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The Discovery of Potent cRaf1 Kinase Inhibitors

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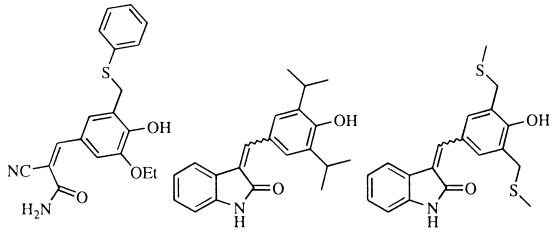
Abstract—A series of benzylidene-1*H*-indol-2-one (oxindole) derivatives was synthesized and evaluated as cRaf-1 kinase inhibitors. The key features of the molecules were the donor/acceptor motif common to kinase inhibitors and a critical acidic phenol flanked by two substitutions. Diverse 5-position substitutions provided compounds with low nanomolar kinase enzyme inhibition and inhibited the intracellular MAPK pathway.

Introduction

Mutation, overexpression, and receptor-mediated hyperactivation of *ras* genes play major roles in the formation of human tumors.[1–5] The protein kinase cRaf1 is required for Ras signal transduction and is the first enzyme in a MAP kinase cascade consisting of the three protein kinases cRaf1, MEK and ERK. Disruption of this cascade at various points inhibits ras signal transduction and transformation in many experimental systems.[1–5] cRaf1 also contributes to tumorigenicity by negatively regulating apoptosis through direct interaction with bcl-2 family members.[6–8] Thus, selective inhibitors of the protein kinase cRaf1 may prove to be effective anticancer agents.



Workers at Kanegafuchi Chem. Ind. Co. reported the signal inhibitory properties of cinnamamides (ST 638) and their "cyclised" derivatives, substituted oxindoles (ST 280 and ST 458).[9] Further studies provided data that suggested that seemingly small substituent changes around a core template could provide remarkably different selectivity and potency profiles in kinase inhibition.[10] The facile synthetic routes and diversity of substituents that could be introduced made this an attractive starting point to discover a cRaf1 kinase inhibitor. In this report, we will describe the cRaf1 SAR of this series of compounds and demonstrate the intracellular inhibition of cRaf1 kinase for representative compounds.

Chemistry

SAR for the benzylidine oxindoles was rapidly elucidated through both solution phase parallel synthetic methods and discrete compound synthesis. The chemical libraries were designed to take advantage of the facile mixed aldol condensation reaction between an aryl aldehyde and a substituted oxindole.[11] Initially we were interested in elucidating the SAR requirements of the benzylidene portion of the molecule. To this end we built focused compound sets utilizing commercially available oxindole and 5-chlorooxindole. Large sets of diversely substituted aryl-aldehydes were reacted in solution phase, parallel synthetic fashion using one equivalent of each monomer in toluene in the presence of catalytic *p*-toluenesulfonic acid at 105 °C for 24 h.

Abbreviations: SAR, structure–activity relationships; EGF(R), endothelial growth factor (receptor); MAPK, mitogen activated protein kinase; ERK, extracellular-signal-regulated kinase; MEK, MAPK/ERK kinase; FGF, fibroblast growth factor.

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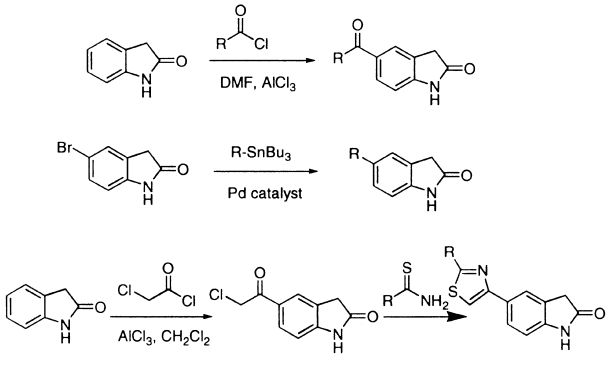
The products were collected by parallel filtration methods to provide more than 450 compounds. The structure and purity of the desired products were evaluated using HPLC and MS analyses.

Once the preferred benzylidine substitution was determined, the emphasis of our synthetic efforts was shifted to elucidating the SAR of the aryl ring of the oxindole and to improving the potency and cellular efficacy of the molecules. Since the synthetic methods have been well described,[12] this discussion will be limited to highlighting the key transformations used to create chemical diversity in the 5-position of the oxindole. Scheme 1 depicts the three principal methods that we used. Selective acylation utilizing a variety of acyl chlorides was achieved by using aluminum chloride with DMF. Palladium-catalyzed couplings were carried out using 5-bromooxindole and aryl stannane reagents. Palladium-catalyzed carbonylations afforded esters that were subsequently hydrolyzed and coupled with diverse amines under standard amide bond forming reactions. Acylation of oxindole with chloroacetylchloride readily afforded the 5-chloromethyl ketone derivative that was subjected to aldol condensation with the desired aromatic aldehyde or treated with thioureas and thioamides to provide functionalized 5-thiazole oxindoles.

Biological Activity

The screening strategy for evaluating cRaf1 inhibitors involved a two step process: (1) use of a cascade assay of three nonhomologous kinases, Raf/MEK/ERK2 and (2) target identification for the potent cascade inhibitors.[13] This strategy provided a facile screen with potency and selectivity information. Over 2000 compounds in the benzylidene oxindole series were evaluated in this assay system and the SAR is summarized in Figure 1. The discussion that follows will move counter-clockwise around the oxindole template of Figure 1 beginning with the NH/carbonyl which is a prototypical donor/acceptor motif found in many protein kinase enzyme inhibitors.[14] Accordingly, *N*-methylation of the amide NH abolished raf kinase inhibition consistent with the reported H-bond pattern of substituted oxindoles bound to FGF receptor tyrosine



Scheme 1. Key synthetic transformations for diverse oxindole monomers.

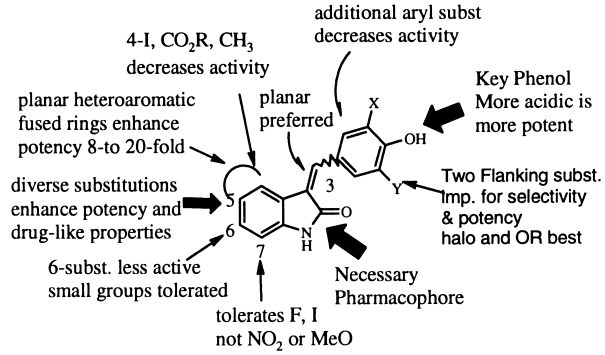


Figure 1. Summary of SAR.

kinase.[15] For optimal potency and selectivity, the benzylidene ring needed to be substituted with a 4-phenol flanked by two substituents. Substitution in the 2-position of the benzylidine aryl ring—for example, methyl, bromo, methoxy, or nitro—rendered the compounds significantly less potent in the enzyme assay.

A significant correlation was found between the acidity of the phenol and the potency of cRaf1 inhibition (Fig. 2). The measured pK_a where X and Y were varied in the 3-(4-hydroxybenzylidine)-5-chloro-oxindole series ranged over greater than 4 units ($X = Y = OMe, pK_a$ 9.8; $X = Y = Cl, pK_a$ 5.5).[16] Compounds with greater acidity were proportionally more potent cRaf1 kinase inhibitors.

The double bond in this series can exist in an E or Z configuration, and both isomers were typically observed in the NMR spectra.[17] However, in the presence of water, the compounds with a pK_a below 7 (corresponding to the more potent inhibitors) rapidly equilibrated to a 1:1 ratio of E- and Z-isomers. We found that for compounds where $X =$ alkoxy and $Y =$ halo (pK_a ~7.0), the equilibrium E:Z ratio was pH dependent. The relative isomer ratio was easily evaluated by HPLC and could be varied by adjusting the pH of the mobile

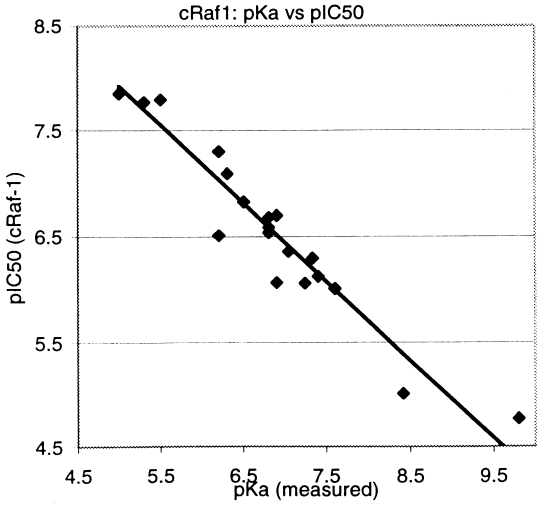
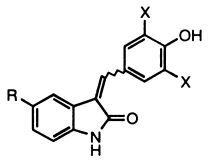


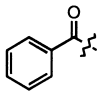
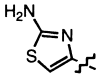
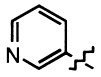
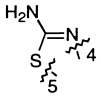
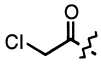
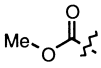
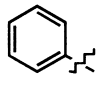
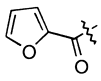
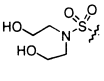
Figure 2. [18].

phase. If the double bond was saturated forming an sp^3 carbon at position 3, the raf kinase inhibition was lost.

Compounds substituted with 4-iodo and 4-methyl groups were synthesized to enforce the Z-configuration in an attempt to determine the active geometric isomer. While these compounds were significantly less active, this may also be explained as due to the introduction of steric hindrance into this area of the binding pocket. Substitution of the 4,5-position with fused ring heterocycles, for example the benzthiazole (**6**) or quinoline analogues, produced compounds which were 8-to 20-fold more potent than the unsubstituted parent (**12**) (see Table 1). The 6- and 7-position could be substituted with halogens and other small groups with moderate improvements in enzyme potency.

Table 1. Representative data



Compds	Structure		Cascade assay	EMAPK[b]
	R	*X*	IC_{50}, μM[a]	%I [cmpd]
1	I	Br	0.009	90% 5 uM
2		Br	0.018	84% 15 uM
3		Br	0.046	94% 15 uM
4		Br	0.011	52% 10 uM
5		Br	0.025	55% 10 uM
6		Br	0.016	66% 15 uM
7		Br	0.065	81% 10 uM
8		Br	0.013	53% 10 uM
9		Cl	0.032	81% 15 uM
10		Cl	0.040	40% 15 uM
11		Br	0.029	0% 15 uM
12	H	Br	1.30	–

[a]Values are means of greater than three experiments for enzyme assay of Raf/MEK/ERK and the target of enzyme inhibition was validated to be cRaf1 kinase.
[b]Cellular evaluation of compounds.[19] Percent inhibition (%I) of MAPK activation at the concentration listed.

The 5-position of the oxindole served as an ideal position to substitute for enhanced potency of the compounds. In addition, the 5-position proved to be remarkably tolerant of a wide variety of functionality including halogens, esters, amides, aryl groups, heteroaryl groups, and acyl groups. A representative set of raf inhibitors is shown in Table 1. Compounds **1**–**11** showed $\geqslant$100-fold selectivity for raf kinase versus CDK1, CDK2, c-src, ERK2, MEK, p38, Tie2, VEGFR2, and c-fms.

The inhibition of cRaf1 kinase activity should result in the down-regulation of MAP kinase activity. A cell-based mechanistic assay, EMAPK, measures the inhibition of EGF stimulated MAP kinase activation.[19] The assay was used to demonstrate that the compounds penetrated the cellular membrane and inhibited the desired target. In general, the 5-aryl, heteroaryl, halo, acyl and ester substitutions were effective intracellular raf kinase inhibitors. Amides and aminosulfonyl groups (e.g. **11**) in the 5-position were significantly less effective. It is important to note that these compounds did not inhibit the upstream target EGFR tyrosine kinase as measured by the lack of inhibition of auto-phosphorylation in the same cellular system.[20]

Conclusion

cRaf1 inhibitors are predicted to be broad-spectrum antitumor agents and have the potential to enhance existing cancer chemotherapies. We have discovered potent cRaf1 kinase inhibitors that contain a pharmacophore composed of an acidic phenol and a donor/acceptor binding group (NH-CO). These inhibitors are effective at blocking the intracellular MAP kinase pathway. The details of the cellular and in vivo efficacy properties of these compounds will be reported in the future.[21]

Acknowledgement

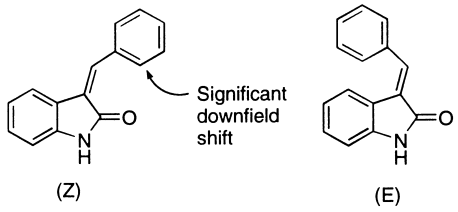
We would like to thank Douglas J. Minick for measuring the pK_a values of our compounds.

References and Notes

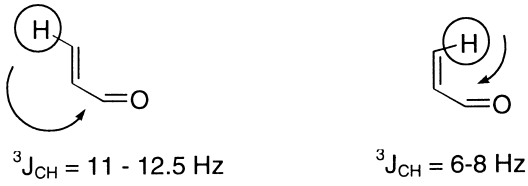
1. Daum, G.; Eisenmann-Tappe, I.; Fries, H.-W.; Troppmair, J.; Rapp, U. R. *TIBS* **1994**, *19*, 474.
2. Avruch, J.; Xian-feng, Z.; Kyriakis, J. M. *TIBS* **1994**, *19*, 279.

3. Moelling, K.; Heimann, B.; Beimling, P.; Rapp, U. R. *Nature* **1984**, *312*, 558.
4. Kolch, W.; Heidecker, G.; Lloyd, P.; Rapp, U. R. *Nature* **1991**, *349*, 426.
5. Monia, B. P.; Johnston, J. F.; Geiger, T.; Muller, M.; Fabbro, D. *Nature Medicine* **1996**, *2 (6)*, 668.
6. Gajewski, T. F.; Thompson, C. B. *Cell* **1996**, *87*, 589.
7. Wang, H.; Rapp, U. R.; Reed, J. C. *Cell* **1996**, *87*, 629.
8. Wang, H.-G.; Takayama, S.; Rapp, U. R.; Reed, J. C. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7063.
9. Shiraishi, T.; Domoto, T.; Imai, N.; Shimada, Y.; Watanabe, K. *Biochem. Biophys. Res. Commun.* **1987**, *147 (1)*, 322.
10. Lackey, K.; McDonald, O. B.; Wood, E.R. In-house unpublished results.
11. A similar approach was undertaken by workers at Sugen in their identification of VEGFR TK inhibitor: Sun, L.; Tran, N.; Tang, F.; Harald, A.; Hirth, P.; McMahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588.
12. Lackey, K. E.; McNutt, R. W., Jr.; Jung, D. K.; Harris, P. A.; Hunter, R. N., III; Veal, J. M.; Dickerson, S.; Peel, M. R. PCT Int. Appl., 144 pp CODEN: PIXXD2. WO 9910325 A1 990304. AN 1999:166598 and references contained therein.
13. McDonald, O. B.; Chen, W. J.; Ellis, B.; Hoffman, C.; Overton, L.; Rink, M.; Smith, A.; Marshall, C. J.; Wood, E. R. *Analytical Biochem.* **1999**, *268*, 318.
14. Kim, S.-H.; Schulze-Gahmen, U.; Brandsen, J.; de Azevedo, W. F. *Prog. Cell Cycle Res.* **1996**, *2*, 137.
15. Mohammadi, M.; McMahon, G.; Sun, L.; Tang, C.; Hirth, P.; Yeh, B. K.; Hubbard, S. R.; Schlessinger, J. *Science* **1997**, *276*, 955.
16. PK_a values were determined using a spectrophotometric method developed in our laboratory. Similar methods are discussed in Albert, A.; Serjeant, E. P. The determination of Ionization Constants, 2nd ed., Edinburgh, T.&A. Constable Ltd., 1971.
17. *E*/*Z* isomers were assigned using ^{1}H NMR chemical shifts, NOE experiments, and 3 bond carbon^{19}-proton coupling constants.

^{1}H NMR trends



^{13}C NMR trends



18. $pIC_{50} = 11.63 - 0.74 * pK_a$; $r^2 = 0.92$, $F = 206.81$, $n = 20$, $P > 0.01$.
19. EMAPK: The EMAPK assay measures the EGF stimulated MAP kinase activation in a cellular assay format. Cells were serum starved for 24 h. cRaf1 inhibitors were pre-incubated with the cells for 60 min. The cells were stimulated with EGF (100 nM) for 5 min. The cells were lysed in SDS polyacrylamide gel sample buffer and the protein samples were resolved by SDS-PAGE. ERK activation was evaluated by Western Blotting using Anti-Phospho ERK (New England BioLabs).
20. EGF receptor autophosphorylation was evaluated in a manner similar to that cited in note 13 using Anti-PY20.
21. Results of a variety of cellular assay systems with a subset of these compounds: Wood, E. R.; Crosby, R. M.; Davenport, E. A.; Gilmer, T. M.; Hunter, B. N.; Keith, B. R.; Lee, A. V.; McDonald, O. B.; Mullin, R. J.; Rusnak, D. W.; Lackey, K. E. *Am. Assoc. Cancer Res.* **1999**, *40*, 724.