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Research Article

Antiproliferative Diarylpyrazole Derivatives as Dual Inhibitors of the ERK Pathway and COX-2

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A series of 3,4-diarylpyrazole-1-carboxamide derivatives was designed and synthesized. A selected group of the target compounds was tested for *in vitro* antiproliferative activities over a panel of 60 cancer cell lines at the National Cancer Institute (NCI, Bethesda, MD, USA) at a single-dose concentration of 10 μM, and the four most active compounds 9a, 9l, 9n, and 10o were further tested in a five-dose testing mode to determine their IC_{50} values over the 60 cell lines. In addition, a selected group of target compounds were tested for inhibitory effect over cyclooxygenase isozymes. Compounds 9a, 9l, 9n, and 10o were also tested for MEK and ERK kinase inhibitory activity using Western blot assay. Compound 10o was selective toward melanoma cell line subpanel, and its antiproliferative activity may be attributed to selective cyclooxygenase-2 inhibition and ERK pathway inhibition.

Key words: 3,4-diarylpyrazole-1-carboxamide, anticancer, cyclooxygenase-2, ERK pathway, pyrazole, vicinal diaryl heterocycle

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Cancer is a major leading cause of death worldwide. 577 190 patients with cancer died, and more than 1.6 million new cancer cases were identified in 2012 only in USA according to the American Cancer Society report.a More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to exceed 13 million in 2030 according to the World Health Organization report.b Despite the extensive efforts and investment in research, the management of human malignancies still constitutes a major challenge for contemporary medicinal chemistry. There has been an urgent need for development of more efficient anticancer agents with minimal side-effects.

The RAS–RAF–MEK–ERK signaling pathway (ERK pathway) plays an important role in tumorigenesis and cancer progression. Abnormal activation of ERK pathway has been linked to uncontrolled cell proliferation, increased cell survival, and tumor progression (1). Sorafenib (Nexavar®, Figure 1), a diarylurea derivative, targets ERK pathway. It inhibits basal phosphorylation of ERK in numerous cancer cell lines *in vitro*, including melanoma cell lines (2). Sorafenib is also a well-known inhibitor of B-RAF. Dysregulated signaling through RAF kinase isoforms has been detected in ~30% of human cancers (3). Constitutive B-RAF activity can be caused by activating oncogenic mutations, such as B-RAF V600E mutation (4). Much interest has been focused on modulation of ERK pathway as a potential strategy for anticancer agents.

Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in the conversion of arachidonic acid to prostaglandins and other eicosanoids. Cyclooxygenase-2 and its product, prostaglandin E_2 (PGE_2), play a crucial role in tumor microenvironment (5). Several reports have shown that PGE_2 and COX-2 have a wide range of effects including induction of cellular proliferation, promotion of angiogenesis, promotion of cancer cell resistance to apoptosis, stimulation of tumor invasion, and suppression of immune responses (6,7). Molecular pathology studies have revealed that COX-2 is overexpressed in cancer and stroma cells during tumor progression, and anticancer chemoradiotherapies induce expression of COX-2 in cancer cells (8). Cyclooxygenase-2 has been proven to be overexpressed in several types of human cancer including melanomas (9–12), colon (13), non-small cell lung (14), intestinal (15), colorectal (16), pancreatic (17), cervical (18), breast (19), endometrial (20), laryngeal (21), papillary thyroid (22), and gastric (23) cancers. Much attention has been focused on COX-2 inhibitors as a beneficial avenue for cancer chemotherapy (7,8). Several

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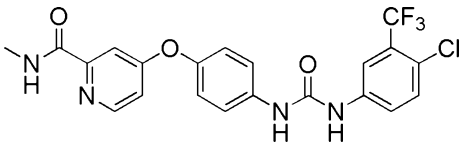


Figure 1: Structure of sorafenib.

experimental and clinical studies have established potent anticancer activity of COX-2 inhibitors such as celecoxib.

Targeting both COX-2 and ERK pathway simultaneously has been reported to produce synergistic antiproliferative activity. ERK1/2 activation or phosphorylation was reported to increase COX-2 protein expression in some cancer cells treated with selective COX-2 inhibitor NS398. The combination of selective COX-2 inhibitor NS398 and MEK inhibitor U0126 produced a synergistic antiproliferative effect at cellular level. Both phosphorylated ERK1/2 and COX-2 protein expression were concentration dependently decreased by combined treatment. Moreover, the combination of selective COX-2 inhibitor and ERK1/2 inhibitor could enhance apoptosis over that by individual treatment (24).

In the present investigation, 54 final compounds were designed and synthesized in rational to the potent inhibitors of ERK pathway A (25), B (26), and C (27) (Figure 2). The target compounds **9a–r**, **10a–r**, **20a–i**, and **21a–i** were designed by introduction of derivatives of the carboxamide side chain of compound B into position 1 of the pyrazole ring of compound A. The phenolic hydroxyl group of A was retained in compounds **10a–r** and **21a–i** and modified into methoxy group in compounds **9a–r** and **20a–i** to examine its effect on the activity. In addition, the furan ring of the lead compound C was isosterically replaced with pyrazole, and the carboxamide chain was modified (Figure 2). The presence of a vicinal diaryl heterocyclic system, 3,4-diarylpyrazole scaffold, can confer COX-2 inhibitory activity of the target compounds, similar to celecoxib (Figure 3). We have previously reported their antiproliferative activity against A375P human melanoma cell line (28–30). Herein, we report their *in vitro* antiproliferative activities against National Cancer Institute (NCI)-60 cancer cell line panel of 9 different cancer types, MEK/ERK kinase inhibitory activity, and their inhibitory effect on cyclooxygenase isozymes: COX-2 and COX-1. The target compounds were rationally designed to

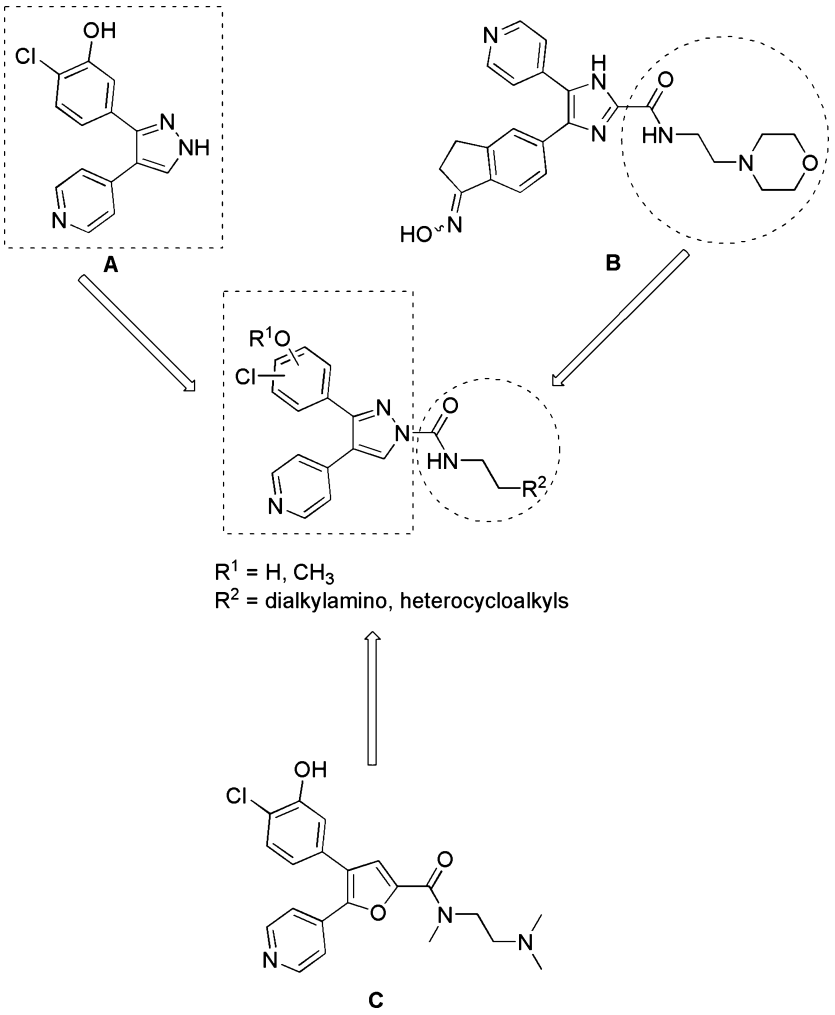


Figure 2: Rational design of the target 3,4-diarylpyrazole-1-carboxamide derivatives.

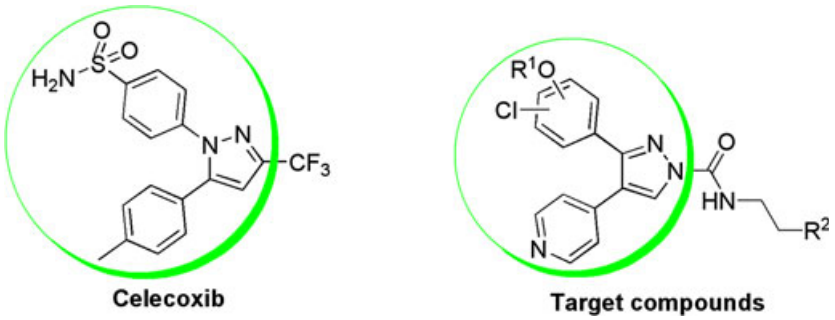


Figure 3: Structures of celecoxib and the target compounds. The vicinal diaryl heterocycle scaffold is essential for cyclooxygenase-2 (COX-2) inhibition.

possess dual mechanisms of antiproliferative activity through COX-2 inhibition and targeting RAS-RAF–MEK–ERK pathway.

Experimental

Synthesis of the target compounds

They were synthesized by the previously reported methods (28–30).

NCI-60 cancer cell line screening

Screening against a panel of 60 cancer cell lines of nine different cancer types was carried out at the NCI, Bethesda, MD, USA,c applying the standard protocol of the NCI.d Sulforhodamine B colorimetric assay method was utilized (31).

Kinase screening

Immunoblot analysis

For immunoblotting, A375P melanoma cells grown to 70–80% confluence were harvested in RIPA lysis buffer and disrupted by sonication and centrifuged at 12 000 rpm for 15 min. The quantity of protein was determined with DC protein assay kit (Bio-Rad Lab., Hercules, CA, USA). Protein samples were subjected to SDS–PAGE and immunoblotted with the appropriate primary antibody overnight at 4 °C. The protein bands were visualized using chemiluminescence detection kit (Amersham HRP Chemiluminescent Substrates; Amersham Biosciences, Piscataway, NJ, USA) after hybridization with the HRP-conjugated secondary antibody from rabbits or mice. The LAS4000-mini (Fujifilm, Tokyo, Japan) was used for chemiluminescence detection.

Compound treatment in A375P cells

To assess the effect of the target compounds on the RAF-1/MEK/ERK signaling pathway, A375P cells were treated with the tested compounds and sorafenib in a dose-dependent way (1, 3, and 10 μM) for 24 h and immunoblotted with antibodies against phospho-MEK1/2, ERK1/2, and β-actin, respectively.

In vitro cyclooxygenase inhibition assay

The ability of the test compounds to inhibit bovine COX-1 and COX-2 was determined using an enzyme immunoassay (EIA; kit catalog number 560101; Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay (ACETM competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μL, 0.1 M Tris–HCl, pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of heme (10 μL) was added 10 μL of various concentrations of test drug solutions (0.01, 0.1, 1, 10, and 50 μM in a final volume of 1 mL). These solutions were incubated for a period of 5 min at 37 °C after which 10 μL of arachidonic acid (100 μM) solution was added and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by EIA. This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells because the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody–PG complex bound to a mouse anti-rabbit monoclonal antibody that was previously attached to the well. The plate was washed to remove any unbound reagents, and then, Ellman's reagent, which contains the substrate to acetylcholine esterase, was added to the well. The product of this enzymatic reaction produced a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, was proportional to the amount of PG tracer bound to the well, which was inversely proportional to the amount of PGs present in the well during the incubation: absorbance $\propto$ [bound PG tracer] $\propto 1/PGs$. Per cent inhibition was calculated by the comparison of compound treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration–inhibition response curve (duplicate determinations).

Results and Discussion

Synthesis

Synthesis of the target compounds 9a–r and 10a–r

The pathway for synthesis of compounds **9a–r** and **10a–r** was reported and discussed in details (Scheme 1) (28,29).

Synthesis of the target compounds 20a–i and 21a–i

The pathway for synthesis of compounds **9a–r** and **10a–r** was reported and discussed in details (Scheme 2) (30).

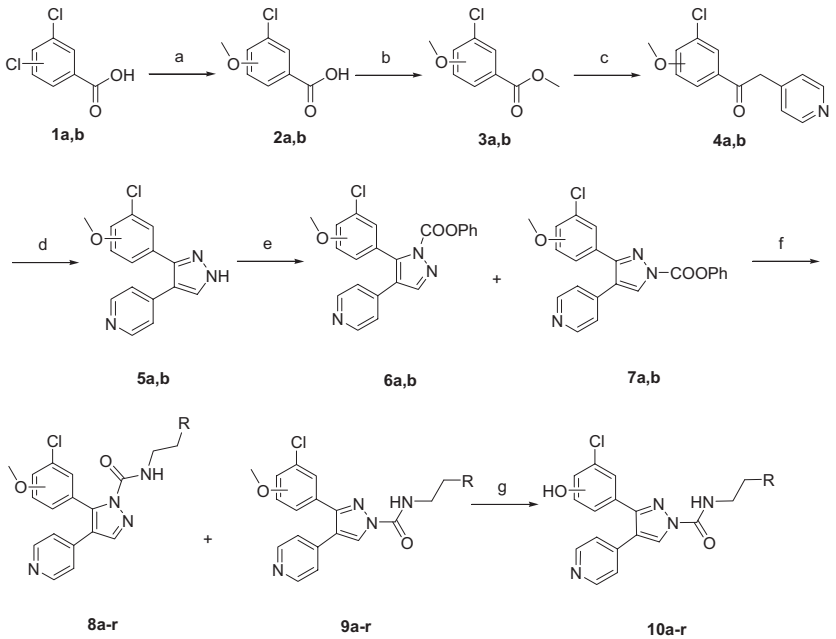
Biological evaluation

Antiproliferative activities of 3,4-diarylpyrazole-carboxamide target compounds against NCI-60 cell line panel

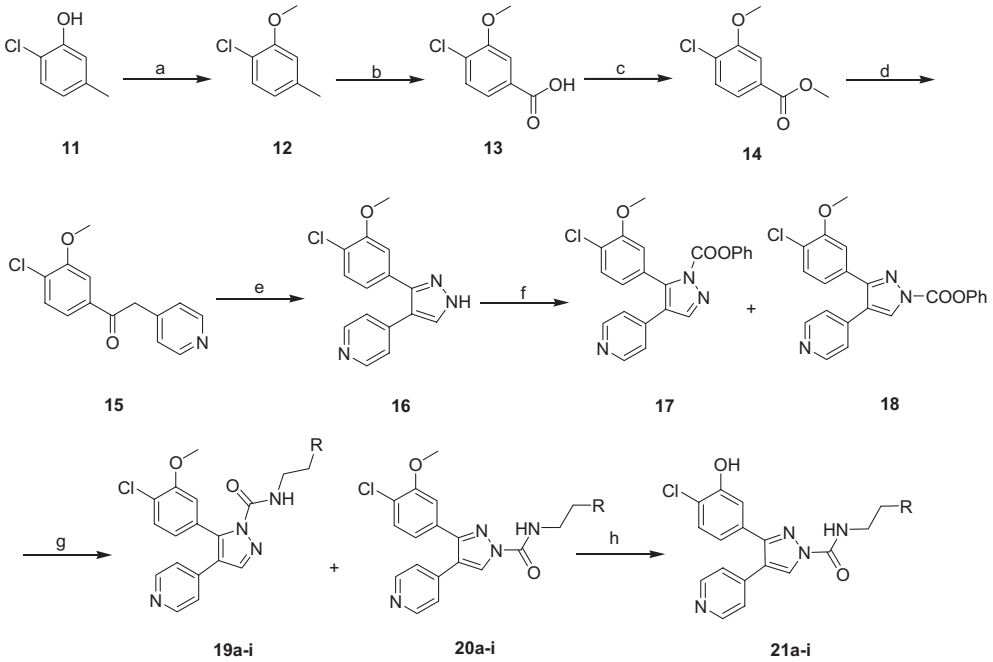
Structures of the target compounds were submitted to NCI, Bethesda, MD, USA,c and the 29 compounds shown in Table 1 were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their antineoplastic activities. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 60 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single-dose concentration of 10 μM, and the percentages of growth inhibition over the 60 tested cell lines were determined. The mean inhibition percentages of all of the tested compounds over the full panel of cell lines are illustrated in Table 1. The results revealed that methoxy derivatives **9j**, **9l**, **9n**, and **20f** showed higher mean%inhibition than the corresponding hydroxyl derivatives **10j**, **10l**, **10n**, and **21f**. In addition, the *para*-chloro compounds were the lowest active compared with *meta*-chloro derivatives. So, *para*-chloro group on the aryl ring at position 3 of the pyrazole moiety is unfavorable for antiproliferative activity of this series of compounds.

The effect of the terminal tertiary amine moiety on the activity was investigated. Compound **9a**, with the highest mean %inhibition, possessing dimethylamino moiety showed higher activity than compounds **9c**, **9d**, **9f**, and **9g** with morpholino, 2′,6′-dimethylmorpholino, *N*-acetylpiperazinyl, and piperidinyl moieties, respectively. In case of 3-chloro-5-methoxyphenyl derivatives **9j–r**, morpholino compound **9l** demonstrated higher mean%inhibition compared with the other analogs. But in cases of 3-chloro-5-hydroxyphenyl and 4-chloro-3-methoxyphenyl analogs, diethyl amino, *N*-methylpiperazinyl, and *N*-acetylpiperazinyl moieties were more favorable for their activities (compounds **10k**, **10n**, **10o**, **20b**, **20e**, and **20f**).

Upon comparing the activities of methoxy and hydroxyl analogs, it was found that the methoxy compounds **9c**, **9j**,



Scheme 1: Reagents and conditions: (a) sodium methoxide, HMPA, 115–120 °C, 15 h; (b) acetyl chloride, MeOH, 0 °C then room temperature, 15 h, 70% (for **3a**), 65.1% (for **3b**); (c) 4-picoline, LHMDS, THF, −25 °C then room temperature, overnight, 50% (for **4a**), 40% (for **4b**); (d) (i) DMF-DMA, room temperature, 18 h, (ii) hydrazine monohydrate, EtOH, room temperature, overnight, 74% (for **5a**), 81% (for **5b**); (e) phenyl chloroformate, TEA, THF, 0 °C, 2 h; (f) appropriate ethanamine, K_2CO_3, CH_2Cl_2, room temperature, 1 h, 16–80% (for **9a–i**), 40–70% (for **9j–r**); (g) $BF_3.Me_2S$, CH_2Cl_2, room temperature, 48 h, 38–63% (for **10a–i**), 41–63% (for **10j–r**).



Scheme 2: Reagents and conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 1 h; (b) $KMnO_4$, C_5H_5N, H_2O, 50 °C, 24 h then room temperature, 13 h, 90%; (c) acetyl chloride, MeOH, 0 °C then room temperature, 15 h, 85%; (d) 4-picoline, LHMDS, THF, −25 °C then room temperature, overnight, 45%; (e) (i) DMF-DMA, room temperature, 18 h, (ii) hydrazine monohydrate, EtOH, room temperature, overnight, 81%; (f) phenyl chloroformate, TEA, THF, 0 °C, 2 h; (g) appropriate ethanamine, K_2CO_3, CH_2Cl_2, room temperature, 1 h, 44–80% (for **20a–i**); (h) $BF_3.Me_2S$, CH_2Cl_2, room temperature, 48 h, 40–61%.

9l, **9n**, and **20f** were more active than the corresponding hydroxyl analogs **10c**, **10j**, **10l**, **10n**, and **21f**. So, it can be concluded that the bulkier and more hydrophobic methoxy group on the phenyl ring at position 3 of the pyrazole ring is more favorable for antiproliferative activity of this series of 3,4-diarylpyrazole-1-carboxamide compounds.

Compounds **9a**, **9l**, **9n**, and **10o** were further tested in a five-dose testing mode to determine their IC_{50} values over the 60 cancer cell lines. The mean IC_{50} values of these four compounds over the nine cancer types are shown in Table 2.

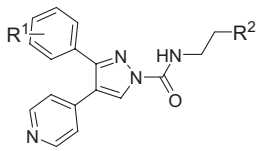
As shown in Table 2, most of the compounds exhibited high potency (in micromolar scale) over all the nine cancer types. Compounds **9a** and **9l** showed broad-spectrum antiproliferative activities over all the nine cancer types with mean IC_{50} <10.5 μM. Compound **9n** was more active against CNS and renal cancer cell lines. While the phenolic compound **10o** possessing *N*-acetylpiperazinyl terminal moiety was more selective for melanoma. Its selectivity index was 1.72 compared with the second most susceptible cancer type, leukemia.

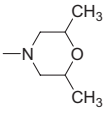
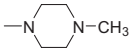
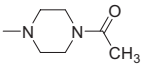
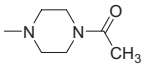
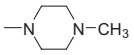
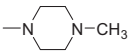
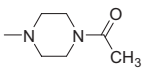
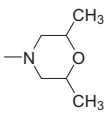
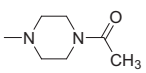
The IC_{50} values of compounds **9a**, **9l**, **9n**, and **10o** tested in five-dose mode over the most sensitive cell line of each subpanel are summarized in Table 3. From these data, we find that most of the IC_{50} values are in micromolar scale. Of special interest, the IC_{50} values of compounds **9l** and **9n** were in submicromolar range over MDA-MB-435 melanoma cell line and EKVX non-small cell lung cancer (NSCLC) cell line, respectively.

MEK/ERK kinase inhibitory activity of compounds 9a, 9l, 9n, and 10o

To study the mechanism of action of 3,4-diarylpyrazole-1-carboxamide derivatives, the four compounds **9a**, **9l**, **9n**, and **10o**, selected for five-dose testing mode over NCI-60 cancer cell line panel, were taken as representative examples of these series of compounds to be tested for their MEK and ERK kinase inhibitory activity. The MEK/ERK-containing A375P cell lysate was treated with three different concentrations of each of the test compounds (1, 3, and 10 μM), and their inhibitory activities were compared with that of sorafenib. The results showed that the tested compounds and sorafenib significantly suppressed phosphorylation of MEK1/2 and ERK1/2 in a dose-dependent manner (Figure 4). Quantification of the results was performed by measuring density of each spot at each concentration, and the inhibition percentages are provided in Table 4. The ERK and MEK activities were inhibited by 87.5% and 80.6%, respectively, with 10 μM of compound **10o** possessing hydroxyl group on the chlorophenyl ring and *N*-acetylpiperazinyl terminal moiety. Moreover, the inhibition percentages expressed by compound **10o** over both ERK and MEK kinases at 1 and 3 μM were higher than those demonstrated by sorafenib at the same concentrations. Compound **10o** showed selectivity toward melanoma subpanel more than

Table 1: Mean inhibition percentages observed with 3,4-diarylpyrazole-1-carboxamide target compounds in single-dose (10 μM) 60-cancer cell line screening



Compound no.	R^1	R^2	Mean%inhibition[a]	Compound no.	R^1	R^2	Mean% inhibition[a]
9a	3-Cl, 4-OMe		62.22	**10k**	3-Cl, 5-OH		25.42
9c	3-Cl, 4-OMe		7.02	**10l**	3-Cl, 5-OH		5.20
9d	3-Cl, 4-OMe		4.85	**10n**	3-Cl, 5-OH		22.39
9f	3-Cl, 4-OMe		26.49	**10o**	3-Cl, 5-OH		25.51
9g	3-Cl, 4-OMe		2.85	**10r**	3-Cl, 5-OH		11.77
9j	3-Cl, 5-OMe		16.48	**20b**	4-Cl, 3-OMe		14.88
9k	3-Cl, 5-OMe		25.33	**20c**	4-Cl, 3-OMe		−2.32
9l	3-Cl, 5-OMe		37.03	**20e**	4-Cl, 3-OMe		28.18
9n	3-Cl, 5-OMe		29.42	**20f**	4-Cl, 3-OMe		18.83
9p	3-Cl, 5-OMe		−2.16	**20g**	4-Cl, 3-OMe		11.94
9r	3-Cl, 5-OMe		7.94	**21c**	4-Cl, 3-OH		−3.62
10c	3-Cl, 4-OH		−7.29	**21d**	4-Cl, 3-OH		−2.97
10g	3-Cl, 4-OH		2.32	**21f**	4-Cl, 3-OH		−9.59
10i	3-Cl, 4-OH		−4.09	**21g**	4-Cl, 3-OH		17.31
10j	3-Cl, 5-OH		10.92				

[a]Mean%inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages are calculated by subtracting the growth percentages from 100.

Table 2: Mean IC_{50} values (μM) of compounds **9a**, **9l**, **9n**, and **10o** over *in vitro* subpanel cancer cell lines[a]

	No. of cell lines in each subpanel	Compound no.			
		9a	**9l**	**9n**	**10o**
Subpanel cancer cell line					
Leukemia	6	6.47	5.06	29.7	7.31
Non-small cell lung cancer	9	6.13	9.96	14.07	15.47
Colon Cancer	7	4.52	4.42	16.61	8.77
CNS Cancer	6	3.25	4.67	7.12	12.77
Melanoma	9	5.90	6.79	11.22	4.25
Ovarian cancer	7	6.28	10.29	13.12	19.16
Renal cancer	8	4.35	5.37	5.93	11.41
Prostate cancer	2	5.78	6.35	15.90	18.95
Breast cancer	6	5.13	5.76	13.12	12.88

[a]Mean IC_{50} values were calculated by dividing the summation of IC_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.

Table 3: IC_{50} values (μM) of the tested compounds **9a**, **9l**, **9n**, and **10o** over the most sensitive cell line of each subpanel

	Compound no.			
	9a	**9l**	**9n**	**10o**
Cancer cell line				
HL-60(TB)[a]	5.30	3.22	25.60	7.79
EKVX[b]	2.23	5.57	0.97	24.60
HT29[c]	2.17	3.98	3.32	3.28
SNB-75[d]	1.68	1.73	1.75	13.50
MDA-MB-435[e]	1.15	0.60	10.40	2.70
OVCAR-8[f]	1.73	7.61	2.94	12.20
RXF-393[g]	2.19	2.67	2.41	3.67
PC-3[h]	4.62	4.24	19.50	13.60
MDA-MB-468[i]	1.95	2.00	10.50	4.59

[a]Leukemia cell line.
[b]Non-small cell lung cancer cell line.
[c]Colon cancer cell line.
[d]CNS cancer cell line.
[e]Melanoma cell line.
[f]Ovarian cancer cell line.
[g]Renal cancer cell line.
[h]Prostate cancer cell line.
[i]Breast cancer cell line.

the other cancer types (Table 2). In addition, compounds **9l** and **9n** were more active against MEK kinase than sorafenib at 1 and 3 μM concentration. Upon investigating the results of compounds **9a**, **9l**, and **10o** over the most sensitive cell line of each cancer subpanel (Table 3), we found that MDA-MB-435 melanoma cell line was the most susceptible for both compounds. These compounds may inhibit melanoma cell proliferation through inhibition of ERK pathway.

In vitro cyclooxygenase inhibition assay

The target 3,4-diarylpyrazole-1-carboxamide derivatives possess a vicinal diaryl heterocyclic scaffold, 3,4-diarylpyrazole.

Table 4: Inhibition percentages expressed by compounds **9a**, **9l**, **9n**, **10o**, and sorafenib over ERK and MEK kinases in Western blot analysis at different concentrations[a]

	% Inhibition					
	ERK			MEK		
Concentration (μM)	1	3	10	1	3	10
Compound no.						
9a	–	19.4	70.6	–	19.3	52.2
9l	–	2.0	55.5	35.9	30.0	82.0
9n	–	28.6	64.8	12.5	27.4	63.1
10o	29.3	62.9	87.5	3.6	67.0	80.6
Sorafenib	15.4	50.2	94.9	–	7.5	80.7

[a]The inhibition percentages were calculated by densitometry of results shown in Figure 4.

Much interest has been given to vicinal diaryl heterocycles as selective COX-2 inhibitors. So, a selected group (29 final compounds) was tested for COX-2 inhibitory activity at molecular level. The best nine compounds in terms of potency against COX-2 were further tested for COX-1 inhibition to determine their selectivity toward COX-2 than COX-1. This part of the work was done among our attempts to investigate the possible mechanism(s) of antiproliferative activities of our target compounds. The results are illustrated in Table 5 (IC_{50}, μM).

The results showed that methoxy derivatives **9l** and **9q** were more potent than the corresponding hydroxyl derivatives **10l** and **10q**. Compound **9l** also showed higher mean%inhibition over the NCI-60 cancer cell line panel than its hydroxyl analog **10l**. This may be attributed to differences in steric and/or electronic effects between methoxy and hydroxyl groups. So, the methoxy group on the chlorophenyl ring may enhance the affinity of the ligand to COX-2 enzyme and hence the potency.

Compound **9q** with terminal 2′-methylpiperidinyl moiety showed higher potency than **9p** with piperidinyl ring. This result together with the higher potency of methoxy derivatives compared with the corresponding hydroxyl derivatives suggests that the bulkier the compound, the more appropriate will be the drug–receptor interaction and hence the higher the COX-2 inhibitory activity.

The effect of substituents on the phenyl ring at position 3 of the pyrazole ring and their positions was investigated. Compounds **9n** and **9q** with 3-chloro-5-methoxyphenyl ring were more potent than **20e** and **20h** possessing 4-chloro-3-methoxyphenyl moiety. Similarly, compound **9l** with 3-chloro-5-methoxyphenyl demonstrated higher potency against COX-2 enzyme and higher mean%inhibition over NCI-60 cancer cell line panel than **9c** with 3-chloro-4-methoxyphenyl ring. 3-Chloro-5-hydroxyphenyl derivatives **10m** and **10q** were more potent than **10h** with 3-chloro-4-hydroxyphenyl and **21d** and **21h** containing

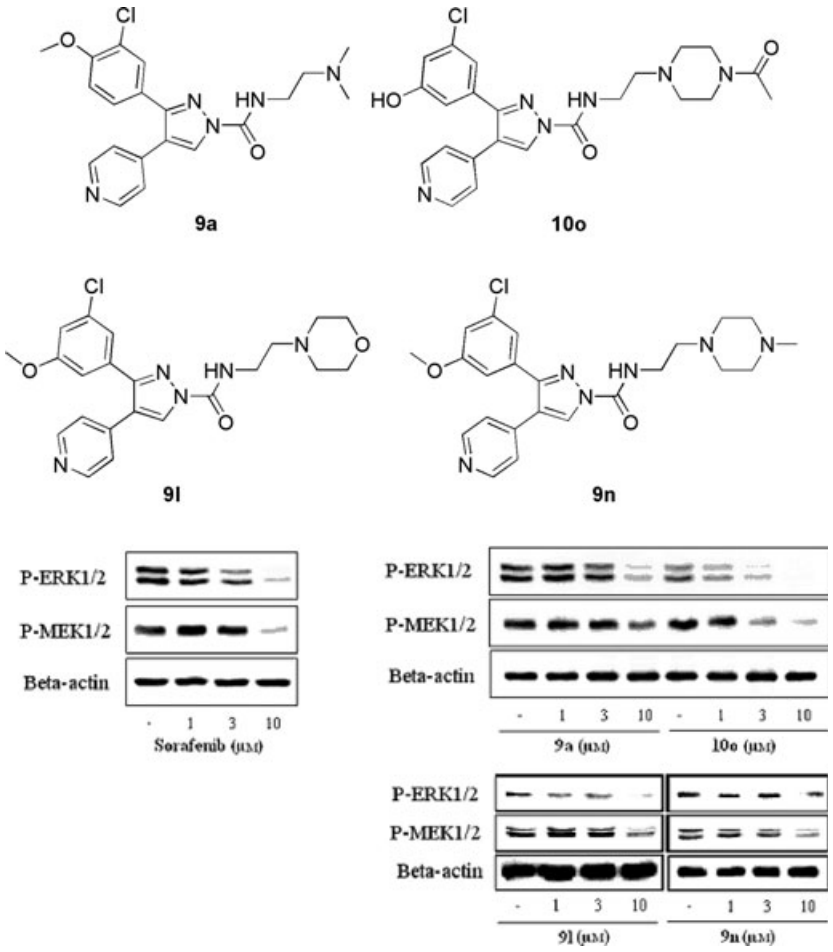


Figure 4: Inhibition of MEK and ERK kinase activities by compounds **9a**, **9l**, **9n**, **10o**, and sorafenib.

4-chloro-3-hydroxyphenyl moiety. However, 3-chloro-4-methoxyphenyl derivatives **9a** and **9g** exerted higher potency than **9p**, **20a**, and **20g**. So, the lowest potency was encountered with 4-chloro-3-methoxyphenyl and 4-chloro-3-hydroxyphenyl rings. It can be concluded that *para*-chloro group on this aryl ring at position 3 of the pyrazole ring is unfavorable for COX-2 inhibitory effect of this series of compounds.

The effect of terminal tertiary amine on biological activities was also investigated. The results were parallel with data obtained over NCI-60 cell line panel. Compound **9a** with dimethylamino terminal moiety demonstrated higher potency against COX-2 and higher mean%inhibition over NCI-60 cell line panel than the corresponding analogs **9c**, **9d**, **9g**, and **9i** possessing morpholino, 2′,6′-dimethylmorpholino, piperidinyl, and pyrrolidinyl moieties, respectively. Compounds **9l** and **9n** possessing morpholino and *N*-methylpiperazinyl moieties, respectively, showed higher potency against COX-2 enzyme and higher mean%inhibition values over NCI-60 cell line panel than compounds **9k** and **9p** with diethylamino and piperidinyl moieties, respectively. On the other hand, compound **10o** with the highest potency against COX-2 and ERK kinase, and with selectivity toward melanoma cells, was better than the corresponding morpholino derivative **10l**.

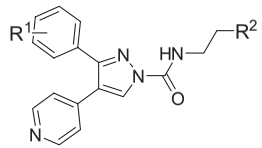
Compounds **9a**, **9g**, **9l**, **9n**, **9q**, **10m**, **10o**, **10q**, and **21b** possessing the most promising results against COX-2 together with celecoxib were further tested for COX-1 inhibition. All the tested compounds did not show any inhibitory activity against COX-1 up to 50 μM concentration. All the nine compounds showed highly promising selectivity indices toward COX-2 than COX-1. Of special interest, compounds **9a**, **9q**, **10o**, and **10q** showed selectivity indices more than 106 comparable to that of celecoxib.

Among all the target compounds, compound **10o** possessing 3-chloro-5-hydroxyphenyl and *N*-acetylpiperazinylmoieties demonstrated the best results. It was equipotent to celecoxib against COX-2. It showed almost the same selectivity index as celecoxib.

Conclusions

A series of 3,4-diarylpyrazole-1-carboxamide derivatives has been designed and synthesized based on our

Table 5: Data of the *in vitro* COX-1/COX-2 enzyme inhibition assay of the target compounds



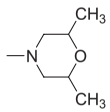
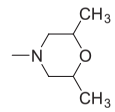
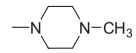
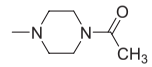
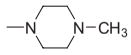
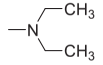
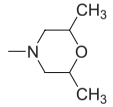
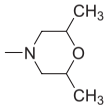
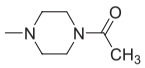
Compound no.	R^1	R^2	IC_{50} (μM)[a] COX-2	IC_{50} (μM)[a] COX-1[b]	S.I.[c]	Compound no.	R^1	R^2	IC_{50} (μM)[a] COX-2	IC_{50} (μM)[a] COX-1[b]	S.I.[c]
9a	3-Cl, 4-OMe		0.45 ± 0.04	>50	>111.11	**10q**	3-Cl, 5-OH		0.47 ± 0.03	>50	>106.38
9c	3-Cl, 4-OMe		>50	–	–	**20a**	4-Cl, 3-OMe		43.0 ± 1.30	–	–
9d	3-Cl, 4-OMe		48.0 ± 1.20	–	–	**20d**	4-Cl, 3-OMe		46.0 ± 2.50	–	–
9g	3-Cl, 4-OMe		1.72 ± 0.16	>50	>29.07	**20e**	4-Cl, 3-OMe		48.2 ± 0.80	–	–
9i	3-Cl, 4-OMe		48.0 ± 0.15	–	–	**20f**	4-Cl, 3-OMe		>50	–	–
9k	3-Cl, 5-OMe		41.0 ± 1.00	–	–	**20g**	4-Cl, 3-OMe		>50	–	–
9l	3-Cl, 5-OMe		1.95 ± 0.23	>50	>25.64	**20h**	4-Cl, 3-OMe		>50	–	–
9n	3-Cl, 5-OMe		1.20 ± 0.09	>50	>41.67	**20i**	4-Cl, 3-OMe		46.8 ± 0.95	–	–
9p	3-Cl, 5-OMe		40.2 ± 0.04	–	–	**21a**	4-Cl, 3-OH		44.4 ± 2.10	–	–

Table 5: continued

Compound no.	R^1	R^2	IC_{50} (μM)[a] COX-2	IC_{50} (μM)[a] COX-1[b]	S.I.[c]	Compound no.	R^1	R^2	IC_{50} (μM)[a] COX-2	IC_{50} (μM)[a] COX-1[b]	S.I.[c]
9q	3-Cl, 5-OMe		0.34 ± 0.02	>50	>147.06	**21b**	4-Cl, 3-OH		1.85 ± 0.10	>50	>27.03
10c	3-Cl, 4-OH		49.2 ± 0.50	–	–	**21c**	4-Cl, 3-OH		>50	–	–
10h	3-Cl, 4-OH		>50	–	–	**21d**	4-Cl, 3-OH		>50	–	–
10l	3-Cl, 5-OH		>50	–	–	**21g**	4-Cl, 3-OH		48.0 ± 2.40	–	–
10m	3-Cl, 5-OH		4.30 ± 0.40	>50	>11.63	**21h**	4-Cl, 3-OH		>50	–	–
10o	3-Cl, 5-OH		0.30 ± 0.02	>50	>166.67		Celecoxib		0.29 ± 0.02	>50	>172.41

COX, cyclooxygenase.

[a]IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1 or COX-2. The IC_{50} values are expressed as means of two determinations ± standard deviation.

[b]No inhibition of COX-1 up to 50 μM.

[c]Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

previous literature studies and as a continuation of our ongoing anticancer development program. Compounds **9a** and **9l** showed broad-spectrum cytotoxicity over all the nine types of cancers tested at the NCI. Compound **9n** was selective for CNS and renal cancer cell line subpanels, while compound **10o** demonstrated selectivity toward melanoma. Compounds **9n** and **10o** are promising leads for future development of potent and selective antiproliferative agents. The IC_{50} values of compounds **9l** and **9n** were in submicromolar scale, 0.60 μM and 0.97 μM over MDA-MB-435 melanoma and EKVX NSCLC cell lines, respectively.

Upon testing the inhibitory effect of the target compounds over COX-2 and COX-1, compound **10o** possessing 3-chloro-5-hydroxyphenyl and *N*-acetylpiperazinyl moieties showed equal potency and selectivity toward COX-2 enzyme as celecoxib. Compounds **9a**, **9l**, **9n**, and **10o** inhibited MEK and ERK kinases in a dose-dependent manner.

Its selectivity toward melanoma subpanel and COX-2 enzyme together with its inhibitory activity toward MEK and ERK kinases make compound **10o** a promising lead for future development of potential anticancer agents. Compound **10o** might exert its antiproliferative activity against melanoma due to dual inhibition of ERK pathway and COX-2.

Acknowledgments

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Conflict of Interest

The authors have declared no conflict of interest.

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Notes

[a]http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf

[b]http://www.who.int/mediacentre/factsheets/fs297/en/

[c]NCI website, www.dtp.nci.nih.gov.

[d]http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html