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Brief Article

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Tri- and Tetrasubstituted Pyrazole Derivates: Regioisomerism Switches Activity from p38MAP Kinase to Important Cancer Kinases

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Supporting Information

ABSTRACT: In the course of searching for new p38α MAP kinase inhibitors, we found that the regioisomeric switch from 3-(4-fluorophenyl)-4-(pyridin-4-yl)-1-(aryl)-1*H*-pyrazol-5-amine to 4-(4-fluorophenyl)-3-(pyridin-4-yl)-1-(aryl)-1*H*-pyrazol-5-amine led to an almost complete loss of p38α inhibition, but they showed activity against important cancer kinases. Among the tested derivatives, 4-(4-fluorophenyl)-3-(pyridin-4-yl)-1-(2,4,6-trichlorophenyl)-1*H*-pyrazol-5-amine (**6a**) exhibited the best activity, with IC_{50} in the nanomolar range against Src, B-Raf wt, B-Raf V600E, EGFRs, and VEGFR-2, making it a good lead for novel anticancer programs.

INTRODUCTION

Pyrazoles represent a class of heterocyclic compounds of significant importance and are considered as extremely versatile building blocks in organic chemistry.[1,2] They are also an important class of compounds in the pharmaceutical industry and medicinal chemistry.[3] However, only a few examples of 1,3,4-trisubstituted pyrazoles are reported in the literature.[4]

In our attempt to investigate the role of the central five-membered heterocyclic core of teardrop-binder based p38α MAP kinase inhibitors such as SB203580[5] (**B**) (Figure 1), we switched from imidazole to oxazole, isoxazole, furan, and pyrrole derivatives, as already described earlier.[6] Furthermore, we investigated the role of exocyclic amino groups in imidazole based inhibitors.[7] Both findings motivated us to aim for 1,3,4-trisubstituted 5-aminopyrazoles.

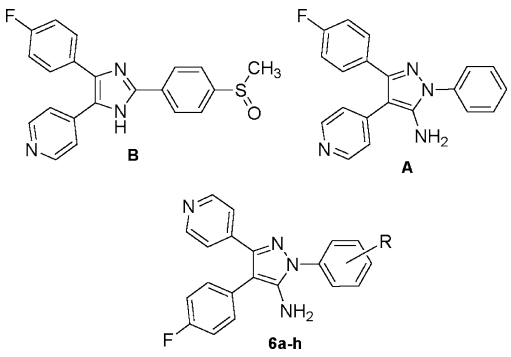

Figure 1. Pyridinylimidazole **B** and pyridinyl substituted pyrazole **A** and **6a**–**h** (regioisomers of **A**, see Table 2).

Aminopyrazoles **A** (Figure 1) were reported by Minami et al. as inhibitors of p38α MAP kinase;[8] however, no biological data on the regioisomer of **A** (switch of substituents in position 3 and 4) are known.

Generally, the regioisomer **A** showed highly efficient inhibition of p38α MAP kinase. Examination of pyrazole **A** showed that it has the same skeleton as **B** of p38α MAP kinase,[6] making a binding mode of **A** similar to SB-like compounds very likely (Figure 2a). In contrast, the regioisomeric switch of substituents in position 3 and 4 should cause issues in the **B**-like interaction pattern (Figure 2b,c). To verify this hypothesis, a series of **B** pyrazole analogues was designed. The interactions of these new compounds with the hinge region and the hydrophobic region (Figure 2b) or the interactions of the central aminopyrazole should be affected (Figure 2c) by the topology of compounds **6**. To prove this, we prepared compounds **6** (regioisomer of **A**) (Figure 1). As expected, the tested derivatives of **6** were inactive against p38α MAP kinase (data not shown). Likewise, docking of the compounds into different structures of p38 yielded only low-scoring poses without hinge binding.

Recently, Liu et al. reported moderate antitumor activity for 1-aryl-5-amino-4-pyrazolecarboxylate derivatives.[9] These results encouraged us to screen the synthesized series of compounds **6a**–**h** against various kinases relevant in cancer.

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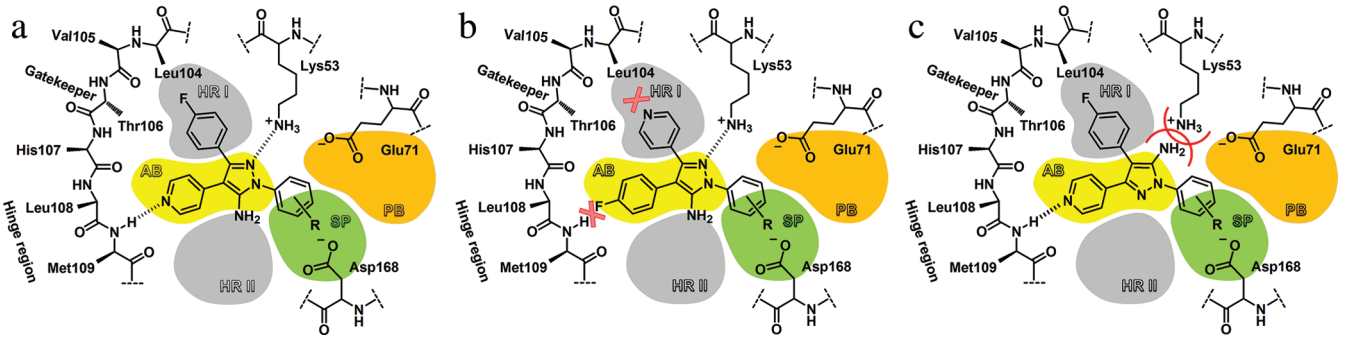


Figure 2. (a) Schematic drawing of suggested relevant interactions between compound **A** and the ATP binding site of p38α. (b,c) Schematic drawing of suggested interaction issues arising from compounds **6** (regioisomer of **A**). The schematic depiction is derived from Traxler et al.[20] AB, adenine binding region; SP, sugar pocket; PB, phosphate binding region; HR I, hydrophobic region I (also called selectivity pocket) adjacent to Thr106; HR II, hydrophobic region II, where the cleft opens to the cytosol.

RESULTS AND DISCUSSIONS

Chemistry. There are several reactions reported for the preparation of pyrazole compounds. Among them, the Knorr pyrazole synthesis is considered as one of the standard methods due to its generality. The reaction leads to the pyrazole derivatives via the condensation of substituted hydrazine with 1,3-dicarbonyl compounds or their derivatives.[10−13] The other method is the 1,3-dipolar cycloaddition of diazoalkanes or nitrile imines with olefins or alkynes.[11] Recently, a novel regioselective synthesis of 1,3,4-trisubstituted pyrazoles has been successfully employed.[4]

Some aminopyrazoles were prepared by reacting aryl hydrazines with 3-methylthio or 3-methoxy-2-cyanoacrylate in ethanol in the presence of sodium ethoxide under reflux.[9,14] The other method is via the reaction of hydrazonyl chlorides with malononitrile in anhydrous ethanol in the presence of sodium ethoxide at room temperature.[14]

The synthesis of the pyrazole compounds **6a−h** and **7−9** is outlined in Scheme 1. 4-Pyridine carbaldehyde (**1**) was reacted with arylhydrazines **2a−h** to furnish hydrazone derivates **3a−h**, followed by chlorination with *N*-chlorosuccinimide (NCS), yielding the hydrazonyl chlorides **4a−h**. To freshly prepared LDA, 4-fluorophenylacetonitrile (**5**) was added, and then subsequently hydrazonyl chlorides **4a−h** were added to the reaction mixture, giving the corresponding aminopyrazole derivatives **6a−h**. Aminopyrazole derivatives **7−9** were synthesized from the reaction of the corresponding hydrazonyl chloride **4a,b** with the respective acetonitrile derivatives **5i,j** in dry ethanol in the presence of sodium ethoxide.

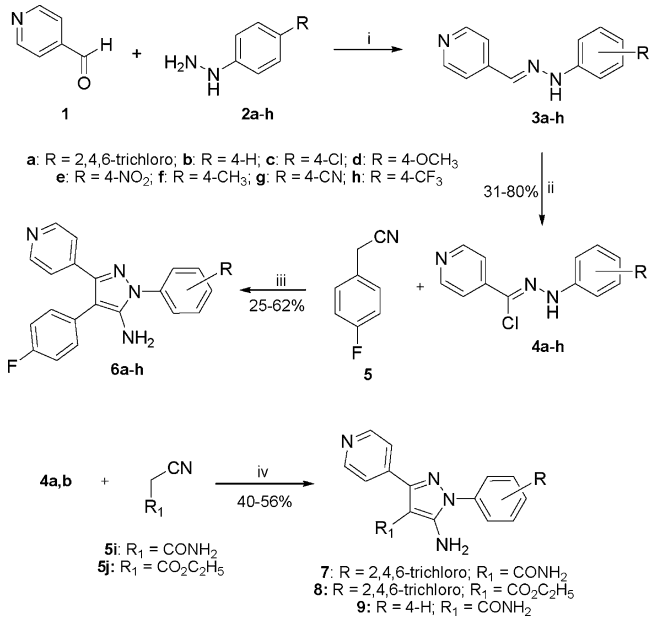
The acid derivative of aminopyrazole **10** was obtained by hydrolysis of its ester compound **8** using an aqueous KOH solution, and it was decarboxylated under reflux conditions in a solution of MeOH/conc HCl to give compound **11** (Scheme 2).

The target compounds **13a,h** were prepared according to a protocol of Deng et al. from the corresponding hydrazone (**3a,h**) with *trans*-*p*-fluoro-ω-nitrostyrene (**12**) in the presence of potassium *tert*-butoxide and TFA in dry THF (Scheme 3).[4]

Biological Studies. No inhibitory effect of compounds **6a−h**, **7−11**, and **13a,h** was found using an isolated p38α MAP kinase assay.[15] This confirms the notion illustrated in Figure 2 proposing that the switch of the substituents in positions 3 and 4 of inhibitor **A** to regioisomer **6** should lead to a dramatic loss of affinity for the p38α ATP binding site.

However, this simple regioisomeric exchange gives rise to significant affinities toward other kinases. Evaluation of lead compound **6a** in 252 different kinases shows inhibition of the

Scheme 1. Synthesis of Pyrazoles 6a−h and 7−9[a]



[a]Reagents and conditions: (i) EtOH, reflux temperature; (ii) dry DMF, NCS; (iii) dry THF, LDA, −78 °C; (iv) dry EtOH, EtONa, 0 °C.

important cancer kinases Src, B-Raf, EGFR, and VEGFR-2 (Table 1). Parallel inhibition of different protein kinases is an approach to increase the effectiveness of protein kinase inhibitors in cancer therapy. This increase of effectiveness can be due to the inhibition of two different cancer related phenotypes such as proliferation (EGFR) and angiogenesis (VEGFR-2).[16] Parallel inhibition of B-Raf and Src can be especially attractive for the treatment of melanoma, since both kinases have been found to be constitutively active in this type of cancer.[17] Since activation of B-Raf in melanoma occurs predominantly by a mutation of valine 600, it is even more intriguing that **6a** also inhibits B-Raf mutant V600E.

Structure−Activity Relationships and Interaction Modeling. A focused library of 14 analogues of **6a** was tested side by side with **6a** against a selected panel of three particularly interesting cancer kinases, B-Raf V600E, Src, and VEGFR-2, to elucidate structure−activity relationships (SAR) in this class of 5-aminopyrazoles (Table 2).

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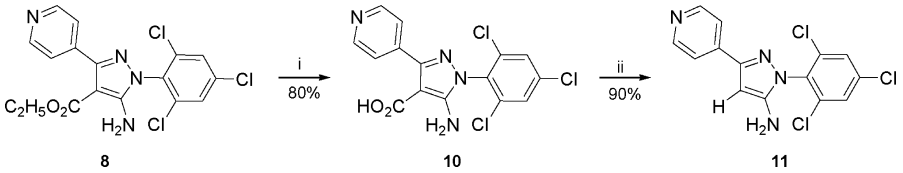
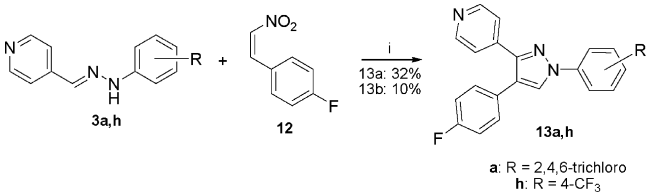
Scheme 2. Decarboxylation of Compound 8[a]



[a]Reagents and conditions: (i) aqueous KOH; (ii) MeOH/conc HCl, reflux temperature.

Scheme 3. General Method for Pyrazole Synthesis (13a,h)[a]



[a]Reagents and conditions: (i) dry THF, *tert*-BuONa, −78 °C, then TFA.

To illustrate and rationalize this SAR information, we have docked these ligands into crystal structures of all three kinases using GOLD v3.2 (CCDC).[18] The proposed binding modes shown for lead compound **6a** in Figure 3 represent top scoring poses for this inhibitor that reflect trends in the measured IC_{50} values rather well.

When comparing inhibitory activities of compounds **6a−h** that comprise variations in substituent R^1, remarkable differences become obvious between the three kinases. While 2,4,6-trichlorophenyl (**6a**) is clearly superior to the unsubstituted (**6b**) or 4-substituted phenyl (**6c−h**) moieties in VEGFR-2 and Src showing activity differences of >35-fold and >17-fold, respectively, this ratio is less pronounced in B-Raf V600E (>1.4-fold). In VEGFR-2 this can be rationalized by the orientation of the ligand placing the R^1 substituent in the hydrophobic region I, which is located between Thr916 and Lys868 (see Figure 3c). This hydrophobic area is just large enough to accommodate both chlorines in the ortho position, as well as substituents in the para position of the phenyl ring. Thus, loss of the chlorines in positions 2 and 6 of the phenyl ring seems detrimental for the inhibitory activity, whereas modifications of position 4 only have minor influence. In contrast, in B-Raf V600E (Figure 3a) R^1 seems to be placed within the hydrophobic region II (HR II), which is located at the entrance of the ATP binding site. Chlorine atoms in positions 2 and 6 are not tightly bound within the rather spacious HR II environment. Substituents in position 4 are oriented toward the water environment of the cytoplasm. This might explain why the inhibitory activity of compounds **6a−h** on B-Raf V600E is barely affected by modifications in the substitution pattern of the phenyl ring. For Src the measured SAR is difficult to explain from the predicted binding mode.

Exchange of the 4-fluorophenyl substitution (R^3) by smaller, nonaromatic, and more hydrophilic substituents such as carbamoyl (**7**, **9**), ethoxycarbonyl (**8**), carboxy (**10**), or hydrogen (**11**) leads to significant reduction or complete loss of activity in all three kinases. As the 4-fluorophenyl ring is either placed in the hydrophobic region I or II in the proposed binding modes, this effect is easily comprehensible.

Absence of the amino group (R^2) in compound **13a** as compared to compound **6a** leads to a 5−10-fold reduction of activity. In all kinases this amino function is directed toward an aspartate of the sugar binding pocket (Asp594 in B-Raf V600E, Asp404 in Src, or Asp1046 in VEGFR-2). Taking protein flexibility into account, direct or water mediated hydrogen bonds might occur. The electrostatic interaction between aromatic amine and aspartate is likely to be weakened by the adjacent aqueous environment. This can explain the moderate decrease of activity. The same exchange of amine to hydrogen in compound **13h** shows similar effects in VEGFR-2 but only small differences in B-Raf and Src when compared to **6h**.

Pharmacological Impact of Inhibitory Profile. Given the variability within and across tumor types, practical medicine has adopted a multifaceted approach to tumor therapy encompassing surgery, radiation, cytotoxics, immune stimulation, and combined noncytotoxic drug therapy. The rationale is clear: tumor variability and propensity to resistance demands variation of selection pressure. The drug discoverer can learn from this that a drug with one or few targets conferring a tumor suppressive effect will quickly select for resistance. Too many targets will lead to limited tolerance, which will limit dose and, thereby, effect.

Empirically driven cancer polypharmacology suggests that activity on a suite of key targets may provide the twin virtues of "cancer spectrum" and "efficacy". Starting with spectrum, all cancers rely on growth factors, however, not the same factors, or their mutants, and to varying degrees. Thus, a substance that is similarly potent on EGFR, VEGFR, PDGFR, and B-Raf will exert broad pressure on growth regulatory pathways without necessarily selecting for any one locus for mutation toward resistance.[19]

Resistance prevention by multitarget effects is, of course, circumvented by generalized resistance via efflux and metabolism. Here, however, fortuitous polypharmacology can also be beneficial in that binding to specific cell stress-related targets also hinders the up-regulation of general defense proteins (e.g., MAPKs, ERKs). This, in turn leaves the tumor vulnerable to cytotoxic substances and local immune reactivation if the kinase inhibitory profile supports rather than inhibits a pro-TH-1 activation response.

Table 1. Inhibitory Profile of 6a against Important Cancer Kinases and Their Mutants

IC_{50} (μM)					
VEGFR-2[a]	Src[a]	B-Raf wt[b]	B-Raf V600E[c]	EGFR wt[a]	EGFR L858R[b]
0.034 ± 0.003	0.399 ± 0.082	0.27	0.592 ± 0.154	0.113 ± 0.052	0.031

[a]Mean ± SEM of three experiments. [b]Value of one experiment. [c]Mean ± SEM of five experiments.

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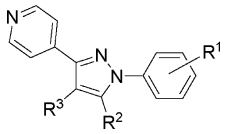
Table 2. Biological Data of Compounds 6a−h, 7−11, and 13a,h



				IC_{50} (μM)		
compd	R^1	R^2	R^3	B-Raf V600E	Src	VEGFR-2
6a	2,4,6-trichloro	NH_2	4-FC_6H_4	0.59	0.40	0.034
6b	4-H	NH_2	4-FC_6H_4	0.90	52	2.4
6c	4-Cl	NH_2	4-FC_6H_4	0.80	23	1.2
6d	4-OCH_3	NH_2	4-FC_6H_4	0.86	23	1.9
6e	4-NO_2	NH_2	4-FC_6H_4	1.4	82	1.4
6f	4-CH_3	NH_2	4-FC_6H_4	0.89	53	2.6
6g	4-CN	NH_2	4-FC_6H_4	1.7	>100	1.3
6h	4-CF_3	NH_2	4-FC_6H_4	1.5	6.7	2.4
7	2,4,6-trichloro	NH_2	$CONH_2$	>100	6.4	31
8	2,4,6-trichloro	NH_2	$CO_2C_2H_5$	>100	>100	>100
9	4-H	NH_2	$CONH_2$	64	19	48
10	2,4,6-trichloro	NH_2	COOH	>100	55	43
11	2,4,6-trichloro	NH_2	H	>100	>100	87
13a	2,4,6-trichloro	H	4-FC_6H_4	2.5	2.0	0.21
13h	4-CF_3	H	4-FC_6H_4	2.7	12	27

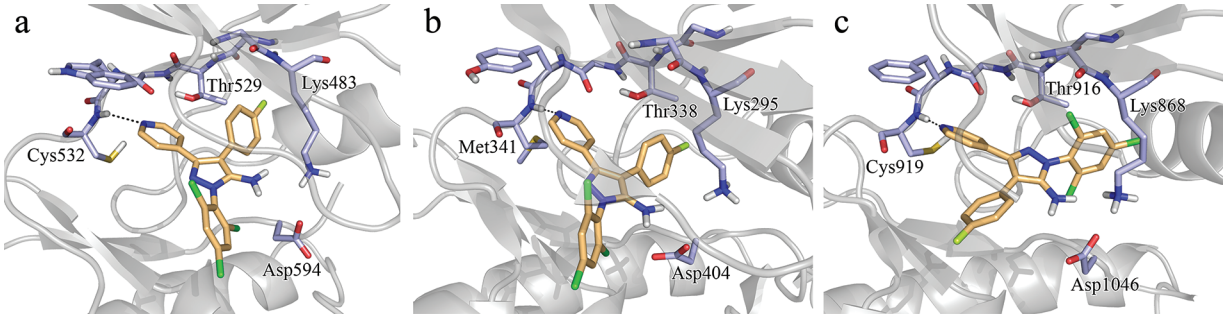


Figure 3. Proposed models of interaction between **6a** and the ATP binding site of B-Raf (a), Src (b), and VEGFR-2 (c). Binding modes are suggested on the basis of docking results using GOLD v3.2 (CCDC). The best scoring poses were carefully checked for consistency with the observed SAR data.

Taking this line, **6a** represents an excellent starting point for a phenomenological lead kinase inhibitor optimization built upon a desirable balance of effects rather than excessive faith in a specific screenable target.

CONCLUSION

In summary, regioisomeric switch completely changed the inhibitory profile of 4-(4-fluorophenyl)-3-(pyridin-4-yl)-1-(aryl)-1*H*-pyrazol-5-amines from p38α MAPK to important cancer kinases, opening a new avenue for structurally novel leads in cancer research. Further studies in cellular systems have to prove the potential benefits of this kinase profile.

EXPERIMENTAL SECTION

General. All commercially available reagents and solvents were used without further purification. NMR data were recorded on a Bruker Spectrospin AC200 or on a Bruker Avance 400 at room temperature. Chemical shifts are reported in ppm relative to the solvent resonance. The purity of the final compounds was determined by HPLC on a Hewlett-Packard HP 1090 series II liquid chromatography using a Betasil C8 column (150 mm × 4.6 mm i.d., dp = 5 μm, Thermo Fisher Scientific, Waltham, MA) at 230 and 254 nm, employing a gradient of 0.01 M KH_2PO_4 (pH 2.3) and methanol as the solvent system with a flow rate of 1.5 mL/min. All final compounds have a purity of >95%.

General Procedure for Synthesis of Amino Pyrazole Derivatives (6a−h). Twenty mmol of LDA was added to dry THF (30 mL) in a three neck flask and cooled to −78 °C. Fourteen mmol of 4-fluorophenyl acetonitrile (5) dissolved in THF (10 mL) was added dropwise, and the reaction mixture was stirred for 45 min. Five mmol of the appropriate hydrazonyl chloride **4a−h** (neat or dissolved in THF) was added slowly to the reaction. After about 1.0 h, the reaction was completed, and the reaction mixture was warmed to room temperature. Water (50 mL), followed by ethyl acetate (50 mL), was added to the reaction mixture, and the organic phase was separated. The aqueous phase was extracted with ethyl acetate (50 mL), and the combined organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to about 5 mL and left overnight, and the product precipitated from the solution. The respective product was filtered off, washed with diethyl ether and/or petroleum ether, and dried. In case the product did not precipitate, the residue was purified by flash chromatography (petroleum ether/ethyl acetate) to yield a pure solid.

4-(4-Fluorophenyl)-3-(pyridin-4-yl)-1-(2,4,6-trichlorophenyl)-1*H*-pyrazol-5-amine (6a). Yield: 35%; pale brown solid; mp 215 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 5.53 (s, br, 2H, NH_2),

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7.12−7.67 (m, 6H), 7.93 (s, 2H), 8.45 (d, J = 6 Hz, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 99.7, 116.1, 122.0, 129.2, 129.3, 132.0, 133.1, 135.9, 136.2, 141.2, 147.2, 147.5, 149.7, 161.5; IR (ATR) 3451, 3293, 3164 (NH_2), 1639 (C═N), 1604, 1573, 1552, 1519 (aromatic rings), 1466, 1212, 972,833 cm^{-1}; EI-HRMS: calcd for $C_{20}H_{12}Cl_3FN_4$ 434.0112, obsd 434.0058.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Computational methods, biochemical protein kinase assays, as well as experimental procedures and analytical data for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

MAP, mitogen-activated protein; Src, sarcoma; B-Raf, serine/threonine-protein kinase; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; NCS, *N*-chlorosuccinimide; LDA, lithium diisopropylamide; TFA, trifluoroacetic acid; ATP, adenosine triphosphate; SAR, structure−activity relationship; HR, hydrophobic region; ERKs, extracellular-signal-regulated kinases; AB, adenine binding region; SP, sugar pocket; PB, phosphate binding region

■ REFERENCES

(1) Sachse, A.; Penkova, L.; Noel, G.; Dechert, S.; Varzatskii, O. A.; Fritsky, I. O.; Meyer, F. Efficient syntheses of some versatile 3,5-bifunctional pyrazole building blocks. *Synthesis* **2008**, 800−806.

(2) Dvorak, C. A.; Rudolph, D. A.; Ma, S.; Carruthers, N. I. Palladium-catalyzed coupling of pyrazole triflates with arylboronic acids. *J. Org. Chem.* **2005**, *70*, 4188−4190.

(3) Kumar, G. G.; Vikas, K.; Vinod, K. Pyrazoles as potential anti-obesity agents. *Res. J. Chem. Environ.* **2011**, *15*, 90−103.

(4) Deng, X. H.; Mani, N. S. Base-mediated reaction of hydrazones and nitroolefins with a reversed regio selectivity: A novel synthesis of 1,3,4-trisubstituted pyrazoles. *Org. Lett.* **2008**, *10*, 1307−1310.

(5) Adams, J. L.; Gallagher, T. F.; Lee, J. Imidazole derivatives and their use as cytokine inhibitors. (SmithKline Beecham Corp., USA. 1993-US674[9314081], 70. WO. 13-1-1993.

(6) Abu Thaher, B.; Koch, P.; Schattel, V.; Laufer, S. Role of the hydrogen bonding heteroatom-Lys53 interaction between the p38 alpha mitogen-activated protein (MAP) kinase and pyridinyl-substituted 5-membered heterocyclic ring inhibitors. *J. Med. Chem.* **2009**, *52*, 2613−2617.

(7) Bracht, C.; Hauser, D. R. J.; Schattel, V.; Albrecht, W.; Laufer, S. A. Synthesis and biological testing of N-aminoimidazole-based p38 alpha MAP kinase inhibitors. *ChemMedChem* **2010**, *5*, 1134−1142.

(8) Minami, N.; Sato, M.; Hasumi, K.; Yamamoto, N.; Keino, K.; Matsui, T.; Kanada, A.; Ohta, S.; Saito, T.; Sato, S.; Asagarasu, A.; Doi, S.; Kobayashi, M.; Sato, J.; Asano, H. Preparation of aminopyrazole derivatives as p38 mitogen-activated protein (p38MAP) kinase inhibitors. [WO2000039116A1], 111. 6-7-2000. Teikoku Hormone Mfg. Co., Ltd.: Japan.

(9) Liu, Y. X.; Liu, S. H.; Li, Y. H.; Song, H. B.; Wang, Q. M. Synthesis and biological evaluation of arylhydrazinocyanoacrylates and N-aryl pyrazolecarboxylates. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2953−2956.

(10) Makino, K.; Kim, H. S.; Kurasawa, Y. Synthesis of pyrazoles. *J. Heterocycl. Chem.* **1998**, *35*, 489−497.

(11) Deng, X. H.; Mani, N. S. Regioselective synthesis of 1,3,5-tri- and 1,3,4,5-tetrasubstituted pyrazoles from N-arylhydrazones and nitroolefins. *J. Org. Chem.* **2008**, *73*, 2412−2415.

(12) Patel, M. V.; Bell, R.; Majest, S.; Henry, R.; Kolasa, T. Synthesis of 4,5-diaryl-1H-pyrazole-3-ol derivatives as potential COX-2 inhibitors. *J. Org. Chem.* **2004**, *69*, 7058−7065.

(13) Peruncheralathan, S.; Ehan, T. A.; Ila, H.; Junjappa, H. Regioselective synthesis of 1-aryl-3,4-substituted/annulated-5-(methylthio)pyrazoles and 1-aryl-3-(methylthio)-4,5-substituted/annulated pyrazoles. *J. Org. Chem.* **2005**, *70*, 10030−10035.

(14) Silvestri, R.; Cascio, M. G.; La Regina, G.; Piscitelli, F.; Lavecchia, A.; Brizzi, A.; Pasquini, S.; Botta, M.; Novellino, E.; Di Marzo, V.; Corelli, F. Synthesis, cannabinoid receptor affinity, and molecular modeling studies of substituted 1-aryl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxamides. *J. Med. Chem.* **2008**, *51*, 1560−1576.

(15) Goettert, M.; Graeser, R.; Laufer, S. A. Optimization of a nonradioactive immunosorbent assay for p38 alpha mitogen-activated protein kinase activity. *Anal. Biochem.* **2010**, *406*, 233−234.

(16) Herbst, R. S.; Johnson, D. H.; Mininberg, E.; Carbone, D. P.; Henderson, T.; Kim, E. S.; Blumenschein, G.; Lee, J. J.; Liu, D. D.; Truong, M. T.; Hong, W. K.; Tran, H.; Tsao, A.; Xie, D.; Ramies, D. A.; Mass, R.; Seshagiri, S.; Eberhard, D. A.; Kelley, S. K.; Sandler, A. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. *J. Clin. Oncol.* **2005**, *23*, 2544−2555.

(17) Smalley, K. S. M.; Herlyn, M. Targeting intracellular signaling pathways as a novel strategy in melanoma therapeutics. *Tumor Prog. Ther. Resist.* **2005**, *1059*, 16−25.

(18) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. *Protein: Struct., Funct., Genet.* **2003**, *52*, 609−623.

(19) Petrelli, A.; Giordano, S. From single- to multi-target drugs in cancer therapy: When aspecificity becomes an advantage. *Curr. Med. Chem.* **2008**, *15*, 422−432.

(20) Traxler, P.; Furet, P. Strategies toward the design of novel and selective protein tyrosine kinase inhibitors. *Pharm. Ther.* **1999**, *82*, 195−206.