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ORIGINAL ARTICLE

Synthesis and *in vitro* biological evaluation of new pyrazole chalcones and heterocyclic diamides as potential anticancer agents

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Abstract Synthesis and characterization of new heterocyclic pyrazole chalcones (**4a–e**) and diamide (**6a–e**) derivatives are described. Pyrazole chalcones were synthesized by the reaction of pyrazole aldehydes and suitable aromatic ketones. Diamides were synthesized by the reaction of phthalic acid and amines. Newly synthesized compounds were characterized by spectral studies and their biological activity was assessed *in vitro* using MCF-7 (human breast adenocarcinoma) and HeLa (human cervical tumor cells) cell lines. Few of the synthesized molecules inhibited the growth of the human breast cancer cell lines and human cervical tumor cell lines at low micromolar to nanomolar concentrations.

1. Introduction

Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells, is a major worldwide health problem. It is a fatal disease standing next to the cardiovascular diseases in terms of morbidity and mortality. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future (Gibbs, 2000; Varmus, 2006). Currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs. Among the currently identified antitumor agents, chalcones represent an important class of molecules that are abundant in edible plants (Modzelewska et al., 2006). Chalcones comprise a class of compounds with important therapeutic potential. The ease of preparation, the potential of oral administration, (Wattenberg

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et al., 1994; Wattenberg, 1995; Baba et al., 2002) and safety (Phillpotts et al., 1984) also support the feasibility of chalcone-based compounds as therapeutic agents. Chalcone and its functionalized derivatives display diverse medicinal properties including anti-inflammatory (Hsie et al., 1998), immunomodulatory (Barfod et al., 2002), anticancer (Kumar et al., 2003), anti-HIV (Artico et al., 1998), antiproliferative (Liu and Go, 2007), and a-glucosidase inhibitory activities (Seo et al., 2005).

A new class of anthranilic diamides has been found to exhibit their action by binding to ryanodine receptors and activating the uncontrolled release of calcium stores (Lahm et al., 2004; Caspar et al., 2004)). Also many of the heterocyclic diamides act as CFTR (Cystic fibrosis transmembrane conductance regulator) modulators (Hirth et al., 2005). Several research groups have been interested in designing various groups of dimeric agents of diverse chemical structure and biological properties, such as echinomycin antibiotics (Tseng et al., 2005; Wakelin and Waring, 1976; Low et al., 1984), 7-H pyridocarbazole derivatives (Pelaprat et al.,1980; Peek et al., 1995), bisanthracyclines (Wakelin, 1986; Chaires et al., 1997), bisnaphthalimides (Bousquet et al., 1995), bisacridines (Kirshenbaum et al., 1994; Moloney et al., 2001), and bisimidazoacridones (Gamage et al., 1999; Kosakowska-Cholody et al., 2005). Many of these turned out to be potent anticancer drugs such as Elinafide (LU79553), bisnaphthalimide that progressed to clinical trials against solid tumors (Hariprakasha et al., 2007).

These findings have motivated us to synthesize biologically active heterocycles, particularly for anticancer class. In our search for new therapeutic agents (Vijesh et al., 2010, 2011; Sunil et al., 2010; Isloor et al., 2012), we have synthesized chalcones, dimeric phthalimide derivatives and performed a first evaluation of their anticancer property. Few molecules have shown significant activity as compared to standard.

2. Experimental

2.1. Materials and methods

Melting points were recorded (uncorrected) on a Buchi Melting Point B-545 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400-MHz and Bruker spectrometers, respectively. IR spectra (in KBr pellets) were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. Elemental analyses were performed on a Thermo Finnigan FLASH EA 1112 CHN analyzer. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F254, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). All the compounds (**4a**–**e**) and (**6a**–**e**) were synthesized in-house from the corresponding commercially available acetophenones, amines and acids.

2.2. General procedure for synthesis of chalcone derivatives *4(a–e)*

3-Substituted-1H-pyrazole-4-carbaldehydes (**3**) were synthesized by the Vilsmayer Haack reaction of semicarbazones as described in the procedure (Vijesh et al, 2011). To a well stirred solution of aldehydes (1 mol), acetophenones (1 mol) in 50% ethanol-water (10 Vol) was added sodium hydroxide (2 mol) at 10 °C and the reaction mixture was stirred at room temperature for 15 hs. After completion of the reaction (confirmed by TLC), the reaction mixture was cooled to 0 °C, diluted with water and the solid separated was filtered, dried to get pure chalcone derivatives **4**(**a**–**n**). Synthesized compounds were recrystallized using ethanol as a solvent.

2.2.1. *(E)-3-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1-(pyridin-2-yl) prop-2-en-1-one (**4a**)*

(TLC, Pet-ether/EtOAc, 1:1, R_f = 0.1), Yield: 62%; Yellow solid; m.p. 157–159 °C. IR (KBr) cm^{-1}: 3320 (NH), 3041 (CH), 1662 (C═N and C═O). ^{1}H NMR (400 MHz, δH, $CDCl_3$): 8.68 (d, 1H, Pyridine-H), 8.2 (s, 1H, Pyrazole-H), 7.72–7.93 (m, 2H, Ar-H), 7.6 (m, 1H, Ar-H), 7.33–7.45 (m, 2H, Ar-H), 7.21 (m, 2H Ar-H), 6.57 (d, 1H, –CO–CH═CH), 5.8 (d, 1H, CH═CH) ppm. MS: *m*/*z* = 294.1 (M + 1). Anal. Calcd for $C_{17}H_{12}FN_3O$: C, 69.62; H, 4.12; N, 14.33. Found: C, 69.65; H, 4.22; N, 14.29.

2.2.2. *(E)-3-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1-(quinolin-2-yl)prop-2-en-1-one (**4b**)*

(TLC, Pet-ether/EtOAc, 8:2, R_f = 0.1), Yield: 74%; Yellow solid; m.p. 168-170 °C. IR (KBr) cm^{-1}: 3318 (NH), 3045 (CH), 1661 (C═N and C═O). ^{1}H NMR (400 MHz, δ H, $CDCl_3$): 8.56 (d, 1H, quinoline-H), 8.2 (m, 4H, Pyrazole-H, Ar-H), 7.70–7.74 (dd, 2H, Ar-H), 7.4 (d, 2H, Ar-H), 7.20–7.33 (m, 2H Ar-H), 6.59 (d, 1H, CH═CH), 5.9 (d, 1H, CH═CH) ppm. MS: *m*/*z* = 344.3 (M + 1). Anal. Calcd for $C_{21}H_{14}FN_3O$: C, 73.46; H, 4.11; N, 12.24. Found: C, 73.40; H, 4.15; N, 12.18.

2.2.3. *(E)-3-(3-(4-fluorophenyl)-1H-pyrazol-4-yl)-1-(5-fluoropyridin-2-yl)prop-2-en-1-one (**4c**)*

(TLC, Pet-ether/EtOAc, 1:1, R_f = 0.2), Yield: 69%; Brown solid; m.p. 150–152 °C. IR (KBr) cm^{-1}: 3324 (NH), 3089 (CH), 1661 (C═N and C═O). ^{1}H NMR (400 MHz, δH, $CDCl_3$): 8.78 (d, 1H, Pyridine-H), 8.09 (s, 1H, Pyrazole-H), 7.82–7.93 (m, 2H, Pyridine-H), 7.44 (m, 2H, Ar-H), 7.23–7.28 (m, 2H, Ar-H), 7.21 (d, 1H, Ar-H) 6.50 (d, 1H, CH═CH), 5.8 (d, 1H, CH═CH) ppm. MS: *m*/*z* = 312.2 (M + 1). Anal. Calcd for $C_{17}H_{11}F_2N_3O$: C, 65.59; H, 3.56; N, 13.50. Found: C, 65.57; H, 3.51; N, 13.45.

2.2.4. *(E)-1-(pyridin-2-yl)-3-(3-(4-(trifluoromethyl)phenyl)-1H-pyrazol-4-yl)prop-2-en-1-one (**4d**)*

(TLC, Pet-ether/EtOAc, 1:1, R_f = 0.2), Yield: 70%; Yellow solid; m.p. 149–151 °C. IR (KBr) cm^{-1}: 3330 (NH), 3040 (CH), 1660 (C═N and C═O). ^{1}H NMR (400 MHz, δH, $CDCl_3$): 8.68 (d, 1H, Pyridine-H), 8.2 (s, 1H, Pyrazole-H), 7.70–7.83 (m, 2H, Pyridine-H), 7.6 (m, 3H, Ar-H), 7.33 (m, 2H, Ar-H), 6.50 (d, 1H, CH═CH), 5.7 (d, 1H, CH═CH) ppm. MS: *m*/*z* = 344.3 (M + 1). Anal. Calcd for $C_{18}H_{12}F_3N_3O$: C, 62.97; H, 3.52; N, 12.24. Found: C, 62.90; H, 3.48; N, 12.04.

2.2.5. *(E)-1-(thiophen-2-yl)-3-(3-(4-(trifluoromethyl)phenyl)-1H-pyrazol-4-yl)prop-2-en-1-one (**4e**)*

TLC, Pet-ether/EtOAc, 1:1, R_f = 0.3), Yield: 76%; Yellow solid; m.p. 135–138 °C. IR (KBr) cm^{-1}: 3328 (NH), 3040 (CH), 1661 (C═N and C═O). ^{1}H NMR (400 MHz, δH,

$CDCl_3$): 8.10 (s, 1H, Pyrazole-H), 7.70–7.83 (m, 2H, Ar-H), 7.50–7.58 (m, 2H, Ar-H), 7.33 (m, 1H, Thiophene-H), 7.2 (dd, 2H, Thiophene-H), 6.45 (d, 1H, CH═CH)), 5.3 (d, 1H, CH═CH) ppm. MS: m/z = 349.0 (M + 1). Anal. Calcd for $C_{17}H_{11}F_3N_2OS$: C, 58.62; H, 3.18; N, 8.04. Found: C, 58.60; H, 3.16; N, 8.08.

*2.2.6. (E)-1-(thiazol-5-yl)-3-(3-(4-(trifluoromethyl)phenyl)-1H-pyrazol-4-yl)prop-2-en-1-one (**4f**)*

TLC, Pet-ether/EtOAc, 1:1, R_f = 0.2), Yield: 76%; Yellow solid; m.p. 135–138 °C. IR (KBr) cm^{-1}: 3324 (NH), 3030 (CH), 1660 (C═N and C═O). ^1H NMR (400 MHz, δH, $CDCl_3$): 8.9 (s, 1H, Thiazole-H), 8.6 (s, 1H, Thiazole-H), 8.10 (s, 1H, Pyrazole-H), 7.70–7.83 (m, 2H, Ar-H), 7.50–7.58 (m, 2H, Ar-H),), 6.45 (d, 1H, CH═CH), 5.4 (d, 1H, CH═CH) ppm. MS: m/z = 350.3 (M + 1). Anal. Calcd for $C_{16}H_{10}F_3N_3OS$: C, 55.01; H, 2.89; N, 12.03. Found: C, 55.11; H, 2.93; N, 12.05.

*2.3. General procedure for synthesis of phthalic acid diamides **6(a–e)***

To a stirred solution of phthalic acid (1 eq) in DMF (10 Vol) were added EDCI (2.4 eq), HOBt (0.8 eq), DIEA (Diisopropylethylamine) (4 eq) and corresponding amines (2 eq) at 0 °C. The reaction mixture was stirred at RT for 18 h. After completion of the reaction (confirmed by TLC), the reaction mixture was poured into ice cold water, and extracted with ethyl acetate. The combined organic layer was dried over sodium sulfate and concentrated using rota evaporator. Crude residue was purified using column chromatography using ethyl acetate as an eluent to get pure diamides **6(a–e)**.

*2.3.1. N-[2-(4-Methoxy-phenyl)-thiazol-5-yl]-N′-[2-(4-methoxy-phenyl)-thiazol-4-yl]-phthalamide (**6a**)*

(TLC, Methanol/DCM, 2:8, R_f = 0.5), Yield: 90%; Off white solid; m.p. 215–218 °C. IR (KBr) cm^{-1}: 3330 (NH), 3036 (CH), 1660 (C═O). ^1H NMR (400 MHz, δH, $CDCl_3$): 8.05 (m,1H, Ar-H), 7.81–7.83 (m, 4H, Ar-H), 7.54–7.69 (m, 5H, Ar-H), 7.28–7.35 (m, 4H, Ar-H, Thioazole-H), 3.89 (s, 6H, -OMe)ppm. MS: m/z = 543.6. (M + 1). Anal. Calcd for $C_{28}H_{22}N_4O_4S_2$: C, 61.98; H, 4.09; N, 10.33. Found: C, 61.78; H, 4.12; N, 10.37.

*2.3.2. N, N′-Bis-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenyl]-phthalamide (**6b**)*

TLC, Methanol/DCM, 2:8, R_f = 0.2), Yield: 88%; Off white solid; m.p. 235–238 °C. IR (KBr) cm^{-1}: 3330 (NH), 3036 (CH), 1660 (C═O). ^1H NMR (400 MHz, δH, $CDCl_3$):7.65 (m, 4H, Ar-H), 7.40–7.48 (m, 2H, Ar-H), 6.8 (s, 2H, Pyrimidine-H), 6.51–6.57 (m, 4H, Ar-H), 2.25 (s, 12H, 4-Me) ppm. MS: m/z = 687.6. (M + 1) Anal. Calcd for $C_{32}H_{30}N_8O_6S_2$: C, 55.96; H, 4.40; N, 16.32. Found: C, 55.92; H, 4.39; N, 16.27.

*2.3.3. N,N′-Bis-(2′-methoxy-biphenyl-2-ylmethyl)-phthalamide (**6c**)*

TLC, Methanol/DCM, 2:8, R_f = 0.6), Yield: 76%; Off white solid; m.p. 185–187 °C. IR (KBr) cm^{-1}: 3333 (NH), 3032 (CH), 1661 (C═O). ^1H NMR (400 MHz, δH, $CDCl_3$): 8.30 (m, 1H, Ar-H), 7.7–7.89 (m, 5H, Ar-H), 7.47–7.55 (m, 4H, Ar-H), 7.35–7.40 (m, 4H, Ar-H), 7.22–7.26 (m, 4H, Ar-H), 7.036 (m, 2H, Ar-H), 4.1 (s, 6H, -OMe), 3.65 (s, 4H, $2CH_2$). MS: m/z = 557.2. (M + 1). Anal. Calcd for $C_{36}H_{32}N_2O_4$: C, 77.68; H, 5.79; N, 5.03. Found: C, 77.78; H, 5.70; N, 5.13.

*2.3.4. N,N′-Bis-[4-(5-methyl-[1,2,4]oxadiazol-3-yl)-phenyl]-phthalamide (**6d**)*

TLC, Methanol/DCM, 2:8, R_f = 0.4), Yield: 74%; Off white solid; m.p. 182–184 °C. IR (KBr) cm^{-1}: 3329 (NH), 3031 (CH), 1659 (C═O). ^1H NMR (400 MHz, δH, $CDCl_3$): 7.87 (dd, 2H, Ar-H), 7.77–7.79 (m, 4H, Ar-H), 7.67–7.70 (m, 6H, Ar-H), 2.51 (s, 6H, -Me) ppm. MS: m/z = 481.6.(M + 1). Anal. Calcd for $C_{26}H_{20}N_6O_4$: C, 64.99; H, 4.20; N, 17.49. Found: C, 64.98; H, 4.19; N, 17.40.

*2.3.5. N,N′-Bis-(5-cyclopropyl-2-phenyl-2H-pyrazol-3-yl)-phthalamide (**6e**)*

(TLC, Methanol/DCM, 2:8, R_f = 0.2), Yield: 70%; Off white solid; m.p. 202–205 °C. IR (KBr) cm^{-1}: 3336 (NH), 3030 (CH), 1664 (C═O). ^1H NMR (400 MHz, δH, $CDCl_3$): 7.83 (dd, 2H, Ar-H), 7.74 (d, 2H, Ar-H) 7.65–7.69 (m, 4H, Ar-H), 7.38–7.50 (m, 4H, Ar-H), 7.22 (d, Ar-H), 5.81 (s, 2H, Ar-H), 1.4 (m, 2H, Cyclopropyl-H), 0.621 (m, 4H, Cyclopropyl-H), 0.37 (m, 4H, Cyclopropyl-H). MS: m/z = 529.3 (M + 1). Anal. Calcd for $C_{32}H_{28}N_6O_2$: C, 72.71; H, 5.34; N, 15.90. Found: C, 72.78; H, 5.35; N, 15.88.

3. Results and discussion

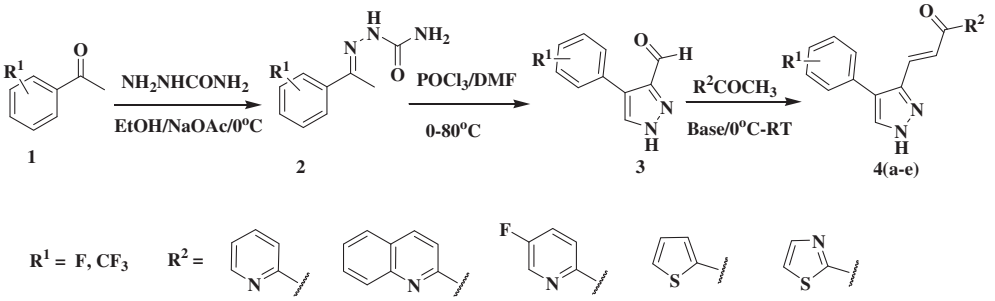
3.1. Chemistry

The synthetic strategy for the preparation of the chalcones is depicted in Scheme 1. Using published methods (Brana et al., 2003; Baraldi et al., 1997), the pyrazole aldehyde **3** was prepared from corresponding acetophenones and semicarbazides and cyclizing the semicarbazone intermediate using phosphorous oxychloride and DMF. A series of chalcone derivatives **4(a–f)** were synthesized in reasonable yields by the condensation of appropriate acetophenones (Baraldi et al., 1997). Diamides were synthesized using phthalic acid and amines (Scheme 2) using EDCI (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and HOBt (Hydroxybenzotriazole) as coupling reagents in 70–90% yield. Formation of both chalcones and diamides was confirmed by ^1H NMR, ^{13}C NMR and mass spectral studies. Spectral data of all the compounds and C, H, N analyses are given in the experimental part. All the newly synthesized compounds were screened for their cytotoxic effect on MCF-7 and HeLa cell lines using MTT assay (Manjula et al., 2010). Among the screened samples, **4c** and **6c** have shown activity with values which are comparable to the standard drug doxorubicin. Remaining compounds did not show any activity, the results are depicted in Table 1.

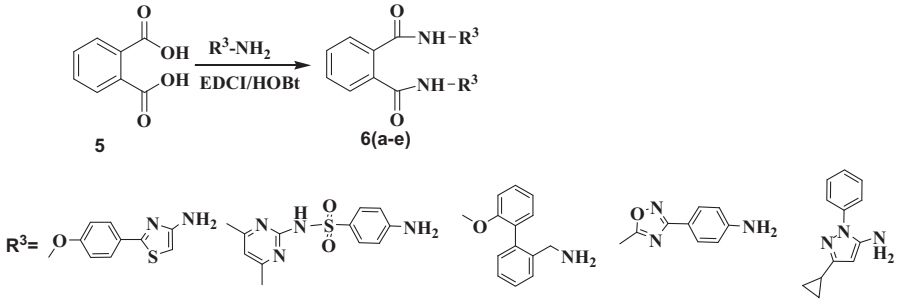
3.2. Anticancer studies

3.2.1. Cell lines

MCF-7 (human breast adenocarcinoma) and HeLa (human cervical tumor cells) cells procured from the National Center for Cell Science, Pune, India were sub-cultured every two to three days and maintained in 25 cm^2 tissue culture flasks (Tarsons Products, Kolkata, India) containing MEM medium supplemented with



Scheme 1 Synthetic route for new pyrazole chalcone derivatives **4(a–c)**.



Scheme 2 Synthetic route for new phthalic acid diamide derivatives **6(a–e)**.

Table 1 IC_{50} value after 24 h drug incubation with HeLa and MCF-7 cell lines by MTT assay.

Drug	IC_{50} (μg/ml)	
	HeLa	MCF-7
4a	26.41 ± 3.54	32.91 ± 2.55
4b	192.86 ± 16.63	178.90 ± 10.84
4c	0.018 ± 0.003	0.047 ± 0.019
4d	40.40 ± 5.60	50.69 ± 6.63
4e	266.78 ± 21.26	219.38 ± 18.80
4f	10.59 ± 3.12	15.72 ± 4.89
6a	81.46 ± 4.47	106.50 ± 9.97
6b	1086.03 ± 32.8	1287.95 ± 54.68
6c	1.41 ± 2.08	4.37 ± 1.71
6d	>1000	>1000
6e	>1000	>1000

The IC_{50} for standard doxorubicin in HeLa and MCF-7 cells was 1.15 ± 0.07 and 1.84 ± 0.05 μg/ml, respectively. All values are expressed as mean ± SEM (n = 3).

10% fetal bovine serum (FBS) and 50 μg/mL gentamicin sulfate at 37 °C in a CO_2 incubator (NuAire, Plymouth, USA) in an atmosphere of humidified 5% CO_2 in 95% air.

3.3. Procedure

The cytotoxic effect of drugs on cancer cells (MCF-7 and HeLa) was assessed by MTT assay (Manjula et al., 2010). In brief, exponentially growing cells (1×10^4 cells/well) were plated in 96-well plates and allowed to adhere for 24 h prior to extract addition. The drugs were dissolved in 0.1% DMSO and then diluted with the medium. The cells were then exposed to different concentrations of the drug (5–200 μg/mL) for 24 h. The cells in the control wells received a medium containing the same volume of DMSO (0.1%). After the incubation, 100 μL of MTT reagent (1 mg/mL in PBS) was added and cells were incubated for an additional 4 h. The formazan produced by the viable cells was solubilized by the addition of 100 μL DMSO. The suspension was placed on a micro-vibrator for 5 min and absorbance was recorded at 540 nm by the plate reader (ELx800, BioTek, VT, USA). The experiment was performed in triplicate. Doxorubicin was used as positive control. The percentage of growth inhibition was calculated with respect to vehicle control using the formula:

$$\%\ \text{Inhibition} = [\{(\text{Control absorbance} - \text{Blank absorbance}) - (\text{Test absorbance} - \text{Blank absorbance})\}\ (\text{Control absorbance} - \text{Blank absorbance})] \times 100$$

4. Conclusions

In this study we have synthesized a new series of pyrazole chalcones, which were characterized by spectral studies and all the synthesized compounds were screened for the *in vitro* anticancer activities, using MCF-7 (human breast adenocarcinoma) and HeLa (human cervical tumor cells) cell lines. Compound **4c** showed the highest inhibition in human MCF-7 and HeLa cell lines, which has 4-fluoro-phenyl and 5-fluoro-pyridin moieties, which has accounted for the highest activity. This compound has the potential to develop as a new class of anticancer agents.

In the second series, new diamides were synthesized and characterized by spectral studies. These compounds were tested for their ability to inhibit the growth in human tumor

cell lines. This series of compounds revealed potent and selective cytotoxicity against human MCF-7 cells and HeLa. The most potent compound **6c**, which has the substituted biphenyl ring has accounted for the increased activity.

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