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Discovery of Heterocyclic Ureas as a New Class of Raf Kinase Inhibitors: Identification of a Second Generation Lead by a Combinatorial Chemistry Approach

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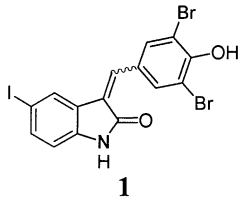
Abstract—Heterocyclic ureas, such as *N*-3-thienyl *N*′-aryl ureas, have been identified as novel inhibitors of raf kinase, a key mediator in the ras signal transduction pathway. Structure–activity relationships were established, and the potency of the screening hit was improved 10-fold to $IC_{50} = 1.7\,\mu M$. A combinatorial synthesis approach enabled the identification of a breakthrough lead ($IC_{50} = 0.54\,\mu M$) for a second generation series of heterocyclic urea raf kinase inhibitors.

The ras signal transduction pathway normally functions to transmit signals from growth factor and cytokine receptors on the cell surface to the nucleus, resulting in the regulation of cell differentiation and division.[1] Activating mutations of ras have been found in nearly one third of all human cancers, including about 50% of colon cancers and 90% of pancreatic cancers.[2] Consequently, inhibition of raf kinase, a downstream effector of ras, has been targeted as a promising strategy for the treatment of cancer.[3,4] In a recent example, certain benzylidene indolinones were found to be potent raf kinase inhibitors (e.g., **1**; $IC_{50} = 9\,nM$), with an acidic phenol moiety flanked by two substituents being critical for optimal potency.[5]

High-throughput screening of our compound collections in a raf kinase assay (cRaf1 isoform) identified the commercially-available[6] 3-thienyl urea **2** as a modest, reversible inhibitor ($IC_{50} = 17\,\mu M$). Although this compound was also identified in our laboratories as a potent inhibitor of p38 kinase (p38 $IC_{50} = 290\,nM$),[7] it was



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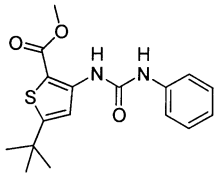

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considered to be worthy of further investigation by virtue of its potential for efficient analoging. At the outset of our work, urea substructures were virtually unknown as kinase inhibitors, with the patent activity being directed at several other compound classes.[8] An exception was a series of 2-thienyl ureas reported to have broad tyrosine kinase inhibitory activity.[9] More recently, however, several series of ureas have been claimed as protein kinase inhibitors.[8a,10]

The initial objective of our program was to explore the SAR of the screening hit **2**, toward improving raf inhibitory potency. Preparation of the ureas was generally straightforward, achieved by reaction of a 3-thienyl amine (or related heterocycle) with phosgene or a phosgene equivalent, followed by treatment with an aniline

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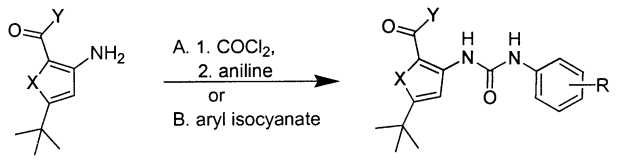
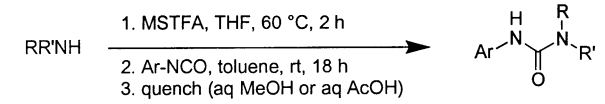


Scheme 1. Synthesis of thienyl, furyl, and pyrrole ureas.

or heterocyclic amine. An alternative procedure involved the reaction of the thienyl amine (or analogue) with an isocyanate (Scheme 1). These procedures, as well as syntheses of analogues of the 2-amino-1-carbomethoxy-5-*t*-butyl thiophene fragment, have been described previously.[7,11]

For the preparation of a combinatorial library of ureas, rapid parallel synthesis was achieved by conducting the amine–isocyanate reaction in anhydrous DMF (80–95 °C, 18 h).[10a] A modular parallel synthesis workstation, incorporating robotic liquid handling and an orbital shaker heating block, was utilized for the preparation of about 1000 analogues.[12] To enable efficient syntheses with amino acids, their hydrates, and amine hydrochlorides, these building blocks were pre-treated in situ with MSTFA [*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide] to effect formation of the trimethylsilyl ester, dehydration, and/or neutralization,[13] and the resulting solutions were then reacted with the

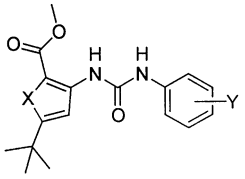


Scheme 2. MSTFA-mediated synthesis of ureas.

isocyanate building blocks. This protocol[14] also provided cleaner urea products from amino alcohols, presumably due to minimizing side reactions with the hydroxy functionality (Scheme 2).

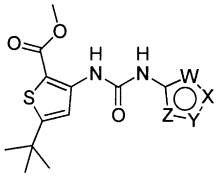
Variation of substituents on the phenyl ring in **2** revealed this as an area for high optimization potential (Table 1).[15] However, while small lipophilic substituents such as methyl and chloro at the *para* position provide an increase in potency (**3** and **4**), the improvement is lost with substituents having greater steric bulk (e.g., **9**–**11**). A variety of electron-donating and -withdrawing substituents also give rise to relatively weak inhibitors. Substitution with methyl or chloro at the *meta* and *ortho* positions provides no improvement in potency, as compared to **2** (e.g., **16**–**20**). Likewise, electron-donating

Table 1. Substitution of the phenyl moiety



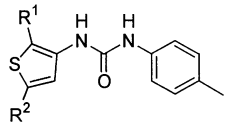
Compd	X	Y	Raf kinase% inhn, 25 μM	Raf kinase IC_{50} (μM)
2	S	H		17
3	S	4-CH_3		1.7
4	S	4-Cl		6.8
5	S	4-F	30	
6	S	4-OH		15
7	S	4-NH_2		18
8	S	4-OCH_3	8	
9	S	4-OPh	34	
10	S	4-CF_3	31	
11	S	4-Et	55	
12	S	4-NHAc	33	
13	S	4-$CONH_2$	22	
14	S	4-COOH	9	
15	S	4-NO2	40	
16	S	3-CH_3		20
17	S	3,4-$(CH_3)_2$		15
18	S	2,4-$(CH_3)_2$	45	
19	S	3-Cl		22
20	S	3,4-Cl_2		20
21	O	4-CH_3		3.0
22	O	3,4-Cl_2		7.7
23	NH	H	43	
24	NH	4-CH_3		3.0
25	NH	3,4-Cl_2		12
26	NCH_3	4-CH_3		5.0

Table 2. Five-membered heterocycle replacements for the phenyl moiety



Compd	W	X	Y	Z	Raf kinase% inhn, 25 μM	Raf kinase IC_{50} (μM)
27	S	C–CH_3	N	N		1.2
28	S	C–Et	N	N		2.0
29	S	C–cyPr	N	N		1.9
30	S	C–*t*Bu	N	N		11
31	S	C–CF_3	N	N		6.6
32	S	C–Ph	N	N	14	
33	S	C–CH_3	CH	N	38	
34	S	C–CH_3	CH	CH		3.7
35	CH	C–CH_3	CH	S		3.1
36	S	CH	CH	CH		11
37	O	C–CH_3	CH	CH	18	
38	N	NH	CH	CH	16	
39	N	N–CH_3	CH	CH		6.7
40	N	N–Et	CH	CH		8.0
41	N	N–*i*Pr	CH	CH		16
42	N	N–*n*Pr	CH	CH		19
43	N	N–Bn	CH	CH		12
44	N	N–Ph	CH	CH	31	
45	N	NH	C–CH_3	CH	13	
46	CH	C–CH_3	N	N–CH_3	17	
47	CH	CH	N	N–Et	32	
48	CH	CH	N	N–Ph	15	
49	CH	C–cyPr	N	N–CH_3	18	
50	CH	N–CH_3	CH	CH	34	
51	CH	N–Et	CH	CH		7.6
52	CH	N–*i*Pr	CH	CH		8.1
53	CH	N–*n*Pr	CH	CH		11
54	CH	N–Bn	CH	CH	36	
55	N	C–SCH_3	NH	N	8	
56	N	C–SCH_3	N–CH_3	N	2	
57	N	C–CH_3	CH	O	18	
58	CH	C–CH_3	O	N	37	
59	CH	C–CH_3	N	O	38	

Table 3. Replacements for the thiophene substituents in **3**



Compd	R^1	R^2	Raf kinase % inhn, 25 μM	Raf kinase IC_{50} (μM)
60	COOEt	*t*Bu		6.0
61	$CONH_2$	*t*Bu	39	
62	$CONHCH_3$	*t*Bu		4.9
63	$CON(CH_3)_2$	*t*Bu		15
64	CH_2NH_2	*t*Bu		13
65	$COCH_3$	*t*Bu	18	
66	Et	*t*Bu	7	
67	H	*t*Bu	32	
68	CH_2NHAc	*t*Bu	2	
69	CH_2NHGly	*t*Bu	46	
70	$COOCH_3$	*i*Pr		4.0
71	$COOCH_3$	*i*Bu	25	
72	$COOCH_3$	CH_2Br		6.0
73	$COOCH_3$	CH_2OH	24	
74	$COOCH_3$	COOH	32	
75	$COOCH_3$	$COOCH_3$	32	
76	$COOCH_3$	NH_2	25	
77	$COOCH_3$	Ph	0	
78	$COOCH_3$	CH_2CH_2Ph	36	

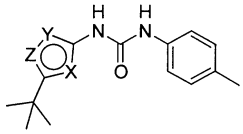
or -withdrawing substituents at the *meta* and *ortho* positions (e.g., OH, NH_2, $CONH_2$, COOH, $COOCH_3$, NHAc, and NO_2) provide relatively ineffective inhibitors ($IC_{50} \geq 20\,\mu M$). Replacement of the thiophene ring by furan or pyrrole results in compounds with comparable potency, and similar SAR (**21**–**26**).

Heterocyclic replacements for the phenyl group were also examined, and several thiadiazoles were found to be more potent than the lead **2**, but not significantly better than analogue **3** (Table 2). As in the phenyl series, the most active analogues are substituted with small lipophilic groups (i.e., **27**–**29**). Closely related thiophenes (**34** and **35**) have comparable potency, while a related furan (**37**) is relatively ineffective. Certain pyrazoles (**39** and **40**) and pyrroles (**51** and **52**) also show moderate activity, while triazoles, isoxazoles, and an oxazole are less active. Overall, for 5-membered heterocycles, only analogues having a small alkyl group in the 3-position (at X in Table 2) and certain heteroatom substitutions exhibit IC_{50} values less than 10 μM.

All replacements explored for the carbomethoxy substituent in **3** resulted in a loss in potency (Table 3), although analogues with a carboethoxy or *N*-methyl amide group (**60** and **62**) have moderate activity. The thiophene 5-*t*-butyl group was also found to be optimal for raf kinase inhibitory activity. Larger lipophilic groups and charged/polar functionalities are not well tolerated, with only the 5-isopropyl (**70**) and 5-bromomethyl (**72**) derivatives having moderate activity.

Several other heterocycles were investigated as replacements for the thiophene ring; examples of analogues related to **3** are given in Table 4. Surprisingly, even analogues that retain the *t*-butyl and carbomethoxy or

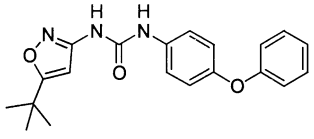
Table 4. Heterocycle replacements for the thiophene ring



Compd	X	Y	Z	Raf kinase % inhn, 25 μM
79	S	C–$COOCH_3$	N	52
80	O	C–$COOCH_3$	N	38
81	N	C–$COOCH_3$	O	16
82	NH	C–COOEt	CH	44
83	N	C–$COOCH_3$	NH	23
84	S	C–CN	CH	46
85	CH	N–CH_3	N	26
86	N	S	CH	25
87	S	N	N	18
88	CH	N	O	44

carboethoxy groups are considerably less potent than **3**. This is in sharp contrast to the related furan and pyrrole analogues (**21**, **24**, and **26**), that are comparable in potency to **3**.

In summary, potency of the hit **2** ($IC_{50} = 17\,\mu M$) was increased 10-fold by 4-methyl substitution on the phenyl ring to give analogue **3** ($IC_{50} = 1.7\,\mu M$). However, despite extensive analoging, characterization of SAR, and identification of compounds with potency comparable to **3**, this series could not be improved beyond the 1 μM IC_{50} barrier. Our combinatorial synthesis program, carried out in parallel during the latter stage of this analoging effort, identified (or re-identified) certain of the more active analogues. More importantly, however, was the identification of **89** as a quite potent inhibitor ($IC_{50} = 0.54\,\mu M$).[17] This result is quite striking, as this 'analogue' of **2** lies outside the SAR established for **2**, which may suggest a different binding orientation for **89**. This represents a prime example of the power of combinatorial chemistry. Using a traditional sequential analoging approach, 'single-point modifications' of the lead **2** (or **3**) provide the relatively inactive analogues **9** and **88**, which would normally provoke a rejection of the 4-phenoxy-phenyl and 5-*tert*-butyl-3-isoxazolyl fragments. In contrast, using a combinatorial chemistry approach, 'multiple-point modifications' are pursued in parallel, and a less biased and greater variety of substituent combinations are examined. As a result, new directions for optimizing potency may be revealed, as demonstrated in this investigation. Indeed, further analoging around **89** rapidly established this compound as the lead for a second generation series of raf kinase inhibitors with significantly enhanced potential.[8a] Our further studies with this class of raf kinase inhibitors ultimately produced a clinical candidate for the treatment of cancer; details will be reported in due course.



89 $IC_{50} = 0.54\,\mu M$

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References and Notes

1. Lowy, D. R.; Willumsen, B. M. *Annu. Rev. Biochem.* **1993**, *62*, 851.
2. Bos, J. L. *Cancer Res.* **1989**, *49*, 4682.
3. Naumann, U.; Eisenmann-Tappe, I.; Rapp, U. R. *Rec. Results Cancer Res.* **1997**, *143*, 237.
4. Monia, B. P.; Johnston, J. F.; Geiger, T.; Muller, M.; Fabbro, D. *Nature Med.* **1996**, *2*, 668.
5. Lackey, K.; Cory, M.; Davis, R.; Frye, S.; Harris, P.; Hunter, R.; Jung, D.; McDonald, B.; McNutt, R.; Peel, M.; Rutkowske, R.; Veal, J.; Wood, E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 223.
6. GK 00687, Maybridge Chemical Company Ltd., Trevillett, Tintagel, Cornwall, UK
7. Redman, A. M.; Johnson, J. S.; Dally, R.; Swartz, S.; Wild, H.; Paulsen, H.; Caringal, Y.; Gunn, D.; Renick, J.; Osterhout, M.; Kingery-Wood, J.; Smith, R. A.; Lee, W.; Dumas, J.; Wilhelm, S. M.; Housley, T. J.; Bhargava, A.; Ranges, G. E.; Shrikhande, A.; Young, D.; Bombara, M.; Scott, W. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 9.
8. (a) Dumas, J. *Exp. Opin, Ther. Pat.* **2001**, *11*, 405. (b) *Exp. Opin. Ther. Pat.* **1998**, *8*, 1749.
9. Tang, P. C.; McMahon, G. PCT Intl. Appl. WO 96/40673. *Chem. Abstr.* **1997**, *126*, 139849.
10. (a) Dumas, J. D.; Sibley, R.; Riedl, B.; Monahan, M. K.; Lee, W.; Lowinger, T. B.; Redman, A. M.; Johnson, J. S.; Kingery-Wood, J.; Scott, W. J.; Smith, R. A.; Bobko, M.; Schoenleber, R.; Ranges, G.; Housley, T. J.; Bhargava, A.; Wilhelm, S. M.; Shrikhande, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2047. (b) Dumas, J. D.; Hatoum-Mokdad, H.; Sibley, R.; Riedl, B.; Scott, W. J.; Monahan, M. K.; Lowinger, T. B.; Brennan, C.; Natero, R.; Turner, T.; Johnson, J. S.; Schoenleber, R.; Bhargava, A.; Wilhelm, S. M.; Housley, T. J.; Ranges, G.; Shrikhande, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2051.
11. Wood, J. E.; Wild, H.; Rogers, D. H.; Lyons, J.; Katz, M.; Caringal, Y.; Dally, R.; Lee, W.; Smith, R. A.; Blum, C. U.S. Patent 6 187 799, 2001; *Chem. Abstr.* **2001**, *134*, 163040.
12. Building blocks were selected 'manually' with the objective of achieving a balance of structural diversity and similarity to the hit **2**. These building blocks included a total of 75 isocyanates and ca. 300 amines, including some custom-prepared compounds. About 1500 syntheses were attempted, and ca. 1000 products were obtained having purities of $>60\%$ (HPLC, 254 nm) and confirmed identities (LC/MS).[10a] These 1000 products were submitted for testing, and key actives that were identified were resynthesized, purified, characterized, and re-tested.
13. MSTFA was selected on the basis of its favorable reactivity, volatility, and relatively low toxicity. For related chemistry achieved by using trimethylsilyl cyanide, see: Anteunis, M. J. O.; Becu, C. *Bull. Chem. Soc. Belg.* **1987**, *96*, 119 and 133.
14. General procedure for MSTFA-mediated synthesis of ureas from amino-alcohols, amino acids, or amine hydrochlorides: Into each reaction vial containing the amine building block (0.1 mmol) in dry THF (0.5 mL) was added MSTFA (0.5 mL), and the reaction mixture was heated at 60 °C for 2 h. After the mixture was cooled to room temperature, the aryl/heteroaryl isocyanate (0.5 mL, 0.2 M in toluene) was added, and the reaction mixture was allowed to stand for 18 h. Aqueous methanol (95%, 2.0 mL) or, preferably, 1:2:1 acetic acid/THF/water (2.0 mL) was then added slowly to quench the reaction. The mixture was allowed to stand for 8 h before the solvents and other volatiles were removed by evaporation.
15. All data reported herein reflect purified and characterized (^{1}H NMR, MS) samples. Raf kinase assay: Raf[16] (cRaf1) was incubated with MEK[16] in 20 mM Tris–HCl, pH 8.2 buffer containing 2 mM 2-mercaptoethanol and 100 mM NaCl. This solution (20 μL) was mixed with 5 μL of water or test compounds diluted with water from 10 mM DMSO stock solutions (1% final DMSO concentration). The kinase reaction was initiated by adding 25 μL [γ-^{33}P] ATP (1000–3000 dpm/pmol) in 80 mM Tris–HCl, pH 7.5, 120 mM NaCl, 1.6 mM DTT, 16 mM $MgCl_2$. The reaction mixtures were incubated at 32 °C, usually for 22 min, and incorporation of ^{33}P into protein was assayed by harvesting the reaction onto phosphocellulose mats, washing away free counts with 1% phosphoric acid, and quantitating phosphorylation by liquid scintillation counting.
16. MacDonald, S. G.; Crews, C. M.; Wu, L.; Driller, J.; Clark, R.; Erikson, R. L.; McCormick, F. *Mol. Cell Biol.* **1993**, *13*, 6615.
17. Lead **89** exhibits *Escherichia coli*-derived p38 kinase $IC_{50} = 360$ nM, that is, similar to the screening hit **2**. p38 Data for many of the other compounds described in this article have been reported previously.[7]