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Discovery of the first known small-molecule inhibitors of heme-regulated eukaryotic initiation factor 2α (HRI) kinase

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ABSTRACT

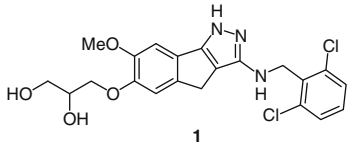
A series of indeno[1,2-*c*]pyrazoles were discovered to be the first known inhibitors of heme-regulated eukaryotic initiation factor 2α (HRI) kinase. The synthesis, structure–activity relationship profile, and in-vitro pharmacological characterization of this inaugural series of HRI kinase inhibitors are detailed.

Oxygen homeostasis is critical to life processes, and multicellular organisms have developed complex mechanisms for the uptake, distribution, and maintenance of oxygen levels. An example of such mechanisms is transport of oxygen from the lungs to various tissues by hemoglobin, which is the primary protein in red blood cells.[1] The role of hemoglobin is to transport oxygen from the lungs to tissues, a function that is facilitated by reversible binding of oxygen to the iron-containing heme moiety of hemoglobin.[2]

Synthesis of hemoglobin is a complex process that involves translation and modifications of globin proteins, heme synthesis, and incorporation of an iron atom into heme.[3,4] Eukaryotic initiation factor 2α (eIF-2α) kinases are important and well characterized regulators of translation in eukaryotic cells.[5–13] Heme-regulated eIF-2α (HRI) kinase is expressed primarily in reticulocytes and in erythroid cells in bone marrow. HRI kinase phosphorylates eIF-2α and initiates a reduction in globin synthesis, thereby regulating the availability of α and β globin chains relative to the amount of heme available for production of hemoglobin. It logically follows that a beneficial level of small-molecule inhibition of HRI kinase should be expected to promote an increased rate of globin production and hemoglobin levels, thereby increasing the oxygen carrying capacity of red blood cells.[9] The discovery of such an HRI kinase inhibitor could hold great promise in the treatment of anemia, a widespread and debilitating condition for which the only currently available treatment options are direct blood transfusion, erythropoietin (EPO), and dietary iron and vitamin supplementation.[14]

With the above model of therapeutic intervention in mind, we initiated an exploratory program to discover and characterize small-molecule inhibitors of HRI kinase. An HRI kinase assay was developed and validated using recombinant full-length human HRI kinase, and subsequent screening led to the discovery of pyrazolindane **1** (Fig. 1).[15,16]

Compound **1** was found to display potent, ATP-competitive HRI kinase inhibition (pIC_{50} = 7.5). Preliminary in-vitro ADME predictors and in-vivo pharmacokinetic parameters showed that this initial screening hit had good stability toward human, dog, and rat liver microsomes ($t_{1/2}$ > 60 min), with human and rat plasma protein binding levels of 87% and 90%, respectively (Table 1). Unfortunately, **1** showed low bioavailability in the rat (ca. 18%) and rapid clearance, with an IV half-life of 1.6 h.



1

Figure 1. Aminopyrazolindane HRI inhibitor from HTS screening.

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Table 1
Preliminary in-vitro and rat pharmacokinetic profiling of **1**

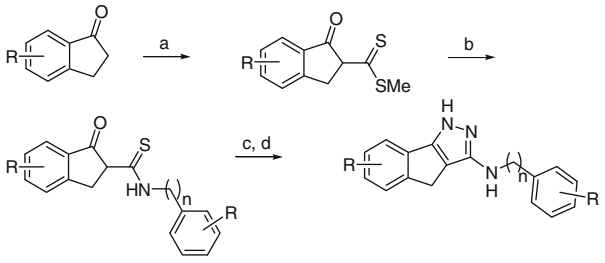
pIC_{50}[a]	Plasma protein binding (%)		Liver microsomal stability ($t_{1/2}$, min)		Pharmacokinetic parameters (rat)[b]		
	Human	Rat	Human	Rat	$t_{1/2}$ (h)	%F	CL (L/kg/h)
7.5	87	90	>60	>60	1.6 ± 0.1	18 ± 6	1.9 ± 0.2

[a] Negative logarithm of the concentration required to achieve 50% enzyme inhibition; value is ±0.3 log units.
[b] Values calculated from three animals.

Given the preliminary profiling data, compound **1** was deemed to be a reasonable starting point from which to undertake a medicinal chemistry program. The general synthesis route for an initial set of analogs is outlined in Scheme 1.

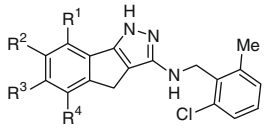
Known or commercially-available indanones were treated with LiHMDS and dimethyl trithiocarbonate to provide thioesters, which were then allowed to react with a variety of amines to provide intermediate thioamides. Alkylation of sulfur with methyl iodide, followed by exposure to excess hydrazine in refluxing ethanol, afforded the final aminopyrazolindane products. We initially evaluated the effects of certain substituents on the indane phenyl ring for specific compounds, as shown in Table 2.

With respect to compound **2**, the removal of all substitution from the indane phenyl ring results in a dramatic loss in activity, and reintroduction of a single methoxy group in the R^1 position of **3** leads to the complete abolition of enzyme inhibition. This lost activity is largely restored by installation of a methoxy group at the R^2 position of **4**, and optimal activity was achieved with methoxy groups at both the R^2 and R^3 positions of **7**. Interestingly, the methylenedioxy analog **8** suffered a 10-fold loss of enzyme inhibitory activity relative to **7**, suggesting that such conformational restriction is detrimental in the tested compounds. With respect to compounds **10–13**, replacement of the R^2 methoxy group with alkyl, amino, halo, and thioether groups all resulted in a substantial loss in activity (e.g., **10**–**13**).



Scheme 1. General synthesis route for aminopyrazolindanes. Reagents and conditions: (a) LiHMDS, $(MeS)_2CS$, THF, 0 °C to rt; (b) amine, EtOH, reflux; (c) MeI, iPr_2NEt, THF; (d) hydrazine, EtOH, reflux.

Table 2
Indane phenyl ring substituent effects



Compd	R^1	R^2	R^3	R^4	pIC_{50}[a]
2	H	H	H	H	5.8
3	OMe	H	H	H	<5
4	H	OMe	H	H	7.3
5	H	H	OMe	H	6.3
6	H	H	H	OMe	6.1
7	H	OMe	OMe	H	7.7
8	H	$-OCH_2O-$		H	6.6
9	H	OMe	H	OMe	6.9
10	H	Et	H	H	6.5
11	H	NMe_2	H	H	6.6
12	H	Br	H	H	6.1
13	H	SMe	H	H	6.0

[a] Negative logarithm of the concentration required to achieve 50% enzyme inhibition. All values are ±0.3 log units.

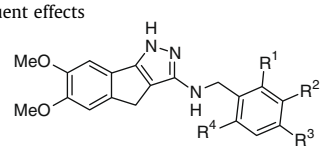
Using the same synthesis route outlined in Scheme 1, next we examined structural changes to the benzylamine moiety (Table 3). Substitution with a chloride or a methyl group at the R^1 and R^4 in compounds **19**–**22** provided high levels of enzyme inhibitory activity, with the dimethyl analog **21** emerging as the most active analog with a pIC_{50} value of 8.1 (IC_{50} = 7.9 nM). An important feature of this series is demonstrated by analogs **17** and **18**, which possess chloride atoms at the *para* (R^3) position. Both these analogs incur a substantial loss in activity relative to their direct comparators **14** and **15**. Methoxy analog **23** also suffers a significant loss in activity.

Having discovered favorable substitution elements for both the phenyl portion of the tricyclic ring and the benzylamine side chain in the tested compounds, we next examined modifications to core ring system that included replacement, homologation, and elimination of the indane methylene group. The synthesis of these compounds is described below.

The synthesis of ketone analog **27** began from known indanedione **24**, which reacts with carbon disulfide and methyl iodide in the presence of potassium fluoride to provide dithioketene acetal **25**.[17] Exposure of **25** to 2,6-dichlorobenzylamine affords vinylogous amide **26**, which is converted to **27** by treatment with hydrazine and subsequent cyclization in refluxing *i*PrOH.

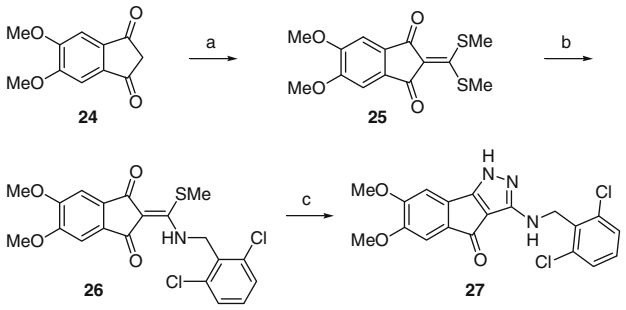
Ether, thioether, and sulfone analogs **31**–**33** were prepared from known benzofuran-3-one **28** and benzothiophen-3-one **29** (Scheme 3).[18] Compounds **28** and **29** were elaborated as described in Scheme 1 to provide benzofuropyrazole **31** and benzothienylpyrazole **32**, respectively. Alternatively, **29** could oxidized using *m*CPBA to afford sulfoxide **30**, which was then processed in a manner analogous to that described in Scheme 2 in order to afford benzothienylpyrazole-*S*,*S*-dioxide **33**.

Table 3
Benzylamine substituent effects

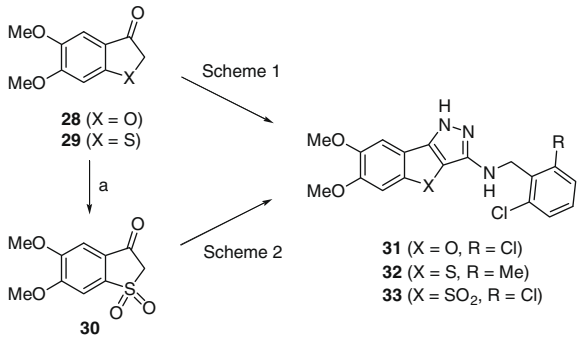


Compd	R^1	R^2	R^3	R^4	pIC_{50}[a]
14	H	H	H	H	6.2
15	Cl	H	H	H	6.7
16	H	Cl	H	H	6.0
17	H	H	Cl	H	5.6
18	Cl	H	Cl	H	5.9
19	Cl	H	H	Cl	7.4
20	Cl	H	H	Me	7.7
21	Me	H	H	Me	8.1
22	Me	H	H	H	7.2
23	OMe	H	H	H	5.5

[a] Negative logarithm of the concentration required to achieve 50% enzyme inhibition. All values are ±0.3 log units.



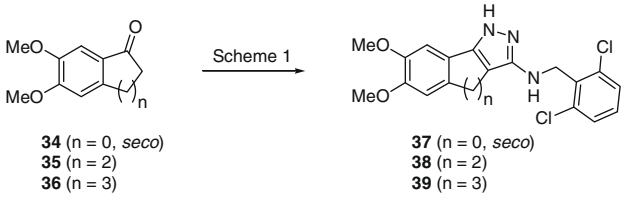
Scheme 2. Synthesis of **33**. Reagents and conditions: (a) CS_2, MeI, CsF, DMSO, 46%; (b) 2,6-dichlorobenzylamine, *i*PrOH, reflux, 95%; (c) hydrazine, *i*PrOH, reflux, 53%.



Scheme 3. Synthesis of ether **31**, sulfide **32**, and sulfone **33**. Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, 74%.

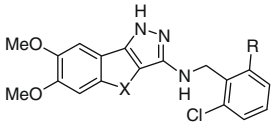
Compounds **37**, **38**, and **39** encompass excision of the central ring, one carbon homologation, and two carbon homologation, respectively (Scheme 4). These compounds were prepared from commercially-available acetophenone **34**, tetralone **35**, or benzocycloheptanone **36** in a manner analogous to that described in Scheme 1.

As shown in Table 4, replacement of the methylene unit of **19** with a ketone group in **27** results in an almost 10-fold increase in enzyme inhibition. Similar replacement of the methylene group with oxygen gives ether **31**, which is essentially isoactive with **19**, while substitution with divalent sulfur incurs a greater than 10-fold loss in activity (i.e., **20** to **32**). This loss of activity is largely regained in sulfoxide analog **33**. In order to examine the purely geometrical aspects of these data, we turn to carbon homologs **38** and **39**. Insertion of either one or two additional methylene units resulted in complete loss of activity, likely without incurring significant electronic perturbation. The total removal of this carbon bridge in seco analog **37** restores a portion of this lost activity. B3LYP/6-31G** optimizations on the cores of **38** and **39** have predicted tautomer-averaged dihedral angles of 13.7° and 20.4°, respectively, between the phenyl and pyrazole rings, while for **19** the two rings are coplanar to within 0.3°.[19] This lack of planarity in **38** and **39** and the concomitant positional shift of the phenyl ring relative to the pyrazole ring is illustrated graphically in



Scheme 4. *Seco* and homologated analogs **37**–**39**.

Table 4
Central ring structure–activity relationship



Compd	X	R	pIC_{50}[a]
27	C=O	Cl	8.2
31	O	Cl	7.2
32	S	Me	6.3
33	SO_2	Cl	7.1
37	–H,H–	Cl	5.6
38	$(CH_2)_2$	Cl	<5
39	$(CH_2)_3$	Cl	<5

[a] Negative logarithm of the concentration required to achieve 50% enzyme inhibition. All values are ±0.3 log units.

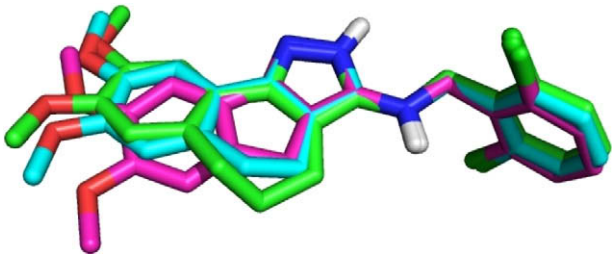


Figure 2. Overlay of energy-minimized structures of **19** (magenta), **38** (cyan), and **39** (green).

Figure 2, where the overlaid, energy-minimized structures of **19**, **38**, and **39** demonstrate the repositioning of the methoxyphenyl portion of these particular molecules.[20] Unfortunately, despite protracted efforts we were unable to obtain a X-ray crystal structure of full-length HRI kinase in either apo or holo form to shed further light on this aspect of our observations. Additional crystallographic efforts using truncated forms of the protein will be reported in due course.

In summary, we have discovered a series of aminopyrazoloindanes that are, to the best of our knowledge, the first known small-molecule inhibitors of HRI kinase. Furthermore, the early in-vitro data for this series was demonstrated to be robust, tractable, and amenable to optimization. Modifications to the central ring of the indane core were examined in detail, ultimately leading to compounds such as **27** that display inhibitory activity against the full-length protein at low nanomolar concentrations. Additional studies of this inaugural series of HRI kinase inhibitors, including selectivity profiling as well as other second-generation studies, will be reported in the near future.

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