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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticancer effects of pongamol derivatives on mitogen signaling and cell cycle kinases

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Abstract A series of oxazole and pyrazole derivatives of pongamol (**1**) were designed and synthesized to examine their anti-cancer activity. The cytotoxicity of these compounds was examined in three different human tumor cell lines, IMR-32, HeLa and Jurkat. Although all compounds tested were quite effective than the pongamol against all the three different types of cancer cell lines examined, the compounds (**2**), (**5**), and (**6**) were found to be the most active compounds of this series.

Keywords *Derris indica* · Chalcones · Pyrazole derivatives · Oxazole · Anticancer activity

Introduction

Cancer is one of the most serious threats against human health in the world (Shewach, 2009). Over the past few decades, extensive research has led to the development of a plethora of chemotherapeutic agents (Cragg and Newman, 2005; Cragg *et al.*, 2009). The limitations of current anticancer drugs and rapid development of drug resistance (Sreedhar and Csermely, 2004; Pechan, 1991; McCubrey *et al.*, 2006) have highlighted the need for the discovery of new anticancer agents, preferably with novel mechanisms of action. To identify new chemical entities for a more effective treatment of cancer, drug designers can follow many strategies, but the crucial decision is always the selection of a suitable starting point from the vast chemical space (Lloyd *et al.*, 2006).

In this respect, natural products evolved as privileged structures (Koehn and Carter, 2005) and biologically prevalidated leads, in other words, as molecules that have probably evolved evolutionarily to exert highly specialized functions. About 74% of anticancer compounds are form both natural or natural product–derived products, indicating potency of these scaffolds (Newman *et al.*, 2003). The unprecedented structures of these molecules make them excellent synthetic targets, and their potent activity against a broad number of therapeutic indications makes these natural products excellent drug lead candidates for new therapeutics (Newman, 2008). In connection with recent investigations of *Derris indica* for value added products (Rao *et al.*, 2009), we have isolated large quantities of pongamol (**1**), which prompted us to synthesize derivatives and screen for potential anticancer activity. Traditionally, the fruits and sprouts of this plant are being used in folk remedies for abdominal tumors in India, the seeds for keloid tumors in Sri Lanka, and a powder derived from the plant for tumors in Vietnam. Literature prevalence revealed that this plant is rich source of Flavonoids and chalcones, which are known for their anti-cancer and anti-oxidant properties (Carcahe-Blanco *et al.*, 2003).

Pongamol (**1**) belongs to the chalcone class family, in which two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system, and the wide variety of pharmacological activities reported includes anti-inflammatory, analgesic, antioxidant, antibacterial, antifungal, and antiprotozoal activities (Gupta and Shaw, 2009).

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Except in the Traditional system of use, no information is available on anticancer activity of pongamol. Oxazoles and pyrazoles are heterocyclic organic compounds that have five-member ring structure. While oxazole contains 3 carbon atoms, an oxygen atom, and 1 nitrogen atoms, pyrazole contains 3 carbon atoms and 2 nitrogen atoms. The drug derivatives of oxazole or pyrazole heterocyclic compounds are reported to enhance drug effectivity (Nakamura *et al.*, 2003; Nawwar *et al.*, 1994). Therefore, our present study was focused to evaluate the anticancer effects of pongamol and its oxazole and pyrazole derivatives. Herein, we report the synthesis and anticancer activity of the pongamol and its derivatives using three human tumor cell lines, neuroblastoma (IMR-32), T-lymphocytes (Jurkat) and cervical cancer cells (HeLa). The rationale to select different cell types was to examine the unique feature and identify the potential target molecule or pathway in cancer cells.

Chemistry

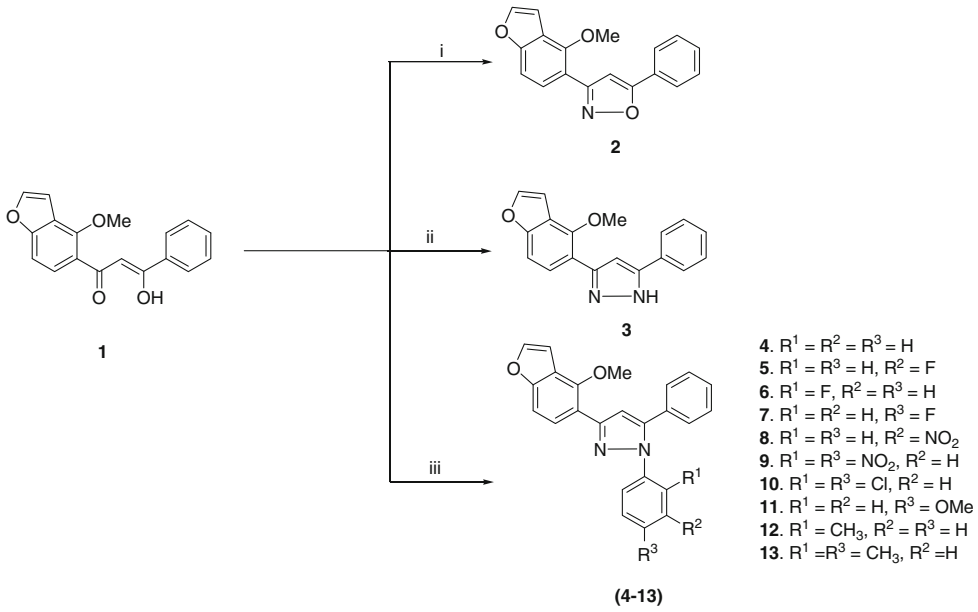
Results and discussion

The designed analogues (**2**–**13**) were synthesized through an acid-catalyzed condensation with hydroxyl amine hydrochloride and various substituted hydrazine hydrochlorides (Scheme 1). The isoxazole derivative (**2**) of pongamol was synthesized by treatment of pongamol with hydroxylamine hydrochloride in acetic acid at 85°C for 4 h in acetic acid. Pyrazole derivatives of pongamol (**3**–**12**) were prepared by heating pongamol (**1**) with various hydrazines for 8 h in acetic acid [hydrazine hydrate for (**3**), phenylhydrazine for (**4**), 3-fluorophenylhydrazine hydrochloride for (**5**), 2-fluorophenylhydrazine hydrochloride for (**6**), 4-fluorophenylhydrazine hydrochloride for (**7**), 3-nitrophenylhydrazine hydrochloride for (**8**), 2,4-dinitrophenylhydrazine hydrochloride for (**9**), 2,4-dichlorophenylhydrazine hydrochloride for (**10**), 4-methoxyphenylhydrazine hydrochloride for (**11**), 2-methylphenylhydrazene hydrochloride for (**12**), and 2,4-dimethyl phenyl hydrazine for (**13**). All the synthesized derivatives were well characterized by using ^{1}H NMR, ^{13}C NMR, and HRESI–MS analyses.

Biological activity

Discussion

The IMR-32 cells (1×10^{6} cells/ml) were treated with varying concentrations of pongamol (**1**) and its synthetic derivatives (**2**–**13**) ranging from 20 to 200 μM for 16 h, and analyzed by MTT assay. The compounds **1**, **4**, **10**, **11**, **13** showed no significant change in cytotoxicity with increased drug concentrations. However, compounds **2**, **3**, **5**, **6**, **7**, **8**, **9**, **12** showed dose-dependent enhancement of cytotoxicity. Among compounds that showed dose-dependent



Scheme 1 Synthesis of pongamol derivatives. Reagents and conditions: (i) Hydroxylamine hydrochloride, Acetic acid, 85°C, 4 h. (ii) hydrazine hydrate. (iii) phenylhydrazine; 3-fluorophenylhydrazine hydrochloride; 2-fluorophenylhydrazine hydrochloride; 4-fluorophenylhydrazine hydrochloride; 3-nitrophenylhydrazine hydrochloride; 2,4-dinitrophenylhydrazine hydrochloride; 2,4-dichlorophenylhydrazine hydrochloride; 4-methoxyphenylhydrazine hydrochloride; 2-methylphenylhydrazene hydrochloride; 2,4-dimethylphenylhydrazene hydrochloride. Acetic acid/8 h, reflux

cytotoxicity, compounds **2**, **5**, and **6** induced a significant dose-dependent cytotoxicity that has reached to a maximum of 80% and minimum of 60% at higher concentrations (Fig. 1a).

Effective anticancer treatments are aimed either to induce cytostasis or apoptosis, therefore drug-induced cell cycle arrest is a pre-requisite for energy-dependent cell killing (Sreedhar *et al.*, 2004; Sreedhar and Csermely, 2004). A dose-dependent cytotoxicity of tumor cells induced by the pongamol (**1**) and its derivatives (**2–13**) therefore suggested a possible cytostatic effect. The IMR-32 tumor cells after drug treatments were analyzed by FACS (fluorescence activated cell sorting), none of the compounds including **2**, **5**, and **6** showed drug-induced cell cycle arrest. However, there is a significant decrease in the cell number in the G1 phase of cell cycle in cells treated with compounds **2**, **5**, **6**, and **12** and the massive decrease in cell number may be related to increased cytotoxicity (Fig. 1b). Interestingly, none of these drugs were also found to be effective even to induce G2-M cell cycle block, on the other hand compounds **1**, **2**, and **5** showed a decrease in G2-M population (Fig. 1c).

To examine whether the pongamol and its derivative drugs-induced effects are cell type specific or not, jurkat and HeLa cancer cells were treated with 200 μM concentration of compounds **1**, **2**, **5**, and **6** compounds similar to IMR-32 and the cytotoxicity was assessed. Unlike IMR-32 which showed 38, 58, 86, and 71 cytotoxicity for compounds **1**, **2**, **5**, and **6**, HeLa showed 23, 26, 28, and 35 and Jurkat showed 17, 31, 35, 45 for the same compounds (Fig. 2a). Although compared to IMR-32 cells, HeLa and Jurkat showed lesser cytotoxicity, it was found to be significant and concentration dependent (data not shown). Cell cycle analysis of HeLa and Jurkat cells both showed drug-induced decrease in cells in both G1 and G2-M phase of cell cycle, which once again may relate to increase in cytotoxicity upon drug treatments (Fig. 2b, c).

Protein kinases are identified as signatures of cancer and blocking the mitogen signaling molecules has emerged as a potential anticancer strategy (Sreedhar *et al.*, 2004; Noble *et al.*, 2004; Gunby *et al.*, 2007). Pongamol (**1**) and its derivatives **2**, **5**, and **6** when tested for anti tyrosine kinase activity failed to show such activity (data not presented), for this reason we examined the effect of these compounds on mitogen signal kinase, ERK1 by immunoblotting. While compound (**2**) was effective in destabilizing the ERK1 in Jurkat, HeLa, and IMR-32, compound (**5**) was effective against IMR-32, and compound (**6**) was effective against HeLa cells. Similarly, targeting the cell cycle using selective cell cycle inhibitor drugs is suggested to be effective anticancer treatment both as single treatment as well as combinatorial drug treatment (Dancey and Sausville, 2003; Lapenna and Giordano, 2009; Malumbres and Barbacid, 2009). Compounds **2**, **5**, and **6** are found to be effective in destabilizing CDK2 in all the three cancer types, Jurkat, HeLa and IMR-32. Since endoplasmic reticulum (ER) stress induces and sensitizes cells for cell cycle independent cytotoxicity (Miki *et al.*, 2009), we found compounds, **1** and **5** induces ER stress which was evidenced by the induction of Grp94 (Fig. 3).

Compared to normal cells, tumor cells are always exposed to high selection pressure and constant stress conditions therefore anticancer drugs that can target either cell cycle or mitogen signaling can be effective in combating cancer (Sreedhar and Csermely, 2004). However, we have examined for effect of all Pongamol (**1**) and its derivatives (**2–13**) in normal fibroblast cells, and found that these drugs do not show any growth inhibitory or cytotoxic effects (data not presented). We are also evaluating the cytotoxic effects of these drugs in primary cultures.

Conclusion

In conclusion, we show that among different oxazole and pyrazole derivatives, the oxazole compound (**2**), pyrazole derivatives with fluorine positional variance **5** and **6** showed enhanced cytotoxicity. Among the Pongamol (**1**) derivatives, compounds **2**, **5**, and **6** though showed differential sensitivity in different cell types in targeting the ERK1 signaling, however, were all effective in targeting CDK2, suggesting a potential role for these drugs in targeting the cell cycle.

Experimental

General

IR spectra were recorded on Nicolet-740 spectrometer with KBr Pellets. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, using TMS as internal standard. The chemical shifts are expressed as (δ) values in parts per million (ppm) and the coupling constants (J) are given in hertz (Hz). Spin multiplicities are described as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were measured on LC-MSD-Trap-SL instrument. Melting points were recorded on Electrothermal 9100 and are uncorrected. Most of the reaction solvents were purified by distillation under nitrogen from the indicating drying agent and used fresh, dichloromethane (calcium hydride), acetone (potassium permanganate). Column chromatography was carried out using silica gel 100–200 mesh (Acme Silica gel) and precoated silica gel plates (Merck, 60 F254) were used for preparative TLC.

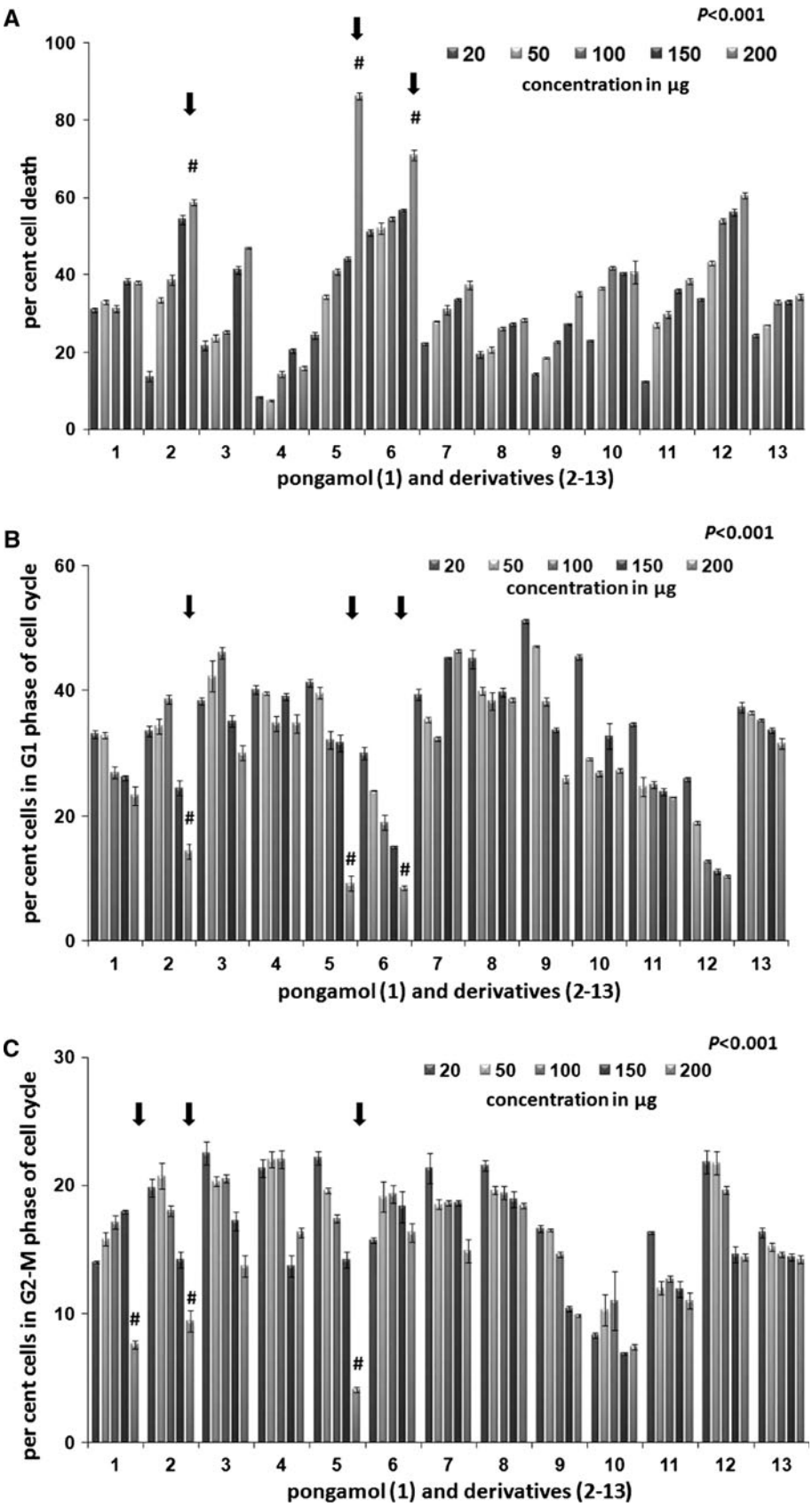


Fig. 1 **a** The IMR32 cells showing the percent cell death induced by pongamol (**1**) and its derivatives (**2**–**13**). Untreated and DMSO-treated cells were used for normalization. **b** Effect of pongamol (**1**) and its derivatives (**2**–**13**) on G1 phase cell cycle arrest of IMR32 cells. Control and DMSO-treated cells were used for reference and compared with drug-induced cytostatic effect. **c** Effect of pongamol (**1**) and its derivatives (**2**–**13**) on G2-M phase cell cycle arrest of IMR32 cells. Control and DMSO-treated cells were used for reference and compared with drug-induced cytostatic effect

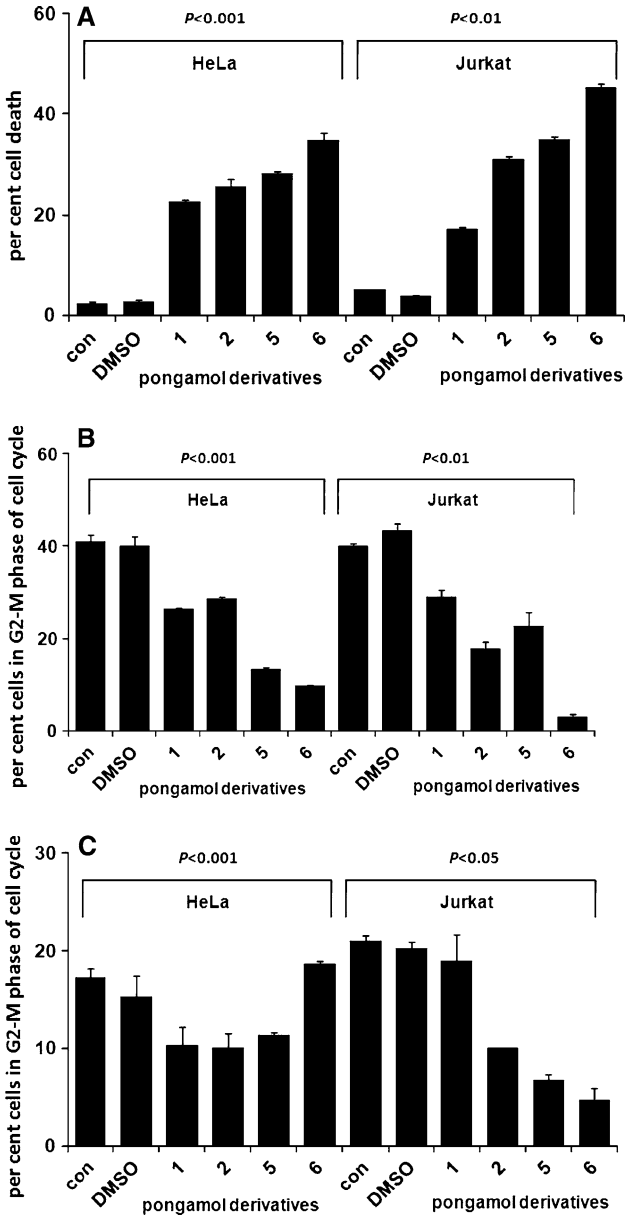


Fig. 2 **a** Cytotoxicity of pongamol (**1**) and its derivatives (**2**, **5**, and **6**) on HeLa and Jurkat tumor cells. Untreated and DMSO-treated cells were used for normalization. **b** Effect of pongamol (**1**) and its derivatives (**2**, **5**, and **6**) on G1 phase cell cycle arrest of HeLa and Jurkat cells. Control and DMSO-treated cells were used for reference and compared with drug-induced cytostatic effect. **c** Effect of pongamol (**1**) and its derivatives (**2**, **5**, and **6**) on G2-M phase cell cycle arrest of HeLa and Jurkat cells. Control and DMSO-treated cells were used for reference and compared with drug-induced cytostatic effect

Extraction and isolation of pongamol from the roots of *Derris indica*

The air dried and powdered roots of *D. indica* (5 kg) were extracted three times with DCM/MeOH (1:1) for 48 h. The combined extracts were concentrated under vacuum.

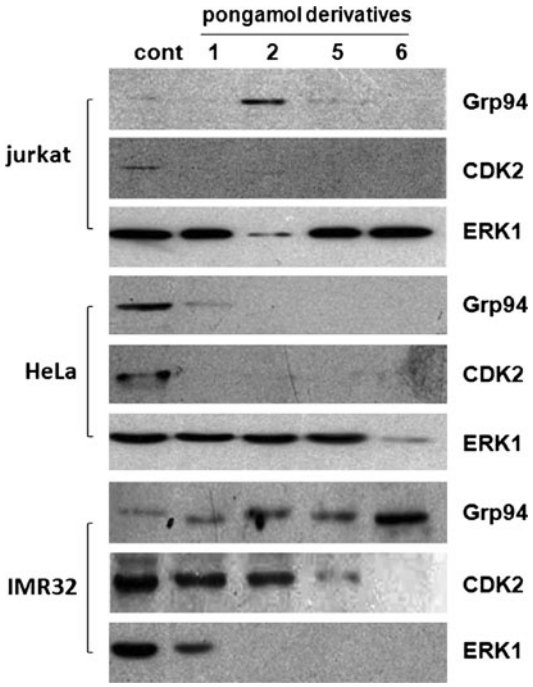


Fig. 3 Immunoblot analysis of grp94, cdk1, and ERK1 proteins from the cell lysates made from pongamol (1) and its derivatives (**2**, **5**, and **6**) treated Jurkat, HeLa, and IMR32 cells

Portion of active DCM/MeOH (1:1) extract (50 g) was subjected to column chromatography (silica gel, 100–200 mesh) using step gradient of hexane, EtOAc, acetone, $CHCl_3$, MeOH to yield six major fractions (F1–F6). Fraction F1 was subjected to repeated silica gel (100–200 mesh) column chromatography (CC) by eluting with EtOAc/hexane (10:90) to yield 1.23 g of pongamol.

General procedure for the preparation of (3-(4-methoxybenzofuran-5-yl)-5-phenylisoxazole (**2**)

The oxazole analogue was prepared by treating pongamol 0.030 g (1 mmol) with hydroxylamine hydrochloride, 0.008 g (1.2 mmol), in acetic acid at 85°C for 4 h (Scheme 1). The reaction mixture was evaporated to dryness; residue was dissolved in dichloromethane and washed with water. The crude product was purified by column chromatography (hexane: ethyl acetate = 95:5) to afford a colorless solid (0.020 g, 72%); mp: 105–106°C; IR (KBr): 1728, 1605, 1468, 1344, 1286, 1235, 1066, 961, 764 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85 (m, H-2 & H-6), 7.64 (d, $J = 8.7$ Hz, H-6′), 7.60 (d, $J = 1.5$ Hz, H-2″), 7.44–7.36 (m, H-3 & H-5′), 7.33–7.26 (m, H-4 & H-5), 6.97 (s, H-3″), 6.87 (s, H-α), 4.17 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7, 156.5, 162.7, 150.1, 144.6, 133.4, 133.8, 128.6, 127.7, 125.6, 124.5, 114.4, 107.4, 104.9, 100.1, 60.4; HRESI–MS calcd for $C_{18}H_{14}N_2O_2$ $[M+H]^+$ 291.0892, found 291.0894.

General procedure for the preparation of *N*-(substituted) phenyl pongamol pyrazole compounds (**3–13**)

Pongamol (0.030 g, 1 mmol) was dissolved in glacial acetic acid (5 ml), and different hydrazine derivatives (1.2 mmol) (hydrazine hydrate, phenylhydrazine, 3-fluoro phenylhydrazine hydrochloride, 2-fluorophenylhydrazine hydrochloride, 4-fluoro phenylhydrazine hydrochloride, 3-nitrophenylhydrazine hydrochloride, 2,4-dinitrophenylhydrazine hydrochloride, 2,4-dichlorophenylhydrazine hydrochloride, 4-methoxyphenylhydrazine hydrochloride, 2-methylphenylhydrazene hydrochloride, 2,4-dimethylphenylhydrazene hydrochloride at 85°C) were added to the solution. The solution was refluxed for 8 h, and then the solvent was removed in vacuo. Residue was dissolved in ethyl acetate and washed with water. Organic portion was collected, dried over sodium sulfate, and concentrated in vacuo. Crude product was further purified by column chromatography. All the products (**3–13**) were prepared by using the same procedure.

3-(4-Methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**3**)

Isolated as a colorless solid (0.022 g, 75%); mp: 129°C; IR (KBr): 3283, 1725, 1656, 1598, 1545, 1496, 1345, 1284, 1230, 1065, 956, 716 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, $J = 8.7$ Hz, H-6′), 7.91–7.83 (m, H-2 & H-6), 7.60 (d, $J = 1.9$ Hz, H-2″), 7.52–7.40 (m, H-3, H-4 & H-5), 7.32 (d, $J = 8.7$ Hz, H-5′), 7.02 (s, H-α), 6.99 (br s, H-3″), 4.21 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.9, 162.9, 157.7, 150.9, 144.4, 129.7, 128.9, 128.8, 126.8, 125.7, 124.1, 107.0, 106.7, 105.2, 104.9, 100.6, 100.2, 59.8; HRESI–MS calcd for $C_{18}H_{14}N_2O_2$ $[M+H]^+$ 291.1128, found 291.1126.

3-(4-Methoxybenzofuran-5-yl)-1,5-diphenyl-1*H*-pyrazole (**4**)

Isolated as a colorless solid. Yield (0.024 g, 65%); mp: 156–157°C; IR (KBr): 3285, 3237, 1657, 1545, 1497, 1437, 1371, 1303, 1239, 997, 755 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.93–7.88 (m, H-2 & H-6), 7.55 (d, $J = 2.3$ Hz, H-2″), 7.43–7.15 (m, H-6′, H-5′, H-3, H-4, H-5, & 5–Ar–H), 6.84 (d, $J = 2.0$ Hz, H-3″), 6.74 (s, H-α), 3.62 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.3, 151.6, 150.8, 144.2, 141.3, 140.8, 133.2, 128.6 (3C), 128.5 (3C), 127.7, 127.3, 126.7, 125.7 (2C), 123.6 (2C), 106.4, 105.9, 105.0, 59.2; HRESI-MS calcd for $C_{24}H_{18}N_2O_2$ $[M+H]^+$ 367.1441, found 367.1443.

1-(3-Fluorophenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**5**)

Isolated as a yellow solid. Yield (0.025 g, 65%); mp: 138°C; IR (KBr): 1691, 1612, 1492, 1462, 1344, 1231, 1188, 1150, 966, 869, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.90–6.82 (m, H-2 & H-6), 7.57 (br s, H-2″), 7.46–7.01 (m, H-6′, H-3, H-4, H-5 & 4-Ar–H), 6.88 (m, H-3″ & H-5′), 6.73 (s, H-α), 3.67 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.2, 160.9, 157.5, 152.0, 150.9, 144.3, 142.6, 141.3, 133.1, 129.6, 129.4, 128.6, 128.0, 127.2, 125.8, 118.7, 115.4, 113.5, 113.2, 111.2, 110.9, 106.9, 106.2, 105.1, 59.2; HRESI-MS calcd for $C_{24}H_{17}FN_2O_2$ $[M+H]^+$ 385.1347, found 385.1356.

1-(2-Fluorophenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**6**)

Isolated as a yellow solid. Yield (0.023 g, 60%); mp: 135–136°C; IR (KBr): 1596, 1556, 1509, 1463, 1345, 1236, 1067, 963, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, $J = 8.6$ Hz, H-6′), 7.61–7.55 (m, H-2″ & H-5′), 7.39–7.21 (m, H-2, H-3, H-4, H-5, H-6 & 3-Ar–H), 7.11–7.04 (m, H-α & Ar–H), 6.94 (dd, $J = 2.2$, 0.9 Hz, H-3″), 4.08 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.2, 157.1, 154.8, 152.4, 151.0, 144.1, 142.9, 133.2, 129.3, 129.2, 128.8, 128.5, 127.8, 127.0, 125.9, 124.0, 118.6, 116.6, 116.3, 115.3, 106.0, 105.6, 105.0, 59.6; HRESI-MS calcd for $C_{24}H_{17}FN_2O_2$ $[M+H]^+$ 385.1347, found 385.1351.

1-(4-Fluorophenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**7**)

Isolated as a yellow solid. Yield (0.026 g, 68%); mp: 139–140°C; IR (KBr): 1641, 1554, 1511, 1465, 1346, 1223, 1151, 1065, 966, 840, 766 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, $J = 8.4$ Hz, H-6′), 7.57 (d, $J = 2.0$ Hz, H-2″), 7.40–7.22 (m, H-2, H-3, H-4, H-5, H-6, 4 Ar–H), 7.08–7.00 (m, H-5′ & H-α), 6.95 (s, H-3″), 4.09 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.4, 156.7, 144.3, 144.0, 128.8, 128.6, 128.5, 128.2, 127.8, 127.3, 126.9, 126.8, 125.8, 125.5, 125.4, 125.3, 115.8, 115.5, 115.2, 108.8, 107.0, 106.4, 106.2, 105.1, 104.9, 60.4, 59.3; HRESI-MS calcd for $C_{24}H_{17}FN_2O_2$ $[M+H]^+$ 385.13547, found 385.1354.

3-(4-Methoxybenzofuran-5-yl)-1-(3-nitrophenyl)-5-phenyl-1*H*-pyrazole (**8**)

Isolated as a yellow viscous liquid. Yield (0.018 g, 45%); IR (KBr): 1659, 1588, 1525, 1483, 1353, 1254, 1151, 1037, 943, 735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.36 (t, $J = 2.2$ Hz, Ar–H). 8.05 (qd, $J = 1.1$, 2.0, 3.0 Hz, Ar–H), 7.92 (m, H-2 & H-6), 7.60 (m, H-2″ & Ar–H), 7.47–7.30 (m, H-3, H-4, H-5, 2 Ar–H), H-6′ & H-5′), 6.86 (dd, $J = 2.2$, 0.9 Hz, H-3″), 6.77 (s, H-α), 4.10 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.9, 151.4, 150.4, 148.5,

144.0, 143.7, 141.2, 130.5, 129.8, 129.3, 128.9, 128.8, 125.5, 121.2, 119.6, 118.2, 110.1, 107.1, 104.9, 60.4; ESI-MS m/z $[M + H]^+$ 411.4.

1-(2,4-Dinitrophenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**9**)

Isolated as a yellow viscous liquid. Yield (0.018 g, 40%); IR (KBr): 1605, 1535, 1500, 1465, 1343, 1238, 1065, 963, 741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.68 (d, $J = 2.0$ Hz, Ar–H). 8.23 (dd, $J = 8.8$, 2.2 Hz, Ar–H), 7.82 (m, H-2 & H-6), 7.59 (d, $J = 2.0$, H-2″), 7.42–7.23 (m, Ar–H, H-6′, H-5′, H-3, H-4 & H-5), 6.94 (br s, H-3″), 6.82 (s, H-α), 3.75 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 154.2, 145.4, 144.8, 144.1, 142.7, 138.7, 132.0, 129.2, 129.0, 128.7, 127.5, 127.0, 126.4, 126.0, 125.5, 120.7, 113.9, 110.2, 107.7, 107.2, 107.1, 104.8, 60.0; HRESI-MS calcd for $C_{24}H_{16}N_4O_6$ $[M + H]^+$ 457.1143, found 457.1122.

1-(2,4-Dichlorophenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**10**)

Isolated as a yellow viscous liquid. Yield (0.024 g, 55%); IR (KBr): 1597, 1492, 1469, 1346, 1235, 1066, 969, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.92 (m, H-2 & H-6), 7.53 (d, $J = 2.2$ Hz, H-2″), 7.48–7.37 (m, H-6′, H-3, H-4, H-5, Aromatic-1H),7.18 (m, H-5′ & 2-Ar–H), 6.96 (d, $J = 2.2$ Hz, H-3″), 6.77 (s, H-α), 3.85 (s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.2, 156.6, 145.4, 144.2, 144.0, 143.2, 137.0, 132.9, 129.9, 128.5, 128.4, 128.2, 127.9, 127.7, 125.7, 125.3, 119.4, 117.8, 114.4, 107.2, 106.8, 105.8, 105.0, 104.8, 60.3; HRESI-MS calcd for $C_{24}H_{16}Cl_2N_2O_2$ $[M+H]^+$ 435.0662, found 435.0665.

3-(4-Methoxybenzofuran-5-yl)-1-(4-methoxyphenyl)-5-phenyl-1*H*-pyrazole (**11**)

Isolated as a yellow viscous liquid. Yield (0.014 g, 35%); IR (KBr): 1596, 1466, 1346, 1242, 1068, 910, 802, 765 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (m, H-2 & H-6′), 7.56 (d, $J = 2.0$ Hz, H-2″), 7.18–7.43 (m, H-5′, H-2, H-3, H-4, H-5, H-6 & 2 Ar–H), 6.86 (d, $J = 2.0$ Hz, H-3″), 6.77 (d, $J = 8.8$ Hz, 2 Ar–H), 6.72 (s, H-α), 3.76 (s, OMe), 3.69 (s, OMe); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 144.1, 134.4, 133.5, 128.5, 127.6, 127.5, 125.8, 125.2, 113.7, 106.1, 106.0, 105.1, 59.5, 55.2; ESI-MS m/z $[M+H]^+$ 397.6.

3-(4-Methoxybenzofuran-5-yl)-5-phenyl-1-o-tolyl-1*H*-pyrazole (**12**)

Isolated as a yellow viscous liquid. Yield (0.016 g, 42%); IR (KBr): 1599, 1500, 1414, 1241, 1186, 1147, 1065, 969, 764 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.89 (d, $J = 8.6$ Hz, H-6′), 7.56 (d, $J = 1.8$ Hz, H-2″), 7.35–7.16 (m, H-5′, H-2, H-3, H-4, H-5, H-6 & 4 Ar–H), 7.09 (s, H-α), 6.95 (d, $J = 1.8$ Hz, H-3″), 4.09 (s, OMe), 2.07 (s, Ar–C$\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.6, 148.9, 151.4, 144.6, 143.9, 139.8, 135.7, 131.0, 130.7, 128.7, 128.4, 127.9, 126.5, 125.6, 119.7, 119.1, 107.0, 106.6, 104.8, 60.4, 29.8; HRESI-MS calcd for $C_{25}H_{20}N_2O_2$ $[M+H]^+$ 381.1596, found 381.1598.

1-(2,4-Dimethylphenyl)-3-(4-methoxybenzofuran-5-yl)-5-phenyl-1*H*-pyrazole (**13**)

Isolated as a yellow viscous liquid. Yield (0.025 g, 57%); IR (KBr): 1596, 1491, 1468, 1345, 1235, 1066, 968, 765 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.91 (m, H-2 & H-6), 7.52 (d, $J = 2.2$ Hz, H-2″), 7.36–7.47 (m, H-6′, H-3, H-4, H-5, Ar–H),7.18 (m, H-5′ & 2 *Ar–H), 6.96 (d, $J = 2.2$ Hz, H-3″), 6.77 (s, H-α), 3.85 (s, OMe), 2.08 (s, Ar–C$\underline{H}_3$), 2.09 (s, Ar–C$\underline{H}_3$). ESI-MS m/z $[M + H]^+$ 394.1.

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References

Carcahe-Blanco EJ, Kang A, Park EJ, Su B, Kardono LBS, Riswan S, Fong HHS, Pezzuto JM, Kinghorn AD (2003) Constituents of the bark of *Pongamia pinnata* with the potential to induce quinone reductase. J Nat Prod 66:1197–1202

Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100:72–79

Cragg GM, Grothaus PG, Newman DJ (2009) Impact of natural products on developing new anti-cancer agents. J Chem Rev 109:3012–3043

Dancey J, Sausville EA (2003) Issues and progress with protein kinase inhibitors for cancer treatment. Nat Rev Drug Discov 2:296–313

Gunby RH, Sala E, Tartari CJ, Puttini M, Gambacorti-Passerini C, Mologni L (2007) Oncogenic fusion tyrosine kinases as molecular targets for anti-cancer therapy. Anticancer Agents Med Chem 7:594–611

Gupta M, Shaw BP (2009) Uses off medicinal plants in panchakarma ayurvedic therapy. Indian J Tradit Knowl 8:372–378

Koehn FE, Carter GT (2005) The evolving role of natural products in drug discovery. Nat Rev Drug Discov 4:206–220

Lapenna S, Giordano A (2009) Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov 8:547–566

Lloyd DG, Golfis G, Knox AJS, Fayne D, Meegan MJ, Oprea TI (2006) Oncology exploration: charting cancer medicinal chemistry space. Drug Discov Today 11:149–159

Malumbres M, Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev 9:153–166

McCubrey JA, Steelman LS, Abrams SL, Lee JT, Chang F, Bertrand FE, Navolanic PM, Terrian DM, Franklin RA, D'Assoro AB, Salisbury JL, Mazzarino MC, Stivala F, Libra M (2006) Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in

malignant transformation and drug resistance. Adv Enzyme Regul 46:249–279

Miki T, Miura T, Hotta H, Tanno M, Yano T, Sato T, Terashima Y, Takada A, Ishikawa S, Shimamoto K (2009) Endoplasmic reticulum stress in diabetic hearts abolishes erythropoietin-induced myocardial protection by impairment of phospho-glycogen synthase kinase-3beta-mediated suppression of mitochondrial permeability transition. Diabetes 58:2863–2872

Nakamura T, Sato M, Kakinuma H, Miyata N, Taniguchi K, Bando K, Koda A, Kameo K (2003) Pyrazole and isoxazole derivatives as new, potent, and selective 20-hydroxy-5,8,11,14-eicosatetraenoic acid synthase inhibitors. J Med Chem 46:5416–5427

Nawwar GA, Swellem RH, Ibrahim AM (1994) Oxazole, pyrazole and piperidine derivatives having an *o*-hydroxyaryl moiety with anticipated molluscicidal activity. Arch Pharm Res 17:66–70

Newman DJ (2008) Natural products as leads to potential drugs: an old process or the new hope for drug discovery? J Med Chem 51:2589–2599

Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 66:1022–1037

Noble MEM, Endicott JA, Johnson LN (2004) Protein kinase inhibitors. Science 303:1713–1928

Pechan PM (1991) Heat shock proteins and cell proliferation. FEBS Lett 280:1–4

Rao RR, Tiwari AK, Reddy PP, Babu KS, Ali AZ, Madhusudana K, Rao JM (2009) New furanoflavanoids, intestinal alpha-glucosidase inhibitory and free-radical (DPPH) scavenging activity from antihyperglycemic root extract of *Derris indica* (Lam.). Bioorg Med Chem 17:5170–5175

Shewach DS (2009) Introduction to cancer chemotherapeutics. Chem Rev 109:2859–2861

Sreedhar AS, Csermely P (2004) Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy. A comprehensive review. Pharmacol Ther 101:227–257

Sreedhar AS, Soti C, Csermely P (2004) Inhibition of Hsp90: a new strategy for inhibiting protein kinases. Biochim Biophys Acta 1697:233–242