Synthesis and *In Vitro* Biological Evaluation of Novel Pyrazole Derivatives as Potential Antitumor Agents

Michael S. Christodoulou,[a,b] Nikolas Fokialakis,[c] Sangkil Nam,[d] Richard Jove,[d] Alexios-Leandros Skaltsounis[c] and Serkos A. Haroutounian[a,*]

[a]*Chemistry Laboratory, Agricultural University of Athens; Iera odos 75, Athens 11855, Greece;* [b]*Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Via Venezian 21, 20133 Milano – Italy;* [c]*Division of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, University of Athens; Panepistimiopolis, Zografou 15771, Athens, Greece; and* [d]*Department of Molecular Medicine, Beckman Research Institute of City of Hope; 1500 East Duarte Road, Duarte, CA 91010, USA*

Abstract: The synthesis of twenty seven novel pyrazole derivatives bearing aryl substituted groups at positions 1 and 3 of the pyrazole structural motif and various functional groups at position 4 is presented. The critical step for their synthesis is the TCT/DMF promoted cyclization of the corresponding hydrazine precursors, which provided the desired pyrazole skeleton. The anticancer properties of the novel pyrazole derivatives were evaluated *in vitro* against human prostate (DU145), melanoma (A2058) and breast cancer (MCF-7) cell lines. Among the compounds tested, pyrazole **5a** and its methoxy derivatives **3d,e** were assayed as the most potent, displaying selective activity against the MCF-7 cell line with IC_{50} values of 14, 10 and 12 μM respectively. Results herein indicate that the reported backbone represents a promising structural lead for further development as antitumor agents.

Keywords: Pyrazole, Antitumor activity, Breast cancer, Melanoma, Prostate cancer.

1. INTRODUCTION

Cancer constitutes one of the most formidable afflictions of the world. Despite significant advances, which have resulted in notable cure rates for various malignancies, cancer is one of the leading causes of death worldwide [1,2]. Since the inhibition of proliferative pathways is considered as an effective strategy to fight cancer, much attention has recently been devoted to the discovery and development of new, more selective anticancer agents [3,4]. In this respect, the incorporation of heterocyclic residues into perspective pharmaceutical lead candidates constitutes an important strategy which provides activity and safety features. Thus, a broad variety of azaheterocycles compounds have been considered as potent antitumor agents, while pyrazoles belong among the most representative five-membered heterocyclic systems [5,6]. Particularly, over the past two decades, pyrazole-containing compounds have received considerable attention, owing to their diverse chemotherapeutic potentials, including versatile antineoplastic activities. Literature survey revealed that various pyrazoles have been identified as potent antimicrobial [7,8], antiviral [9,10] and anticancer agents [11-13], in addition to their capability to exert remarkable anticancer effects through the inhibition of different types of enzymes that play important roles in cell division [14-16]. Extensive studies have been devoted to arylpyrazole derivatives such as Celecoxib, a well-know cyclooxygenase-2 inhibitor [17,18], which has recently been shown to display antitumor activity against human breast [19], lung [20] and prostate cancer [21].

Intrigued by our interest to contribute to these studies we envisioned the synthesis of novel pyrazole derivatives bearing substituted aryl groups at the positions 1 and 3 of the pyrazole ring and a variety of functional groups on C-4. The critical step for their synthesis is the 2,4,6-trichloro[1,3,5] triazine (TCT)/DMF [22] promoted cyclization of the hydrazine substrates which provided the desired pyrazole derivatives backbone. The *in vitro* anticancer activities of these compounds were evaluated against three characteristic human cancer cell lines, namely DU145 prostate cancer, A2058 melanoma cancer and MCF-7 breast cancer cell lines.

2. EXPERIMENTAL

2.1. General Methods

All reactions were carried out in oven-dried glassware under argon atmosphere. All starting materials were purchased from Aldrich, while the solvents used were purified by distillation prior to use. Solvent mixtures employed in chromatography have been reported as volume to volume ratios. Thin layer chromatography (TLC) was performed on Merck pre-coated aluminium sheets of silica gel 60 F_{254}, while the products visualization was accomplished by UV absorbance at 254 nm and/or spraying an alcoholic solution of anisaldehyde and heating. Flash column chromatography was performed on silica gel (35-70 μm) purchased from

*Address correspondence to this author at the Department of Sciences, Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece; Tel: +30 2105294247; Fax: +30 2105294265; E-mail: sehar@aua.gr

1875-6638/12 $58.00+.00

SDS. Melting points were determined on a Stuart apparatus (SMP3) and are uncorrected. IR spectra were recorded on a Thermo electron corporation Nicolet 6700 FT-IR spectrometer in dichloromethane in ZnSe round windows. ^{1}H NMR and ^{13}C NMR spectra were recorded in the indicated solvents on Bruker DRX-400 and DRX-200 spectrometers at 400 and 50 MHz respectively. Chemical shifts (δ) for proton and carbon resonances are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard. MS spectra were recorded using the electrospray ionisation (ESI) technique on a Thermo Accela LC TSQ Quantum Access MS-MS spectrometer. The syntheses of compounds **1a-e, 2a-e, 3a-e, 4b-e, 5a-e** were performed in accordance with previously reported procedures [23].

2.2. Synthesis of 1-phenyl-2-(1''-phenylethylidene) Hydrazine (1f)

To a stirred solution of acetophenone (2.4 mL, 20 mmol) in 35 mL of ethanol and acetic acid (1.5 mL), was added a solution of phenylhydrazine hydrochloride (3.5 g, 24 mmol) and trimethylamine (4.2 mL, 30 mmol) in 40 mL of ethanol and stirred overnight at room temperature. The resulting precipitate was filtered and washed with ethanol and diethylether to provide the hydrazine product **1f** as a white solid (95% yield). IR (film) ν: 3349 cm^{-1}; ^{1}H NMR (DMSO): δ = 9.30 (1H, s, N*H*), 7.81 (2H, d, *J* 5.6 Hz, H-2''' and H-6'''), 7.40-4.26 (7H, m, H-2', H-3', H-3''', H-4''' and H-5'''), 6.78 (1H, m, H-4'), 2.28 (3H, s, H-2''); ^{13}C NMR (DMSO): δ = 146.5 (C-1''), 141.0 (C-1'), 139.9 (C-1'''), 129.3 (C-3''' and C-5'''), 128.7 (C-3'), 127.9 (C-4'''), 125.6 (C-2''' and C-6'''), 119.3 (C-4'), 113.3 (C-2'), 13.26 (C-2''); MS: 211.20 (M+H$^+$). Anal. Calcd for $C_{14}H_{14}N_2$: C, 79.97; H, 6.71; N, 13.32; Found: C, 79.84; H, 6.57; N 13.47.

2.3. Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (2f)

2,4,6-Trichloro-[1,3,5]-triazine, TCT (11.2 g, 60 mmol) was added to dimethylformamide (8.30 mL) and stirred at room temperature to produce a white solid. After the consumption of TCT (20 min, revealed by TLC), 2.63 g of hydrazine **1f** (12.5 mmol) in DMF (32 mL) were added and the stirring was continued for 2h. The reaction mixture was cooled to 0 °C and quenched with water. The organic phase was extracted twice with EtOAc and the combined organic extracts were washed with saturated Na_2CO_3, brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc, 7:3) yielded compound **2f** as a white solid (85% yield). mp 146-147 °C; IR (film) ν: 1672 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 10.1 (1H, s, C*H*O), 8.57 (1H, s, H-5), 7.85 (2H, d, *J* 8.0 Hz, H-2'), 7.82 (2H, d, *J* 10.4 Hz, H-2'' and H-6''), 7.56-7.52 (5H, m, H-3', H-4', H-3'', and H-5''), 7.42 (1H, t, *J* 7.2 Hz, H-4''); ^{13}C NMR ($CDCl_3$): δ = 185.2 (*C*HO), 154.8 (C-3), 139.0 (C-1'), 131.3 (C-1''), 131.0 (C-5), 129.7 (C-3'), 129.3 (C-4''), 129.0 (C-3'' and C-5''), 128.8 (C-2'' and C-6''), 128.0 (C-4'), 122.5 (C-4), 120.0 (C-2'); MS: 249.18 (M+H$^+$). Anal. Calcd for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28; Found: C, 77.29; H, 4.68; N 11.42.

2.4. Synthesis of Esters 3f-h

2.4.1. (E)-ethyl 3-(1',3'-diphenyl)-1'H-pyrazol-4'-yl) Acrylate (3f)

To a stirred solution of pyrazolocarboxaldehyde **2f** (1.07 g, 4.3 mmol) in 14 mL of acetonitrile, $Ph_3PCHCO_2CH_2CH_3$ (1.6 g, 4.7 mmol) was added and the mixture was refluxed overnight. The reaction mixture was quenched with 60 mL ethyl acetate, washed with H_2O, brine, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (hexane/EtOAc, 8:2) to afford the ester **3f** as a white solid (80% yield). mp 133-134 °C; IR (film) ν: 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 8.26 (1H, s, H-5'), 7.79 (2H, d, *J* 7.2 Hz, H-2''' and H-6'''), 7.76 (1H, d, *J* 16 Hz, H-3), 7.70 (2H, d, *J* 7.2 Hz, H-2''), 7.53-7.44 (5H, m, H-3'', H-4'', H-3''' and H-5'''), 7.36 (1H, t, *J* 6.8 Hz, H-4'''), 6.30 (1H, d, *J* 16 Hz, H-2), 4.26 (2H, q, *J* 6.8 Hz, OC*H*$_2$CH$_3$), 1.34 (3H, t, *J* 6.8 Hz, OCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$): 167.0 (C-1), 153.3 (C-3'), 139.5 (C-1''), 135.1 (C-3), 132.6 (C-4'), 129.5 (C-3'' and C-1'''), 128.8 (C-3''' and C-5'''), 128.7 (C-2''' and C-6'''), 128.6 (C-4'''), 127.2 (C-4''), 126.4 (C-5'), 119.3 (C-2''), 117.6 (C-2), 60.38 (O*C*H$_2$CH$_3$), 14.36 (OCH$_2$*C*H$_3$); MS: 319.33 (M+H$^+$). Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80; Found: C, 75.63; H, 5.59; N 8.97.

2.4.2. (E)-methyl 3-(1'-(4''-methoxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl) Acrylate (3g)

To a stirred solution of pyrazolocarboxaldehyde **2a** (0.42 g, 1.5 mmol) in 5.0 mL of acetonitrile, $Ph_3PCHCO_2CH_3$ (0.60 g, 1.8 mmol) was added and the mixture was refluxed overnight. The reaction mixture was quenched with 40 mL of ethyl acetate, washed with H_2O, brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc, 8:2) provided compound **3g** as a white solid (80% yield). mp 149-150 °C; IR (film)ν: 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 8.16 (1H, s, H-5'), 7.75 (1H, d, *J* 16 Hz, H-3), 7.68 (4H, d, *J* 8.8 Hz, H-2'', H-2''' and H-6'''), 7.51 (2H, t, *J* 7.2 Hz, H-3''' and H-5'''), 7.45 (1H, t, *J* 7.2 Hz, H-4'''), 7.01 (2H, d, *J* 8.8 Hz, H-3''), 6.28 (1H, d, *J* 16 Hz, H-2), 3.88 (3H, s, C4''-OC*H*$_3$), 3.80 (3H, s, OC*H*$_3$); ^{13}C NMR ($CDCl_3$): 167.5 (C-1), 158.8 (C-4''), 153.0 (C-3'), 135.5 (C-3), 133.2 (C-1''), 132.3 (C-4'), 128.8 (C-3''' and C-5'''), 128.7 (C-2''' and C-6'''), 128.5 (C-1'''), 126.4 (C-4'''), 121.0 (C-2''), 117.2 (C-5'), 116.8 (C-2), 114.6 (C-3''), 55.61 (C4''-O*C*H$_3$), 51.57 (O*C*H$_3$); MS: 335.33 (M+H$^+$). Anal. Calcd for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38; Found: C, 71.63; H, 5.61; N 8.52.

2.4.3. (E)-methyl 3-(1',3'-diphenyl-1'H-pyrazol-4'-yl) Acrylate (3h)

The ester **3h** was prepared from pyrazolocarboxaldehyde **2f** according to the previously described procedure. Purification by flash column chromatography (hexane/EtOAc, 8:2) furnished **3h** as a white solid (80% yield). mp 109-110 °C; IR (film) ν: 1712 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 8.26 (1H, s, H-5'), 7.79 (2H, d, *J* 7.6 Hz, H-2''' and H-6'''), 7.76 (1H, d, *J* 16 Hz, H-3), 7.70 (2H, d, *J* 7.2 Hz, H-2''), 7.53-7.44 (5H, m, H-3'', H-4'', H-3''', and H-5'''), 7.36 (1H, t, *J* 7.2 Hz, H-4'''), 6.30 (1H, d, *J* 16 Hz, H-2), 3.80 (3H, s, OC*H*$_3$); ^{13}C

NMR ($CDCl_3$): 167.5 (C-1), 153.3 (C-3'), 139.5 (C-1''), 135.4 (C-3), 132.2 (C-4'), 129.6 (C-3''), 128.8 (C-3''' and C-5'''), 128.7 (C-2''' and C-6'''), 128.6 (C-1'''), 127.2 (C-4'''), 126.4 (C-5'), 119.3 (C-2''), 117.6 (C-4''), 117.1 (C-2), 51.61 (OCH_3); MS: 305.29 ($M+H^+$). Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20; Found: C, 74.73; H, 5.51; N 9.38.

2.5. Synthesis of Thioesters 4a, f-h

2.5.1. (E)-O-Ethyl 3-(1'-(4''-methoxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl)prop-2-enethioate (4a)

To a stirred solution of ester **3a** (80 mg, 0.23 mmol) in 3.0 mL of toluene, Lawesson's reagent (0.74 g, 1.8 mmol) was added and the mixture was refluxed overnight. The solvent was removed under vacuum and the remaining residue was purified by flash column chromatography (hexane/EtOAc, 8:2) to provide the thioester **4a** as yellow oil (70% yield). IR (film) ν: 1251 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.19 (1H, s, H-5'), 7.78 (1H, d, *J* 16 Hz, H-3), 7.72-7.68 (4H, m, H-2'', H-2''' and H-6'''), 7.52 (2H, t, *J* 7.2 Hz, H-3''' and H-5'''), 7.46 (1H, t, *J* 7.2 Hz, H-4'''), 7.02 (2H, d, *J* 8.4 Hz, H-3''), 6.91 (1H, d, *J* 16 Hz, H-2), 4.63 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 3.88 (3H, s, OCH_3), 1.46 (3H, t, *J* 7.2 Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$): 203.5 (C-1), 158.8 (C-4''), 153.5 (C-3'), 133.1 (C-1''), 132.4 (C-4'), 131.3 (C-3), 128.7 (C-2''', C-3''' and C-5'''), 128.5 (C-1'''), 128.4 (C-4'''), 126.2 (C-5'), 121.0 (C-2''), 117.6 (C-2), 114.6 (C-3''), 67.66 (OCH_2CH_3), 55.61 (OCH_3), 13.82 (OCH_2CH_3); MS: 365.34 ($M+H^+$). Anal. Calcd for $C_{21}H_{20}N_2O_2S$: C, 69.20; H, 5.53; N, 7.69; Found: C, 69.32; H, 5.61; N 7.76.

2.5.2. (E)-O-Ethyl 3-(1',3'-diphenyl-1'H-pyrazol-4'-yl)prop-2-enethioate (4f)

Thioester **4f** was obtained from **3f** according to the previously described procedure. Purification by flash column chromatography (hexane/EtOAc, 8:2) furnished **4f** as a yellow oil (75% yield). IR (film) ν: 1265 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.29 (1H, s, H-5'), 7.80 (2H, d, *J* 8.0 Hz, H-2''' and H-6'''), 7.79 (1H, d, *J* 16 Hz, H-3), 7.78 (2H, d, *J* 8.0 Hz, H-2''), 7.53-7.48 (5H, m, H-3'', H-4'', H-3''' and H-5'''), 7.37 (1H, t, *J* 7.2 Hz, H-4'''), 6.92 (1H, d, *J* 16 Hz, H-2), 4.64 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 1.46 (3H, t, *J* 7.2 Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$): 203.4 (C-1), 153.8 (C-3'), 139.4 (C-1''), 132.3 (C-4'), 131.1 (C-3), 129.6 (C-3''), 129.0 (C-1'''), 128.8 (C-3''' and C-5'''), 128.7 (C-2''' and C-6'''), 128.6 (C-4'''), 128.5 (C-4''), 127.2 (C-5'), 126.1 (C-2), 119.3 (C-2''), 67.72 (OCH_2CH_3), 13.84 (OCH_2CH_3); MS: 335.27 ($M+H^+$). Anal. Calcd for $C_{20}H_{18}N_2OS$: C, 71.83; H, 5.42; N, 8.38; Found: C, 71.97; H, 5.31; N 8.16.

2.5.3. (E)-O-Methyl 3-(1'-(4''-methoxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl)prop-2-enethioate (4g)

Compound **4g** was obtained from ester **3g** according to the previously described procedure. Purification by flash column chromatography (hexane/EtOAc, 8:2) gave **4g** as a yellow oil (80% yield). IR (film) ν: 1251 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.19 (1H, s, H-5'), 7.78 (1H, d, *J* 16 Hz, H-3), 7.70 (2H, d, *J* 7.2 Hz, H-2''' and H-6'''), 7.69 (2H, d, *J* 8.8 Hz, H-2''), 7.53 (2H, t, *J* 7.2 Hz, H-3''' and H-5'''), 7.47 (1H, t, *J* 7.2 Hz, H-4'''), 7.02 (2H, d, *J* 8.8 Hz, H-3''), 6.93 (1H, d, *J* 16 Hz, H-2), 4.18 (3H, s, OCH_3), 3.88 (3H, s, C4''-OCH_3); ^{13}C NMR ($CDCl_3$): 203.2 (C-1), 158.8 (C-4''), 153.6 (C-3'), 133.1 (C-1''), 132.4 (C-4'), 131.3 (C-3), 128.8 (C-1''', C-2''', C-3''', C-5''' and C-6'''), 128.6 (C-4'''), 128.0 (C-5'), 126.2 (C-2), 121.0 (C-2''), 114.6 (C-3''), 58.50 (O-CH_3), 55.62 (C4''-OCH_3); MS: 351.28 ($M+H^+$). Anal. Calcd for $C_{20}H_{18}N_2O_2S$: C, 68.55; H, 5.18; N, 7.99; Found: C, 68.69; H, 5.31; N 7.76.

2.5.4. (E)-O-Methyl 3-(1',3'-diphenyl-1'H-pyrazol-4'-yl)prop-2-enethioate (4h)

Thioester **4h** was obtained from compound **3h** according to the previously described procedure. Purification by flash column chromatography (hexane/EtOAc, 8:2) produced **4h** as a yellow oil (80% yield). IR (film) ν: 1247 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 8.29 (1H, s, H-5'), 7.80 (2H, d, *J* 7.2 Hz, H-2''' and H-6'''), 7.77 (1H, d, *J* 16 Hz, H-3), 7.72 (2H, d, *J* 8.8 Hz, H-2''), 7.55-7.46 (5H, m, H-3'', H-4'', H-3''', and H-5'''), 7.37 (1H, t, *J* 7.2 Hz, H-4'''), 6.95 (1H, d, *J* 16 Hz, H-2), 4.18 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$): 203.1 (C-1), 153.9 (C-3'), 139.4 (C-1''), 132.3 (C-4'), 131.1 (C-3), 129.6 (C-3''), 128.8 (C-3''' and C-5'''), 128.7 (C-2''' and C-6'''), 128.6 (C-1'''), 128.3 (C-4'''), 127.3 (C-5'), 126.2 (C-2), 119.3 (C-2''), 117.9 (C-4''), 58.53 (OCH_3); MS: 321.30 ($M+H^+$). Anal. Calcd for $C_{19}H_{16}N_2OS$: C, 71.22; H, 5.03; N, 8.74; Found: C, 71.37; H, 5.17; N 8.86.

2.6. Demethylation of Ester 3g

2.6.1. (E)-Methyl 3-(1'-(4''-hydroxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl) Acrylate (5g)

To a stirred solution of ester **3g** (0.19 g, 0.57 mmol) in CH_2Cl_2 (18 mL) at -78°C, BBr_3 (2.8 mmol, 1.0 M in CH_2Cl_2, 2.8 mL) was added drop wise and stirring was continued at -78°C for 1 h. The reaction was allowed to reach the room temperature and stirred for additional 16 h. at room temperature. Then, the reaction mixture was cooled to 0°C and quenched with MeOH and HCl 1N. The organic phase was extracted twice with ethyl acetate and the combined organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuum. Purification by flash column chromatography (hexane/EtOAc, 7:3) provided **5g** as a white solid (85% yield). mp 209-210 °C; IR (film) ν: 1670 cm^{-1}; ^1H NMR (acetone-d6): δ = 8.85 (1H, s, H-5'), 7.79 (2H, d, *J* 8.8 Hz, H-2''), 7.71 (1H, d, *J* 16 Hz, H-3), 7.70 (2H, d, *J* 7.6 Hz, H-2''' and H-6'''), 7.56 (2H, t, *J* 7.6 Hz, H-3''' and H-5'''), 7.50 (1H, t, *J* 7.6 Hz, H-4'''), 7.01 (2H, d, *J* 8.8 Hz, H-3''), 6.44 (1H, d, *J* 16 Hz, H-2), 3.73 (3H, s, OCH_3); ^{13}C NMR (acetone-d6): 166.8 (C-1), 156.6 (C-3' and C-4''), 135.0 (C-3), 132.8 (C-4'), 132.4 (C-1''), 128.6 (C-3''' and C-5'''), 128.5 (C-2''' and C-6'''), 128.4 (C-4'''), 128.0 (C-1'''), 127.3 (C-5'), 120.6 (C-2''), 116.6 (C-2), 115.8 (C-3''), 50.69 (OCH_3); MS: 321.28 ($M+H^+$). Anal. Calcd for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74; Found: C, 71.41; H, 5.18; N 8.97.

2.7. General Procedure for the Synthesis of Amides 8a-e

2.7.1. (E)-N,N-diethyl-3-(1'-(4''-methoxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl) Acrylamide (8a)

Diethylamine (0.4 mL, 3.8 mmol), was added drop wise into a solution of carboxylic acid **6a** (0.35 g, 1.1 mmol) in

acetonitrile (12 mL) and stirred at room temperature for 30 min. Then, 0.53 g of TBTU (1.6 mmol) were added and the stirring was continued until all starting material was consumed. The reaction mixture was quenched with brine and extracted twice with ethyl acetate. The combined organic extracts were washed successively with HCl (1N), water, 5% aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum to a solid residue. The latter was washed with DEE and dried over P_2O_5 to provide amide **8a** as a white solid (80% yield). mp 159-160 °C; IR (film) ν: 1654 cm^{-1} **^{1}H NMR** ($CDCl_3$): δ = 8.12 (1H, s, H-5'), 7.77 (1H, d, *J* 16 Hz, H-3), 7.70 (2H, d, *J* 7.2 Hz, H-2''' and H-6'''), 7.69 (2H, d, *J* 8.8 Hz, H-2''), 7.47 (2H, t, *J* 7.2 Hz, H-3''' and H-5'''), 7.41 (1H, t, *J* 7.2 Hz, H-4'''), 7.00 (2H, d, *J* 8.8 Hz, H-3''), 6.59 (1H, d, *J* 16 Hz, H-2), 3.87 (3H, s, OC*H*$_3$), 3.47 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 3.35 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 1.17 (6H, t, *J* 7.2 Hz, NCH$_2$C*H*$_3$); **^{13}C NMR** ($CDCl_3$): 165.8 (C-1), 158.6 (C-4''), 152.5 (C-3'), 133.3 (C-4'), 132.9 (C-1''), 132.7 (C-3), 128.8 (C-2''' and C-6'''), 128.6 (C-3''' and C-5'''), 128.3 (C-1'''), 126.6 (C-4'''), 120.9 (C-2''), 118.1 (C-5'), 117.1 (C-2), 114.6 (C-3''), 55.60 (O*C*H$_3$), 42.75 (N*C*H$_2$CH$_3$), 40.23 (N*C*H$_2$CH$_3$), 13.85 (NCH$_2$*C*H$_3$); MS: 376.36 (M+H$^+$). Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.57; H, 6.71; N, 11.19; Found: C, 73.78; H, 6.93; N 11.34.

2.7.2. (E)-N,N-diethyl-3-(1'-(4''-methoxyphenyl)-3'-(3'''-methoxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (8b)

Acid **6b** was converted to amide **8b** as described previouly (white solid, 85% yield). mp 111-112 °C; IR (film) ν: 1645 cm^{-1}; **^{1}H NMR** ($CDCl_3$): δ = 8.09 (1H, s, H-5'), 7.76 (1H, d, *J* 16 Hz, H-3), 7.68 (2H, d, *J* 8.8 Hz, H-2''), 7.36 (1H, t, *J* 8.0 Hz, H-5'''), 7.28-7.24 (2H, m, H-2''' and H-6'''), 6.98 (2H, d, *J* 8.8 Hz, H-3''), 6.96 (1H, d, *J* 8.0 Hz, H-4'''), 6.59 (1H, d, *J* 16 Hz, H-2), 3.86 (3H, s, C3'''-OC*H*$_3$), 3.85 (3H, s, C4''-OC*H*$_3$), 3.46 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 3.33 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 1.16 (6H, t, *J* 7.2 Hz, NCH$_2$C*H*$_3$); **^{13}C NMR** ($CDCl_3$): 165.8 (C-1), 159.8 (C-3'''), 158.6 (C-4''), 152.4 (C-3'), 134.2 (C-4'), 133.2 (C-1''), 132.5 (C-3), 129.7 (C-1'''), 126.6 (C-5'''), 121.3 (C-5'), 120.9 (C-2''), 118.1 (C-6'''), 117.2 (C-4'''), 114.5 (C-3''), 114.4 (C-2'''), 113.8 (C-2), 55.56 (C4''-O*C*H$_3$), 55.32 (C3'''-O*C*H$_3$), 42.15 (N*C*H$_2$CH$_3$), 40.98 (N*C*H$_2$CH$_3$), 13.36 (NCH$_2$*C*H$_3$) 13.24 (NCH$_2$*C*H$_3$); MS: 406.13 (M+H$^+$). Anal. Calcd for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36; Found: C, 71.22; H, 6.94; N 10.48.

2.7.3. (E)-N,N-diethyl-3-(1'-(4''-methoxyphenyl)-3'-(4'''-methoxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (8c)

According to the general procedure, amide **8c** was obtained from acid **6c** as white solid (85% yield). mp 141-142 °C; IR (film) ν: 1645 cm^{-1}; **^{1}H NMR** ($CDCl_3$): δ = 8.09 (1H, s, H-5'), 7.76 (1H, d, *J* 16 Hz, H-3), 7.68 (2H, d, *J* 8.8 Hz, H-2''' and H-6'''), 7.64 (2H, d, *J* 8.0 Hz, H-2''), 7.01 (2H, d, *J* 8.0 Hz, H-3''), 7.00 (2H, d, *J* 8.8 Hz, H-3''' and H-5'''), 6.60 (1H, d, *J* 16 Hz, H-2), 3.87 (6H, s, C4''-OC*H*$_3$ and C4'''-OC*H*$_3$), 3.48 (2H, q, *J* 6.8 Hz, NC*H*$_2$CH$_3$), 3.37 (2H, q, *J* 6.8 Hz, NC*H*$_2$CH$_3$), 1.19 (6H, t, *J* 7.2 Hz, NCH$_2$C*H*$_3$); **^{13}C NMR** ($CDCl_3$): 165.8 (C-1), 159.8 (C-4'''), 158.5 (C-4''), 152.4 (C-3'), 133.3 (C-4'), 132.9 (C-1''), 130.0 (C-2''' and C-6'''), 126.4 (C-3), 125.3 (C-1'''), 120.9 (C-2''), 117.9 (C-5'), 116.8 (C-2), 114.5 (C-3''' and C-5'''), 114.1 (C-3''), 55.61 (C4''-O*C*H$_3$), 55.38 (C4'''-O*C*H$_3$), 42.15 (N*C*H$_2$CH$_3$), 41.02 (N*C*H$_2$CH$_3$), 15.06 (NCH$_2$*C*H$_3$) 13.29 (NCH$_2$*C*H$_3$); MS: 406.13 (M+H$^+$). Anal. Calcd for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36; Found: C, 71.24; H, 6.95; N 10.49.

2.7.4. (E)-N,N-diethyl-3-(1'-(4''-methoxyphenyl)-3'-(3''',4'''-dimethoxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (8d)

According to the general procedure, amide **8d** was obtained from acid **6d** and purified by flash column chromatography using EtOAc as eluent (white solid, 80% yield). mp 137-138 °C; IR (film) ν: 1650 cm^{-1}; **^{1}H NMR** ($CDCl_3$): δ = 8.09 (1H, s, H-5'), 7.78 (1H, d, *J* 16 Hz, H-3), 7.68 (2H, d, *J* 8.8 Hz, H-2''), 7.26 (1H, d, *J* 1.6 Hz, H-2'''), 7.22 (1H, dd, *J* 8.4 Hz *J* 1.6 Hz, H-6'''), 7.00 (2H, d, *J* 8.8 Hz, H-3''), 6.96 (1H, d, *J* 8.4 Hz, H-5'''), 6.60 (1H, d, *J* 16 Hz, H-2), 3.96 (3H, s, C3'''-OC*H*$_3$), 3.94 (3H, s, C4'''-OC*H*$_3$), 3.86 (3H, s, C4''-OC*H*$_3$), 3.47 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 3.36 (2H, q, *J* 7.2 Hz, NC*H*$_2$CH$_3$), 1.18 (6H, t, *J* 7.2 Hz, NCH$_2$C*H*$_3$); **^{13}C NMR** ($CDCl_3$): 165.8 (C-1), 158.6 (C-4''), 152.4 (C-3'), 149.3 (C-3'''), 149.1 (C-4'''), 133.3 (C-4'), 132.8 (C-1''), 126.5 (C-1'''), 125.6 (C-3), 121.6 (C-5'), 120.9 (C-2''), 118.0 (C-6'''), 117.0 (C-5'''), 114.6 (C-3''), 111.7 (C-2'''), 111.3 (C-2), 56.01 (C4''-O*C*H$_3$), 55.98 (C3'''-O*C*H$_3$), 55.58 (C4'''-O*C*H$_3$), 42.20 (N*C*H$_2$CH$_3$), 41.00 (N*C*H$_2$CH$_3$), 15.00 (NCH$_2$*C*H$_3$) 13.26 (NCH$_2$*C*H$_3$); MS: 436.43 (M+H$^+$). Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65; Found: C, 69.11; H, 6.92; N 9.79.

2.7.5. (E)-N,N-diethyl-3-(1'-(4''-methoxyphenyl)-3'-(2''',5'''-dimethoxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (8e)

Amide **8e** was obtained as a white solid (80% yield) from acid **6e** according to the general procedure and flash column chromatography purification (hexane/EtOAc, 3:7). mp 138-139 °C; IR (film) ν: 1651 cm^{-1}; **^{1}H NMR** ($CDCl_3$): δ = 8.08 (1H, s, H-5'), 7.68 (2H, d, *J* 9.2 Hz, H-2''), 7.58 (1H, d, *J* 16 Hz, H-3), 7.02 (1H, d, *J* 2.4 Hz, H-6'''), 6.99 (2H, d, *J* 9.2 Hz, H-3''), 6.97 (1H, d, *J* 8.8 Hz, H-3'''), 6.96 (1H, dd, *J* 8.8 Hz *J* 2.4 Hz, H-4'''), 6.37 (1H, d, *J* 16 Hz, H-2), 3.87 (3H, s, C5'''-OC*H*$_3$), 3.81 (3H, s, C2'''-OC*H*$_3$), 3.79 (3H, s, C4''-OC*H*$_3$), 3.43 (2H, q, *J* 6.8 Hz, NC*H*$_2$CH$_3$), 3.24 (2H, q, *J* 6.8 Hz, NC*H*$_2$CH$_3$), 1.11 (6H, t, *J* 6.8 Hz, NCH$_2$C*H*$_3$); **^{13}C NMR** ($CDCl_3$): 166.0 (C-1), 158.5 (C-4''), 153.7 (C-3'), 151.7 (C-5'''), 149.8 (C-2'''), 133.4 (C-4'), 132.8 (C-1''), 128.6 (C-3), 126.6 (C-5'), 120.8 (C-2''), 119.4 (C-1'''), 116.7 (C-4'''), 116.0 (C-3'''), 115.6 (C-6'''), 114.5 (C-3''), 112.6 (C-2), 56.36 (C4''-O*C*H$_3$), 55.86 (C5'''-O*C*H$_3$), 55.57 (C2'''-O*C*H$_3$), 42.12 (N*C*H$_2$CH$_3$), 40.89 (N*C*H$_2$CH$_3$), 14.87 (NCH$_2$*C*H$_3$) 13.24 (NCH$_2$*C*H$_3$); MS: 436.27 (M+H$^+$). Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65; Found: C, 69.13; H, 6.93; N 9.81.

2.8. General Procedure for the Demethylation of Amides 8a, b, d, e

2.8.1. (E)-N,N-diethyl-3-(1'-(4''-hydroxyphenyl)-3'-phenyl-1'H-pyrazol-4'-yl) acrylamide (9a)

To a stirred solution of amide **8a** (0.20 g, 0.53 mmol) in CH_2Cl_2 (16 mL) at -78°C, BBr_3 (2.7 mmol, 1.0 M in CH_2Cl_2, 5 equiv per protective group) was added drop wise and stirred at -78°C for 1 h. The reaction was allowed to reach

the room temperature and stirred for additional 16 h. The reaction mixture was cooled to 0°C and quenched with the addition of MeOH and HCl 1N. Then, the organic phase was extracted twice with EtOAc and the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting residue was purified by flash column chromatography (hexane/EtOAc, 3:7) to provide **9a** as a white solid (85% yield). mp 246-247 °C; IR (film) ν: 1642 cm^{-1}; **^{1}H NMR** (DMSO): δ = 9.02 (1H, s, H-5'), 7.72 (2H, d, *J* 8.4 Hz, H-2''), 7.61 (2H, d, *J* 6.8 Hz, H-2''' and H-6'''), 7.53 (2H, t, *J* 6.8 Hz, H-3'' and H-5'''), 7.49 (1H, d, *J* 16 Hz, H-3), 7.48 (1H, t, *J* 6.8 Hz, H-4'''), 6.96 (1H, d, *J* 16 Hz, H-2), 6.93 (2H, d, *J* 8.4 Hz, H-3''), 3.42 (4H, q, *J* 7.2 Hz, NCH_2CH_3), 1.15 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 1.06 (3H, t, *J* 7.2 Hz, NCH_2CH_3); **^{13}C NMR** (DMSO): 165.2 (C-1), 156.9 (C-4''), 151.7 (C-3'), 133.0 (C-4'), 132.0 (C-1''), 131.8 (C-3), 129.2 (C-2''' and C-6'''), 128.8 (C-3''', C-4''' and C-5'''), 128.1 (C-1'''), 120.9 (C-2''), 117.9 (C-5'), 117.8 (C-2), 116.3 (C-3''), 41.84 (NCH_2CH_3), 15.68 (NCH_2CH_3), 13.68 (NCH_2CH_3); MS: 362.30 ($M+H^+$). Anal. Calcd for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63; Found: C, 73.33; H, 6.24; N 11.81.

2.8.2. (E)-N,N-diethyl-3-(1'-(4''-hydroxyphenyl)-3'-(3'''-hydroxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (9b)

Amide **8b** was demethylated according to the previously described procedure to provide after purification by flash column chromatography (Hexane/EtOAc, 4:6) the deprotected amide **9b** as a white solid (85% yield). mp 233-234 °C; IR (film) ν: 1650 cm^{-1}; **^{1}H NMR** (CD_3OD): δ = 8.58 (1H, s, H-5'), 7.64 (1H, d, *J* 16 Hz, H-3), 7.62 (2H, d, *J* 8.8 Hz, H-2''), 7.31 (1H, t, *J* 8.0 Hz, H-5'''), 7.01 (1H, s, H-2'''), 7.07 (1H, d, *J* 8.0 Hz, H-6'''), 6.93-6.87 (3H, m, H-3'' and H-4'''), 6.83 (1H, d, *J* 16 Hz, H-2), 3.47 (4H, q, *J* 7.2 Hz, NCH_2CH_3), 1.18 (6H, t, *J* 7.2 Hz, NCH_2CH_3); **^{13}C NMR** (CD_3OD): 166.9 (C-1), 157.5 (C-3'''), 156.8 (C-4''), 152.4 (C-3'), 133.8 (C-4'), 132.8 (C-3), 132.0 (C-1''), 129.5 (C-1'''), 127.9 (C-5'''), 121.0 (C-5' and C-2''), 119.7 (C-6'''), 116.3 (C-4'''), 115.5 (C-2'''), 115.3 (C-3''), 115.2 (C-2), 42.16 (NCH_2CH_3), 40.98 (NCH_2CH_3), 13.81 (NCH_2CH_3) 12.04 (NCH_2CH_3); MS: 378.15 ($M+H^+$). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13; Found: C, 70.29; H, 6.01; N 11.28.

2.8.3. (E)-N,N-diethyl-3-(1'-(4''-hydroxyphenyl)-3'-(3''',4'''-hydroxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (9d)

According to the general procedure, deprotected amide **9d** was obtained from amide **8d** after purification by flash column chromatography (EtOAc) and solvent evaporation as a white solid (85% yield). mp 257-258 °C; IR (film) ν: 1643 cm^{-1}; **^{1}H NMR** (CD_3OD): δ = 8.71 (1H, s, H-5'), 7.74 (1H, d, *J* 16 Hz, H-3), 7.71 (2H, d, *J* 9.2 Hz, H-2''), 7.24 (1H, d, *J* 2.0 Hz, H-2'''), 7.08 (1H, dd, *J* 8.0 Hz *J* 2.0 Hz, H-6'''), 6.99 (1H, d, *J* 16 Hz, H-2), 6.98 (2H, d, *J* 9.2 Hz, H-3''), 6.96 (1H, d, *J* 8.0 Hz, H-5'''), 3.47 (4H, q, *J* 7.2 Hz, NCH_2CH_3), 1.21 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 1.14 (3H, t, *J* 7.2 Hz, NCH_2CH_3); **^{13}C NMR** (CD_3OD): 165.1 (C-1), 156.8 (C-4''), 149.2 (C-3'), 147.8 (C-3'''), 147.6 (C-4'''), 132.9 (C-4'), 132.0 (C-1''), 125.4 (C-1'''), 124.2 (C-3), 120.7 (C-2''), 118.8 (C-5'), 118.3 (C-6'''), 117.9 (C-5'''), 116.4 (C-3''), 115.7 (C-2'''), 114.6 (C-2), 41.78 (NCH_2CH_3), 15.12 (NCH_2CH_3) 13.68 (NCH_2CH_3); MS: 394.35 ($M+H^+$). Anal. Calcd for $C_{22}H_{23}N_3O_4$: C, 67.16; H, 5.89; N, 10.68; Found: C, 67.31; H, 5.74; N 10.83.

2.8.4. (E)-N,N-diethyl-3-(1'-(4''-hydroxyphenyl)-3'-(2''',5'''-hydroxyphenyl)-1'H-pyrazol-4'-yl) Acrylamide (9e)

According to the general procedure, deprotected amide **9e** was obtained from amide **8e** after purification by flash column chromatography (hexane/EtOAc, 4:6) and solvent evaporation as a white solid (85% yield). mp 263-264 °C; IR (film) ν: 1650 cm^{-1}; **^{1}H NMR** (DMSO): δ = 8.90 (1H, s, H-5'), 7.66 (2H, d, *J* 8.8 Hz, H-2''), 7.35 (1H, d, *J* 16 Hz, H-3), 6.91 (2H, d, *J* 8.8 Hz, H-3''), 6.79 (1H, d, *J* 7.6 Hz, H-3'''), 6.76 (1H, d, *J* 16 Hz, H-2), 6.71 (1H, d, *J* 2.4 Hz, H-6'''), 6.70 (1H, dd, *J* 7.6 Hz *J* 2.4 Hz, H-4'''), 3.36 (4H, q, *J* 6.8 Hz, NCH_2CH_3), 1.10 (3H, t, *J* 6.8 Hz, NCH_2CH_3), 1.10 (3H, t, *J* 6.8 Hz, NCH_2CH_3); **^{13}C NMR** (DMSO): 165.3 (C-1), 156.7 (C-4''), 150.3 (C-3'), 150.2 (C-5'''), 148.1 (C-2'''), 133.0 (C-4'), 132.0 (C-1''), 127.5 (C-3), 120.6 (C-2''), 120.3 (C-1'''), 119.1 (C-5'), 117.3 (C-4'''), 117.0 (C-3'''), 116.9 (C-6'''), 116.6 (C-2), 116.3 (C-3''), 41.84 (NCH_2CH_3) 15.65 (NCH_2CH_3) 13.71 (NCH_2CH_3); MS: 394.36 ($M+H^+$). Anal. Calcd for $C_{22}H_{23}N_3O_4$: C, 67.16; H, 5.89; N, 10.68; Found: C, 67.32; H, 5.72; N 10.85.

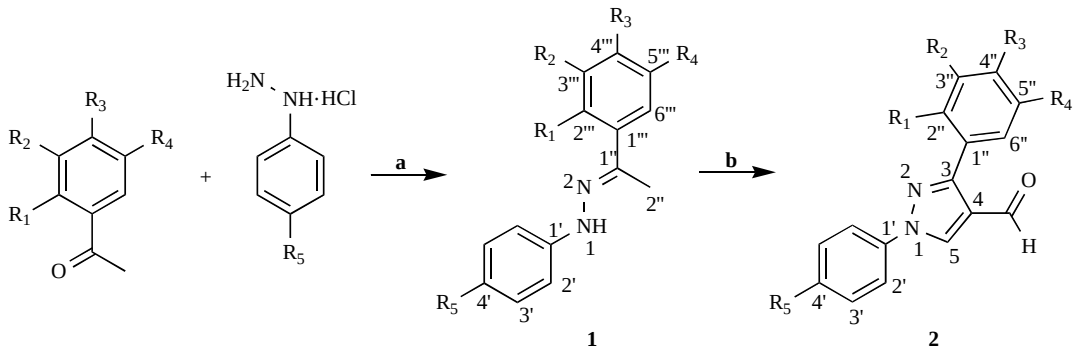
2.9. Antitumor Activity Assays

The human cancer cell lines for A2058 melanoma, MCF-7 breast cancer and DU145 prostate cancer were obtained from ATCC. They were cultured in RPMI-1640 medium containing 10% fatal bovine serum (FBS), 100 units/ml of penicillin, and 100 µg/ml streptomycin. All cells were maintained in a 5% CO_2 atmosphere at 37 °C. To determine the viability of the cells, MTS assays were performed as described by the supplier (Promega; Madison, WI). Briefly, cells (5,000/well) were seeded in 96-well plates and incubated overnight at 37 °C in 5% CO_2. Cells were treated for 48 h with 10 or 20 µM of each compound. Dimethyl sulfoxide (DMSO) was used as the vehicle control. Cell viability was determined by tetrazolium conversion to its formazan dye and absorbance of formazan was measured at 490 nm using an automated ELISA plate reader. The production of formazan dye was directly proportional to the number of living cells. Each experiment was done in quadruplicate.

3. RESULTS AND DISCUSSION

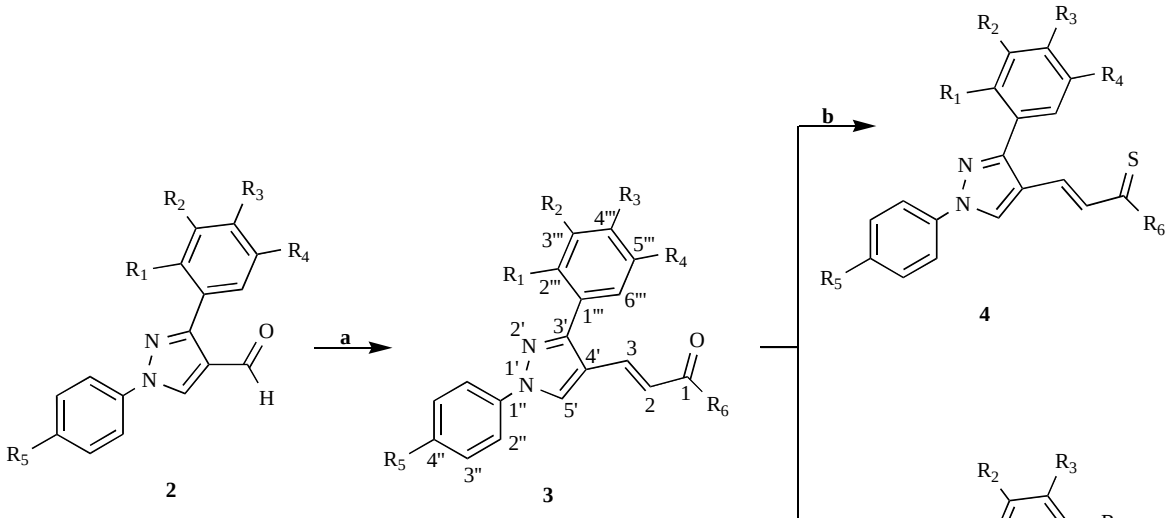
3.1. Chemical Synthesis

The synthesis of pyrazolocarbaldehydes **2** was accomplished as outlined in Scheme **1**. More specifically, the acetophenone substrates were condensed with p-methoxyphenylhydrazine(or phenylhydrazine) hydrochlorides to provide efficiently the hydrazines **1a-f**. The latterupon treatment with an iminium salt {prepared by reacting the 2,4,6-trichloro[1,3,5]triazine (TCT, cyanuric chloride) with DMF} produced the corresponding pyrazolocarbaldehydes **2** in good overall yields (48-80%). The Wittig olefination of carbaldehydes **2** with the stabilized phosphoranes gave in excellent yields (80-90%) the α,β unsaturated esters **3**, which by Lawesson's reagent were transformed to the corresponding



1a, 2a R_1= H, R_2= H, R_3= H, R_4= H, R_5 = OCH_3
1b, 2b R_1= H, R_2= OCH_3, R_3= H, R_4= H, R_5 = OCH_3
1c, 2c R_1= H, R_2= H, R_3= OCH_3 , R_4= H, R_5 = OCH_3
1d, 2d R_1= H, R_2= OCH_3, R_3= OCH_3, R_4= H, R_5 = OCH_3
1e, 2e R_1= OCH_3, R_2= H, R_3= H, R_4= OCH_3, R_5 = OCH_3
1f, 2f R_1= H, R_2= H, R_3= H, R_4= H, R_5 = H

Scheme 1. Reagents and Conditions: **(a)** Acetic acid, Et_3N, Ethanol; **(b)** TCT, Dichloromethane.



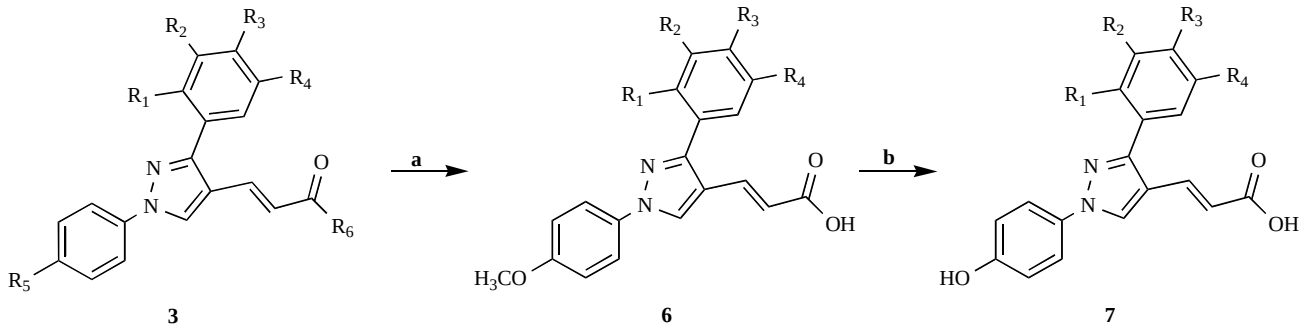
3a, 4a R_1= H, R_2= H, R_3= H, R_4= H, R_5 = OCH_3, R_6 = OCH_2CH_3
3b, R_1= H, R_2= OCH_3, R_3= H, R_4= H, R_5 = OCH_3, R_6 = OCH_2CH_3
3c, R_1= H, R_2= H, R_3= OCH_3, R_4= H, R_5 = OCH_3, R_6 = OCH_2CH_3
3d, R_1= H, R_2= OCH_3, R_3= OCH_3, R_4= H, R_5 = OCH_3, R_6 = OCH_2CH_3
3e, R_1= OCH_3, R_2= H, R_3= H, R_4= OCH_3, R_5 = OCH_3, R_6 = OCH_2CH_3
3f, 4f R_1= H, R_2= H, R_3= H, R_4= H, R_5 = H, R_6 = OCH_2CH_3
3g, 4g R_1= H, R_2= H, R_3= H, R_4= H, R_5 = OCH_3, R_6 = OCH_3
3h, 4h R_1= H, R_2= H, R_3= H, R_4= H, R_5 = H, R_6 = OCH_3

5a R_1= H, R_2= H, R_3= H, R_4= H, R_6 = OCH_2CH_3
5b R_1= H, R_2= OH, R_3= H, R_4= H, R_6 = OCH_2CH_3
5c R_1= H, R_2= H, R_3= OH, R_4= H, R_6 = OCH_2CH_3
5d R_1= H, R_2= OH, R_3= OH, R_4= H, R_6 = OCH_2CH_3
5e R_1= OH, R_2= H, R_3= H, R_4= OH, R_6 = OCH_2CH_3
5g R_1= H, R_2= H, R_3= H, R_4= H, R_6 = OCH_3

Scheme 2. Reagents and conditions: (a) Wittig reagents, Acetonitrile, reflux; (b) Lawesson's reagent, Toluene, Reflux; (c) BBr_3, Dichloromethane, -78 °C.

α,β unsaturated thioesters **4** (70-80%). Finally, demethylation of the latter in the presence of BBr_3 afforded the corresponding phenol derivatives **5** (Scheme **2**).

Esters **3** were hydrolyzed almost quantitavely furnishing the acids **6**, which were subsequently demethylated in the presence of BBr_3 (compounds **7a-e**, Scheme **3**). On the other hand, acids **6** were converted in very good yields (80-85%) to amides **8** by reaction with diethylamine in the presence of the uronium coupling reagent TBTU. Finally, their BBr_3 promoted demethylation provided efficiently the corresponding deprotected amides **9** (Scheme **4**).

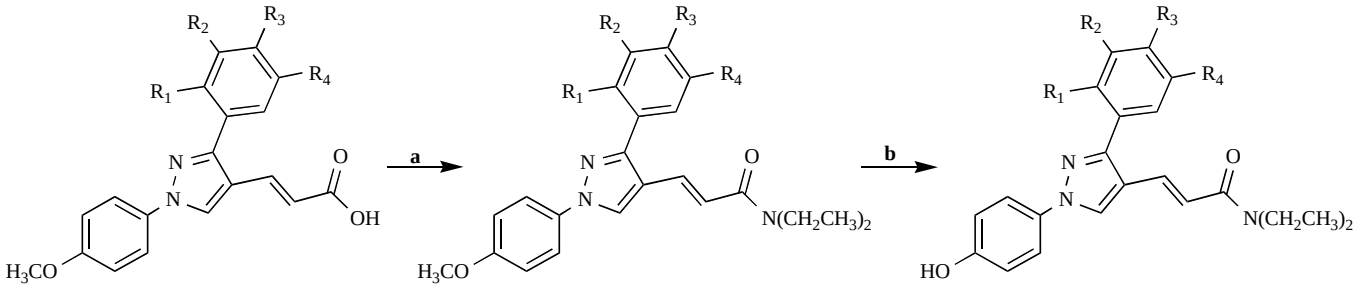


3 **6** **7**

6a R_1= H, R_2= H, R_3= H, R_4= H
6b R_1= H, R_2= OCH_3, R_3= H, R_4= H
6c R_1= H, R_2= H, R_3= OCH_3, R_4= H
6d R_1= H, R_2= OCH_3, R_3= OCH_3, R_4= H
6e R_1= OCH_3, R_2= H, R_3= H, R_4= OCH_3

7a R_1= H, R_2= H, R_3= H, R_4= H
7b R_1= H, R_2= OH, R_3= H, R_4= H
7c R_1= H, R_2= H, R_3= OH, R_4= H
7d R_1= H, R_2= OH, R_3= OH, R_4= H
7e R_1= OH, R_2= H, R_3= H, R_4= OH

Scheme 3. Reagents and Conditions: (a) Ethanol, DMSO, NaOH; (b) BBr_3, Dichloromethane, -78 °C.



6 **8** **9**

8a R_1= H, R_2= H, R_3= H, R_4= H
8b R_1= H, R_2= OCH_3, R_3= H, R_4= H
8c R_1= H, R_2= H, R_3= OCH_3, R_4= H
8d R_1= H, R_2= OCH_3, R_3= OCH_3, R_4= H
8e R_1= OCH_3, R_2= H, R_3= H, R_4= OCH_3

9a R_1= H, R_2= H, R_3= H, R_4= H
9b R_1= H, R_2= OH, R_3= H, R_4= H
9d R_1= H, R_2= OH, R_3= OH, R_4= H
9e R_1= OH, R_2= H, R_3= H, R_4= OH

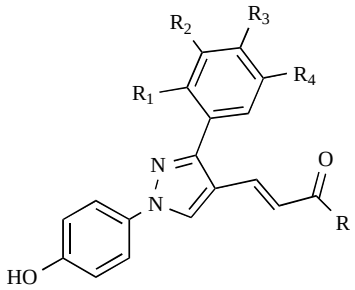
Scheme 4. Reagents and Conditions: (a) Diethylamine, TBTU, Acetonitrile; (b) BBr3, dichloromethane, -78 °C.

3.2. Antitumor Activity

In order to investigate the antitumor properties of the novel synthetic pyrazole derivatives against human cancer cells, we have determined the activities of the hydroxy compounds **5a-e**, **7a-e** and **9a,b,d,e** against human A2058 melanoma, MCF-7 breast and DU145 prostate cancer cells at 20 μM concentration for 48 h. The respective results are presented in Table **1** indicating that the acid derivatives **7** are inactive against all cancer cell lines except compounds **7c** and **7e** which reduced cell viability by 47% and 61% respectively in DU145 prostate cancer cell lines. Similarly, amides **9** displayed very low reduction in the viability of all cancer cell lines except compound **9b**, which reduced cell viability by 50% at the MCF-7 breast cancer cell line. On the contrary, esters **5** showed good efficiency for the reduction of cell viability against all tested cancer cell lines (Table **1**). In respect to their substitution pattern, it is evident that the introduction of a hydroxy group at R_2 or R_3 positions of their B-phenyl moieties (esters **5b** and **5c** respectively) results in the decrease of their activity without differentiating their cell viabilities. On the other hand, the introduction of two hydroxy groups at both R_2 and R_3 made ester **5d** totally inactive against all cancer cell lines. As is depicted in Table **1**, the most active -among all tested esters- was compound **5a**, which bears only hydrogens in the B-phenyl moiety. This compound displayed the highest reduction of cell viability in the MCF-7 cancer cell line (79%), while further studies revealed that ester **5a** exhibits an IC_{50} value of 14μM against MCF-7 breast cancer cell line (Fig. **1**).

Based on the aforementioned results, we exploited the modification of ester **5a** scaffold and synthesized the compounds **3f-h**, **4a,f-h** and **5g**. The biological evaluation results for these molecules and their precursors **3a-e** against human A2058 melanoma, MCF-7 breast and DU145 prostate cancer cells at 10 μM concentrations for 48 h are presented in Table 2. It is noticeable that the transformation of ethylester to the corresponding methylester **5g** resulted in the complete loss of activity. Similar findings were obtained for the methoxy precursor of ester **5a** which was also totally inactive. In addition, the displacement of the oxygen with a sulphur atom (thioesters **4a,f-h**) did not increase the activity, while esters **3f,h** which bear a hydrogen atom in the place of the hydroxy group of ester **5a** on their A-phenyl moiety exhibited only moderate activity, which was lower when compared to the activity of ester **5a**. The introduction of a methoxy group in the positions R_1, R_2, R_3 and R_4 of B-phenyl ring derived the

Table 1. Cell Viability Upon Treatment[a] for 48h at 37 °C for Compounds 5a-e, 7a-e and 9a,b-d,e



Compound	R	R_1	R_2	R_3	R_4	Cell Viability (% Control)		
						A2058	DU145	MCF-7
5a	OCH_2CH_3	H	H	H	H	38±3*	32±2*	21±2*
5b	OCH_2CH_3	H	OH	H	H	60±4*	38±3*	50±4*
5c	OCH_2CH_3	H	H	OH	H	61±4*	39±3*	54±3*
5d	OCH_2CH_3	H	OH	OH	H	>100	85±5*	≥100
5e	OCH_2CH_3	OH	H	H	OH	52±4*	50±4*	88±5*
7a	OH	H	H	H	H	95±5*	72±5*	>100
7b	OH	H	OH	H	H	≥100	>100	>100
7c	OH	H	H	OH	H	>100	53±3*	>100
7d	OH	H	OH	OH	H	>100	94±5*	>100
7e	OH	OH	H	H	OH	88±5*	39±3*	77±5*
9a	$N(CH_2CH_3)_2$	H	H	H	H	76±5*	67±4*	72±5*
9b	$N(CH_2CH_3)_2$	H	OH	H	H	68±3*	79±4*	50±2*
9d	$N(CH_2CH_3)_2$	H	OH	OH	H	>100	91±5*	92±5*
9e	$N(CH_2CH_3)_2$	OH	H	H	OH	75±4*	62±4*	≥100*

[a]Cells (5,000 cells/each well) were treated with 20 μM of **5a-e**, **7a-e** and **9a,b-d,e** for 48h at 37 °C. Cell viability was determined as described in the Experimental Methods.

*P< 0.05, compared with control.

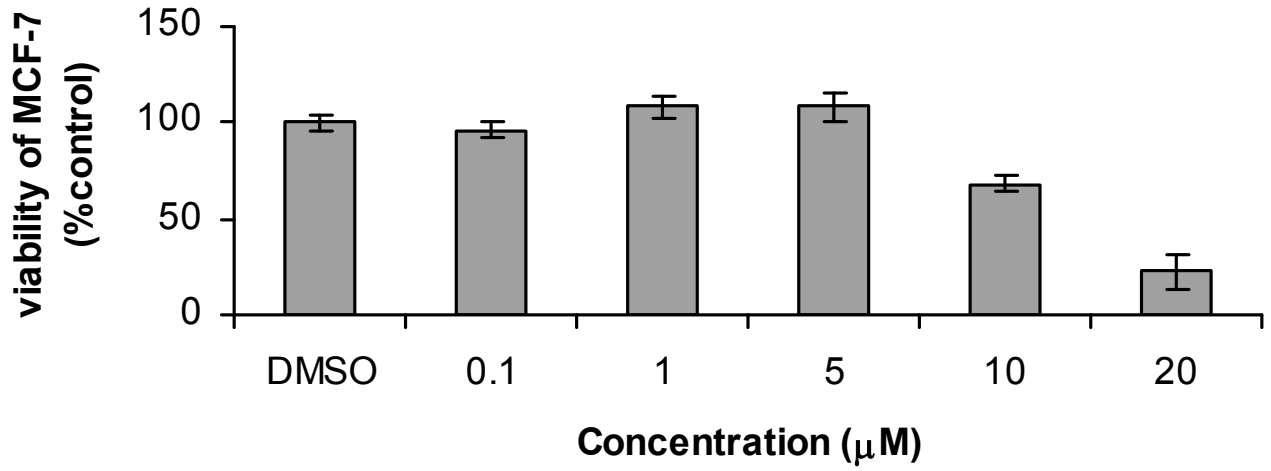
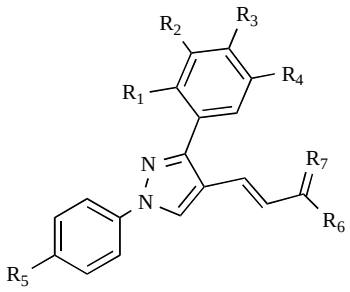


Fig. (1). Viability of MCF-7 Cancer cell line treated with ester **5a** at Concentrations 0.1, 1, 5, 10, 20 μM. The IC_{50} of ester **5a** was 14 μM.

Table 2. Cell Viability Upon Treatment[a] for 48h at 37 °C for Compounds 3a-h, 4a,f-h, 5g and 8a-e



Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Cell Viability (% Control)		
								A2058	DU145	MCF-7
3a	H	H	H	H	OCH_3	OCH_2CH_3	O	95±5*	82±5*	> 100
3b	H	OCH_3	H	H	OCH_3	OCH_2CH_3	O	85±5*	64±4*	81±5*
3c	H	H	OCH_3	H	OCH_3	OCH_2CH_3	O	76±5*	81±5*	93±5*
3d	H	OCH_3	OCH_3	H	OCH_3	OCH_2CH_3	O	69±4*	62±4*	50±3*
3e	OCH_3	H	H	OCH_3	OCH_3	OCH_2CH_3	O	90±5*	77±4*	59±3*
3f	H	H	H	H	H	OCH_2CH_3	O	91±5*	80±5*	79±5*
3g	H	H	H	H	OCH_3	OCH_3	O	81±5*	75±5*	83±5*
3h	H	H	H	H	H	OCH_3	O	88±5*	78±5*	68±4*
4a	H	H	H	H	OCH_3	OCH_2CH_3	S	99±5*	94±5*	>100
4f	H	H	H	H	H	OCH_2CH_3	S	>100	88±5*	93±5*
4g	H	H	H	H	OCH_3	OCH_3	S	88±5*	83±5*	>100
4h	H	H	H	H	H	OCH_3	S	98±5*	87±5*	92±5*
5g	H	H	H	H	OH	OCH_3	O	74±5*	62±4*	≥100

[a] Cells (5,000 cells/each well) were treated with 10 μM of **-h**, **4a,f-h**, **5g** and **8a-e** for 48h at 37 °C. Cell viability was determined as described in the Experimental Methods.
*$P < 0.05$, compared with control.

compounds **3b** and **3c**, which displayed very low inhibition capacity, while the introduction of a second methoxy group (compounds **3d** and **3e**) increased considerably their activity. It must be pointed out that compounds **3d** and **3e** were determined as the most potent which are acting selectively against the MCF-7 breast cancer cells (Table **2**), exhibiting IC_{50} values of 10 and 12 μM respectively.

4. CONCLUSION

The study herein demonstrates that various novel synthetic small molecules bearing the pyrazole moiety display potent antitumor activities against human cancer cells. In particular, the hydroxy-pyrazole derivative **5a** was determined to act selectively against MCF-7 breast cancer cell line displaying an IC_{50} value of 14 μM. The introduction of methoxy substituents on this pyrazole backbone enhanced the anticancer activity and compounds **3d** and **3e** exhibited IC_{50} values of 10 and 12 μM respectively against the same cell line, indicating that this pyrazole structural motif represents a promising structural backbone for further development.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENT

Declared none.

REFERENCES

[1] Eckhardt, S. Recent progress in the development of anticancer agents. *Curr. Med. Chem.*, **2002**, *2*, 419-39.

[2] Lee, C.W.; Hong, D.H.; Han, S.B.; Jong, S.-H.; Kim, H.C.; Fine, R.L.; Lee, S.-H.; Kim, H.M. A novel stereo-selective sulfonylurea, 1-[1-(4-aminobenzoyl)-2,3-dihydro-1H-indol-6-sulfonyl]-4-phenyl-imidazolidin -2-one, has antitumor efficacy in *in vitro* and *in vivo* tumor models. *Biochem. Pharmacol.*, **2002**, *64*, 473-80.

[3] Hu, W.-P.; Yu, H.-S.; Chen, Y.-R.; Tsai, Y.-M.; Chen, Y.-K.; Liao, C.-C.; Chang, L.-S.; Wang, J.-J. Synthesis and biological evaluation of thiobenzanilides as anticancer agents. *Bioorg. Med. Chem.*, **2008**, *16*, 5295-302.

[4] Kemnitzer, W.; Jiang, S.; Wang, Y.; Kasibhatla, S.; Crogan-Grundy, C.; Bubenik, M.; Labrecque, D.; Denis, R.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S.X. Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers

using a cell- and caspase-based HTS assay. Part 5: Modifications of the 2- and 3-positions. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 603-07.

[5] Elguero, J. In *Pyrazoles, Comprehensive Heterocyclic Chemistry II*; Katritzky, A.R.; Rees, C.W.; Scriven, E.F.V., Ed.; Elsevier Science Ltd.: Oxford, **1996**; pp. 1-75.

[6] Varvounis, G.; Fiamegos, Y.; Pilidis, G. Pyrazol-3-ones. Part III: reactivity of the ring substituents. *Adv. Heterocycl. Chem.*, **2001**, *80*, 75-165.

[7] Kumar, V.; Aggarwal, R.; Tyagi, P.; Singh, S.P. Synthesis and antibacterial activity of some new 1-heteroaryl-5-amino-4-phenyl-3-trifluoromethylpyrazoles. *Eur. J. Med. Chem.*, **2005**, *40*, 922-27.

[8] Brana, M.F.; Gradillas, A.; Ovalles, A.G.; Lopez, B.; Acero, N.; Llinares, F.; Mingarro, D.M. Synthesis and biological activity of N,N-dialkylaminoalkylsubstituted bisindolyl and diphenyl pyrazolone derivatives. *Bioorg. Med. Chem.*, **2006**, *14*, 9-16.

[9] Storer, R.; Ashton, C.J.; Baxter, A.D.; Hann, M.M.; Marr, C.L.P.; Mason, A.M.; Mo, C.-L.; Myers, P.L.; Noble, S.A.; Penn, H.R.; Wier, N.G.; Niall, G.; Woods, J.M.; Coe, P.L. The Synthesis and Antiviral Activity of 4-Fluoro-1-β-D-ribofuranosyl-1Hpyrazole-3-carboxamide. *Nucleosides Nucleotides*, **1999**, *18*, 203-16.

[10] Allen, S.H.; Johns, B.A.; Gudmundsson, K.S.; Freeman, G.A.; Boyd, F.L.J.; Sexton, C.H.; Selleseth, D.W.; Creech, K.L.; Moniri, K.R. Synthesis of C-6 substituted pyrazolo[1,5-a]pyridines with potent activity against herpesviruses. *Bioorg. Med. Chem.*, **2006**, *14*, 944-54.

[11] Baraldi, P.G.; Beria, I.; Cozzi, P.; Geroni, C.; Espinosa, A.; Gallo, M.A.; Entrena, A.; Bingham, J. P. Cinnamoyl nitrogen mustard derivatives of pyrazole analogues of tallimustine modified at the amidino moiety: design, synthesis, molecular modeling and antitumor activity studies. *Bioorg. Med. Chem.*, **2004**, *12*, 3911-21.

[12] Park, H.-J.; Lee, K.; Park, S.-J.; Ahn, B.; Lee, J.-C.; Cho, H.Y.; Lee, K.-I. Identification of antitumor activity of pyrazole oxime ethers. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 3307-12.

[13] Ortega, M.A.; Montoya, M.E.; Zarranz, B.; Jaso, A.; Aldana, I.; Leclerc, S.; Meijerc, L.; Monge, A. Pyrazolo[3,4-b]quinoxalines. A New Class of Cyclin-Dependent Kinases Inhibitors. *Bioorg. Med. Chem.*, **2002**, *10*, 2177-84.

[14] Warshakoon, N.C.; Wu, S.; Boyer, A.; Kawamoto, R.; Renock, S.; Xu, K.; Pokross, M.; Evdokimov, A.G.; Zhou, S.; Winter, C.; Walter, R.; Mekel, M. Design and synthesis of a series of novel pyrazolopyridines as HIF 1-a prolyl hydroxylase inhibitors. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 5687-90.

[15] Huang, S.; Lin, R.; Yu, Y.; Lu, Y.; Connolly, P.J.; Chiu, G.; Li, S.; Emanuel, S.L.; Middleton, S.A. Synthesis of 3-(1H-benzimidazol-2-yl)-5-isoquinolin-4-ylpyrazolo[1,2-b]pyridine, a potent cyclin dependent kinase 1 (CDK1) inhibitor. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 1243-5.

[16] Zhu, G.-D.; Gong, J.; Gandhi, V.B.; Woods, K.; Luo, Y.; Liu, X.; Guan, R.; Klinghofer, V.; Johnson, E.F.; Stoll, V.S.; Mamo, M.; Li, Q.; Rosenberg, S.H.; Giranda, V.L. Design and synthesis of pyridine–pyrazolopyridine-based inhibitors of protein kinase B/Akt. *Bioorg. Med. Chem.*, **2007**, *15*, 2441-52.

[17] Hashimoto, H.; Imamura, K.; Haruta, J.-I.; Wakitani, K. 4-(4-Cycloalkyl/aryl-oxazol-5-yl)benzenesulfonamides as selective cyclooxygenase-2 inhibitors: enhancement of the selectivity by introduction of a fluorine atom and identification of a potent, highly selective, and orally active COX-2 inhibitor JTE-522. *J. Med. Chem.*, **2002**, *45*, 1511-7.

[18] Ranatunge, R.R.; Earl, R.A.; Garvey, D.S.; Janero, D.R.; Letts, L.G.; Martino, A.M.; Murty, M.G.; Richardson, S.K.; Schwalb, D.J.; Young, D.V.; Zemtseva, I.S. 3-(2-Methoxytetrahydrofuran-2-yl)pyrazoles: a novel class of potent, selective cyclooxygenase-2 (COX-2) inhibitors. *Bioorg. Med. Chem. Lett*., **2004**, *14*, 6049-52.

[19] Blumenthal, R.D.; Waskewich, C.; Goldenberg, D.M.; Lew, W.; Flefleh, C.; Burton, J. Chronotherapy and Chronotoxicity of the Cyclooxygenase-2 Inhibitor, Celecoxib, in Athymic Mice Bearing Human Breast Cancer Xenografts. *Clin. Cancer Res.*, **2001**, *7*, 3178-85.

[20] Liu, X.; Yue, P.; Schönthal, A.H.; Khuri, F.R.; Sun, S.-Y. Cellular FLICE-Inhibitory Protein Down-regulation Contributes to Celecoxib-Induced Apoptosis in Human Lung Cancer Cells. *Cancer Res.*, **2006**, *66*, 11115-11119.

[21] Pruthi, R.S.; Derksen, J.E.; Moore, D.; Carson, C.C.; Grigson, G.; Watkins, C.; Wallen, E. Phase II Trial of Celecoxib in Prostate-Specific Antigen Recurrent Prostate Cancer after Definitive Radiation Therapy or Radical Prostatectomy. *Clin. Cancer Res.*, **2006**, *12*, 2172-7.

[22] De Luca, L.; Giacomelli, G.; Masala, S.; Porcheddu, A. A mild procedure for the preparation of 3-aryl-4-formylpyrazoles. *Synlett*, **2004**, 2299-2302.

[23] Christodoulou, M.S.; Liekens, S.; Kasiotis, K.M.; Haroutounian, S.A. Novel pyrazole derivatives: synthesis and evaluation of anti-angiogenic activity. *Bioorg. Med. Chem.* **2010**, *18*, 4338-4350.

Received: March 08, 2011 Revised: November 11, 2011 Accepted: November 22, 2011