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Synthesis and biological evaluation of novel (4 or 5-aryl)pyrazolyl-indoles as inhibitors of interleukin-2 inducible T-cell kinase (ITK)

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ABSTRACT

Interleukin-2 inducible T-cell kinase (ITK) is one of five kinases that belong to the Tec kinase family that plays an important role in T-cell and mast cell signaling. Various reports point to a role of ITK in the treatment of allergic asthma. For example, it was shown that mice lacking ITK have reduced airway hyperresponsiveness, inflammation and tracheal responses in an allergic asthma model. In this article, we disclose novel ITK inhibitors based on (4 or 5-aryl)pyrazolyl-indole scaffold that were also found to be selective for ITK over other kinases like IRK, CDK2, GSK3ß and PKA.

1. Introduction

Interleukin-2 inducible T-cell kinase (ITK) is one of five kinases that belong to the Tec kinase family, which is primarily expressed in hematopoietic cells and participates in major immunological processes.[1] ITK plays an important role in T-cell and mast cell signaling.[1] Number of reports point to a role of ITK in the treatment of allergic asthma. For example, it was shown that mice lacking ITK have reduced airway inflammation in an allergic asthma model,[2] reduced airways hyperresponsiveness to allergen challenge and reduced tracheal responses to cholinergic challenge.[3] In response to allergen challenge with OVA (ovalbumin), ITK knockout mice showed reduced lung inflammation, eosinophils infiltration and mucous production.[4] A new dimension of the contribution of ITK to T-cell activation in HIV replication has been investigated recently. Studies using siRNA specific for ITK as well as a reported ITK inhibitor BMS509744 in primary human $CD4^+$ T cells suggested that inhibition of ITK blocks HIV replication by affecting multiple stages of the HIV life cycle, including viral entry, transcription from the viral LTR, and virion assembly or release.[5] Although a number of companies have disclosed potent inhibitors of ITK,[6,7] no ITK inhibitor has been tested in any clinical trials. Pyrazolyl-indole derivatives have been reported as aurora kinase inhibitors.[8] Our hit finding activities along with molecular modeling studies predicted that (4 or 5-aryl)pyrazolyl-indole would serve as a good scaffold for designing inhibitors of ITK. In this article, we wish to disclose novel and selective ITK inhibitors based on (4 or 5-aryl)pyrazolyl-indole scaffold.

2. Chemistry

Target compounds were synthesized as shown in Schemes 1–5. As illustrated in Scheme 1, the synthesis of 4-phenylpyrazolyl-indole scaffold began with benzyl cyanide, which on treatment with *N*,*N*-dimethylformamide dimethyl acetal gave the acrylonitrile **1**, which on reflux with hydrazine hydrochloride furnished the desired 3-amino-4-phenyl pyrazole. Diazotization of **2** with aqueous $NaNO_2$ under acidic conditions followed by treatment with aqueous KI provided the iodopyrazole **3**, which was treated with di-*t*-butyl dicarbonate to yield the Boc-protected iodopyrazole **4**. Reacting **4** with 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid under Suzuki coupling conditions furnished the ester **5**, which was subsequently hydrolyzed to acid **6**. The acid **6** was coupled with various amines using standard peptide coupling conditions to give target compounds **7–13**.

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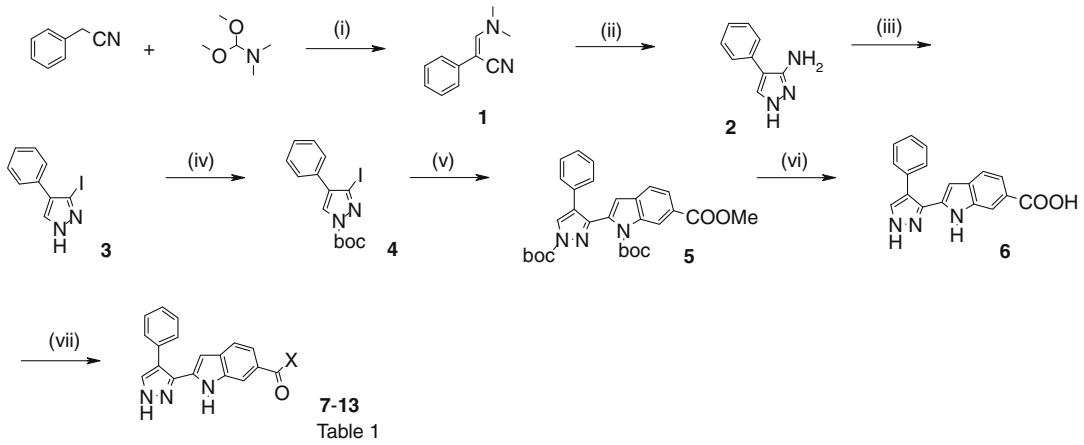
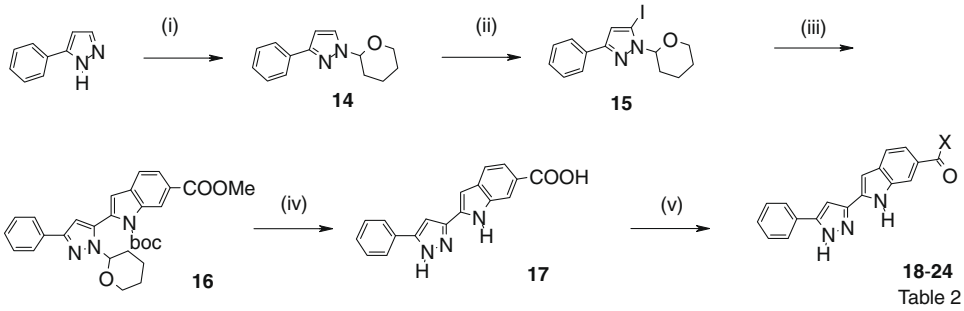
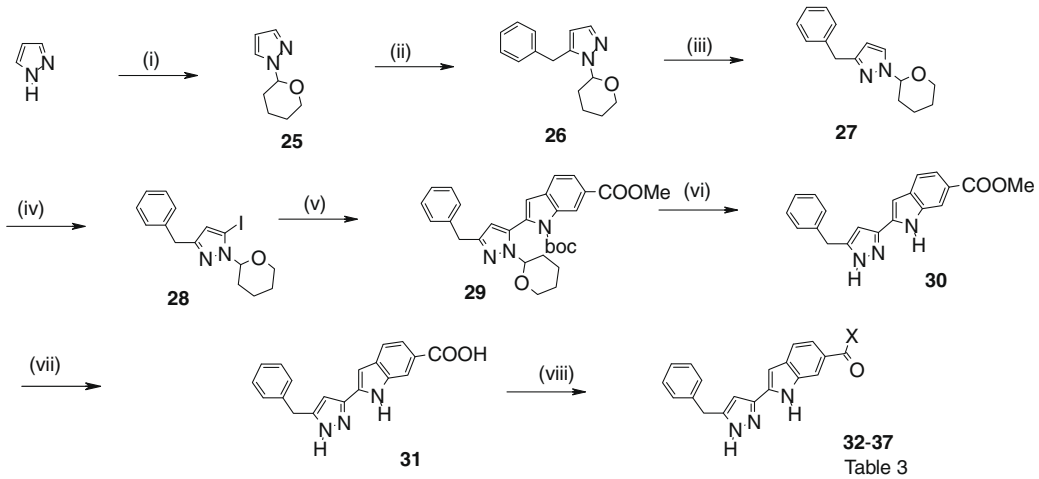


Scheme 1. Reagents and conditions: (i) DMF, 100 °C (75%); (ii) H_2NNH_2·HCl, ethanol, reflux (65%); (iii) aq $NaNO_2$, KI, concd H_2SO_4, MeOH (41%); (iv) $(Boc)_2O$, Et_3N, DCM (99%); (v) 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid, $Pd(dppf)Cl_2$, dioxane, Cs_2CO_3, 33%; (vi) KOH, ethanol, reflux (64%); (vii) amine, EDCI, HOBt, $NaHCO_3$, DMF.



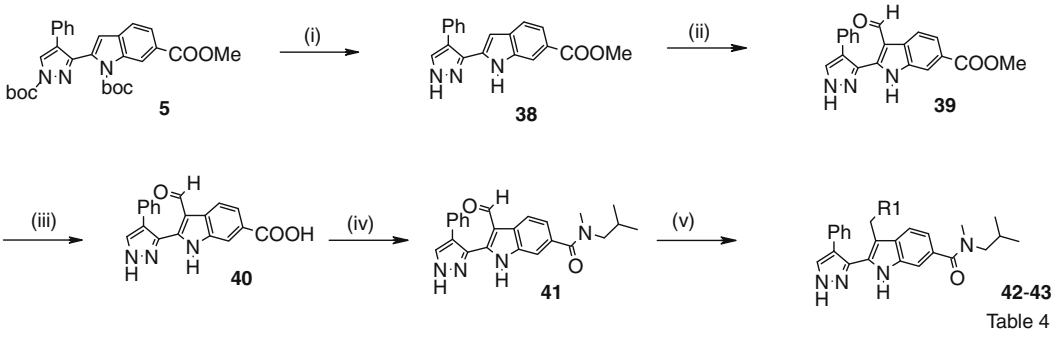
Scheme 2. Reagents and conditions: (i) DHP, TFA, toluene, reflux (98%); (ii) *n*-BuLi, I_2, THF, −78 °C (61%); (iii) $Pd(dppf)Cl_2$, dioxane, 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid, Cs_2CO_3, 32% (iv) KOH, ethanol, reflux, then acid (aq HCl) workup (55%); (v) amine, EDCI, HOBt, $NaHCO_3$, DMF.



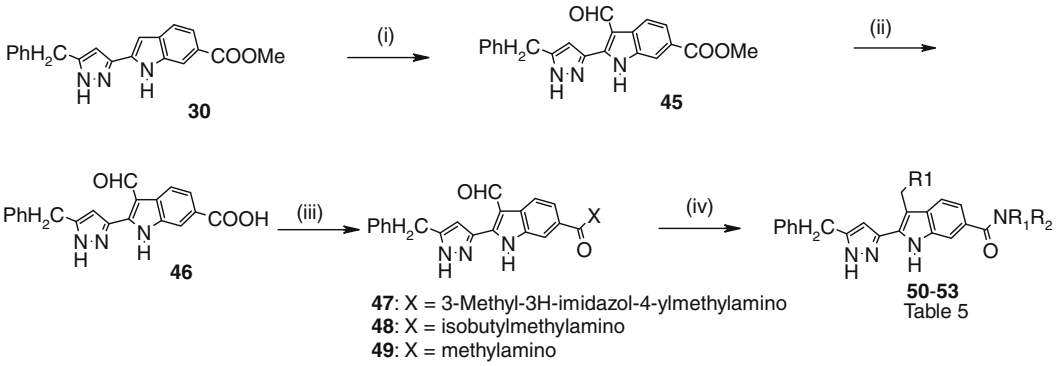
Scheme 3. Reagents and conditions: (i) DHP, TFA, toluene, reflux (98%); (ii) *n*-BuLi, BnBr, THF, −78 °C (38%); (iii) ethereal HCl, then DHP, TFA, toluene, reflux, 83%; (iv) *n*-BuLi, I_2, THF, −78 °C (41%); (v) $Pd(dppf)Cl_2$, dioxane, Cs_2CO_3, 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid, 31% (vi) 4 M HCl in dioxane, rt, then K_2CO_3/MeOH, 85%; (vii) aqueous KOH, EtOH, reflux, 89%; (viii) amine, EDCI, HOBt, $NaHCO_3$, DMF.

Commercially available 3-phenyl-1*H*-pyrazole was regioselectively protected as THP aminal derivative **14** that was iodinated to furnish the iodopyrazole **15**, which on coupling with 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid gave the ester **16** (Scheme 2). Hydrolysis of the ester **16** provided acid **17**, which was coupled with various amines as before to furnish target compounds **18**–**24**.

As shown in Scheme 3, pyrazole was converted to its THP derivative **25**, which on treatment with *n*-BuLi and benzyl bromide furnished the benzyl pyrazole **26** in moderate yield. Removal of the



Scheme 4. Reagents and conditions: (i) HCl in dioxane (97%); (ii) DMF, $POCl_3$, (67%); (iii) KOH, ethanol, reflux (45%); (iv) *N*-isobutyl-*N*-methylamine, EDCI, $NaHCO_3$, HOBt, DMF; (v) amine, Et_3N, AcOH, THF, $NaBH_4$.



Scheme 5. Reagents and conditions: (i) DMF, $POCl_3$, (69%); (ii) KOH, ethanol, reflux (69%); (iii) amine, EDCI, HOBt, $NaHCO_3$, DMF; (iv) amine, Et_3N, AcOH, THF, $NaBH_4$.

THP group, followed by reflux with DHP under acidic conditions furnished the other regioisomeric benzyl pyrazole **27**.[9] A similar sequence of events as before, which included iodination, Suzuki coupling, hydrolysis and finally amide formation, provided the target compounds **32**–**37**.

Suzuki coupling product **5** from Scheme 1 was treated with 4 M HCl in dioxane to remove the Boc groups (Scheme 4). The resulting ester **38** was formylated at the indole-3 position selectively using $POCl_3$/DMF complex to furnish the aldehyde **39**, which on hydrolysis followed by coupling the acid **40** with *N*-methyl-*N*-isobutyl amine afforded the amide **41**. This amide was then subjected to reductive amination with various primary amines using standard conditions to afford indole-3-substituted derivatives **42**–**43** (Scheme 4, Table 4). On the other hand, amide **41** on reduction with $NaBH_4$ in CH_3OH furnished the alcohol **44**.

Scheme 5 depicts a similar sequence of reactions to afford indole-3-substituted derivatives in the 5-benzylpyrazole series. Thus, the ester **30** from Scheme 3 was formylated regioselectively at indole 3-C position. The ester group in the resulting aldehyde **45** was hydrolyzed to give the acid **46** that was further coupled with three different amines to yield the amides **47**–**49**. Each of these amides was then subjected to reductive amination with different amines to afford the target compounds **50**–**53** (Scheme 5, Table 5). Reduction of **47** with $NaBH_4$ in CH_3OH afforded the alcohol **54**. A common feature of some of the final compounds in Schemes 1–5 is the appearance of NMR signals due to tautomerism in the pyrazole ring.

3. Results and discussion

3.1. Molecular modeling studies

Our hit finding activities showed that pyrazolyl-indoles are inhibitors of ITK. Docking studies of various pyrazolyl-indole derivatives with ITK indicated a common mode of binding which involved three hydrogen bonds in the hinge region of ITK between: (i) N–*H* of the pyrazole ring with the carbonyl of Glu436 of ITK, (ii) N of pyrazole and –NH of Met438 of ITK and (iii) N–*H* of the indole ring with the carbonyl of Met438 of ITK. Additionally the amide chain points towards the solvent accessible region. In ITK, Phe435 acts as a gatekeeper residue by guarding access to a hydrophobic pocket that is located adjacent to the ITK active site.[10] Molecular modeling studies predicted that arylpyrazolyl-indoles or arylmethylpyrazolyl-indoles, which have an aryl ring on the pyrazole ring, would have an additional binding interaction: π–π stacking between Phe435 and the aryl ring on the pyrazole. It was also apparent from docking that among the three series examined, 5-benzylpyrazolyl-indoles show the best binding to ITK, and was followed in the decreasing order by 4-phenylpyrazolyl-indoles and 5-phenylpyrazolyl-indoles. Compounds **13**, **24** and **34** have same amide residue (*N*-methyl-*N*-isobutyl) but belong to the 4-phenyl, 5-phenyl and 5-benzyl series, respectively. Figures 1a and c illustrate the comparative docking of **13**, **24** and **34**, respectively onto ITK. The docking score of **24** (5-phenyl series) was the lowest followed by that of **13** (4-phenyl series) while the docking score of **34** (5-benzyl series) was the highest. As shown in Figures 1a and b, the orientation of the corresponding phenyl ring with respect to Phe435 of ITK is different in **13** and **24**. As depicted in Figure 1a for compound **13**, as the 4-phenyl ring on the pyrazole is too far to engage in effective π–π stacking interaction with Phe435 of ITK, the molecule prefers to form three H-bonding interactions with the hinge region of ITK as described before. In case of **24** (Fig. 1b), it seems that the phenyl ring is closer and oriented favorably for a possible π–π stacking interaction with Phe435 of ITK and in doing so loses one of the three potential H-bonding interactions with the hinge region. On the other hand, Figure 1c clearly shows that in case of **34** which contains a benzyl group on 5-C atom of the

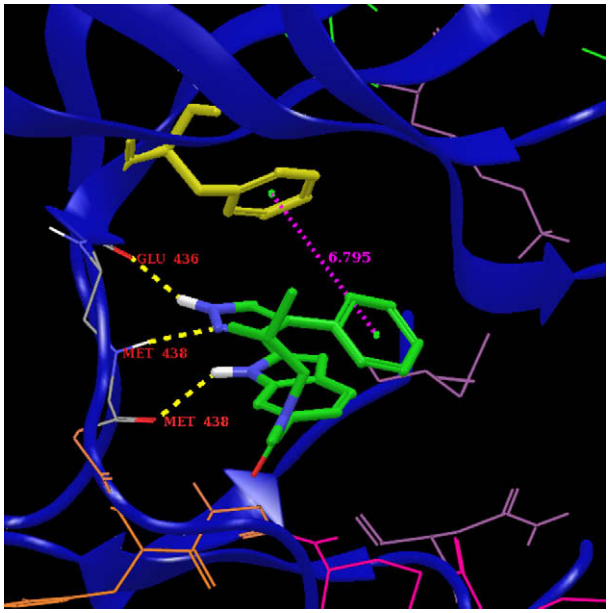


Figure 1a. Compound **13** docked into the ATP-binding pocket of ITK showing three potential H-bonding interactions with hinge region and unfavorable orientation of the phenyl ring for a π–π interaction with Phe435.

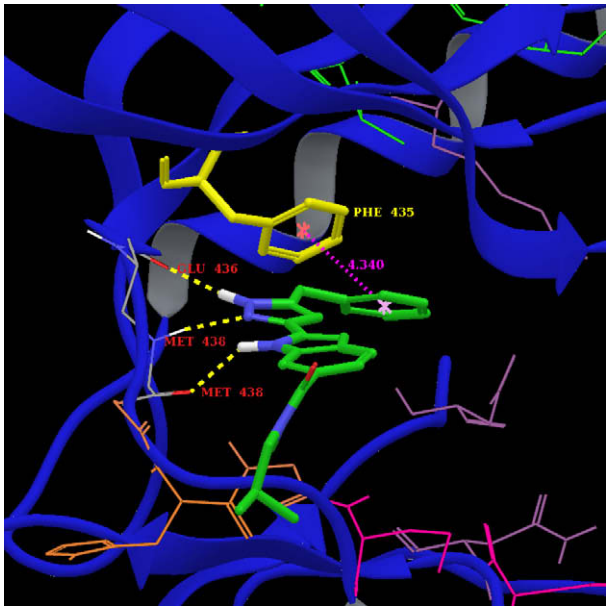


Figure 1c. Compound **34** docked into the ATP-binding pocket of ITK. The ligand makes three hydrogen-bonding interactions (dashed yellow lines) with the backbone atoms of Glu 436 and Met 438 of the ITK hinge. The 5-benzyl ring of **34** makes a favorable π–π stack with the gatekeeper Phe435.

pyrazole nucleus, the methylene group imparts some degree of flexibility so that the phenyl ring of the benzyl group can orient itself for a favorable π–π stacking interaction with Phe435 of ITK while still engaging in the three possible H-bonding interactions with the hinge region. When the above docking exercise was repeated for compounds **10**, **21** and **32** which belong to the 4-phenyl, 5-phenyl and 5-benzyl series, respectively, and also have the same amide substituent, similar modes of binding were predicted with **32** showing the best docking score followed by **10** and **21** in decreasing order. Armed with these docking predictions, it was decided to synthesize members from all three series and measure their potencies for inhibition of ITK.

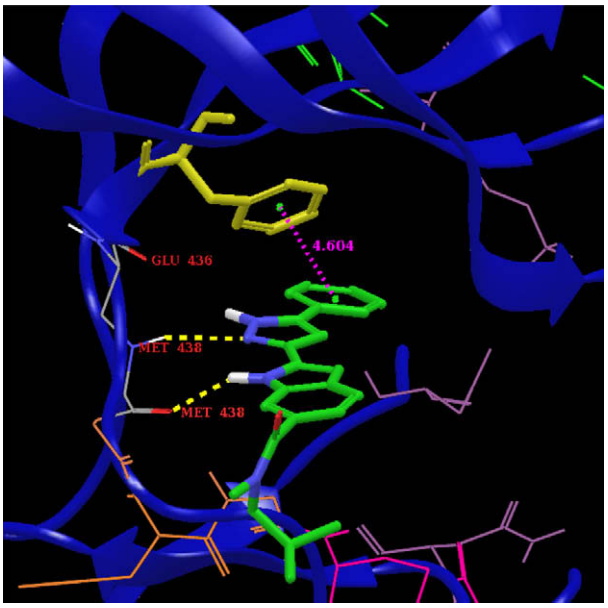


Figure 1b. Compound **24** docked into the ATP-binding pocket of ITK. While the phenyl ring is positioned properly for a favorable π–π stack with the gatekeeper Phe435, only two possible H-bonding interactions with the hinge region are predicted.

Increases in potencies of ITK inhibitors have been achieved by designing inhibitors that by going around the gatekeeper residue bind to the amino acid residues (e.g., Lys391 and Asp500) in the kinase specificity pocket (KSP).[6b] We reasoned that incorporation of functional motifs of suitable length and H-bonding potential in the 3-C position of the indole ring of our inhibitors should offer access to the amino acid residues in the KSP and that this may result in increase in potency. Accordingly, a number of analogues of the three series discussed above were designed that had diverse types of substitutions of varying lengths and functionality. Introduction of bulky substituent in the immediate vicinity of indole-3-C atom was avoided as it might have changed the torsion angle between the indole and pyrazole rings due to steric repulsion, which in turn could have affected the H-bonding interaction with the hinge region. In the 5-benzyl series, presence of flexible benzyl group allowed introduction of various groups in the 3-C position while maintaining the π–π stacking with the gatekeeper residue and the three hinge interactions. The torsion angle as discussed above changed minimally, with 4.3° as the maximum change predicted. As depicted in Figure 2, docking studies of **53** with ITK revealed a torsion angle of 8.1° when compared to the value of 6°, the torsion angle in the unsubstituted derivative **36**. Docking of remaining analogues in Table 5 with ITK predicted similar results. Interestingly the presence of –OH group in **53** provides for a favorable H-bonding interaction with Lys391.

In case of 5-phenyl series, the rigidity imparted by presence of 5-phenyl group allowed only smaller aliphatic residues similar to those present in compounds **43** and **44** (docking data not shown), with minimal change in the torsion angle as discussed above. However, there is a loss of one H-bonding interaction in the hinge region compared to two such interactions for the unsubstituted compound. In contrast, introduction of substituent in the 3-C position of 4-phenyl series results in a flipped dock pose within the active pocket, loss of π–π stacking and all potential hinge interactions (docking data not shown). Changes in torsion angle by as much as 151° were predicted.

Insulin receptor tyrosine kinase (IRK) is structurally homologous to ITK but whereas ITK has phenylalanine in the gatekeeper

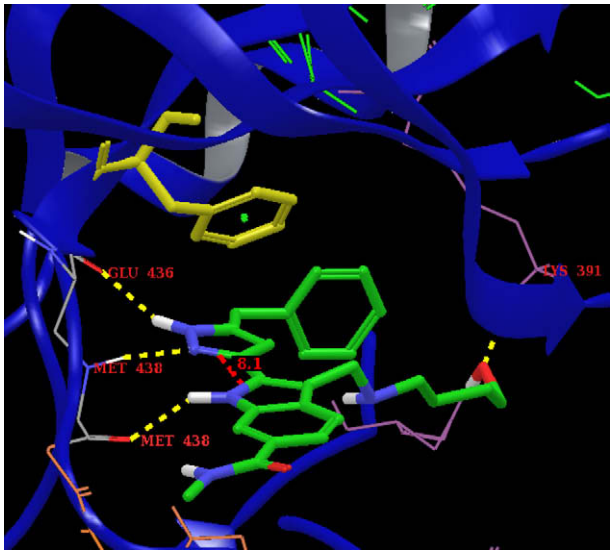


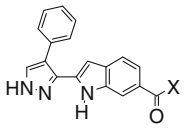
Figure 2. Compound **53** docked into the ATP-binding pocket of ITK. Note the H-bonding interaction with Lys391.

position, in IRK this role is played by a methionine residue. Hence it was hypothesized that selectivity for ITK over IRK can be achieved by designing molecules that have an aryl ring in close proximity to Phe435 of ITK. In fact, it has been suggested in literature that improvement in potency and selectivity can be achieved by modulating interactions with the gatekeeper residue, as this residue varies in different kinases.[10,11] On the other hand, in the case of ITK recent reports have detailed efforts to achieve increased potency and selectivity by maximizing interactions with amino acid residues present in KSP.[6b] For therapeutic reasons selectivity over kinases controlling the cell cycle or the cell metabolism is required and for this reason we measured inhibitory activity on IRK, CDK2, GSK3ß and PKA.

3.2. In vitro activity

Compounds **7**–**13** were evaluated for their inhibitory activity against recombinant full length ITK using an IMAP-based assay and the IC_{50} values obtained are summarized in Table 1. Some of these compounds inhibited ITK at sub-micromolar level (compounds **10**–**13**) and compound **13** is the most potent member in this series with an IC_{50} value of 0.33 μM. Next, compounds **18**–**24** were assayed against ITK as described before to determine the effect of changing the position of the phenyl ring from C-4 to C-5 of the pyrazole ring. As seen from Table 2, this resulted in diminished activity in all cases, with as much as fivefold loss in potency (compare compounds **8** with 1**9**, and **11** with **22**). When the 5-benzylpyrazolyl-indole scaffold, obtained by replacing the phenyl group on C-5 of the pyrazole ring with benzyl group, was subjected to docking studies with ITK, better binding was predicted. Thus, as illustrated in Table 3, compounds **32** and **34** showed submicromolar inhibition of ITK and the most potent member in this series was compound **34** with IC_{50} value of 0.56 μM. The 5-phenylpyrazolyl-indole derivatives were least potent against ITK in accordance with the predictions of the docking studies, whereas, as opposed to the predictions, 4-phenylpyrazolyl-indoles were generally more potent than 5-benzylpyrazolyl-indoles. It should also be noted that in each series the most potent compound was an amide with *N*-isobutylmethylamine (compounds **13**, **24** and **34**). As there was no improvement in potency by changing the position of the phenyl group or by substitution with benzyl group, further substitution at the indole 3-C atom was explored. Molecular modeling studies with 3-indole substituted derivatives predicted interactions in

Table 1
Inhibitory activity of 4-phenyl-1*H*-pyrazol-3-yl-indole series



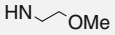
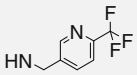
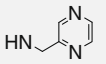
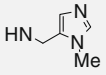
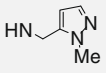
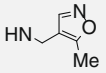
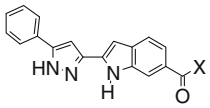
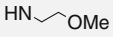
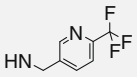
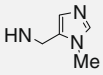
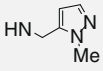
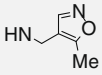
Compound	X	ITK IC_{50} (μM)	IRK IC_{50} (μM)	CDK2 IC_{50} (μM)	GSK3ß IC_{50} (μM)	PKA IC_{50} (μM)
7		2.51				
8		2.00				
9		1.1				
10		0.79	7.08	0.63	0.54	10.0
11		0.63	9.33	0.16	1.26	7.08
12		0.63	5.75	0.4	2.29	5.01
13		0.33	>10	3.16	6.76	Not active

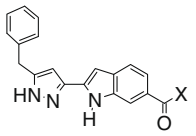
Table 2
Inhibitory activity of 5-phenyl-1*H*-pyrazol-3-yl-indole series



Compound	X	IC_{50} (μM)
18		3.16
19		10.0
20		4.36
21		1.26
22		3.16
23		1.82
24		0.85

the kinase specificity pocket[6b] (KSP) of ITK. In the 4-phenylpyrazolyl-indole series, compounds **42**–**44** were synthesized (Scheme 4); but when tested against ITK showed appreciable reduction in the activity against ITK (Table 4; compare compounds **42**–**44** with **13**). This was in line with the predictions of docking studies. In the 5-phenyl series, analogues with similar 3-C substitution as in **44** were synthesized but contrary to docking predictions were found to be inactive (data not shown).When similar substitutions were carried out in the 3-C position of the indole moiety in the 5-benzylpyrazoly-indole series (Scheme 5), almost no improvement in

Table 3
Inhibitory activity of 5-benzyl-1*H*-pyrazol-3-yl-indole series



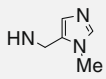
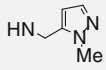
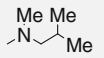
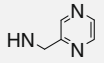
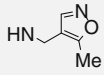
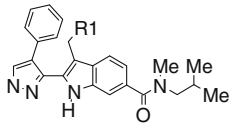
Compound	X	ITK IC_{50} (μM)	IRK IC_{50} (μM)	CDK2 IC_{50} (μM)	GSK3ß IC_{50} (μM)	PKA IC_{50} (μM)
32		0.63	5.01	31.6	Not active	Not active
33		1.00	20.0	12.6	Not active	Not active
34		0.56	>100	Not active	Not active	Not active
35		2.06				
36	HNMe	1.63				
37		2.04				

Table 4
Inhibitory activity of 3-indole substituted 4-phenyl-1*H*-pyrazol-3-yl-indole series



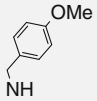
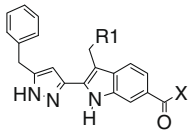
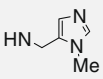
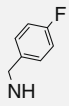
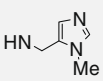
Compound	R1	IC_{50} (μM)
42		20.4
43	$NH(CH_2)_3OH$	>100
44	OH	>100

Table 5
Inhibitory activity of 3-indole substituted 5-benzyl-1*H*-pyrazol-3-yl-indole series



Compound	X	R1	IC_{50} (μM)
50			0.63
51			0.50
52	HNMe		5.0
53	HNMe	$NH(CH_2)_4OH$	50.1
54		OH	1.58

potency was observed (Table 5, compare compounds **50** and **54** with **32** and compound **51** with **34**). Rather, in the 3-C position of indole, introduction of polar straight chain residue led to 30-fold loss in potency (compare compound **53** with **36**), whereas, aromatic residues did not affect the potency appreciably. In case of **53**, this directly contradicts the prediction based on modeling studies that showed potential interaction with Lys391 and the reason behind this discrepancy is unclear at this point.

Some of the most potent members from 4-phenylpyrazolyl-indole and 5-benzylpyrazolyl-indole scaffolds (Tables 1 and 3) were tested against kinases such as IRK, GSK3ß, CDK2 and PKA. As shown in Table 1, compounds **10**–**12** that belong to the 4-phenyl-pyrazolyl-indole series are equipotent towards ITK, CDK2 or GSK3ß, but are comparatively less active against IRK and PKA. Only compound **13** from the same series is selective for ITK when compared to the four other kinases. Pleasingly three compounds from the 5-benzyl-pyrazolyl-indole series show much greater preference for ITK (Table 3, compounds **32**–**34**). Of particular note is compound **34**, which is about 200-fold less active towards IRK, while being completely inactive against CDK2, GSK3ß, PKA and AKT1. It is instructive to note that the most potent compounds from the 4- or 5-phenylpyrazolyl-indole as well as 5-benzylpyrazolyl-indole series that have the *N*-isobutyl-*N*-methylamine group as part of the amide were the most selective against other kinases (compounds **13**, **24** and **34**, respectively). Comparison of the amide chains present in **13**, **24** and **34** (*N*-isobutyl-*N*-methylamine) with those in the rest of the compounds in Tables 1–3 reveal an important difference: tertiary amide group in **13**, **24** and **34** versus secondary amide groups in rest of the compounds in Tables 1–3. As noted before, the amide group is directed towards the solvent accessible region. The tertiary amide group in compounds **13**, **24** and **34** has a methyl group on its amide nitrogen atom and hence can form hydrogen bonds with the solvent molecules through its carbonyl oxygen only whereas a secondary amide group should be able to utilize both its carbonyl oxygen as well as the amide hydrogen for hydrogen bonding with solvent. It should be noted that most potent compounds (**13**, **24** and **34**) in each series have more lipophilic group on the amide nitrogen, although this may incur a price by a decrease in solubility. Based on this, it seems that the binding to the water-accessible region is detrimental to the potency. Further, presence or absence of a glycine residue immediately after the hinge region in kinases has been reported to be responsible for difference in the shape of the solvent accessible region influencing selectivity.[12] However, both ITK and IRK have the corresponding glycine residue present in the solvent accessible region, but differ in the gatekeeper residue. ITK has phenylalanine as a gatekeeper residue whereas methionine plays the same role in IRK. Comparison of selectivity of **13** and **34** for ITK inhibition over IRK reveals that although both have the same amide residue which occupies the water-accessible region, these compounds differ in the corresponding group that will interact with the gatekeeper residue. Docking studies predicted that by design the phenyl group in the 5-benzyl series is better placed for effective π–π stacking with the gatekeeper residue (Phe435) in ITK than the corresponding phenyl group in the 4-phenyl series. This fact along with the finding that **34** is a more selective inhibitor (>178-fold selectivity over IRK) of ITK than **13** (30-fold selectivity over IRK) supports our hypothesis that interaction with the gatekeeper residue is energetically more relevant than the interaction in the solvent accessible region.

4. Conclusion

In summary, two novel series containing aryl substituted pyrazolyl-indoles that act as selective inhibitors of ITK were identified. These compounds were designed with a phenyl group on the

pyrazole ring of the inhibitors so that the phenyl ring can engage into a π–π interaction with the gatekeeper residue Phe435 in ITK. The 4-phenylpyrazolyl-indole series had generally the most potent compounds (compound **13**), whereas the 5-benzylpyrazolyl-indole series displayed more selectivity for ITK (compound **34**). Submicromolar inhibitors of ITK (compounds **13** and **34**) which are selective for ITK over IRK were discovered. In fact, compound **34** is completely inactive against kinases like CDK2, GSK3ß, PKA and AKT1. Although substitution in the 3-C position of the indole part did not improve potency or selectivity, there is still scope for substitution in the phenyl group on the pyrazole ring that extends new groups beyond the gatekeeper residue Phe435. Investigations to probe whether these modifications result in enhanced potency and or selectivity are in progress and will be reported in due course.

5. Experimental

5.1. General

All reagents were obtained from commercial sources and used without further purification unless stated otherwise. THF was distilled from sodium-benzophenone. Flash column chromatography was performed with Rankem Silica Gel (230–400 mesh size or 40–63 μm particle size). Thin layer chromatography (TLC) was performed on Merck pre-coated TLC aluminum sheets with Silica Gel 60 F254. The ^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer, and chemical shifts (δ) are given in ppm and are referenced to the corresponding solvent residual peak. The spectral splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet, br, broad. The LC/ES-MS analysis was performed using a Thermo Finnigan LCQ Advantage Max spectrometer. GC–MS analysis was carried out on a Thermo Focus GC spectrometer. High-resolution mass spectra were obtained using electrospray ionization (ESI) technique on a LC-MSD/TOF system by Agilent.

5.2. (*Z*)-3-Dimethylamino-2-phenyl-acrylonitrile (1)

A solution of benzyl cyanide (10 g, 85.36 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (17 ml, 15.25 g, 128 mmol) in DMF (40 ml) was stirred at 100 °C for 12 h. The cooled reaction mixture was diluted with water and the resulting precipitate was filtered and dried to give the title compound (11.0 g, 75%). ^{1}H NMR (DMSO-d_6) δ 7.43 (s, 1H), 7.33–7.25 (m, 4H), 7.10–7.04 (m, 1H), 3.19 (s, 6H); GC–MS (EI) *m/z* 172.10 [M$^+$].

5.3. 4-Phenyl-1*H*-pyrazol-3-ylamine (2)

To a solution of **1** (2.0 g, 11.61 mmol) in EtOH (10 ml) was added hydrazine monohydrochloride (6 g, 87.6 mmol) and the reaction mixture was refluxed overnight. The reaction mixture was concentrated, neutralized with aqueous saturated $NaHCO_3$ and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (50:50 v/v) as eluent to yield the title compound (1.2 g, 65%). ^{1}H NMR (DMSO-d_6) δ 7.79 (s, 1H), 7.51 (dt, 2H, *J* = 8.1, 1.8 Hz), 7.35 (t, 2H, *J* = 7.8 Hz), 7.16 (dt, 1H, *J* = 7.8, 0.6 Hz), 3.17 (s, 2H); GC–MS (EI) *m/z* 159.11 [M$^+$].

5.4. 3-Iodo-4-phenyl-1*H*-pyrazole (3)

To an ice-cooled solution of **2** (2.0 g, 12.56 mmol) in MeOH (20 ml) was added aqueous H_2SO_4 (40 ml, 50% v/v). To the reaction mixture was then added aqueous $NaNO_2$ (0.87 g, 12.6 mmol, 12 ml H_2O) and the reaction mixture was stirred at 0 °C for 1 h. An aqueous solution of KI (5.83 g, 35.12 mmol) was added dropwise, the reaction was further stirred at 0 °C for 1 h, diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (70:30 v/v) as eluent to afford the title compound (1.4 g, 41%). ^{1}H NMR (DMSO-d_6) δ 13.41 (s, 1H), 7.97 (d, 1H, *J* = 1.8 Hz), 7.57–7.54 (m, 2H), 7.42 (t, 2H, *J* = 7.5 Hz), 7.31 (tt, 1H, *J* = 7.5, 1.8 Hz); GC–MS (EI) *m/z* 269.88 [M$^+$].

5.5. 3-Iodo-4-phenyl-pyrazole-1-carboxylic acid *tert*-butyl ester (4)

To a reaction mixture containing **3** (0.5 g, 1.85 mmol) and Et_3N (0.30 ml, 0.22 g, 2.17 mmol) in CH_2Cl_2 (10 ml) was added di-*tert*-butyl dicarbonate (0.48 g, 2.2 mmol). The reaction mixture was stirred at room temperature for 24 h, quenched with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (70:30 v/v) as eluent to furnish the title compound (0.67 g, 99%). ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1H), 7.59 (dt, 2H, *J* = 6.9, 1.8 Hz), 7.49–7.44 (m, 2H), 7.41 (dt, 1H, *J* = 6.9, 1.5 Hz), 1.60 (s, 9H); GC–MS (EI) *m/z* 269.95 [M−Boc+H]$^+$.

5.6. 2-(1-*tert*-Butoxycarbonyl-4-phenyl-1*H*-pyrazol-3-yl)-indole-1,6-dicarboxylic acid 1-*tert*-butyl ester 6-methyl ester (5)

A pressure bottle containing a solution of **4** (1.3 g, 3.51 mmol) and 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid (1.68 g, 5.26 mmol) in 1,4-dioxane (40 ml) was purged with nitrogen for 3 min. To it was added in succession Cs_2CO_3 (3.43 g, 10.52 mmol) and Pd(dppf)Cl_2 (0.29 g, 0.35 mmol), the reaction mixture was purged with nitrogen again for 3 min and the reaction bottle was sealed. The reaction mixture was heated at 80 °C for 6 h, cooled to room temperature, diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc as eluent to yield the title compound (0.6 g, 33%). ^{1}H NMR (DMSO-d_6) δ 8.79 (t, 1H, *J* = 0.6 Hz), 8.76 (s, 1H), 7.92 (dd, 1H, *J* = 8.1, 1.5 Hz), 7.80 (d, 1H, *J* = 8.1 Hz), 7.29–7.23 (m, 5H), 7.06 (d, 1H, *J* = 0.9 Hz), 3.90 (s, 3H), 163 (s, 9H), 1.20 (s, 9H); LC–ESMS *m/z* 517.80 [M+H]$^+$.

5.7. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (6)

A solution of **5** (0.32 g, 0.62 mmol) and KOH (0.14 g, 2.5 mmol) in MeOH–H_2O (14 ml, 6:1 v/v) was refluxed for 12 h. The reaction mixture was concentrated in vacuo, acidified with aqueous saturated NH_4Cl solution and the resulting precipitate was filtered and dried to furnish the title compound (0.12 g, 64%). ^{1}H NMR (DMSO-d_6) δ 13.35 (s, 1H), 11.74 (s, 1H), 8.04 (s, 1H), 7.97 (s, 1H), 7.55 (d, 1H, *J* = 8.7 Hz), 7.47–7.38 (m, 5H), 7.36–7.32 (m, 1H), 6.33 (s, 1H); LC–ESMS *m/z* 304.20 [M+H]$^+$.

5.8. General procedure for amide formation

To the solution of a carboxylic acid (0.23 mmol) and amine (0.46 mmol) in DMF (2.5 ml) were added EDC (0.35 mmol), HOBt (0.35 mmol) and $NaHCO_3$ (0.8 mmol). The reaction mixture was stirred at room temperature for 24 h, diluted with water and extracted with EtOAc. The combined extracts were washed with

water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography (typically MeOH–CH_2Cl_2, 5:95 v/v) to afford the desired amide.

5.8.1. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (2-methoxy-ethyl)-amide (7)

^{1}H NMR (DMSO-d_6) δ 13.28 (s, 1H), 11.63 (s, 1H), 8.36 (s, 1H), 8.00 (s, 1H), 7.92 (s, 1H), 7.43–7.33 (m, 7H,), 6.27 (s, 1H), 3.48–3.40 (m, 4H), 3.27 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 361.16574 [M+H]$^+$ (Calcd for $C_{21}H_{21}N_4O_2$: 361.1659).

5.8.2. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (6-trifluoromethyl-pyridin-3-ylmethyl)-amide (8)

^{1}H NMR (DMSO-d_6) δ 13.30 (s, 1H), 11.69 (s, 1H), 9.06 (t, 1H, J = 5.7 Hz), 8.75 (s, 1H), 8.02 (d, 1H, J = 1.5 Hz), 8.00 (s, 1H), 7.97 (s, 1H), 7.90 (s, 1H), 7.87 (s, 1H), 7.49–7.34 (m, 6H), 6.28 (s, 1H), 4.60 (d, 2H, J = 5.7 Hz); LC–HRMS (ESI$^+$) *m/z* 462.15376 [M+H]$^+$ (Calcd for $C_{25}H_{19}F_3N_5O$: 462.15362).

5.8.3. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (pyrazin-2-ylmethyl)-amide (9)

^{1}H NMR (DMSO-d_6) δ 13.29 (s, 1H), 11.68 (s, 1H), 9.05 (s, 1H), 8.62 (d, 1H, J = 1.5 Hz), 8.59 (dd, 1H, J = 2.1, 1.5 Hz), 8.53 (d, 1H, J = 2.7 Hz), 7.99 (s, 1H), 7.97 (s, 1H), 7.52 (d, 1H, J = 8.4 Hz), 7.46–7.39 (m, 5H), 7.35–7.33 (m, 1H), 6.28 (s, 1H), 4.62 (d, 2H, J = 5.7 Hz); LC–HRMS (ESI$^+$) *m/z* 395.16114 [M+H]$^+$ (Calcd for $C_{23}H_{19}N_6O$: 395.16149).

5.8.4. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (10)

^{1}H NMR (DMSO-d_6) δ 13.31 (s, 1H), 11.66 (s, 1H), 8.72 (t, 1H, J = 6.0 Hz), 8.00 (s, 1H), 7.95 (s, 1H), 7.55 (s, 1H), 7.49–7.47 (m, 1H), 7.46–7.42 (m, 3H), 7.42–7.38 (m, 2H), 7.37–7.34 (m, 1H), 6.84 (s, 1H), 6.27 (s, 1H), 4.47 (d, 2H, J = 5.4 Hz), 3.64 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 397.17709 [M+H]$^+$ (Calcd for $C_{23}H_{21}N_6O$: 397.17714).

5.8.5. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (2-methyl-2*H*-pyrazol-3-ylmethyl)-amide (11)

^{1}H NMR (DMSO-d_6) δ 13.30 (s, 1H), 11.68 (s, 1H), 8.85 (t, 1H, J = 5.7 Hz), 8.00 (d, 1H, J = 1.5 Hz), 7.95 (s, 1H), 7.47 (d, 1H, J = 1.5 Hz), 7.46–7.39 (m, 4H), 7.37–7.32 (m, 2H), 7.31 (d, 1H, J = 1.5 Hz), 6.28 (d, 1H, J = 1.2 Hz), 6.16 (d, 1H, J = 1.8 Hz), 4.52 (d, 2H J = 5.7 Hz), 3.85 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 397.17699 [M+H]$^+$ (Calcd for $C_{23}H_{21}N_6O$: 397.17714).

5.8.6. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (5-methyl-isoxazol-4-ylmethyl)-amide (12)

^{1}H NMR (DMSO-d_6) δ 13.29 (s, 1H), 11.66 (s, 1H), 8.75 (t, 1H, J = 5.7 Hz), 8.40 (s, 1H), 8.00 (s, 1H), 7.92 (s, 1H), 7.45–7.41 (m, 5H), 7.38–7.33 (m, 2H), 6.26 (d, 1H, J = 1.5 Hz), 4.25 (d, 2H, J = 5.4 Hz), 2.44 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 398.16087 [M+H]$^+$ (Calcd for $C_{23}H_{20}N_5O_2$: 398.16115).

5.8.7. Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (13)

^{1}H NMR ($CDCl_3$) δ 10.34 (s, 1H), 7.61 (s, 1H), 7.53 (s, 1H), 7.49–7.47 (m, 2H), 7.44–7.37 (m, 3H), 7.05 (d, 1H, J = 6.9 Hz), 6.48 (s, 1H), 3.45–3.30 (m, 2H), 3.15 (s, 3H), 1.93 (m, 1H), 1.01 (s, 3H), 0.72 (s, 3H); LC–ESMS *m/z* 373.20 [M+H]$^+$.

5.9. 5-Phenyl-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazole (14)

To a solution of 3-phenyl-1*H*-pyrazole (0.9 g, 6.24 mmol) in toluene (6 ml) was added trifluoroacetic acid (0.025 ml, 0.037 g, 0.32 mmol). The mixture was heated to 80 °C and to it was added 3,4-dihydro-2*H*-pyran (0.60 ml, 0.55 g, 6.58 mmol). After stirring the reaction mixture overnight at 80 °C, the toluene was evaporated. The resulting residue was diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (80:20 v/v) to afford the title compound (1.4 g, 98%). ^{1}H NMR (DMSO-d_6) δ 7.93 (d, 1H, J = 2.4 Hz), 7.82 (d, 1H, J = 1.2 Hz), 7.80–7.79 (m, 1H), 7.43–7.3 (m, 2H), 7.30 (tt, 1H, J = 7.2, 1.8 Hz), 6.77 (d, 1H, J = 2.7 Hz), 5.43 (dd, 1H, J = 9.9, 2.4 Hz), 3.98–3.91 (m, 1H), 3.68–3.60 (m, 1H), 2.19–2.07 (m, 1H), 2.00–1.90 (m, 2H), 1.75–1.65 (m, 1H), 1.59–1.51 (m, 2H); LC–ESMS *m/z* 228.93 [M+H]$^+$.

5.10. 3-Iodo-5-phenyl-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazole (15)

To a stirred solution of **14** (1.8 g, 7.88 mmol) in anhydrous THF (12 ml) at −78 °C was added 1.6 M *n*-BuLi (5.4 ml, 8.64 mmol) and the reaction mixture was stirred at −78 °C for 30 min. A solution of I_2 (2.2 g, 8.66 mmol) in THF (12 ml) was added dropwise at −78 °C, the reaction mixture was warmed to room temperature over 2 h and stirred for 3 h. The reaction mixture was quenched at 0 °C by a saturated aqueous solution of $NaHSO_3$ and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (80/20, v/v) as eluent to yield the title compound (1.7 g, 61%). ^{1}H NMR (DMSO-d_6) δ 7.80 (dt, 2H, J = 6.6, 1.5 Hz), 7.44–7.38 (m, 2H), 7.36–7.30 (m, 1H), 7.07 (s, 1H), 4.43 (dd, 1H, J = 9.6, 2.7 Hz), 3.97–3.93 (m, 1H), 3.67–3.59 (m, 1H), 2.45–2.30 (m, 1H), 2.06–1.99 (m, 1H), 1.95–1.88 (m, 1H), 1.79–1.63 (m, 1H), 1.60–1.51 (m, 2H); GC–MS (EI) *m/z* 353.92 [M$^+$].

5.11. 2-[5-Phenyl-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazol-3-yl]-indole-1,6-dicarboxylic acid 1-*tert*-butyl ester 6-methyl ester (16)

A pressure bottle containing a solution of **15** (2.0 g, 5.65 mmol) and 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid (2.13 g, 6.67 mmol) in 1,4-dioxane (20 ml) was purged with nitrogen for 3 min. To it was added in succession Cs_2CO_3 (4.62 g, 14.18 mmol) and Pd(dppf)Cl_2 (0.46 g, 0.56 mmol), the reaction mixture was purged with nitrogen again for 3 min and the reaction bottle was sealed. The reaction mixture was heated at 80 °C for 6 h, cooled to room temperature, diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (80/20, v/v) as eluent to yield the title compound (0.9 g, 32%). ^{1}H NMR (DMSO-d_6) δ 8.87 (t, 1H, J = 0.6 Hz), 7.90 (dt, 2H, J = 8.4, 1.5 Hz), 7.86–7.81 (m, 2H), 7.47–7.41 (m, 2H), 7.37–7.31 (m, 1H), 7.04 (d, 1H, J = 0.9 Hz), 7.02 (s, 1H), 5.17 (dd, 1H, J = 9.6, 2.1 Hz), 3.91 (s, 3H), 3.88–3.84 (m, 1H), 3.42–3.37 (m, 1H), 2.33–2.26 (s, 1H), 1.98–1.83 (m, 2H), 1.65–1.40 (m, 3H), 1.30 (s, 9H); LC–ESMS *m/z* 501.87 [M+H]$^+$ (Calcd for $C_{29}H_{32}N_3O_5$).

5.12. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (17)

A solution of **16** (0.6 g, 1.20 mmol) and KOH (0.21 g, 3.74 mmol) in EtOH–H_2O (14 ml, 6:1 v/v) was refluxed for 12 h. The reaction mixture was concentrated in vacuo and to the residue at 0 °C was added ethereal HCl. After 3 h at room temperature, the reaction mixture was concentrated, neutralized by aqueous saturated $NaHCO_3$ solution and extracted with EtOAc. The combined layers were dried over Na_2SO_4 and concentrated to give the title

compound (0.2 g, 55%). ^{1}H NMR (DMSO-d_6) δ 12.01 (br, 1H), 8.1 (s, 1H), 7.86 (s, 1H), 7.83 (s, 1H), 7.64 (d, 1H, J = 8.1 Hz), 7.58 (t, 1H, J = 8.1 Hz), 7.49 (t, 2H, J = 8.1 Hz), 7.37 (t, 1H, J = 7.2 Hz), 7.19 (s, 1H), 6.88 (s, 1H); LC–ESMS m/z 304.13 $[M+H]^+$ (Calcd for $C_{18}H_{14}N_3O_2$).

5.13. General procedure

5.13.1. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (2-methoxy-ethyl)-amide (18)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.50 (s, 1H), 11.76 (s, 1H), 8.39 (s, 1H), 7.95 (s, 1H), 7.82 (d, 2H, J = 7.5 Hz), 7.60–7.48 (m, 4H), 7.41 (d, 1H, J = 7.8 Hz), 7.18 (s, 1H), 6.83 (s, 1H), 3.49–3.41 (m, 4H), 3.28 (s, 3H); LC–HRMS (ESI$^+$) m/z 361.16578 $[M+H]^+$ (Calcd for $C_{21}H_{21}N_4O_2$: 361.1659).

5.13.2. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (6-trifluoromethyl-pyridin-3-ylmethyl)-amide (19)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.51 (s, 1H), 11.83 (s, 1H), 9.11 (s, 1H), 8.76 (d, 1H, J = 1.8 Hz), 8.02 (dd, 1H, J = 8.1, 1.8 Hz), 7.99 (s, 1H), 7.89 (d, 1H, J= 8.1 Hz), 7.84 (s, 1H), 7.82 (s, 1H), 7.59 (s, 2H), 7.49 (t, 2H, J = 7.5 Hz), 7.38 (d, 1H, J = 6.3 Hz), 7.18 (s, 1H), 6.88 (s, 1H), 4.62 (d, 2H, J = 5.7 Hz); LC–HRMS (ESI$^+$) m/z 462.1534 $[M+H]^+$ (Calcd for $C_{25}H_{19}F_3N_5O$: 462.15362).

5.13.3. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (pyrazin-2-ylmethyl)-amide (20)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.51 (s, 1H), 11.80 (s, 1H), 9.08 (s, 1H), 8.65 (s, 1H), 8.60 (dd, 1H, J = 2.7, 1.5 Hz), 8.54 (d, 1H, J = 2.7 Hz), 8.00 (s, 1H), 7.82 (d, 2H, J = 7.5 Hz), 7.64 (s, 1H), 7.58 (s, 1H), 7.51 (t, 2H, J = 7.5 Hz), 7.39 (t, 1H, J = 7.5 Hz), 7.19 (s, 1H), 6.85 (s, 1H), 4.64 (d, 2H, J = 5.7 Hz), LC–HRMS (ESI$^+$) m/z 395.16084 $[M+H]^+$ (Calcd for $C_{23}H_{19}N_6O$: 395.16149).

5.13.4. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (21)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.33 (br s, 1H), 11.78 (s, 1H), 8.74 (s, 1H), 7.96 (s, 1H), 7.84 (d, 1H, J = 1.5 Hz), 7.81 (s, 1H), 7.55–7.52 (m, 3H), 7.49 (t, 2H, J = 7.5 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.77 (s, 1H), 6.86 (s, 1H), 6.83 (s, 1H), 4.48 (d, 2H, J = 4.5 Hz), 3.64 (s, 3H); LC–HRMS (ESI$^+$) m/z 397.1771 $[M+H]^+$ (Calcd for $C_{23}H_{21}N_6O$: 397.17714).

5.13.5. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (2-methyl-2*H*-pyrazol-3-ylmethyl)-amide (22)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.41 (br s, 1H), 11.87 (s, 1H), 8.89 (t, 1H, J = 5.7 Hz), 7.97 (s, 1H), 7.84 (d, 1H, J = 3.0 Hz), 7.82 (s, 1H), 7.57–7.56 (m, 2H), 7.49 (t, 2H, J = 7.5 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.31 (d, 1H, J = 2.1 Hz), 7.18 (s, 1H), 6.82 (s, 1H), 6.17 (d, 1H, J = 1.8 Hz), 4.54 (d, 2H, J = 5.4 Hz), 3.85 (s, 3H); LC–HRMS (ESI$^+$) m/z 397.17645 $[M+H]^+$ (Calcd for $C_{23}H_{21}N_6O$: 397.17714).

5.13.6. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (5-methyl-isoxazol-4-ylmethyl)-amide (23)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.51 (s, 1H), 11.78 (s, 1H), 8.78 (t, 1H, J = 5.7 Hz), 8.41 (s, 1H), 7.94 (s, 1H), 7.83 (d, 1H, J = 1.5 Hz), 7.80 (s, 1H), 7.57 (s, 1H), 7.54 (s, 1H), 7.51 (t, 2H, J = 8.1 Hz), 7.45–7.37 (m, 1H), 7.19 (d, 1H, J = 1.8 Hz), 6.82 (d, 1H, J = 1.8 Hz), 4.26 (d, 2H, J = 5.7 Hz), 2.45 (s, 3H); LC–HRMS (ESI$^+$) m/z 398.16079 $[M+H]^+$ (Calcd for $C_{23}H_{20}N_5O_2$: 398.16115).

5.13.7. 2-(5-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (24)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.37 (br s, 1H), 11.74 (s, 1H), 7.84 (d, 1H, J = 1.5 Hz), 7.81 (s, 1H), 7.56 (d, 1H, J = 8.4 Hz), 7.49 (t, 2H, J = 7.5 Hz), 7.40 (d, 1H, J = 1.5 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.15 (s, 1H), 7.00 (d, 1H, J = 7.8 Hz), 6.85 (s, 1H), 3.42–3.40 (m, 2H), 2.95 (s, 3H), 2.00 (s, 1H), 0.91–0.78 (br, 6H); LC–HRMS (ESI$^+$) m/z 373.2022 $[M+H]^+$ (Calcd for $C_{23}H_{25}N_4O$: 373.20229).

5.14. 1-(Tetrahydro-pyran-2-yl)-1*H*-pyrazole (25)

A solution of pyrazole (12.0 g, 176.26 mmol) and trifluoroacetic acid (2 ml, 3.06 g, 26.83 mmol) in toluene (35 ml) was heated to 80 °C and to it was added 3,4-dihydro-2*H*-pyran (15.7 ml, 15.57 g, 185.1 mmol). After stirring the reaction mixture at 80 °C for 2 h, the toluene was evaporated. The resulting residue was diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc as eluent to afford the title compound (24.1 g, 90%) as a pale yellow liquid. ^{1}H NMR (DMSO-d_6) δ 7.86 (1H, d, J = 2.4 Hz), 7.47 (1H, d, J = 1.5 Hz), 6.29 (1H, d, J = 1.8 Hz), 5.39 (1H, dd, J = 10.2, 2.7 Hz), 3.94–3.87 (1H, m), 3.65–3.57 (1H, m), 2.13–2.02 (1H, m), 1.96–1.84 (2H, m), 1.73–1.60 (1H, m), 1.56–1.47 (2H, m); GC–MS (EI) m/z 151.91 $[M^+]$.

5.15. 5-Benzyl-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazole (26)

To a stirred solution of **25** (20 g, 131.4 mmol) in anhydrous THF (700 ml) at −78 °C was added *n*-BuLi (83 ml, 131.8 mmol) and the reaction mixture was stirred at −78 °C for 1 h. Benzyl bromide (22.7 g, 132.2 mmol) was added dropwise at −78 °C and the reaction mixture was warmed to room temperature over 3 h and stirred overnight. The reaction mixture was quenched at 0 °C by ice-cold water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc as eluent to yield the title compound (12.0 g, 38%). ^{1}H NMR (DMSO-d_6) δ 7.37 (d, 1H, J = 1.8 Hz), 7.34–7.29 (m, 2H), 7.25–7.20 (m, 4H), 5.95 (d, 1H, J = 1.5 Hz), 5.36 (dd, 1H, J = 9.9, 2.7 Hz), 4.06 (s, 2H), 3.87–3.80 (m, 1H), 3.61–3.53 (m, 1H), 2.31–2.18 (m, 1H), 1.80–1.72 (m, 1H), 1.62–1.45 (m, 4H); GC–MS (EI) m/z 242.02 $[M^+]$.

5.16. 3-Benzyl-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazole (27)

To a solution of **26** (0.6 g, 2.48 mmol) in MeOH (5 ml) at 0 °C was added ethereal HCl (15 ml). The reaction mixture was then stirred overnight at room temperature and then concentrated in vacuo. The residue was neutralized with aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. To the resulting residue were added trifluoroacetic acid (0.01 ml, 0.015 g, 0.13 mmol) and toluene (10 ml). The mixture was heated to 80 °C and to it was added 3,4-dihydro-2*H*-pyran (0.25 ml, 0.23 g, 2.74 mmol). After stirring the reaction mixture overnight at 80 °C, the toluene was evaporated. The resulting residue was diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc (80:20 v/v) to afford the title compound (0.5 g, 83%). ^{1}H NMR (DMSO-d_6) δ 7.74 (d, 1H, J = 2.4 Hz), 7.30–7.17 (m, 5H), 6.06 (d, 1H, J = 2.4 Hz), 5.30 (dd, 1H, J = 10.5, 2.7 Hz), 3.93–3.87 (m, 1H), 3.86 (s, 2H), 3.63–3.54 (m, 1H), 2.10–2.01 (m, 1H),

1.95–1.82 (m, 2H), 1.71–1.57 (m, 1H), 1.55–1.46 (m, 2H); GC–MS (EI) *m/z* 242.02 [M^+] (Calcd for $C_{15}H_{18}N_2O$).

5.17. 3-Benzyl-5-iodo-1-(tetrahydro-pyran-2-yl)-1*H*-pyrazole (28)

To a stirred solution of **27** (4.2 g, 17.3 mmol) in anhydrous THF (15 ml) at −78 °C was added *n*-BuLi (11 ml, 17.6 mmol) and the reaction mixture was stirred at −78 °C for 30 min. A solution of I_2 (4.5 g, 17.7 mmol) in THF (10 ml) was added dropwise at −78 °C, the reaction mixture was warmed to room temperature over 2 h and stirred for 3 h. The reaction mixture was quenched at 0 °C by a saturated aqueous solution of $NaHSO_3$ and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc as eluent to yield the title compound (2.6 g, 41%). ^{1}H NMR (DMSO-d_6) δ 7.30–7.16 (m, 5H), 6.31 (s, 1H), 5.30 (dd, 1H, J = 10.2, 2.7 Hz), 3.93–3.87 (m, 1H), 3.86 (s, 2H), 3.62–3.53 (m, 1H), 2.35–2.22 (m, 1H), 2.01–1.93 (br m, 1H), 1.87–1.79 (m, 1H), 1.75–1.61 (m, 1H), 1.55–1.46 (m, 2H); GC–MS (EI) *m/z* 367.99 [M^+].

5.18. 2-[5-Benzyl-2-(tetrahydro-pyran-2-yl)-2*H*-pyrazol-3-yl]-indole-1,6-dicarboxylic acid 1-*tert*-butyl ester 6-methyl ester (29)

A pressure bottle containing a solution of **28** (2.5 g, 6.79 mmol) and 1-(*t*-butoxycarbonyl)-6-(methoxycarbonyl)-1*H*-indol-2-ylboronic acid (3.24 g, 10.15 mmol) in 1,4-dioxane (30 ml) was purged with nitrogen for 3 min. To it was added in succession Cs_2CO_3 (6.62 g, 20.32 mmol) and [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (0.39 g, 0.48 mmol), the reaction mixture was purged with nitrogen again for 3 min and the reaction bottle was sealed. The reaction mixture was heated at 80 °C for 6 h, cooled to room temperature, diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexanes–EtOAc as eluent to yield the title compound (1.1 g, 31%). ^{1}H NMR (DMSO-d_6) δ 8.80 (t, 1H, J = 0.9 Hz), 7.88 (dd, 1H, J = 8.1, 1.5 Hz), 7.76 (d, 1H, J = 8.1 Hz), 7.34–7.26 (m, 4H), 7.23–7.16 (m, 1H), 6.92 (d, 1H, J = 0.6 Hz), 6.27 (s, 1H), 5.04 (dd, 1H, J = 9.9, 2.4 Hz), 3.92 (s, 2H), 3.89 (s, 3H), 3.87–3.79 (m, 2H), 2.26–2.15 (m, 1H), 1.90–1.82 (m, 1H), 1.80–1.70 (br, 1H), 1.60–1.35 (m, 3H), 1.19 (s, 9H); LC–ESMS *m/z* 432.40 [M−THP+H]$^+$.

5.19. 2-(5-Benzyl-2*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid methyl ester (30)

To **29** (1.1 g, 2.13 mmol) at 0 °C was added 4 M HCl in 1,4-dioxane (15 ml). The reaction mixture was stirred overnight and then concentrated in vacuo. To the crude solid were added K_2CO_3 (0.67 g, 4.85 mmol) and MeOH (10 ml) and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was acidified at 0 °C with 1 N HCl to pH 3, the resulting precipitate was filtered and dried to give pure the title compound (0.6 g, 85%). ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H), 8.01 (d, 1H, J = 0.9 Hz), 7.58 (d, 1H, J = 1.2 Hz), 7.57 (s, 1H), 7.36–7.29 (m, 4H), 7.26–7.20 (m, 2H), 6.80 (dd, 1H, J = 2.1, 0.9 Hz), 6.51 (s, 1H), 4.02 (s, 2H), 3.84 (s, 3H); LC–ESMS *m/z* 332.07 [M+H]$^+$.

5.20. 2-(5-Benzyl-2*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (31)

To a solution of **30** (0.6 g, 1.81 mmol) in EtOH (10 ml) was added 5% aqueous KOH (0.203 g, 3.62 mmol in 4 ml H_2O) and the reaction mixture was refluxed overnight. The reaction mixture was then concentrated in vacuo, acidified with 1 N HCl and the resulting precipitate was filtered to afford the title compound (0.51 g, 89%). ^{1}H NMR (DMSO-d_6) δ 11.73 (s, 1H), 7.99 (d, 1H, J = 0.9 Hz), 7.56 (d, 1H, J = 1.5 Hz),7.54 (s, 1H), 7.34–7.33 (m, 1H), 7.32–7.29 (m, 3H), 7.26–7.21 (m, 1H), 6.77 (d, 1H, J = 1.2 Hz), 6.49 (s, 1H), 4.01 (s, 2H); LC–ESMS *m/z* 318.13 [M+H]$^+$.

5.21. General procedure

5.21.1. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (32)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 12.92 (s, 1H), 11.64 (s, 1H), 8.70 (s, 1H), 7.90 (s, 1H), 7.54 (s, 1H), 7.49 (s, 2H), 7.37–7.28 (m, 4H), 7.25–7.21 (m, 1H), 6.82 (s, 1H), 6.71 (s, 1H), 6.48 (s, 1H), 4.47 (d, 2H, J = 5.4 Hz), 4.02 (s, 2H), 3.63 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 411.19334 [M+H]$^+$ (Calcd for $C_{24}H_{23}N_6O$: 411.19279).

5.21.2. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (2-methyl-2*H*-pyrazol-3-ylmethyl)-amide (33)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 12.93 (s, 1H), 11.66 (s, 1H), 8.84 (s, 1H), 7.90 (s, 1H), 7.50 (s, 2H), 7.34 (s, 1H), 7.32–7.30 (m, 4H), 7.29 (d, 1H, J = 1.8 Hz), 6.72 (s, 1H), 6.48 (s, 1H), 6.15 (d, 1H, J 1.5 Hz), 4.51 (d, 2H, J = 5.7 Hz), 4.02 (s, 2H), 3.83 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 411.19234 [M+H]$^+$ (Calcd for $C_{24}H_{23}N_6O$: 411.19279).

5.21.3. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (34)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 12.90 (s, 1H), 11.50 (s, 1H), 7.49 (d, 1H, J = 8.4 Hz), 7.34–7.29 (m, 5H), 7.26–7.22 (m, 1H), 6.96 (d, 1H, J = 8.4 Hz), 6.69 (s, 1H), 6.45 (s, 1H), 4.02 (s, 2H), 3.42–3.40 (m, 2H), 2.93 (s, 3H), 1.99 (s, 1H), 0.85 (br, 6H); LC–HRMS (ESI$^+$) *m/z* 387.21812 [M+H]$^+$ (Calcd for $C_{24}H_{27}N_4O$: 387.21794).

5.21.4. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (pyrazin-2-ylmethyl)-amide (35)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR ($CDCl_3$) δ 10.51 (s, 1H), 8.65 (s, 1H), 8.44 (s, 2H), 8.06 (s, 1H), 7.60–7.53 (m, 2H), 7.45 (dd, 1H, J = 8.4, 1.5 Hz), 7.28–7.25 (m, 3H), 7.24–7.21 (m, 2H), 6.70 (s, 1H), 6.39 (s, 1H), 5.47 (br s, 1H), 4.79 (d, 2H, J = 5.1 Hz), 4.02 (s, 2H); LC–HRMS (ESI$^+$) *m/z* 409.17741 [M+H]$^+$ (Calcd for $C_{24}H_{21}N_6O$: 409.17714).

5.21.5. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid methylamide (36)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 12.91 (s, 1H), 11.62 (s, 1H), 8.28 (s, 1H), 7.87 (s, 1H), 7.48 (s, 2H), 7.36–7.28 (m, 4H), 7.26–7.23 (m, 1H), 6.70 (s, 1H), 6.48 (s, 1H), 4.02 (s, 2H), 2.79 (d, 3H, J = 4.5 Hz); LC–HRMS (ESI$^+$) *m/z* 331.15508 [M+H]$^+$ (Calcd for $C_{20}H_{19}N_4O$: 331.15534).

5.21.6. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (5-methyl-isoxazol-4-ylmethyl)-amide (37)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 12.92 (s, 1H), 11.64 (s, 1H), 8.74 (t, 1H, J = 6.3 Hz), 8.40 (s, 1H), 7.88 (s, 1H), 7.48 (s, 2H), 7.36–7.22 (m, 5H), 6.71 (s, 1H), 6.48 (s, 1H), 4.25 (d, 2H, J = 5.7 Hz), 4.02 (s, 2H), 2.44 (s, 3H); LC–HRMS (ESI$^+$) *m/z* 412.17724 [M+H]$^+$ (Calcd for $C_{24}H_{22}N5O_2$: 412.1768).

5.22. 2-(4-Phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid methyl ester (38)

To a solution of **5** (0.5 g, 0.97 mmol) in MeOH (12 ml) was added aqueous KOH (0.17 g, 3.03 mmol, in 2 ml H_2O) and the reaction mixture was at room temperature for 3 h. It was then concentrated, acidified with 1 N HCl and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated to give the title compound (0.3 g, 97%). ^{1}H NMR (DMSO-d_6) δ 13.36 (s, 1H), 11.79 (s, 1H), 8.06 (s, 1H), 7.99 (s, 1H), 7.56 (d, 1H, J = 8.1 Hz), 7.50–7.38 (m, 5H), 7.34 (d, 1H, J = 6.9 Hz), 6.32 (s, 1H), 3.84 (s, 3H); LC–ESMS m/z 318.13 $[M+H]^+$.

5.23. 3-Formyl-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid methyl ester (39)

To DMF (1.1 g 15.1 mmol) at 0 °C was added phosphorous oxychloride (2.32 g, 15.1 mmol). The reaction mixture was stirred at room temperature for 1 h and to it was then slowly added a solution of **38** (0.48 g, 1.51 mmol) in DMF (2 ml). After stirring for 3 h at 70 °C, the reaction was quenched with H_2O and the resulting solid was filtered to afford the title compound (0.35 g, 67%). ^{1}H NMR (DMSO-d_6) δ 13.75 (s, 1H), 12.65 (s, 1H), 9.59 (s, 1H), 8.29 (s, 1H), 8.19 (d, 1H, J = 8.7 Hz), 8.07 (s, 1H), 7.84 (dd, 1H, J = 8.7, 1.5 Hz), 7.30 (d, 1H, J = 1.5 Hz), 7.30–7.26 (m, 3H), 7.25–7.23 (m, 1H), 3.87 (s, 3H); LC–ESMS m/z 346.13 $[M+H]^+$.

5.24. 3-Formyl-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid (40)

Prepared from **39** by the procedure in Section 5.7 for compound **6**.

^{1}H NMR (DMSO-d_6) δ 13.70 (s, 1H), 12.41 (s, 1H), 9.59 (s, 1H), 8.31 (d, 1H, J = 1.5 Hz), 8.12 (d, 1H, J = 7.8 Hz), 7.43 (s, 1H), 7.31 (s, 1H), 7.30–7.26 (m, 3H), 7.25–7.23 (m, 1H), 7.22–7.20 (m, 1H); LC–ESMS m/z 332.13 $[M+H]^+$.

5.25. 3-Formyl-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (41)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.69 (s, 1H), 12.41 (s, 1H), 9.58 (s, 1H), 8.30 (d, 1H, J = 1.5 Hz), 8.12 (d, 1H, J = 7.8 Hz), 7.94 (s, 1H), 7.42 (s, 1H), 7.30–7.26 (m, 5H), 2.95 (br, 2H), 2.93 (s, 3H), 1.98 (m, 1H), 0.83 (br, 6H); LC–ESMS m/z 401.20 $[M+H]^+$.

5.26. General procedure

5.26.1. 3-[(4-Methoxy-benzylamino)-methyl]-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (42)

To a solution of **41** (0.1 g, 0.25 mmol) in MeOH (8 ml) was added 4-methoxybenzylamine (0.035 g, 0.25 mmol) followed by catalytic amount of glacial acetic acid. The reaction was stirred at room temperature for 12 h, cooled to 0 °C and treated with sodium borohydride (0.014 g, 0.37 mmol). After stirring at 0 °C for 15 min, the reaction was quenched with water and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with CH_3OH/CH_2Cl_2 (5:95) as eluent to yield the title compound (0.022 g, 17%). ^{1}H NMR (DMSO-d_6) δ 11.34 (s, 1H), 8.12 (s, 1H), 7.70 (d, 1H, J = 8.1 Hz), 7.32 (s, 1H), 7.29–7.25 (m, 4H), 7.18 (dd, 1H, J = 8.7, 4.5 Hz), 7.11 (d, 2H, J = 8.7 Hz), 7.04 (d, 1H, J = 7.5 Hz), 6.82 (d, 2H, J = 8.7 Hz), 3.72 (s, 3H), 3.71 (s, 2H), 3.54 (s, 2H), 3.42–3.40 (m, 2H, obscured by H_2O signal), 2.94 (s, 3H), 1.98 (m, 1H), 0.84 (br, 6H); LC–HRMS (ESI^+) m/z 522.28607 $[M+H]^+$ (Calcd for $C_{32}H_{36}N_5O_2$: 522.28635).

5.26.2. 3-[(3-Hydroxy-propylamino)-methyl]-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (43)

Prepared by the procedure in Section 5.26.1 for compound **42**.

^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1H), 7.68 (s, 1H), 7.59 (d, 1H, J = 8.1 Hz), 7.45–7.40 (m, 4H), 7.39–7.35 (m, 1H), 7.24 (s, 1H), 7.14 (d, 1H, J = 8.4 Hz), 4.08 (s, 2H), 3.83 (t, 2H, J = 5.7 Hz), 3.38 (br, 2H), 3.17 (br, 3H), 2.95 (t, 3H, J = 6.0 Hz), 1.85 (t, 3H, J = 6.3 Hz), 1.67 (s, 1H), 0.87 (d, 6H, J = 6.9 Hz); LC–HRMS (ESI^+) m/z 460.27014 $[M+H]^+$ (Calcd for $C_{27}H_{34}N_5O_2$: 460.2707).

5.26.3. 3-Hydroxymethyl-2-(4-phenyl-1*H*-pyrazol-3-yl)-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (44)

To a solution of **41** (15 mg, 0.037 mmol) in CH_3OH at 0 °C was added $NaBH_4$ (3 mg, 0.08 mmol) and the reaction was stirred at room temperature for 30 min. The reaction was quenched with water (5 ml) and extracted with EtOAc. The combined extracts were washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with CH_3OH/CH_2Cl_2 (8:92) as eluent to yield the title compound (13 mg, 85%). ^{1}H NMR (DMSO-d_6) δ 7.98 (s, 1H), 7.81 (d, 1H, J = 8.1 Hz), 7.40 (s, 1H), 7.31–7.26 (m, 3H), 7.25 (s, 1H), 7.22–7.19 (m, 1H), 7.18–7.12 (m, 1H), 4.66 (s, 2H), 3.44–3.43 (m, 1H), 3.24–3.23 (m, 1H), 3.07 (s, 3H), 2.00–1.97 (m, 1H), 1.02 (d, 3H, J = 5.1 Hz), 0.75 (d, 3H, J = 6.0 Hz); LC–HRMS (ESI^+) m/z 403.21255 $[M+H]^+$ (Calcd for $C_{24}H_{27}N_4O_2$: 403.21285).

5.27. 2-(5-Benzyl-2*H*-pyrazol-3-yl)-3-formyl-1*H*-indole-6-carboxylic acid methyl ester (45)

To DMF (0.33 g, 4.52 mmol) at 0 °C was added phosphorous oxychloride (0.69 g, 4.52 mmol). The reaction mixture was stirred at room temperature for 1 h and to it was then slowly added a solution of **30** (0.6 g, 1.81 mmol) in DMF (2 ml). After stirring for 4 h at room temperature, the reaction was quenched with H_2O, the resulting solid was filtered and dried to afford the title compound (0.45 g, 69%). ^{1}H NMR (DMSO-d_6) δ 12.65 (s, 1H), 10.56 (s, 1H), 8.47 (br s, 1H), 8.23 (d, 1H, J = 8.1 Hz), 8.06 (s, 1H), 7.80 (dd, 1H, J = 8.4, 1.5 Hz), 7.38–7.32 (m, 4H), 7.28–7.23 (m, 1H), 6.78 (s, 1H), 4.09 (s, 2H), 3.87 (s, 3H); LC–ESMS m/z 360.13 $[M+H]^+$.

5.28. 5.282-(5-Benzyl-2*H*-pyrazol-3-yl)-3-formyl-1*H*-indole-6-carboxylic acid (46)

To a solution of **45** (0.45 g, 1.25 mmol) in EtOH (10 ml) was added 5% aqueous KOH (0.3 g, 5.34 mmol in 6 ml H_2O) and the reaction mixture was refluxed overnight. The reaction mixture was then concentrated in vacuo, acidified with 1 N HCl and the resulting precipitate was filtered to afford the title compound (0.3 g, 69%). ^{1}H NMR (DMSO-d_6) δ 12.59 (s, 1H), 10.56 (s, 1H), 8.21 (d, 1H, J = 8.1 Hz), 8.05 (s, 1H), 7.79 (dd, 1H, J = 8.1, 1.5 Hz), 7.35–7.32 (m, 4H), 7.28–7.23 (s, 1H), 6.77 (s, 1H), 4.09 (s, 2H); LC–ESMS m/z 346.20 $[M+H]^+$.

5.29. General procedure

5.29.1. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-formyl-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (47)

Prepared by the general procedure as in Section 5.8, yield = 0.28 g, 69%.

^{1}H NMR (DMSO-d_6) δ 13.44 (s, 1H), 12.48 (s, 1H), 10.55 (s, 1H), 8.86 (t, 1H, J = 5.4 Hz), 8.16 (d, 1H, J = 8.1 Hz), 7.95 (s, 1H), 7.70 (t, 1H, J = 8.4 Hz), 7.57 (s, 1H), 7.38–7.32 (m, 4H), 7.28–7.24 (m, 1H),

6.85 (s, 1H), 6.74 (s, 1H), 4.48 (d, 2H, *J* = 5.4 Hz), 4.08 (s, 2H), 3.64 (s, 3H); LC–ESMS *m/z* 439.27 $[M+H]^+$.

5.29.2. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-formyl-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (48)

Prepared by the general procedure as in Section 5.8, yield = 0.51 g, 85%

^{1}H NMR (DMSO-d_6) δ 13.43 (s, 1H), 12.37 (s, 1H), 10.56 (s, 1H), 8.16 (d, 1H, *J* = 8.1 Hz), 7.40 (s, 1H), 7.36 (d, 1H, *J* = 0.9 Hz), 7.35 (s, 1H), 7.33–7.32 (m, 2H), 7.28–7.22 (m, 1H), 7.18–7.14 (m, 1H), 6.72 (d, 1H, *J* = 2.1 Hz), 4.08 (s, 2H), 3.42–3.40 (m, 2H), 2.92 (s, 3H), 1.99 (s, 1H), 0.85 (br, 6H); LC–ESMS *m/z* 415.20 $[M+H]^+$.

5.29.3. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-formyl-1*H*-indole-6-carboxylic acid methylamide (49)

Prepared by the general procedure as in Section 5.8.

^{1}H NMR (DMSO-d_6) δ 13.45 (s, 1H), 12.48 (s, 1H), 10.56 (s, 1H), 8.44 (d, 1H, *J* = 4.2 Hz), 8.16 (d, 1H, *J* = 8.1 Hz), 7.93 (s, 1H), 7.68 (dd, 1H, *J* = 8.4, 1.5 Hz), 7.39–7.32 (m, 4H), 7.28–7.22 (m, 1H), 6.75 (d, 1H, *J* = 2.1 Hz), 4.08 (s, 2H), 2.80 (d, 3H, *J* = 4.5 Hz); LC–ESMS *m/z* 359.20 $[M+H]^+$.

5.30. General procedure

5.30.1. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-morpholin-4-ylmethyl-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (50)

Prepared by the procedure in Section 5.26.1 for compound **42**.

^{1}H NMR (DMSO-d_6) δ 13.01 (s, 1H), 11.55 (s, 1H), 8.71 (s, 1H), 7.88 (s, 1H), 7.67 (s, 1H), 7.64 (s, 1H), 7.54 (s, 1H), 7.34–7.31 (m, 4H), 7.26–7.22 (m, 1H), 6.82 (s, 1H), 6.51 (s, 1H), 4.47 (dd, 2H, *J* = 4.8, 2.7 Hz), 4.40 (t, 1H, *J* = 5.7 Hz), 4.02 (s, 2H), 3.77 (s, 2H), 3.62 (s, 3H), 3.55–3.46 (m, 3H), 2.45–2.34 (m, 4H); LC–HRMS (ESI^+) *m/z* 510.26122 $[M+H]^+$ (Calcd for $C_{29}H_{32}N_7O_2$: 510.2612).

5.30.2. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-[(4-fluoro-benzylamino)-methyl]-1*H*-indole-6-carboxylic acid isobutyl-methyl-amide (51)

Prepared by the procedure in Section 5.26.1 for compound **42**.

^{1}H NMR (DMSO-d_6) δ 11.47 (s, 1H), 7.57 (d, 1H, *J*= 8.4 Hz), 7.40–7.34 (m, 3H), 7.33–7.31 (m, 3H), 7.30–7.21 (m, 2H), 7.13 (t, 2H, *J* = 8.4 Hz), 6.99 (dd, 1H, *J* = 8.4, 1.5 Hz), 6.42 (s, 1H), 4.04 (s, 2H), 4.01 (s, 2H), 3.79 (s, 2H), 3.42–3.40 (m, 2H, obscured by H_2O signal), 2.93 (s, 3H), 1.98 (br, 1H), 0.85 (br, 6H); LC–HRMS (ESI^+) *m/z* 524.28197 $[M+H]^+$ (Calcd for $C_{32}H_{35}FN_5O$: 524.28202).

5.30.3. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-{[(pyridin-4-ylmethyl)-amino]-methyl}-1*H*-indole-6-carboxylic acid methylamide (52)

Prepared by the procedure in Section 5.26.1 for compound **42**.

^{1}H NMR (DMSO-d_6) δ 12.95 (br s, 1H), 11.51 (s, 1H), 8.47 (d, 2H, *J* = 5.7 Hz), 8.31 (d, 1H, *J* = 4.5 Hz), 7.85 (s, 1H), 7.55 (d, 1H, *J* = 8.4 Hz), 7.48 (dd, 1H, *J* = 8.1, 1.2 Hz), 7.34–7.33 (m, 3H), 7.32–7.30 (m, 3H), 7.26–7.21 (m, 2H), 6.47 (s, 1H), 4.01 (overlapping s, 4H), 3.74 (s, 2H), 2.79 (d, 3H, *J* = 4.5 Hz); LC–HRMS (ESI^+) *m/z* 451.22343 $[M+H]^+$ (Calcd for $C_{27}H_{27}N_6O$: 451.22409).

5.30.4. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-[(4-hydroxy-butylamino)-methyl]-1*H*-indole-6-carboxylic acid methylamide (53)

Prepared by the procedure in Section 5.26.1 for compound **42**.

^{1}H NMR (DMSO-d_6) δ 11.57 (s, 1H), 8.32 (d, 1H, *J* = 4.5 Hz), 7.84 (s, 1H), 7.63 (d, 1H, *J* = 8.4 Hz), 7.50 (dd, 1H, *J* = 8.7, 1.5 Hz), 7.34–7.32 (m, 3H), 7.26–7.20 (m, 2H), 6.88 (s, 1H), 6.50 (s, 1H), 5.32 (br t, 1H), 4.02 (s, 2H), 2.79 (d, 3H, *J* = 4.5 Hz), 2.73–2.71 (m, 1H), 2.59 (t, 2H, *J* = 6.3 Hz), 2.53 (m, 2H), 2.27 (m, 1H), 2.03–1.97 (m, 1H), 1.50–1.43 (m, 4H); LC–HRMS (ESI^+) *m/z* 432.23967 $[M+H]^+$ (Calcd for $C_{25}H_{30}N_5O_2$: 432.2394).

5.30.5. 2-(5-Benzyl-1*H*-pyrazol-3-yl)-3-hydroxymethyl-1*H*-indole-6-carboxylic acid (3-methyl-3*H*-imidazol-4-ylmethyl)-amide (54)

Prepared by the procedure in Section 5.26.3 for compound **44**.

^{1}H NMR (DMSO-d_6) δ 13.1 (br s, 1H), 11.52 (s, 1H), 8.73 (t, 1H, *J* = 5.7 Hz), 7.89 (s, 1H), 7.64 (d, 1H, *J* = 8.4 Hz), 7.53 (dd, 1H, *J* = 8.4, 1.5 Hz), 7.52 (s, 1H), 7.35–7.30 (m, 4H), 7.26–7.21 (m, 1H), 6.82 (d, 1H, *J* = 1.2 Hz), 6.54 (s, 1H), 4.93 (s, 1H), 4.81 (s, 2H), 4.46 (d, 2H, *J* = 5.1 Hz), 4.04 (s, 2H), 3.63 (s, 3H); LC–HRMS (ESI^+) *m/z* 441.20352 (Calcd for $C_{25}H_{25}N_6O_2$: 441.20335).

5.31. Methods for biological assays

5.31.1. ITK enzymatic activity (IMAP assay)

GST-ITK full length was from Invitrogen (PV3875). Substrate was FAM-Blk peptide (5-FAM-EFPIYDFLPAKKK-NH2). Reaction conditions were as following: 10 ng ITK/well in a final volume of 20 μl of final reaction buffer (20 mM Hepes pH 7.4; 2.5 mM $MgCl_2$; 0.01% Tween; 5 mM DTT; 0.05% BSA) containing 500 nM substrate, 6 μM ATP. The reaction time was 2 h and the binding time was 2 h. The reaction was stopped by addition of 60 μl binding solution (Progressive Binding IMAP system, 60% buffer A: 40% buffer B, beads 1:1000, Molecular Devices). Signal was detected with an envision reader (PE) by measuring fluorescence polarization at 485 nm/535 nm.

5.31.2. Insulin receptor (InsR) enzymatic activity (IMAP assay)

The enzyme was a GST-fusion protein of the recombinant catalytic domain (AA 987-1381) expressed in Sf21 cells infected with recombinant Baculovirus. Substrate was FAM-peptide (5-FAM-KKSRGDYMTMQIG-NH2). The reaction conditions were as following: 25 ng InsR/well in a final volume of 20 μl of final reaction buffer (50 mM Tris pH 7.5; 10 mM $MnCl_2$; 5 mM DTT; 0.01% BSA) containing 100 nM substrate, 3 μM ATP. The reaction time was 80 min and the binding time was 2 h. The reaction was stopped by addition of 60 μl binding solution (Progressive Binding IMAP system, 85% buffer A: 15% buffer B, beads 1:400, Molecular Devices). Signal was detected with an envision reader (PE) by measuring fluorescence polarization at 485 nm/ 535 nm.

5.31.3. PKA enzymatic activity (IMAP assay)

His-PKA (catalytic domain) was from Panvera. Substrate was FAM- peptide (5-FAM-GRTGRRNSI-NH2). The reaction conditions were as following: 100 pg PKA/well in a final volume of 20 μl of final reaction buffer (10 mM Tris pH 7.2; 10 mM $MgCl_2$; 0.05% NaN_3; 1 mM DTT; 0.1% BSA) containing 100 nM substrate, 1 μM ATP. The reaction time was 40 min and binding time was 2 h. The reaction was stopped by addition of 60 μl binding solution (Progressive Binding IMAP system, 100% buffer A, beads 1:400, Molecular Devices). Signal was detected with an envision reader (PE) by measuring fluorescence polarization at 485 nm/ 535 nm.

5.31.4. Akt1 enzymatic activity (IMAP assay)

His-Akt1 full length was from Upstate. Substrate was FAM- peptide (5-FAM-GRTGRRNSI-NH2). The reaction conditions were as following: 200 pg Akt1/well in a final volume of 20 μl of final reaction buffer (10 mM Tris pH 7.2; 10 mM $MgCl_2$; 0.05% NaN_3; 1 mM DTT; 0.1% BSA) containing 100 nM substrate, 20 μM ATP. The reaction time was 1 h and the binding time was 2 h. The reaction was stopped by addition of 60 μl binding solution (Progressive Binding IMAP system, 100% buffer A, beads 1:400, Molecular Devices). Signal was detected with an envision reader (PE) by measuring fluorescence polarization at 485 nm/ 535 nm.

5.31.5. CDK2 enzymatic activity (IMAP assay)

GST-CDK2/CyclinE full length was from ProQinase. Substrate was FAM- peptide (5-FAM-GGGPATPKKAKKL-OH). The reaction conditions were as following: 2 ng CDK2/well in a final volume of 20 μl of final reaction buffer (10 mM Tris pH 7.2; 10 mM $MgCl_2$; 0.05% NaN_3; 1 mM DTT; 0.1% BSA) containing 100 nM substrate, 30 μM ATP. The reaction time was 40 min and the binding time was 1 h. The reaction was stopped by addition of 60 μl binding solution (Progressive Binding IMAP system, 100% buffer A, beads 1:400, Molecular Devices). Signal was detected with an envision reader (PE) by measuring fluorescence polarization at 485 nm/535 nm.

5.31.6. GSK3ß enzymatic activity (ATP consumption assay)

GST-GSK3ß full length was from ProQinase. Enzymatic activity was measured using the Easylite–Kinase Detection Assay System from PE. The substrate was the N-terminus biotinylated peptide [YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE] from Upstate. The reaction conditions were as following: 100 ng GSK3/well in a final volume of 20 μl of final reaction buffer (50 mM Hepes pH 7.5; 3 mM $MgCl_2$; 3 mM $MnCl_2$; 0.05% BSA; 1 mM DTT) containing 500 nM substrate, 200 nM ATP. The reaction time was 60 min at room temperature in dark. The reaction was stopped by addition of 20 μl Easylite reagent. Luminescence was detected with an Envision reader (PE).

5.32. Molecular modeling

Molecular docking studies were performed using Maestro, version 8.5, and Glide, version 5.0 (Schrodinger,L.L.C.). The crystal structure of ITK complexed with thiophene-2-carboxylic acid [1-(2-carbamoyl-ethyl)-5-(cyclohexanecarbonyl-methyl-amino)-1*H*-benzoimidazol-2-yl]-amide inhibitor was used as a template for the Glide docking. Among several ITK crystal structures bound with inhibitors, this structure was selected because of the structural similarity of the bound thiazolyl benzimidazole ligand with the pyrazolyl-indole derivatives under study.

The crystal structure of ITK was optimized for docking using the Protein Preparation Wizard provided by Schrodinger. This involved assignment of bond orders, addition of hydrogens and capping of uncapped termini. The hydrogen-bonding network was optimized by reorienting all the hydroxyl groups and the amide groups of Asn and Gln. Appropriate states and orientations of the imidazole ring in the His residues were selected. Finally the whole structure was refined with a constrained minimization to 0.3 rmsd using the Optimised Potentials for Liquid Simulations (OPLS) force field.

The pyrazolyl-indoles were constructed using the fragment dictionary of Maestro 8.5 and geometry optimized under the OPLS force field using the Polak-Ribiere Conjugate gradient protocol as implemented in Macromodel v. 9.6. The gradient convergence threshold was set to 0.001.

Partial atomic charges for compounds as well as the protein were assigned according to the OPLS-AA force field. The standard precision (SP) Glide docking method was used to dock all the compounds into the catalytic site of ITK. Grids for Glide docking were calculated using the bound inhibitor as the reference of the catalytic site in ITK. Upon completion of each docking calculation, a maximum of 10 poses per ligand were generated. The top-scored pose was chosen using the Glidescore (Gscore) function.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.056.

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