBr) 1,664 (CO), cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 3H), 3.25–3.31 (d, $J = 4.6$ Hz, 1H), 3.75–3.87 (m, 7H), 5.54–5.58 (q, $J = 4.6$ Hz, 1H), 6.33–6.40 (m, 3H), 7.49–7.55 (m, 2H), 7.82–7.87 (m, 3H), 7.91 (s, 1H), 8.05–8.07 (dd, $J = 7.1$ Hz, $J = 1.6$ Hz, 1H) MS [EI, M+] calcd for $C_{23}H_{22}N_2O_3$ *m/z* 374.4324, found 374.7421.

Determination of cytotoxicity

Cytotoxic effect of synthesized acetylated pyrazolines were assessed using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [31]. Exponentially growing cells were seeded in triplicate in 96-well plates at a concentration of 5 × 103 cells/well [100 μL of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % fetal bovine serum (FBS) as medium]. After 24 h, upon attachment of the cells, the medium was replaced with medium containing gradient concentrations of synthesized compounds. For this purpose, test compounds were dissolved in 0.1 % dimethyl sulfoxide (DMSO), and diluted with

the medium. The cells were then exposed to different concentrations of the pyrazolines ranging from 0.1 to 100 μg/mL and incubated for 72 h. Cells in the control wells received the same volume of medium containing 0.1 % DMSO. After 72 h, the medium was flicked off and cell cultures were incubated with 100 μL of MTT reagent (2 mg/mL) for 4 h at 37 °C.

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