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Substituted benzimidazoles: A novel chemotype for small molecule *h*KSP inhibitors

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ABSTRACT

Substituted benzimidazoles were profiled as inhibitors of kinesin spindle protein (KSP), an increasingly important target for the development of anticancer drugs. This series demonstrated the monoastral phenotypic response and was found to be active in both enzymatic and cellular-based assays.

Kinesin spindle protein, known as KSP or *Hs* Eg5, plays a critical role in mitotic spindle assembly and function. A small-molecule KSP inhibitor would potentially arrest mitosis by preventing normal bipolar spindle formation,[1] inducing apoptosis or cell death following the well-documented monoastral phenotype[2] visible under microscopic magnification. As a result, KSP has become a significant target for the development of cancer therapeutic drugs[3] with the potential to overcome the mechanism-based side effects of the microtubule-targeting taxanes and vinca alkaloids, such as neuropathy.[4] Herein, we describe the use of substituted benzimidazoles as a novel chemotype for the inhibition of *h*KSP which demonstrated potency in both in vitro (endpoint) and cell-based (Alamar blue cellular proliferation) assays,[5] while exhibiting positive cooperative binding[6a] with Cytokinetics' clinical quinazolinone series.[6b]

Hit optimization of a 2-aminoimidazole identified through high-throughput screening (HTS) was commenced using both mixture-based libraries as well as single, purified compounds. Utilizing this twofold approach along with our proprietary affinity-based selection mass spectrometric automated ligand identification system (ALIS),[7] mixtures of compounds (hundreds per mixture) containing a diverse set of building blocks were screened for KSP binding, the most active of which were subjected individually to enzymatic and cell-based assays. A solid-phase synthetic strategy adapted from the well-documented polymer-supported routes in the literature[8] was utilized for the mixture-based and the majority of parallel discrete compound preparation (Scheme 1). This versatile approach to benzimidazoles **6** allowed for rapid diversification at three positions (**6**, R1, R2, and R3). Beginning with either Wang (for X = O) or Rink (for X = NH) resin, nitrobenzoic acid **1** was bound to the solid-phase support to give **2** as the corresponding ester or amide, respectively. S_NAr displacement of fluoride by primary amines provided nitroanilines **3**, which were reduced with tin(II) chloride to give 1,2-diamines **4**. The benzimidazole core was subsequently formed by cyclization with isothiocyanates to generate **5**, the resin cleavage of which provided **6** in high purity. Methyl ester variants (**6**, R1 = $-CO_2Me$) were obtained by esterification of the corresponding carboxylic acid with trimethylsilyldiazomethane following cleavage from the resin. Synthesis of secondary and tertiary amides at the R1 position, the former of which had been demonstrated on solid phase beginning with reductive amination of an aldehyde-based resin with various amines,[8c] were prepared by amidation of the carboxylic acids post-cleavage.

The commercial sources of isothiocyanates used to diversify the R3 position of **6** were rapidly exhausted, thus necessitating the need for a facile synthetic approach to these building blocks. Penso and Albanese demonstrated the conversion of trifluoromethylacetamides to isothiocyanates,[9] which was adapted to a one-pot procedure to fit our needs (Scheme 2).

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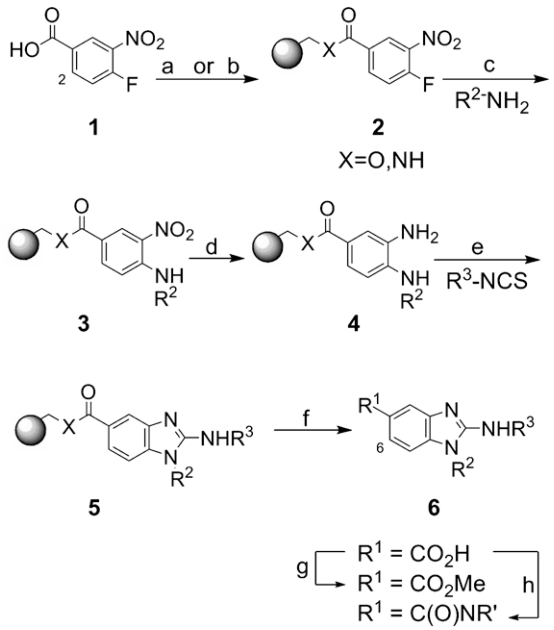
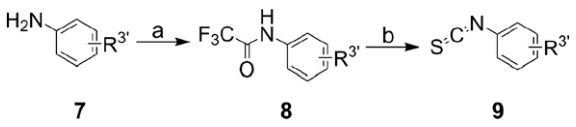


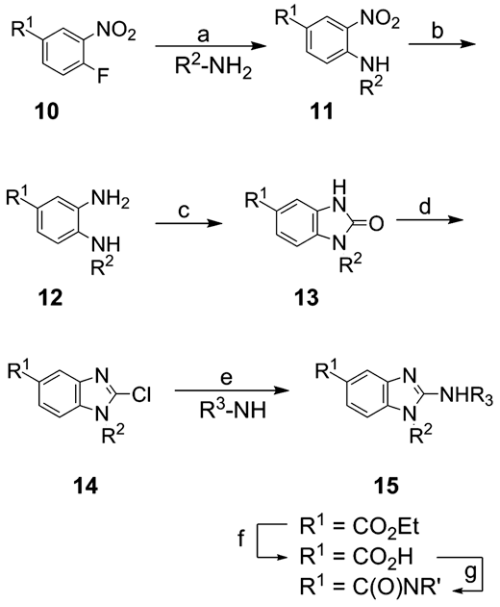
Scheme 1. Solid-phase synthesis of benzimidazoles. (a) X = O Wang resin, *N,N′*-diisopropylcarbodiimide, DMAP; (b) X = NH $(COCl)_2$, DMF then Rink resin, *i*-Pr_2NEt; (c) *i*-Pr_2NEt; (d) $SnCl_2$; (e) *N,N′*-diisopropylcarbodiimide; (f) TFA, H_2O; (g) TMS–CHN_2; (h) PS-CDI resin, HOBt, *i*-Pr_2NEt, HNR′.



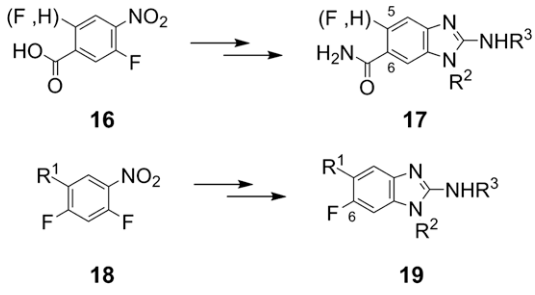
Scheme 2. One-pot synthesis of isothiocyanates from anilines.[9] (a) TFAA, pyr; (b) CS_2, NaOH, K_2CO_3.

While the conversion of anilines to arylisothiocyanates following this route was successful in most cases, the synthesis of aliphatic isothiocyanates failed and therefore the introduction of aliphatic substituents on the exocyclic nitrogen of the 2-aminobenzimidazole scaffold required an alternative methodology. Aliphatic R3 substituents on compounds **6** which were not commercially available as the corresponding isothiocyanates were necessarily introduced through S_NAr of 2-chlorobenzimidazoles **14** to give the corresponding 2-aminobenzimidazoles **15** (Scheme 3). Beginning with 4-fluoro-3-nitrobenzoic acid ethyl ester (**10**, R1 = CO_2Et), S_NAr displacement of fluoride with primary amines gave **11** in analogous fashion to the solid phase approach previously illustrated. 1,2-Diamines **12**, formed by hydrogenation of nitroanilines **11**, were converted to 2-chlorobenzimidazoles **14** in a two-step procedure.[10] Thermal chloride displacement[10b] with amines provided **15**, in which the R1 ethyl ester could be converted to the corresponding carboxylic acid by HCl-promoted hydrolysis and to various amides by subsequent amidation with EDC/HOBt or PS-CDI/HOBt.

Regioisomeric variants of these 2-aminoimidazoles, in which the amide carbonyl is shifted to C6 (Scheme 4, **17**) were prepared under identical conditions to those described in Scheme 1, beginning with the corresponding 3-fluoro-4-nitrobenzoic acid (**16,** –H). While 2,5-difluoro-4-nitrobenzoic acid (**16**, –F) underwent selective displacement of the 5-fluoro substituent by the R2 amines en route to C5 fluorobenzimidazoles **17**, with 2,4-difluoro-5-nitrobenzoic acid (**18**, R1 = CO_2H) it was necessary to modify the reaction conditions to avoid bis-fluoride displacement. Consequently, the sequence was transitioned to solution-phase



Scheme 3. Synthesis of 2-aminobenzimidazoles containing aliphatic R3 substituents. (a) *i*-Pr_2NEt; (b) H_2, Pd–C; (c) CDI, Et_3N, 55 °C; (d) $POCl_3$, neat; (e) DMSO, *i*-Pr_2NEt, 100 °C; (f) 6 N HCl, reflux; (g) EDC, HOBt, *i*-Pr_2NEt, HNR′ or PS-CDI, HOBt, *i*-Pr_2NEt, HNR′.



Scheme 4. Synthesis of regioisomeric 2-aminobenzimidazoles.

(R1 = CO_2Et) and the S_NAr step was performed at 0 °C which selectively led to 6-fluorobenzimidazoles **19**.

One of the initial screening hits, compound **6a**, was used as a starting point for the optimization beginning at the R2 position (Table 1). With an IC_{50} = 7.4 μM in the in vitro endpoint assay, this compound displayed monoaster formation at 100 μM concentration. The benzylic functionality was required for enzymatic activity and substitution at the benzylic methylene was not tolerated—simple introduction of an additional methyl group as in **6b** resulted in a total loss of activity. The *ortho* substituent on the aromatic ring proved to be important for potency as well. Replacing the trifluoromethyl moiety with a ring-nitrogen or a methoxy substituent, as in compounds **6d** and **6e**, respectively, resulted in a substantial loss in potency. However, the 1,2-dimethoxy variant **6f** proved to be well-tolerated with an IC_{50} = 5.0 μM and also showed monoaster forma-

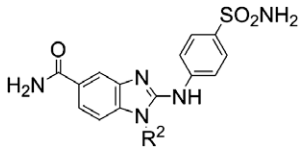
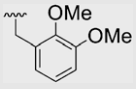
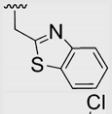
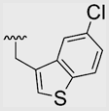
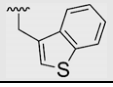


Figure 1. 2-Aminobenzimidazole with R^2 substituents (see Table 1).

Table 1
In vitro endpoint assay data and SAR at the R^2 position (refer to Fig. 1).

Compound	R^2	IC_{50} (μm)[5]
6a		7.4
6b		>20
6c		>20
6d		>20
6e		20
6f		5.0
6g		>20
6h		19.0
6i		2.1

tion in cells at a concentration of 100 μM. Benzothiophenes linked through C3, when properly substituted, were found to be interchangeable groups with the *ortho*-trifluoromethylphenyl of **6a**, resulting in IC_{50} = 19 μM for chloro-substituted **6h** and IC_{50} = 2.1 μM for unsubstituted **6i**. Benzothiazole **6g**, while structurally similar to benzothiophene **6i** but linked through C2, was not tolerated with an IC_{50} > 20 μM.

While keeping the R2 *ortho*-trifluoromethylbenzyl substituent constant, the left- and right-hand portions of the molecule were profiled (Table 2). Modifying the *para*-substituent (R3′) on the anilino-ring led to significant gains in potency. Replacing the sulfonamide (**6a**) with methyl sulfone (**6j**) improved the in vitro activity from 7.4 to 4.8 μM. The corresponding methyl ether (**6k**) and thioether (**6m**) proved even more potent at 2.5 μM and 0.5 μM, respectively. The addition of a fluoro substituent *ortho* to the *p*-methoxy group improved the activity comparing **6k** to **6l** (2.5 μM vs 1.3 μM) and **6p** to **6q** (>20 μM vs 2.3 μM).

The left hand portion of the 2-aminoimidazole core proved less amenable to modification. In general, the carboxylic acids (X = OH, **6n**–**r**) were found to be less potent than the corresponding primary amides discussed above (X = NH_2, **6a**, **6j**–**m**) with R3′ as SMe again being the most potent at 1.4 μM. Both **6k** and **6m** showed cellular activity with IC_{50}'s of 19 and 16 μM, respectively, in the 48 h Alamar blue assay as well as displaying the monoastral phenotype at 25 μM. Not only were the methyl esters (X = OMe, **6s**–**u**) in general less potent than the corresponding carboxylic acids and primary amides, these derivatives also failed to follow the SAR trend at the R3′ position. In both the primary amide (see **6a**) and the carboxylic acid (see **6n**) series, the R3′ sulfonamide was the

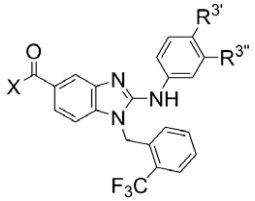


Figure 2. 2-Aminobenzimidazoles in Table 2.

Table 2
In vitro data and SAR at X and R3 positions (refer to Fig. 2)

Compound	X	R3′	R3″	IC_{50} (μM)[5]
6a	$-NH_2$	$-SO_2NH_2$	H	7.4
6j	$-NH_2$	–SO2Me	H	4.8
6k	$-NH_2$	–OMe	H	2.5
6l	$-NH_2$	–OMe	F	1.3
6m	$-NH_2$	–SMe	H	0.52
6n	–OH	$-SO_2NH_2$	H	>20
6o	–OH	–SO2Me	H	6.9
6p	–OH	–OMe	H	>20
6q	–OH	–OMe	F	2.3
6r	–OH	–SMe	H	1.4
6s	–OMe	$-SO_2NH_2$	H	2.2
6t	–OMe	–OMe	H	13.4
6u	–OMe	–SMe	H	33.4
6v	–NHMe	–OMe	H	5.2
6w	–NHMe	$-SO_2NH_2$	H	9.2
6x	$-NMe_2$	$-SO_2NH_2$	H	16.3
6y	$-N(CH_2)_4$	$-SO_2NH_2$	H	>20
6z	–NH–Ph(*o*-OMe)	$-SO_2NH_2$	H	2.4
6aa	–NH–Ph(o–OMe)	–OMe	H	6.0

least potent and the thiomethylether the most potent. The reverse was true in the methyl ester series with sulfonamide **6s** and thioether **6u** having IC_{50} values of 2.2 and 33 μM respectively. The majority of amides synthesized which were larger than the primary amide (X = NH_2, ~30 compounds) were found to be less potent than the corresponding primary amides, as illustrated by comparing compounds **6a** (7.4 μM) with **6x** and **6y** (16.3 and >20 μM, respectively). However, two substitutions were tolerated at this position. *N*-Methyl amides **6v** and **6w** demonstrated small loses in potency compared to the analogous primary amides **6k** and **6a** (2.5–5.2 and 7.4–9.2 μM, respectively). The *ortho*-methoxy-aniline-based amides **6z** and **6aa** showed mixed results compared to the corresponding primary amides, with **6aa** losing activity versus **6k** (2.5 to 6.0 μM) and **6z** showing a modest improvement over **6a** (7.4–2.4 μM).

With the apparent need for a polar group on the distal side of the R3 ring identified, the spacer between the 2-aminobenzimidazole core and this group was probed (Fig. 3, Table 3). Small, non-aromatic heterocycles such as aminopyrrolidine **15a** failed to display any in vitro activity. Similarly, adding an additional one (**15b**, **15c**, and **15d**) or two (**15e**) methylene spacer while keeping the active *para*-substituted phenyl ring in place also resulted in significant potency loss. The synthesis of more than 50 related compounds illustrated that the anilino ring was a requirement for both enzymatic and cellular potency.

With the periphery of the benzimidazole template profiled, further substitution of the bicycle was examined (Fig. 4). Moving the primary amide from C5 in **6a** to C6 in **17a** and **17b** resulted in complete loss of in vitro activity. 6-Fluoro derivative **19a**, a regioisomer of inactive **17b**, retained the potency of the parent compound (**6a**) at 7.9 μM, indicating that C6 may be amenable to further substitution provided that the C5 carbonyl remains intact. The C2 amino functionality found in all compounds discussed thus far proved

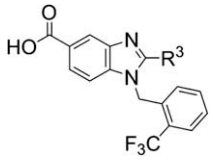
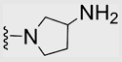
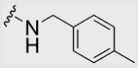
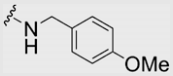
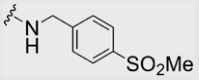
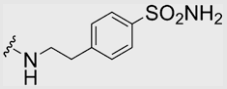
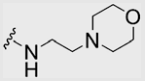


Figure 3. 2-Aminobenzimidazole with aliphatic R3 substituents (see Table 3).

Table 3
In vitro endpoint assay data for aliphatic R3 substituents

Compound	R3	IC50 (μM)[5]
15a		>20
15b		>20
15c		>20
15d		>20
15e		>20
15f		>20

to be vital as well since replacing it with a methylene linker in **20a** and **20b** led to potency loss.

A DMPK analysis of selected compounds from this series showed poor oral exposure in the rat, but a promising profile in cytochrome P450 inhibition (Table 4). Compounds **6k** and **6m** demonstrated good permeability in the Caco-2 assay.

As compounds identified from mixture-based, solid-phase benzimidazole libraries as *h*KSP inhibitors, this series appears to be non-competitive with ADP and monastrol.[3a] Conclusive evidence of multiple compounds in this series showing binding affinity enhancement of quinazolinone-based Ispinesib analogs to KSP through positive cooperative allosteric binding has been discovered and will be reported in a separate communication. Cell-based activity has been observed in 48 h and 7-day Alamar blue assays and monoaster formation was observed for the most active compounds. Kinesin counterscreens indicated all compounds discussed herein are selective for *h*KSP over Kif3B and nKHC (with in vitro IC_{50} values >50 μM). Selectivity over HSET proved to be more of a challenge but several compounds demonstrated moderate to good selectivity in the corresponding biochemical assays, such as **6a** (fivefold), **6o** (sevenfold), **6m** (eightfold), **6l** (11-fold), and **6i** (>24-fold). Each benzimidazole combinatorial position has been explored, and intuitive compounds are currently under investigation.

Acknowledgments

The authors acknowledge Drs. Noriyuki Kawahata and Tong Wang for their early work on this project.

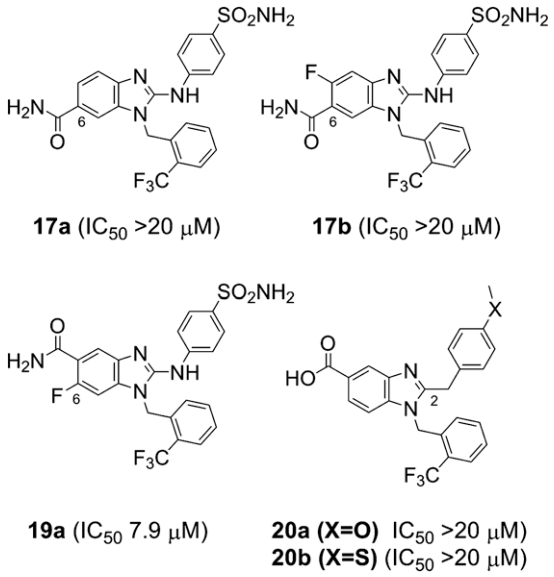


Figure 4. Benzimidazoles with varying substitution patterns and their in vitro endpoint assay IC_{50} values.[5]

Table 4
DMPK profile of selected compounds

Compound	Rat AUC (nM h)[a]	P450 3A4 (co/pre)[b]	P450 2D6 (co/pre)[b]	P450 2C9 (co/pre)[b]	Caco-2 (nm/s)[c]
6j	0	>30/>20	>30/>15	15/>8	18
6k	600	>30/>15	>30/>15	8/>4	670
6m	150	>30/<15	>30/>15	14/>7	530

[a] Rat oral exposure over 6 h at a dose of 10 mpk.
[b] Reported as IC_{50} in μM.
[c] Caco-2 permeability.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.040.

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5. Each compound assayed in the in vitro endpoint assay was tested in duplicate ($n = 2$) with %CV's of <10. Further details of the in vitro and cellular proliferation assays can be found in Supplementary data, as well as the

corresponding patent application: Shipps, G. W., Jr.; Ma, Y.; Lahue, B. R.; Seghezzi, W.; Herbst, R.; Chuang, C.-C.; Annis, D. A.; Kirt-Ley, M. WO2008153701(A1).
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