Imidazole- and Benzimidazole-Based Inhibitors of the Kinase IspE: Targeting the Substrate-Binding Site and the Triphosphate-Binding Loop of the ATP Site


Keywords: Drug design / Inhibitors / Ligand design / Molecular recognition

The enzymes of the mevalonate-independent biosynthetic pathway to isoprenoids are attractive targets for the development of new drug candidates, in particular against malaria and tuberculosis, because they are present in major human pathogens but not in humans. Herein, the structure-based design, synthesis, and biological evaluation of a series of inhibitors featuring a central imidazole or benzimidazole scaffold for the kinase IspE from E. coli, a model for the corresponding malarial enzyme, are described. Optimization of the binding preferences of the hydrophobic sub-pocket at the substrate-binding site allowed IC₅₀ values in the lower micromolar range to be reached. Structure–activity relationship studies using a 1,2-disubstituted imidazole central core revealed that alicyclic moieties fit the sub-pocket better than acyclic aliphatic and aromatic residues. The phosphate-binding region in the ATP-binding site of IspE, a neutral glycine-rich loop, was addressed for the first time by an additional vector attached to the central core. Polar functional groups, such as trifluoromethyl or nitriles, were introduced to undergo orthogonal dipolar interactions with the amide groups in the loop. Alternatively, small hydrogen-bond-accepting heterocyclic residues, capable of binding to the convergent NH groups in the loop, were explored. The biological data showed slightly improved inhibitory potency in some cases and confirmed the challenges in addressing, with gain in binding affinity, the highly water-exposed sections of enzyme active sites, such as the glycine-rich loop of IspE.

Introduction

Discovered in the early 1990s, the non-mevalonate pathway is a biosynthetic route to assemble the universal isoprenoid precursors, isopentenyl diphosphate (IPP; 1) and dimethylallyl diphosphate (DMAPP; 2), in seven enzymatic steps starting from pyruvate (3) and d-glyceraldehyde 3-phosphate (4) (Scheme 1).[1] The enzymes of this pathway are essential in plants[2] and many human pathogens, including the tuberculosis-causing Mycobacterium (M.) tuberculosis (M.) and the causative agent of malaria, Plasmodium (P.) falciparum.[3] In humans, in contrast, isoprenoids are exclusively assembled through the mevalonate-dependent biosynthetic route, with acetyl coenzyme A serving as the only carbon source. Thus, the enzymes of the non-mevalonate pathway are widely recognized as attractive targets for the generation of lead compounds with potential as selective antibacterial, antimalarial, or agrochemical agents featuring novel modes of action, which is a fundamental issue that needs to be overcome to fight fast-developing resistance.[5]

The fourth enzyme and only kinase involved in the non-mevalonate pathway, IspE [4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME) kinase, EC 2.7.1.148], catalyzes the ATP- and Mg²⁺-dependent phosphorylation of the tertiary alcohol of CDP-ME (5) to give 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate (CDP-ME2P, 6; Scheme 1).[6] Due to the lack of structural information on IspE orthologues from pathogens until the recent report of X-ray crystal structures of the M. tuberculosis enzyme,[6b] our initial efforts in mapping the molecular recognition properties of IspE used the enzyme from Escherichia (E.) coli as a model system. In our previous studies, we extensively explored the binding preferences of the small hydrophobic and the ribose sub-pockets of the substrate-binding site.[6g,7]

Herein, we report the structure-based design, synthesis, and biological evaluation of imidazole- and benzimidazole-based inhibitors targeting the phosphate recognition site –
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Scheme 1. The non-mevalonate biosynthetic pathway to isoprenoid precursors, i.e., isopentenyl diphosphate (IPP; 1) and dimethylallyl diphosphate (DMAPP; 2). DXS: 1-deoxy-d-xylulose 5-phosphate synthase (EC 2.2.1.7); IspC: 1-deoxy-d-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267); IspD: 4-diphosphocytidyl-2C-methyl-d-erythritol 4-phosphate synthase (EC 2.7.7.60); IspE: 4-diphosphocytidyl-2C-methyl-d-erythritol kinase (EC 2.7.1.148); IspF: 2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12); IspG: 2C-methyl-d-erythritol 2,4-cyclodiphosphate reductase (EC 1.17.7.1); IspH: (2E)-1-hydroxy-2-methyl-2-butenyl 4-diphosphate reductase.

Results and Discussion

Ligand Design

Molecular modeling was performed with the software MOLOC[9] using the X-ray crystal structure of E. coli IspE in complex with CDP-ME (5) and the ATP-analogue adenosine-5′-(γ-imino)triphosphate (AMP-PNP, PDB accession code 1OJ4, 2.0 Å resolution).[6c] The active site of E. coli IspE can be divided into three main pockets, corresponding to the cytidine- and methylerythritol-binding regions for the recognition of substrate 5, and the adenosine-binding pocket at the other end of the active site (Figure 1).

In our previous studies, we have addressed the cytidine-binding pocket with a 1,5-disubstituted cytosine scaffold stacking between the aromatic side chains of Tyr25 and Phe185, similar to the natural substrate. Saturated five-membered heterocycles such as 2-tetrahydrothienyl or 2-tetrahydrofuranyl moieties were shown to address the ribose sub-pocket best.[6g,7b] Modeling revealed the presence of a small, hydrophobic sub-pocket lined by Leu15, Leu28, and Phe185 adjacent to the cytidine-binding pocket. Appropriate filling, achieved with small lipophilic residues perfectly oriented into the cavity by a propargylic sulfonamide moiety in its preferred conformation (staggered with the N-lone pair bisecting the O=S=O moiety), allowed inhibitory constants ($K_i$) down to the triple-digit nanomolar range to be obtained.[7a]

The introduction of an additional third vector towards the glycine-rich loop required the installation of a new central core in the ligands. Modeling suggested a 1,2,4-trisubstituted imidazole ring as an appropriate scaffold to direct the three exit vectors, with the heterocycle stacking on the polarized side chain of Asp141 (Figure 2).

We first investigated the appropriate filling of the hydrophobic sub-pocket by introducing different lipophilic substituents at imidazole-C-2. Subsequently, the triphosphate-binding region of E. coli IspE was addressed by the introduction of pharmacophores linked to position C-4 of the imidazole moiety by a methylene spacer. In IspE, a glycine-rich loop (Gly101–Ser108; Figure 1) is responsible for binding the ATP-triphosphate through multiple hydrogen bonds with the converging backbone NH groups, without the involvement of basic amino acids or metal cations.[6c,6f,8] This confers a certain lipophilic character to the loop, which, together with a balance between preorganization and the ability to wrap around a guest, makes it appealing for increasing binding affinity, also taking advantage of a potential bisubstrate inhibition mode.[10] In addition to hydrogen-bond-accepting heterocycles such as tetrazole or triazole, we considered small and negatively polarized functional groups, such as nitriles and trifluoromethyl groups, as phosphate surrogates suitable for this neutral glycine-rich loop, due to their ability to undergo favorable orthogonal dipolar interactions.[11]
Figure 1. Schematic view of the active site of *E. coli* IspE depicting the different pockets, of which the inhibitors described here address the cytidine-binding pocket, the hydrophobic sub-pocket, and the phosphate-binding loop.

Figure 2. Design strategy of new ligands bearing a 1,2,4-trisubstituted imidazole central core and proposed binding mode of the imidazole fragment at the active site of *E. coli* IspE (PDB code: 1OJ4) as modeled with MOLOC. Distances are given in Å units. Color code: gray C enzyme, green C ligand, blue N, red O, yellow S. If not otherwise stated, this color code is used throughout.

**Optimization of the Binding towards the Sub-Pocket**

Ligands (±)-7-(±)-12 (see Table 1 for the structures) were synthesized in two or three steps, as illustrated in Scheme 2 for compound (±)-10 [for the syntheses of the other ligands, see the Supporting Information, Scheme S1]. In the first step, cyclohexanecarbaldehyde (13) was treated with glyoxal and ammonium hydrogen carbonate in water, following a literature procedure, to afford the 2-substituted imidazole 14. Alkylation of imidazole 14 with propargyl bromide gave alkyne 15, which was subsequently cross-coupled with the previously described 1-thiolanylated 5-iodocytosine (±)-16 under Sonogashira conditions to provide the desired ligand (±)-10 in moderate yield.
Scheme 2. Synthesis of ligands (±)-10 and (±)-17. Reagents and conditions: (a) Glyoxal, NH₄HCO₃, H₂O, 25 °C, 18 h, 25 %; (b) propargyl bromide, Cs₂CO₃, DMF , 0 to 25 °C, 9 h, 28 %; (c) [PdCl₂(PPh₃)₂], CuI, Et₃N, DMF , 25 °C, 3.5 h, 25 %; (d) Me₂NSO₂Cl, Et₃N, CH₂Cl₂, 25 °C, 14 h, 62 %; (e) i. nBuLi, THF , –78 °C, 45 min; ii. DMF , –78 to 25 °C, 2 h, 83 %; (f) Me ₃SiCF₃, nBu₄NF , THF , 0 to 25 °C, 2 h, 73 %; (g) NaH, MeI, DMF , 0 to 25 °C, 16 h, 85 %; (h) 5 % aq. HCl, 25 °C, 7 h, 90 %; (i) propargyl bromide, Cs₂CO₃, DMF , 0 to 25 °C, 8 h, 48 %; (j) (±)-16, [PdCl₂(PPh₃)₂], CuI, Et₃N , DMF , 25 °C, 7 h, 47 %.

Table 1. Structures and inhibitory activities of ligands (±)-7-(±)-12 against E. coli IspE.

<table>
<thead>
<tr>
<th>Inhibitor R</th>
<th>clogD at pH 8.0</th>
<th>IC₅₀ [μM]</th>
<th>Kᵢ [μM]</th>
</tr>
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<tbody>
<tr>
<td>(±)-7 H</td>
<td>1.87</td>
<td>&gt;500</td>
<td>-</td>
</tr>
<tr>
<td>(±)-8 nPr</td>
<td>2.54</td>
<td>83</td>
<td>48</td>
</tr>
<tr>
<td>(±)-9 iBu</td>
<td>2.89</td>
<td>80</td>
<td>46</td>
</tr>
<tr>
<td>(±)-10 cyclohexyl</td>
<td>3.50</td>
<td>12</td>
<td>6.9</td>
</tr>
<tr>
<td>(±)-11 cyclopentyl</td>
<td>2.97</td>
<td>15</td>
<td>8.6</td>
</tr>
<tr>
<td>(±)-12 Ph</td>
<td>3.42</td>
<td>144</td>
<td>83</td>
</tr>
</tbody>
</table>

[a] The clogD values were calculated with the Advanced Chemistry Development (ACD/Labs) Software v. 12.5 (1994–2012, ACD/Labs). [b] Kᵢ values were calculated from IC₅₀ values and Kᵢ = 150 μM using the Cheng–Prusoff equation.[10]

The inhibitory potency towards E. coli IspE was found to be strongly dependent on the substituent filling the hydrophobic sub-pocket (Table 1). Compound (±)-7, unsubstituted at imidazole-C-2, did not display any significant inhibition at 500 μM, whereas an additional substituent at C-2 resulted in a substantial gain in binding affinity. Linear or branched alkyl chains, such as n-propyl and isobutyl, led to IC₅₀ values in the double-digit micromolar range for compounds (±)-8 and (±)-9.

Replacement of the alkyl chains with saturated alicyclic moieties in inhibitors (±)-10 and (±)-11 resulted in an additional five- to sixfold enhancement in activity, with IC₅₀ values of 12 and 15 μM, respectively. Interestingly, the phenyl-substituted analogue (±)-12 was found to be substantially less potent (IC₅₀ value = 144 μM), indicating that

The half-maximal (50 %) inhibitory concentration (IC₅₀) values were determined in an enzyme-coupled assay using pyruvate kinase and lactate dehydrogenase as auxiliary enzymes to enable photometric monitoring (see Scheme S2).[13] For the phosphorylation of the substrate CDP-ME (5), IspE consumes ATP, which is then regenerated by pyruvate kinase dephosphorylating phosphoenol pyruvate. The resulting pyruvate is reduced to l-lactate by the lactate dehydrogenase using NADH as reducing agent. The consumption of NADH is monitored photometrically at 340 nm. This photometric assay has previously been proven to work effectively for structurally related compounds by comparison with a direct NMR spectroscopic assay.[13]
the inhibitory activity increase of compounds (±)-10 and (±)-11 is not solely a result of increasingly favorable partitioning (increasing clogD values), but also of a better fit of the respective substituents into the sub-pocket (Figure 3).

Figure 3. Close-up of the binding mode of (±)-10 in the hydrophobic sub-pocket of E. coli IspE as modeled with MOLOC (PDB code: 1OJ4). Distances in Å.

Addressing the Phosphate-Binding Loop

The cyclohexyl substituent was maintained as the moiety filling the hydrophobic sub-pocket, due to the superior binding affinity of (±)-10 compared with the other compounds included in Table 1. Therefore, the new series of compounds was based on the structure of inhibitor (±)-10 bearing an additional vector at imidazole-C-4 for targeting the glycine-rich P-loop.

The introduction of the additional substituent in ligands (±)-17–(±)-23 (see Table 2 for the structures) was achieved through the organolithium-mediated formylation of the imidazole moiety and subsequent chain extension by intergroup conversion of the resulting aldehyde. As an example, the synthesis of compound (±)-17 is shown in Scheme 2. The 2-substituted imidazole 14 was protected with the ortho-directing N,N-dimethylsulfamoyl group using the corresponding sulfamoyl chloride and triethylamine as base to give 24 in moderate yield.\textsuperscript{[14]} Lithiation of imidazole 24 with nBuLi, followed by reaction with N,N-dimethylformamide (DMF), afforded 5-formylimidazole 25 as a single regioisomer in very good yield.\textsuperscript{[15]} Aldehyde 25 was then treated with the Ruppert–Prakash reagent\textsuperscript{[16]} and tetrabutylammonium fluoride to afford alcohol (±)-26 as a racemate in good yield. Methylation of the secondary alcohol and subsequent cleavage of the protecting group of (±)-27 under acidic conditions gave key intermediate (±)-28. The imidazole moiety was then N-alkylated with propargyl bromide using cesium carbonate as base, affording a mixture of regioisomers, which was separated by column chromatography to provide the desired 1,2,4-trisubstituted imidazole (±)-29 in moderate yield. Finally, Sonogashira cross-coupling with 5-iodocytosine (±)-16 yielded the target compound (±)-17 in 47% yield. All other 1,2,4-trisubstituted imidazoles were prepared starting from aldehyde 25 as a common precursor following similar strategies.

The nitrile functional group of ligands (±)-18 and (±)-19 were introduced by Horner–Wadsworth–Emmons reaction; the common precursor of dinitriles (±)-20 and (±)-21 was obtained by Knoevenagel condensation (see Scheme S3). Reduction of the aldehyde functional group to give the corresponding alcohol and subsequent activation by treatment with thionyl chloride allowed the introduction by nucleophilic substitution of the 1H-tetrazole and 1,2,3-1H-triazole moieties in ligands (±)-22 and (±)-23, respectively (see Scheme S4).

Table 2. Structures and inhibitory activities of ligands (±)-17–(±)-23 against E. coli IspE.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>R</th>
<th>clogD\textsuperscript{[a]} at pH 8.0</th>
<th>IC\textsubscript{50} [µM]</th>
<th>K\textsubscript{m} [µM]</th>
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<td>4.42</td>
<td>9.9</td>
<td>5.7</td>
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<td></td>
<td>2.71</td>
<td>107</td>
<td>61</td>
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</tbody>
</table>

[a] The clogD values were calculated with the Advanced Chemistry Development (ACD/Labs) Software v. 12.5 (1994–2012, ACD/Labs). [b] K\textsubscript{m} values were calculated from IC\textsubscript{50} values and K\textsubscript{m} = 150 µM using the Cheng–Prusoff equation.\textsuperscript{[20]}

According to molecular modeling studies, all the compounds in this series should establish favorable orthogonal dipolar interactions with the peptide bonds of the glycinerich loop (see Figure S1). Only the trifluoromethyl derivative (±)-17, however, was found to be slightly more active than the parent compound (±)-10 unsubstituted at imidazole-C-4. This might indicate that the methylene linker carrying the phosphate surrogate is too flexible to efficiently position the pharmacophore in the phosphate-binding re-
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gion. This flexibility allows phosphate surrogates, in particular the more polar versions, to orient towards bulk water rather than binding to the loop.

Benzimidazole-Based Ligands

The next series of compounds was designed with the specific aim of establishing a more direct and preorganized access to the glycine-rich loop. For this purpose, the imidazole core was extended to include a benzimidazole moiety. In this way, the methylene linker could be omitted and the tendency of the phosphate mimic to orient toward the bulk solvent should thereby be attenuated. Molecular modeling studies suggested that positions C-4 and C-5 of the benzimidazole core were well-suited for targeting the glycine-rich loop, depending on the phosphate surrogate chosen.

Benzimidazoles (±)-30–(±)-34 (see Figure 4 for the structures) were synthesized by applying the strategy described previously for the imidazole-based ligands. This involved construction of the heterocyclic core and its eventual modification by intergroup conversion, followed by N-alkylation and final Sonogashira cross-coupling. As an example, the synthesis of 4-alkoxybenzimidazole (±)-34 is shown in Scheme 3 (for the syntheses of the other benzimidazole-derived ligands, see Scheme S5). Reductive cyclization of 2-amino-3-nitrophenol (35) with aldehyde 13 in the presence of sodium dithionite according to a literature procedure gave 2,4-disubstituted benzimidazole 36 in 80% yield.[17] Treatment of alcohol 36 with 2-propanol under Mitsunobu conditions provided ether 37 in moderate yield after recrystallization from ethyl acetate. Whereas the alkylation of 2,5-disubstituted benzimidazoles afforded a ca. 1:1 mixture of regioisomers, usually separable with column chromatography with the structural assignment based on 2D NMR spectroscopy, the outcome of the alkylation of 4-alkoxybenzimidazole 37 with sodium hydride and propargyl bromide proved to be strongly dependent on the solvent (see Figure S2). In DMF, the undesired and sterically more hindered 1,2,7-trisubstituted benzimidazole 38 was the major isomer formed (ratio 38/39 = 4:1), indicating electronic rather than steric control of the reaction. The regioselectivity of the alkylation was inverted in tetrahydrofuran (THF), in which a 1:3 ratio of 38/39 was observed. The ratio in favor of the desired isomer was significantly improved (38/39 = 1:5) in a less polar, noncoordinating solvent, such as dichloromethane. This observation supports a mechanism involving coordination of the Na+ cation to the deprotonated nitrogen atom of the benzimidazole.
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Scheme 3. Synthesis of ligand (±)-34 and ORTEP plot at 100 K (atomic displacement parameters are shown at the 50% probability level) of the X-ray crystal structure of (±)-34. For the sake of clarity, solvent molecules are omitted and only one position of the disordered tetrahydrothienyl ring and isopropyl group are shown. Reagents and conditions: (a) 1 m aq. Na2S2O4, EtOH, 70 °C, 5 h, 80%; (b) PPh3, diisopropyl azodicarboxylate, iPrOH, THF, 25 °C, 2 h, 49%; (c) propargyl bromide, NaH, CH2Cl2, 0 to 25 °C, 24 h, 44%; (d) (±)-16, [PdCl2(PPh3)2], CuI, iPr2NEt, DMF, 25 °C, 24 h, 36%.

Scheme of azole moiety assisted by the proximal alkoxy group. As a consequence, the N-3 position is blocked, and alkylation is directed to position N-1. In polar solvents such as DMF, the solvent competes for Na+ complexation, and electronic control of the regioselectivity is established. Sonogashira cross-coupling reaction of 1,2,4-isomer 39 with 5-iodocytosine (±)-16 finally afforded ligand (±)-34 in moderate yield.

Crystals of benzimidazole (±)-34 suitable for X-ray crystal structure analysis were grown by slow evaporation of a CH2Cl2/MeOH solution (Scheme 3), subsequent measurements confirmed the correct assignment of the regioisomers formed in the alkylation reaction. In the crystal structure, the isopropoxy substituent is disordered and adopts two orientations. In both conformations, however, the O(10)–C(11) bond is almost antiperiplanar to the C(4)–C(9) bond, with torsion angles \( |\tau(C8–C9–O10–C11)| \) of 7° and 2°, respectively, allowing conjugation of the oxygen atom with the aromatic system. The propargylic unit of (±)-34 is perpendicular to the aromatic ring, as expected, with a torsion angle \( \tau(C2–N1–C20–C21) = 100° \). These conformational preferences were corroborated by searches in the Cambridge Structural Database (CSD)[18] performed on similar molecular fragments (see Figure S3).

The inhibitory activities of ligands (±)-30–(±)-34 against E. coli IspE were determined by using the photometric assay described above and are summarized in Figure 4. The unsubstituted control compound (±)-30 displayed an \( IC_{50} \) value of 17 μM, which is in a similar range to that of the corresponding imidazole (±)-10 (Table 1). Introduction of a CF3 group at position C-5 of the benzimidazole core resulted in a slight loss of potency for (±)-31, with an \( IC_{50} \) value of 25 μM. The more polar nitrile (±)-32 (\( IC_{50} = 13 \mu{M} \)) was found to be slightly more potent. Interestingly, the trend is reversed as compared to the imidazole-based inhibitors with a flexible linker, in which activity was lost with the introduction of more polar residues and was highest for the CF3 derivative (see Table 1). With an \( IC_{50} \) value of 85 μM, phosphate (±)-33 is a weaker inhibitor than control compound (±)-30. The dramatic difference in clogD values [(±)-30: 5.97; (±)-33: –0.37], however, suggests that the expected energetic gains from phosphate binding to the glycine-rich loop, in analogy to the \( \beta \)-phosphate of AMP-PNP in the cocrystal structure (see Figure S4), might well be overcompensated for by the highly unfavorable partitioning. The lipophilic isopropoxy counterpart (±)-34 is inactive at inhibitor concentrations below 500 μM, despite strain-free binding suggested by the modeling. This finding also supports attractive polar interactions of the substituents in (±)-31–(±)-33 with the P-loop.

Conclusions

We have used the structure-based approach to design ligands featuring trisubstituted imidazole or benzimidazole central cores to address the cytidine-binding pocket, the hydrophobic sub-pocket, and the glycine-rich loop at the active site of the kinase IspE from E. coli. The syntheses of
the inhibitors relied on the construction of the heterocyclic moieties and their subsequent regioselective functionalization, such as by formation of imidazoles and alkylation of 4-alkoxybenzimidazoles. Biological evaluation of the ligands demonstrated that appropriate filling of the hydrophobic sub-pocket is essential to gain binding affinity towards IspE, as this cavity represents one of the few lipophilic environments of the otherwise highly polar active site. Addressing the water-exposed phosphate-binding region requires careful choice of the linker. Whereas flexible linkers are not appropriate to orient polar phosphate surrogates into the loop, they seem to be better tolerated for apolar phosphate mimics. We are currently focused on further enhancing the degree of preorganization of the ligands, which should allow additional binding affinity in the glycine-rich loop to be gained. The study has provided valuable new insight into the molecular recognition properties at the active site of IspE. The fact that the best biological activities only reach into the lower micromolar IC50-range, despite convincing molecular modeling predictions, underlines once more the challenges of efficiently addressing highly polar binding sites with synthetic ligands.

Experimental Section

In vitro Assays, Materials: [1,3,4,513C3]-Diphosphocytidyl-2C-methyl-o-erythritol (CDP-ME, 5) was prepared as described earlier.[13] E. coli IspE was prepared according to a published procedure.[6a] NADH and phosphoenolpyruvate potassium salt (PEP) were purchased from Biomol; ATP, the pyruvate kinase, and the lactate dehydrogenase from Sigma–Aldrich.

Enzyme-Coupled Photometric Assay for IC50 Determination: Assays were conducted in 96-well plates (Nunc, Cat. No 781602) with transparent flat-bottoms. Assay mixtures were prepared as described previously[13] with some minor modifications: A solution containing 100 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 2 mM dihydrothreitol, 2.5 mM potassium phosphoenolpyruvate, 2 mM ATP, 0.46 mM NADH, 1 U of lactate dehydrogenase, 1 U of pyruvate kinase, and the lactate dehydrogenase from Sigma–Aldrich.

Chemistry. General: For a detailed description of the chemicals, analytical equipment, and experimental methods used, see the Supporting Information. In the following, the general synthetic procedures and experimental details for the syntheses of all compounds shown in Scheme 1 and Scheme 3 are described. All other synthetic and analytical protocols are included in the Supporting Information. For the atom numbering used for the assignment of the 1H NMR spectra, see the spectra appended in the Supporting Information.

N-Alkylation of Imidazoles. General Procedure A: A solution of the imidazole (1.0 equiv.) in DMF (7.7 mL/mmol) was treated with Cs2CO3 (1.1 equiv.) and the alkyl halide (1.0 equiv.) at 0 °C. The suspension was stirred at 0 °C for 0.5 h and at 25 °C for 6–20 h and concentrated. The residue was treated with water and extracted twice with CH2Cl2. The combined organic phases were washed with water (3 ×) and brine, dried with MgSO4, filtered, and concentrated. The residue was purified by column chromatography (CC).

Sonogashira Cross-Coupling Reaction. General Procedure B: A solution of 5-iodocytosine (±)-16 (1.0 equiv.), the acetylene derivative (1.0–3.0 equiv.), and the base (2.0–3.0 equiv.) in anhydrous DMF was deoxygenated thoroughly by bubbling with argon for 45 min. [PdCl2(PPh3)2] (0.01–0.10 equiv.) and CuI (0.02–0.20 equiv.) were added and the suspension was stirred at 25 °C for 1.48 h. The resulting suspension was filtered through a plug of SI03 (washing with CH2Cl2/MeOH, 92:8) and concentrated. The residue was purified by CC. If Et3N was used in the eluting solvent, the fractions containing the desired product were concentrated, dissolved in CH2Cl2, washed with water (2 ×) and brine (2 ×), dried with MgSO4, and the solvents evaporated.

N-Alkylation of Benzimidazoles. General Procedure C: A solution of the benzimidazole (1.0 equiv.) in DMF, THF, or CH2Cl2 (7.7 mL/mmol) was treated with NaH (60% dispersion in oil, 1.1 equiv.) and stirred at 25 °C for 1 h. The alkyl halide (1.0 equiv.) was added at 0 °C. The suspension was stirred at 0 °C for 0.5 h and at 25 °C for 6–20 h and the solvents evaporated. The residue was treated with water and extracted twice with CH2Cl2. The combined organic phases were washed with water (3 ×) and brine, dried with MgSO4, filtered, and concentrated. The residue was purified by CC.

2-Cyclohexyl-1H-imidazole (14): A solution of NH4HCO3 (2.82 g, 35.6 mmol) and 40% sq. glyoxal (2.0 mL, 17.8 mmol). The mixture was stirred at 25 °C for 18 h, diluted with water (80 mL), and extracted with CH2Cl2/PrOH (3:1, 3 × 100 mL). The combined organic phases were washed with brine (150 mL), dried with MgSO4, filtered, and concentrated. CC (SiO2; CH2Cl2/MeOH, 95:5–90:10) gave 14 (0.66 g, 25%) as a yellow-beige solid, m. p. 136 °C (decomp.). 1H NMR (300 MHz, CDCl3): δ = 1.22–1.44 (m, 3 H, 4-Heq and 3'-Heq), 1.46–1.58 (m, 2 H, 2'-Heq and 3'-Heq), 1.69–1.75 (m, 1 H, 4'-Heq), 1.80–1.86 (m, 2 H, 3, 3'-Heq), 2.03–2.10 (m, 2 H, 2'-Heq and 3'-Heq), 2.75 (tt, J = 11.5, 3.6 Hz, 1 H, 1'-Heq), 6.95 (s, 2 H, 4-H and 5-H) ppm. NH not visible. 13C NMR (100 MHz, CDCl3/CDOD, 97.3): δ = 25.78, 25.97 (2 C), 31.77 (2 C), 35.75, 120.76 (2 C), 152.62 ppm. IR (ATR): ν = 3049 (w), 2928 (m), 2852 (w), 1674 (w), 1571 (m), 1480 (w), 1435 (m), 1384 (m), 1283 (w), 1182 (w), 1152 (w), 1134 (w), 1098 (s), 1074 (m), 1012 (w), 976 (m), 913 (m), 882 (s), 818 (s), 756 (s), 756 (m), 739 (s), 708 (s), 689 (cm–1). HRMS (EI): calcd. for C9H12N2: [M]+ 150.1151, found 150.1151 (25%); calcd. for C6H8N2: [M – C6H5]+ 95.0604, found 95.0604 (100%); calcd. for C9H12N2: [M – C6H5]+ 82.0525, found 82.0520 (34%).

2-Cyclohexyl-1-prop-2-ynyl-1H-imidazole (15): Iodide 14 (220 mg, 1.47 mmol) was treated with Cs2CO3 (525 mg, 1.61 mmol) and propargyl bromide (130 μL, 1.47 mmol) in DMF (11 mL) according to General Procedure A. The suspension was stirred at 25 °C for 9 h. CC (SiO2; pentane/EtOH, 9:1 → 7:1) gave 15 (77 mg, 28%) as a yellow solid. δ = 0.31 (SiO2; pentane/EtOH, 9:1, m. p. 46–52 °C). 1H NMR (300 MHz, CDCl3): δ = 1.31–1.43 (3 H, 3, 3'-Heq and 3'-Heq), 1.59–1.74 (3 m, 3 H, 2', 6'-Heq and 4'-Heq), 1.82–1.93 (3 m, 4 H, 3, 3'-Heq and 3'-Heq), 2.64 (tt, J = 11.6, 3.4 Hz, 1 H, 1'-Heq), 4.64 (dd, J = 2.6 Hz, 2 C, NCH3), 6.93 and 6.96 (2 d, 2d, J = 1.4 Hz, 2 H, 4-H and 5-H) ppm. 13C NMR (75 MHz, CDCl3): δ = 25.99, 26.53 (2 C), 31.94 (2 C), 35.14, 35.94, 73.86, 77.30, 118.41, 127.19, 151.63 ppm. IR (ATR): ν = 2321 (w), 3200 (w), 3104 (w), 2928 (m), 2853 (m), 2116 (w), 1674 (w), 1523 (w), 1452 (m), 1376 (w), 1337 (w), 1284 (w), 1230 (m), 1128 (w), 1152 (w), 1134 (m), 1098 (s), 1074 (m), 1012 (w), 976 (m), 913 (m), 882 (s), 818 (s), 756 (s), 756 (m), 739 (s), 708 (s), 689 (cm–1). HRMS (EI): calcd. for C9H14N2: [M]+ 150.1151, found 150.1151 (25%); calcd. for C6H8N2: [M – C6H5]+ 95.0604, found 95.0604 (100%); calcd. for C9H12N2: [M – C6H5]+ 82.0525, found 82.0520 (34%).
was added dropwise over 40 min to a solution of 24 (9.01 g, 35.50 mmol) in THF (160 mL) at –78 °C. The resulting solution was stirred at –78 °C for 45 min, treated dropwise with DMF (13.0 mL, 168.0 mmol), and stirred at –78 °C for 1 h. The solution was slowly warmed to 25 °C over 1 h, stirred at 25 °C for 1 h, cooled to –78 °C, and treated with sat. aq. NaHCO3 (30 mL). The residue was diluted with water (20 mL) at 25 °C and extracted with CH2Cl2 (2 × 50 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried with MgSO4, filtered, and concentrated. CC (SiO2; pentane/EtOAc, 3:1) gave 25 (8.31 g, 83%) as a yellow solid. Rf = 0.53 (SiO2; pentane/EtOAc, 1:1). m.p. 94–96 °C. 1H NMR (300 MHz, CDCl3): δ = 1.23–1.43 (m, 3 H, 4'-Hax and 3',5'-Hax), 1.63–1.77 (m, 3 H, 2',6'-Hax and 4'-Hax), 1.85–1.97 (m, 4 H, 3',5'-Hax and 2',6'-Hax), 2.92 (s, 6 H, Me), 3.27 (tt, J = 11.6, 3.3 H, 1 H, 1'-H), 7.78 (s, 1 H, 4'-H), 10.05 (s, 1 H, CHO) ppm. 13C NMR (100 MHz, CDCl3): δ = 26.53, 26.19 (2 C), 32.54 (2 C), 37.96, 38.14 (2 C), 132.92, 138.04, 160.63, 180.47 ppm. IR (ATR): ʋ = 3102 (w), 2939 (m), 2854 (m), 1761 (m), 1538 (s), 1458 (s), 1414 (s), 1389 (s), 1249 (m), 1164 (s), 1094 (s), 1047 (s), 962 (s), 888 (m), 781 (m), 763 (m), 720 (s), 630 (w) cm⁻¹. HRMS (EI): 318.1483 ppm (100%); calcd. for C12H16N2NaOS⁺ [M+Na]⁺ 308.1039, found 308.1038 (13%); calcd. for C12H14NO3S⁺ [M⁺]⁺ 286.1220, found 286.1219 (49%); 206.1646 (30%). C12H14NO3S (285.4) ppm: C 50.51, H 6.71, N 14.72; found C 50.52, H 6.67, N 14.61.

(2)-2-Cyclohexyl-N,N-dimethyl-2,2,2-trifluoroo-1-hydroxymethyl-1H-imidazole-1-sulfonamide (12): A solution of Alkyne 15 (54 mg, 0.29 mmol) was treated with 5-iodosocyno (3-10): Alkyne 15 (54 mg, 0.29 mmol) was treated with 5-iodosocyno (3-10); 1270 (m), 1226 (m), 1179 (w), 1160 (w), 1100 (m), 970 (w), 923 (w), 871 (w), 809 (w), 778 (s), 721 (m) cm⁻¹. 13C NMR (75 MHz, CDCl3): δ = 125.77, 26.36 (2 C), 28.29, 31.89 (2 C), 33.42, 35.91, 38.88, 66.11, 76.88, 88.99, 93.89, 112.87, 136.64, 144.76, 154.56, 164.18 ppm. IR (ATR): δ = 3104 (sh. w), 2926 (m), 2851 (w), 2231 (w), 1637 (s), 1482 (s), 1447 (m), 1401 (m), 1261 (m), 1297 (m), 1257 (m), 1226 (m), 1179 (w), 1160 (w), 1000 (m), 970 (w), 923 (w), 890 (w), 778 (s), 721 (m) cm⁻¹. HRMS (MALDI-3HPA): calcd. for C29H25N5NaOS⁺ [M+Na]⁺ 432.4211, found 422.4420 (2%); calcd. for C29H25Na(NO3)- [MNa⁺] 405.1672, found 406.1670 (3%); calcd. for C29H24NaO4S⁺ [M+Na⁺] 384.1853, found 384.1852 (100%); calcd. for C29H24NaO4S⁺ [M–C5H8]+ 298.1662 (14%).

2-Cyclohexyl-N,N-dimethyl-1H-imidazole-1-sulfonamide (24): N,N-Dimethylsulfamoyl chloride (6.3 mL, 58.6 mmol) was added to a solution of 14 (8.00 g, 53.3 mmol) and Et3N (8.5 mL, 61.2 mmol) in CH2Cl2 (80 mL). The suspension was stirred at 25 °C for 14 h, filtered (washing with CH2Cl2), and the filtrate was concentrated. CC (SiO2; pentane/EtOAc, 2:1) gave 24 (8.48 g, 62%) as a white solid. Rf = 0.30 (SiO2; pentane/EtOAc, 1:1). m.p. 95–97 °C.

1H NMR (300 MHz, CDCl3): δ = 1.25–1.43 (m, 3 H, 4'-Hax and 3',5'-Hax), 1.59–1.74 (m, 3 H, 2',6'-Hax and 4'-Hax), 1.83–1.97 (m, 4 H, 3',5'-Hax and 2',6'-Hax), 2.88 (s, 6 H, Me), 3.14 (tt, J = 11.7, 3.4 Hz, 1 H, 1'H), 6.95 (d, J = 1.7 Hz, 1 H, 4'-H), 7.17 (d, J = 1.7 Hz, 1 H, 5'H) ppm. 13C NMR (75 MHz, CDCl3): δ = 25.84, 26.43 (2 C), 32.65 (2 C), 37.23, 38.17 (2 C), 118.95, 127.22, 153.88 ppm. IR (ATR): ʋ = 3102 (w), 2949 (w), 2853 (m), 1673 (w), 1533 (w), 1482 (m), 1466 (m), 1458 (m), 1446 (m), 1415 (m), 1388 (s), 1345 (w), 1321 (w), 1298 (w), 1261 (m), 1226 (w), 1186 (m), 1174 (s), 1163 (s), 1152 (s), 1133 (s), 1093 (s), 1072 (m), 1047 (s), 999 (m), 961 (s), 909 (m), 887 (m), 856 (w), 817 (w), 765 (w), 737 (m), 721 (s), 708 (s) cm⁻¹. HRMS (EI): calcd. for C12H14N2O3⁺ [M+H+] 257.1192, found 257.1199 (7%); calcd. for C12H14N2O3⁺ [M+Na+] 279.0986, found 279.1000 (2%); calcd. for C12H14N2O3⁺ [M+Me2NSO2]⁺ 299.1157, found 299.1160 (5%).
40 mL). The combined organic phases were washed with water (3 x 100 mL) and brine (100 mL), dried with MgSO4, filtered, and concentrated. (CC: SiO2; pentane/EtOAc, 5:1 → 2:1) gave (±)-27 (440 mg, 85%) as an off-white solid. Rf = 0.44 (SiO2; pentane/ EtOAc, 7:3), m.p. 76–78 °C. 1H NMR (300 MHz, CDCl3): δ = 1.26–1.43 (m, 3 H, 4'-Ha and 3',5'-Ha), 1.59–1.76 (m, 3 H, 2',6'-Ha and 4',5'-Ha). 1.85–1.93 (m, 4 H, 3',5'-Heq and 2',6'-Heq).

(±)-28: A suspension of (±)-27 (300 mg, 0.81 mmol) in 5% aq. HCl (10 mL) was stirred at 55 °C for 7 h. The mixture was cooled to 0°C, neutralized with sat. aq. NaHCO3, and extracted with CH2Cl2 (3 x 20 mL). The combined organic phases were washed with brine (30 mL), dried with MgSO4, and concentrated. (CC: SiO2; pentane/EtOAc, 2:1 → 1:1) gave (±)-28 (191 mg, 90%) as a white crystalline solid, m.p. 154–155 °C. 1H NMR (300 MHz, CDCl3): δ = 1.25–2.68, 26.23 (2 C), 32.58, 32.64, 36.72 (2 C), 36.82, 38.58, 72.84 [q, 2(J,C,F) = 31.2 Hz]. 123.84 [q, 1(J,C,F) = 283.2 Hz, 125.53 Hz (br. s, 1 H, 2',6'-Ha), 133.11 [br. q, 3(J,C,F) = 35.5 Hz]. 157.35 ppm. 19F NMR (376 MHz, CDCl3): δ = –74.31 [d, 3(J,H,F) = 6.4 Hz] ppm. IR (ATR): ν = 3152, 2931 (w), 2859 (w), 1570 (w), 1497 (w), 1475 (w), 1461 (w), 1417 (w), 1389 (m), 1364 (m), 1347 (m), 1262 (m), 1239 (w), 1208 (w), 1159 (s), 1128 (s), 1113 (m), 1099 (m), 1084 (t), 1015 (s), 969 (m), 856 (m), 844 (m), 767 (m), 726 (s), 705 (s), 640 (w) cm^-1.

HRMS (EI): calcd. for C12H18F3N2O+ [M+H]+ 263.1366, found 263.1362 (100%).

19F NMR (376 MHz, CDCl3): δ = –76.25 [d, 3(J,H,F) = 6.5 Hz] ppm.

IR (ATR): ν = 3007 (w), 2932 (m), 2856 (w), 2123 (w), 1504 (w), 1450 (w), 1385 (m), 1378 (w), 1271 (m), 1165 (s), 1120 (s), 1101 (s), 1018 (w), 971 (w), 891 (w), 869 (w), 838 (w), 806 (w), 698 (cm^-1). HRMS (ESI): calcd. for C13H19F4N4O2+ [M + H]+ 312.1552, found 312.1552 (100%).
was stirred at 25 °C for 2 h and concentrated. CC (SiO₂: pentane/EtOAc, 2:1) and recrystallization in EtOAc gave 37 (410 mg, 49 %) as a white solid. Rₜ = 0.57 (SiO₂: pentane/EtOAc, 1:1), m.p. 216 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.18–1.48 (m, 3 H, 4'-H₈ax and 3',5'-H₈ax), 1.40 (d, J = 6.0 Hz, 6 H, Me), 1.60–1.89 (m, 5 H, 2',6'-H₈ax, 4'-H₈ax, and 3',5'-H₈ax), 2.13–2.19 (m, 2 H, 2',6'-H₈ax), 2.91 (br. s, 1 H, 1'-H), 4.72 (br. s, 1 H, OCH), 6.66 (d, J = 8.1 Hz, 1 H, 5'-H), 7.10 (t, J = 8.0 Hz, 1 H, 6'-H), 7.29 (br. s, 1 H, 7'-H), 9.07 (br. s, 1 H, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 22.26 (2 C), 25.83, 26.04 (2 C), 31.93 (2 C, 38.57, 70.30, 104.86, 122.21, 157.85 ppm, four aromatic signals are not visible. IR (ATR): ν = 3675 (w), 2975 (m), 2926 (m), 2852 (m), 1618 (w), 1601 (m), 1593 (m), 1495 (w), 1444 (m), 1416 (m), 1380 (m), 1370 (m), 1357 (m), 1322 (m), 1292 (w), 1265 (m), 1242 (s), 1176 (w), 1140 (w), 1106 (s), 1088 (m), 1068 (s), 1045 (s), 993 (m), 891 (w), 856 (m), 782 (m), 767 (w), 735 (s), 669 (w), 632 (w), 609 (w) cm⁻¹. HRMS (EI): calcd. for C₂₂H₂₄N₂O₂+ [M]+ 381.1727, found 381.1725, 381.1729 (39 %); calcd. for C₁₉H₁₇N₂O⁺ [M – C₃H₆]+ 294.1257, found 294.1260 (30 %); calcd. for C₁₆H₁₈N₂O⁺ [M – C₃H₆ – C₄H₇]+ 216.1257, found 216.1251 (25 %); calcd. for C₁₃H₉N₂O⁺ [M – C₃H₆]+ 216.1251 (25 %); calcd. for C₁₆H₂₀N₂O⁺ [M – C₃H₆ – C₄H₇]+ 254.1405 (30 %); calcd. for C₁₃H₁₆N₂O⁺ [M – C₃H₆]+ 254.1405 (30 %); calcd. for C₁₆H₂₀N₂O⁺ [M – C₃H₆ – C₄H₇]+ 302.1648, found 302.1650 (75 %); calcd. for C₁₉H₁₇N₂O⁺ [M – C₃H₆]+ 254.1414, found 254.1405 (30 %); calcd. for C₁₆H₁₈N₂O⁺ [M – C₃H₆ – C₄H₇]+ 199.0867, found 199.0866 (100 %); C₁₉H₂₁N₂O⁺ (396.2238, found 406.2231 (66 %).

Supporting Information (see footnote on the first page of this article): Figures (modeling, regioselective alkylation, CSD searches) and schemes (biological assay, synthones not shown in the main manuscript) referred to in this article, materials and general methods, synthetic and analytical protocols, X-ray diffraction data, NMR spectra of new compounds.

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Imidazole- and Benzimidazole-Based Inhibitors of the Kinase IspE


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