Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01208 • Publication Date (Web): 20 Oct 2015

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Hit Optimization of 5-Substituted-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamides: Potent Glycogen Synthase Kinase-3 (GSK-3) Inhibitors with In Vivo Activity in Model of Mood Disorders

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ABSTRACT

Novel treatments for bipolar disorders, with improved efficacy and broader spectrum of activity are urgently needed. Glycogen synthase kinase 3β (GSK-3β) has been suggested to be a key player in the pathophysiology of bipolar disorders. A series of novel GSK-3β

inhibitors having the common *N*-[(1-alkylpiperidin-4-yl)methyl]-1*H*-indazole-3-carboxamide scaffold were prepared taking advantage of an X-ray co-crystal structure of compound **5** with GSK-3β. We probed different substitutions at the indazole 5-position and at the piperidine-nitrogen to obtain potent ATP-competitive GSK-3β inhibitors with good cell activity. Among the compounds assessed in the *in vivo* PK experiments, **14i** showed, after i.p. dosing, encouraging plasma PK profile and brain exposure, as well as efficacy in a mouse model of mania. Compound **14i** was selected for further *in vitro/in vivo* pharmacological evaluation, in order to elucidate the use of ATP-competitive GSK-3β inhibitors as new tools in the development of new treatments for mood disorders.

INTRODUCTION

Bipolar disorders are severe psychiatric conditions characterized by swings between depressive episodes (anhedonia, cognitive disorders, and suicidal thoughts) and periods of elevated mood (hyperarousal, irritability and hallucinations). Bipolar disorder affects about 1% of the world population, with symptoms generally emerging in young adults with comparable prevalence in males and females. According to the current literature lithium remains the best available mood stabilizer, but efficacy is primarily during the manic phase, while additional supportive therapy is required to control the depressive phase.[1] Anticonvulsant agents, such as valproic acid and lamotrigine or atypical antipsychotic drugs such as olanzapine and quetiapine have some efficacy, although limited to particular patient sub-groups. Moreover, current medications have little efficacy in preventing relapse (low remission rate) and have significant side-effects. For these reasons there is a significant medical need to identify more effective and safer drugs, acting on both the acute and global course of the illness.[1]

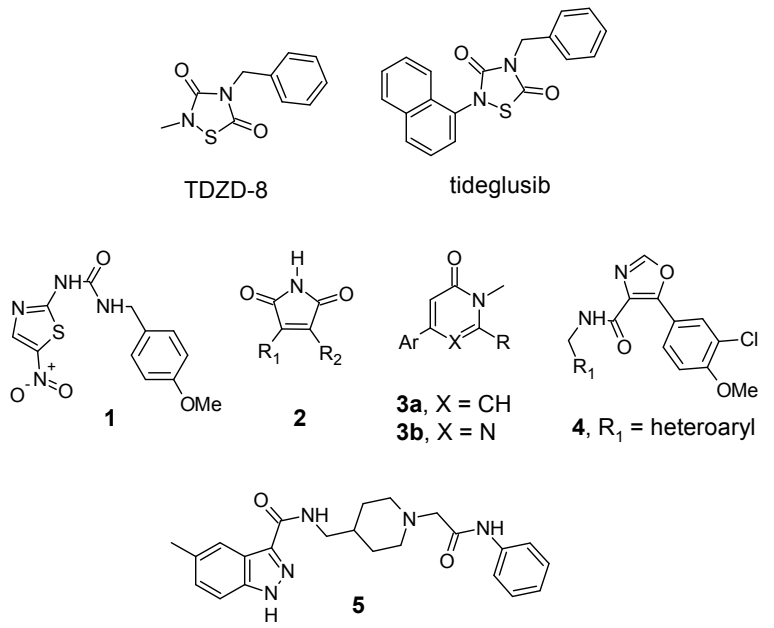
Recent literature has pointed out a significant role for glycogen synthase kinase 3β (GSK-3β) in the pathophysiology of bipolar disorders.[2] Dysregulation of the enzyme has been proposed as a potential driver of the disease and strong support for this has been the discovery of an inherited mutation of the GSK-3 gene which leads to over-activation of the enzyme, causing psychiatric illnesses in human.[3] Moreover both lithium, and to lesser extent valproic acid, are inhibitors of the GSK-3β signaling cascade.[4,5,6]

GSK-3 is a constitutively active multi-functional serine/threonine protein kinase discovered in the late 1970s.[7] In humans, GSK-3 is encoded by two different and independent genes, generating GSK-3α and GSK-3β proteins, with molecular weights of about 51 and 47 kDa, respectively. The two isoforms share nearly identical sequences in their kinase domains (95% homology of the catalytic domain) while differing substantially in sequence elsewhere in the protein.[8] Like other kinases, GSK-3β transfers phosphate groups from ATP to specific substrates, usually proteins. After phosphorylation, the substrate undergoes a functional change, by which kinases can mediate various biological functions. In the central nervous system, GSK-3β is widely expressed and has a fundamental role, in neuronal signaling, regulating the Wnt pathway (by β-catenin deactivation) and tau protein function.[3, 8]

In the last decade, several novel classes of adenosine triphosphate (ATP) competitive and non-ATP competitive GSK-3β inhibitors have been reported.[10] Among the non-ATP competitive inhibitors, 4-benzyl-1,2,4-thiadiazolidine-3,5-diones such as TDZD-8 or tideglusib (Chart 1) showed good kinase inhibition (IC_{50} = 2 μM and 0.06 μM, respectively) and good efficacy in many animal models but failed to exhibit clinical benefit in Alzheimer's disease clinical trial.[11] Reported ATP competitive inhibitors such as N-(1,3-thiazol-2-yl)urea (**1**)[12], 3,4-diaryl-maleimides and derivatives (**2**),[13] 6-heteroaryl pyridones (**3a**) and pyrimidinones (**3b**),[14] 5-aryl-4-carboxamide-1,3-oxazoles (**4**),[15] shared a standard H-bond

pattern interaction with the hinge region of the binding site, involving one hydrogen bond acceptor and one or two hydrogen bond donor sites. Despite good *in vitro* potency and in some cases favorable PK properties, few of these GSK-3β inhibitors have showed efficacy in preclinical *in vivo* models and none of them have progressed to the clinical phase for mood-disorder related indications.[16,17,18]

Chart 1. Structures of GSK-3β inhibitors



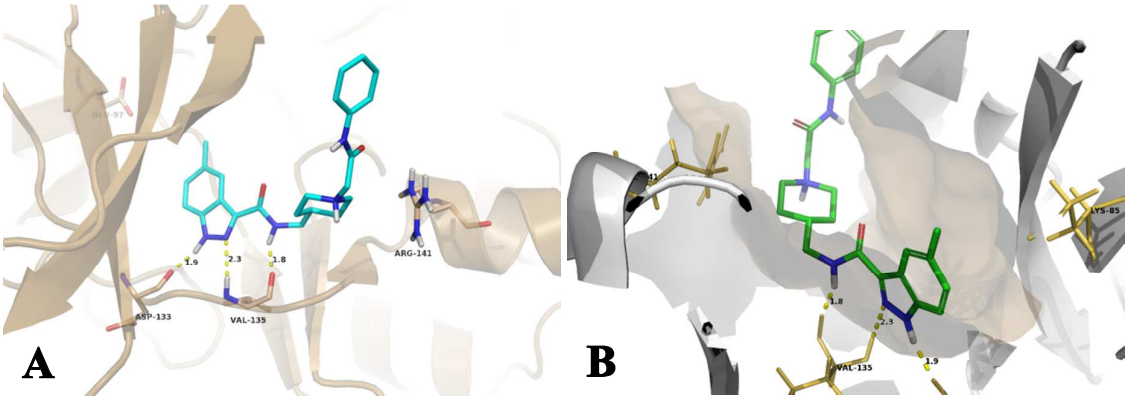
In the present paper, our effort to identify novel GSK-3β inhibitors acting as mood-stabilizers in animal models is described. The chemical program was initiated, following the identification of the 1H-indazole-3-carboxamide hit compound **5** (after a virtual screening campaign described in a separate paper)[19,20]. The x-ray structure of GSK-3β co-crystallized with this ligand, helped us to design novel *N*-[(1-alkylpiperidin-4-yl)methyl]-1*H*-indazole-3-carboxamides as potent GSK-3β enzyme inhibitors. Compounds demonstrated robust activity in cell-based model while selected compounds showed appropriate pharmacokinetic properties for *in vivo* efficacy studies. In the absence of rodent *in vivo* models of bipolar

disorders, the most promising compounds were tested in the low-dose amphetamine hyper-motility test, a well-established preclinical model of mania. Early results of these efficacy experiments are reported here.

RESULTS AND DISCUSSION

Initial Hit Finding Results. Following a structure-based hit discovery program,[19] we identified a series of *N*-[(1-alkylpiperidin-4-yl)methyl]-1*H*-indazole-3-carboxamides showing GSK-3β inhibition with low micromolar IC_{50}'s. One of these preliminary hits (**5**, Chart 1) with IC_{50} = 0.64 μM was successfully co-crystallised with GSK-3β revealing key interaction features between the enzyme and inhibitor. (Figure 1).

Figure 1. X-ray crystal structure of compound **5** in the GSK-3β ATP binding site: (**A**) view of the hinge region interactions, (**B**) view from the inner cavity toward the hinge region and solvent accessible area.



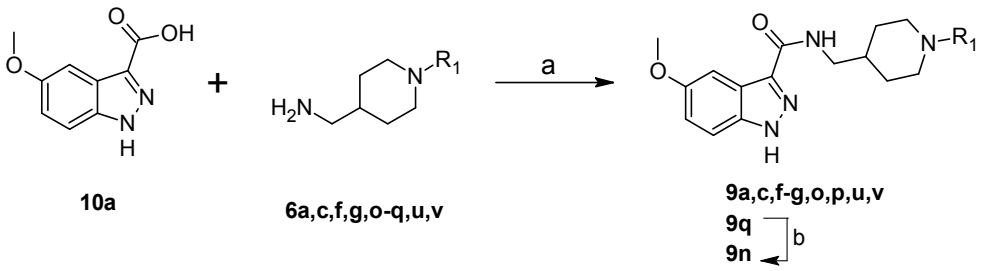
Figures 1A and 1B show the key interactions between compound **5** and amino acid residues of the enzyme ATP binding pocket. The indazole moiety is located at the adenine binding site and its two nitrogen atoms form hydrogen bonds with the backbone carbonyl group of Asp133 and with the backbone NH of Val135 of the hinge region. The backbone carbonyl of Val135 also appears to be involved in a hydrogen bond interaction with the NH of the

carboxamide group of compound **5** while the piperidine nitrogen is oriented toward the guanidine group of Arg141. This configuration orients the terminal phenylacetamide moiety toward the external solvent accessible part of the binding pocket. The apparent lack of tight interactions between protein and ligand in this broad section of the binding site, could be useful for modulating physico-chemical properties of the molecules without significantly influencing the inhibitory potency. Interestingly, the indazole 5-methyl group is directed toward the inner cavity of the ATP binding pocket suggesting opportunities for additional polar interactions with Lys85 and Asp200 or lipophilic interaction with other proximal residues (Figure 1B).

The encouraging initial *in vitro* results together with the knowledge of the protein structure information, obtained from the X-ray analysis of co-crystallized **5** with GSK-3β, supported an extensive hit to lead medicinal chemistry program around the hit series core scaffold. The analysis of the crystal structure together with already available SAR information for a bioisosteric class of GSK-3β inhibitors,[21] suggested focus on two areas of interest: the indazole 5-substituent and piperidine *N*-substitution. Both, as previously indicated, providing opportunities to probe positive interaction with the protein and to modulate physicochemical properties.

Chemistry. The compounds described in this paper were prepared according to the general routes outlined in Schemes 1-4. The 5-methoxyindazol-3-carboxamide derivatives **9a,c,f,g,o-q,u,v** (Table 1) were readily prepared by a direct hydroxybenzotriazole (HOBt) - *N*,*N*'-dicyclohexylcarbodiimide (DCC) mediated carboxylic acid - amine coupling reaction, between 5-methoxy-indazol-3-carboxylic acid **10a** and the corresponding 1-(piperidin-4-yl)methane amines **6** (Scheme 1).

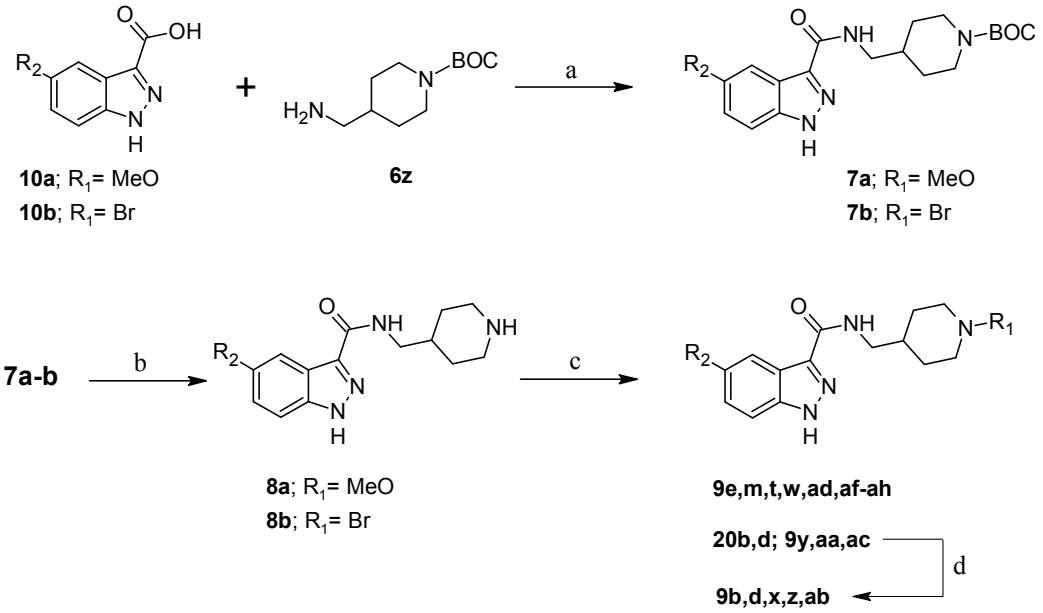
Scheme 1.[a] Synthesis of indazole-3-carboxamide derivatives **9a,c,f,g,n-q,u,v**



[a] Reagents and conditions: a) HOBt, DCC, DMF, r.t., 39–67%; b) H_2, 10% Pd/C, 80 °C, 37%.

The remaining derivatives **9** were synthesized by piperidine *N*-alkylation of the 5-methoxy-1*H*-indazole-3-carboxamide key intermediate **8a** (Scheme 2). For carboxylic acid derivatives **9b**, **9d**, **9x**, **9z** and **9ab** alkaline hydrolysis of the corresponding ester produced the final compounds.

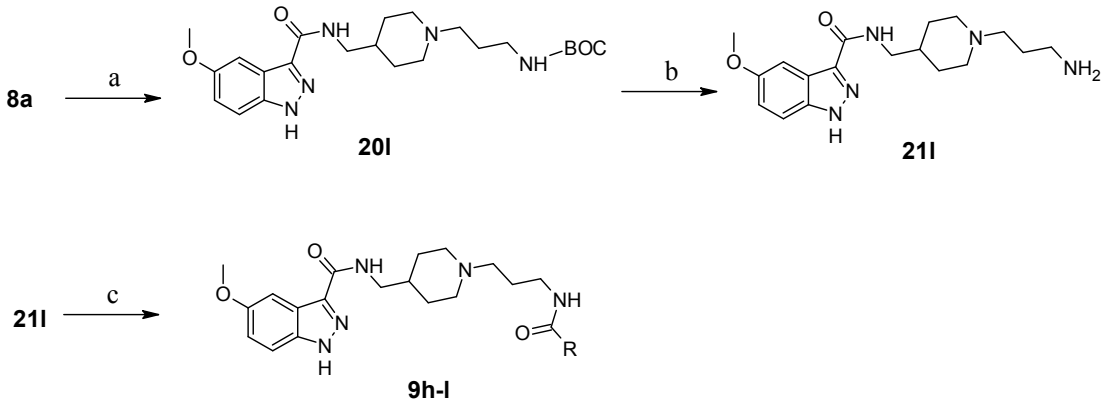
Scheme 2.[a] Synthesis of indazole-3-carboxamide derivatives **9b,d,e,m,t,w-z,aa-ah**



[a] Reagents and conditions: a) HOBt, DCC, DMF, 0 °C, 2h -> r. t., o.n., 87 – 96%; b) 2M HCl-EtOH, MeOH, r.t., 3h, 76-89%; c) R_1-X, K_2CO_3, DMF, 80 °C, 1h, 11-59%; d) acq. NaOH, MeOH, Δ, o.n., 43-87% .

The acyl amides **9h-l** were prepared starting from the intermediate **8a** after N-alkylation with tert-butyl *N*-(3-bromopropyl)carbamate, subsequent de-protection and acylation with the appropriate acyl chloride (Scheme 3).

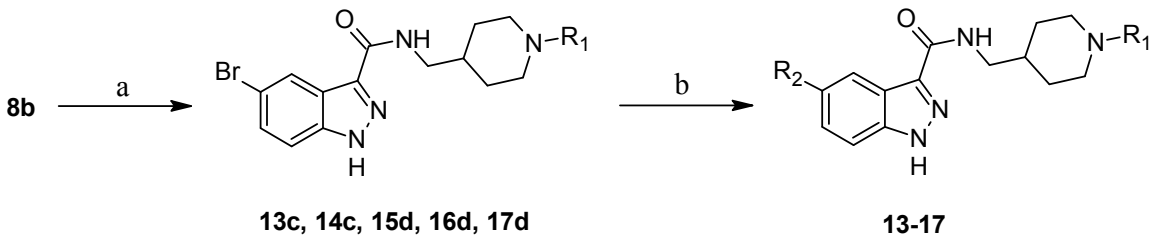
Scheme 3.[a] Synthesis of amide derivatives **9h-l**



[a] Reagents and conditions: a) BOC-NH$(CH_2)_3$Br, TEA, MEK, 80 °C, 1h; b) TFA, DCM, r. t.; o.n., 63%; c) R-COCl, DMSO, DCM, r. t., 2h, 17-69%.

Compounds with different indazole 5-substituents were generally prepared following the route depicted in Scheme 4. The key intermediate 5-bromo-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide **8b** was piperidine *N*-alkylated with the appropriate alkyl halide, to give the 5-bromoindazole-3-carboxamide intermediates **13c**, **14c**, **15d**, **16d** and **17d**, which were then coupled with a range of boronic acids or esters, to afford the target compounds **13-17** (see Table 2 for structures).

Scheme 4.[a] Synthesis of derivatives **13-17**

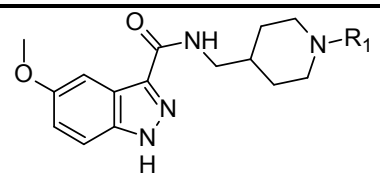


[a] Reagents and conditions: a) R_1-X, K_2CO_3, DMF, 80 °C, 1h, 16-62%; b) $R_2B(OH)_2$ or $R_2B(OR)_2$, Pd(dppf)Cl_2, Cs_2CO_3, dioxane/H_2O (3:1), μ-wave (45'@ T = 160 °C, max power 300 W), 15-45%.

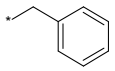
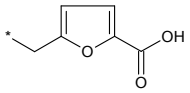
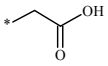
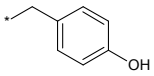
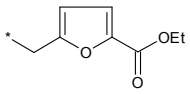
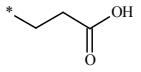
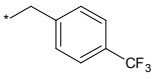
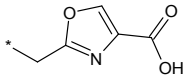
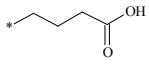
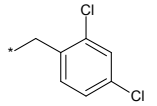
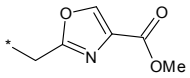
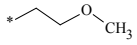
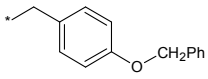
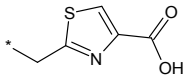
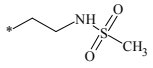
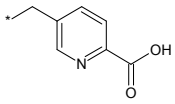
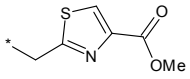
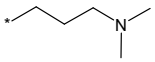
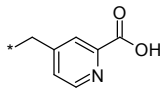
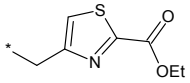
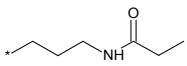
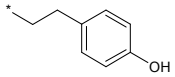
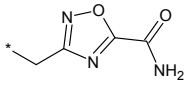
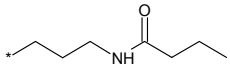
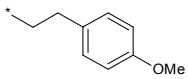
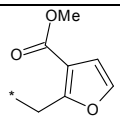
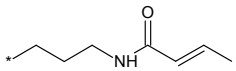
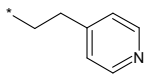
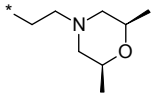
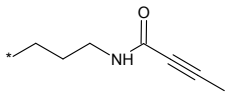
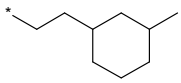
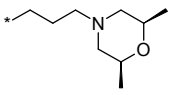
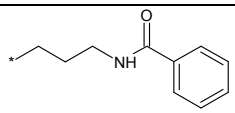
Structure-Enzymatic Activity Relationship Analysis. All the molecules prepared were tested in the GSK-3β ATP-ase enzymatic assay where the human recombinant enzyme GSK-3β was incubated in the presence of compounds or vehicle in a reaction buffer containing ATP plus the un-phosphorylated specific substrate peptide. Substrate phosphorylation was measured and percent inhibition calculated relative to a positive control. The half maximal inhibitory concentration values (IC_{50}) were determined by non-linear regression analysis of the inhibition curves.

Focusing initially on the piperidine substitution of the 5-methoxy-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide derivatives (Table 1), straight chain *N*-alkylcarboxylic acids showed some improvement in activity with an increase in potency of two-fold for the acetyl and propionyl derivatives (**9b** and **9c**) and 5-fold for the longer butyryl derivative **9d**. Substituents bearing a terminal neutral polar group (e.g., methoxyethyl **9e**, methansulfonyl ethyl amine **9f**) did not significantly affect kinase inhibitory activity relative to the parent methyl analogue (**9a**). Some, particularly more lipophilic, alkyl amides such as the derivatives **9k** and **9l** did give sub-micromolar activity.

Table 1. GSK-3β activity of 5-methoxy-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide derivatives



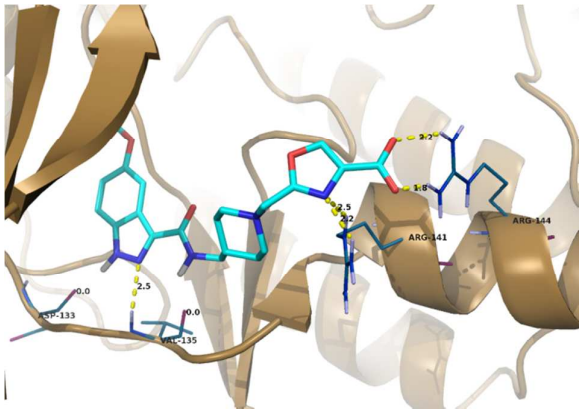
Inhibitory Potencies (IC_{50})[a] vs Human GSK3β								
ID	**R_1**	**IC_{50} (μM)**	**ID**	**R_1**	**IC_{50} (μM)**	**ID**	**R_1**	**IC_{50} (μM)**

9a	Me	1.20	**9m**		0.25	**9x**		0.40
9b		0.67	**9n**		0.40	**9y**		0.33
9c		0.69	**9o**		0.64	**9z**		0.07
9d		0.23	**9p**		0.31	**9aa**		0.21
9e		0.87	**9q**		0.35	**9ab**		0.05
9f		1.00	**9r**[b]		0.32	**9ac**		0.13
9g		1.70	**9s**		0.27	**9ad**		0.95
9h		1.13	**9t**		0.35	**9ae**		0.49
9i		0.75	**9u**		0.56	**9af**		0.23
9j		1.51	**9v**		0.51	**9ag**		1.40
9k		0.53	**9w**		0.36	**9ah**		2.10
9l		0.58						

[a] IC_{50} values were calculated from data points obtained as averages of duplicate wells. Table with complete list of IC_{50} and 95% confidence intervals is showed in supplementary information file (Table S. I. 1). [b] Sodium salt.

Alkylaryl or alkylpyridyl analogues did show some improvements in the inhibitory potency. For example, benzyl derivatives (**9m-q**), phenyl-ethyl derivatives (**9t-u**) and encouragingly the more polar pyridine-methyl derivatives (**9r-s**) and the pyridine-ethyl derivative (**9v**), all have IC_{50} values between 0.64 μM and 0.25 μM, despite the different nature of the inserted substituents. Esters and carboxylic acid substituted methylene 5-ring heteroaryl analogues showed significantly improved target affinity, with IC_{50}'s of 70 and 50 nM respectively with the 2-methylen-1,3-oxazole-4-carboxylic acid **9z** and its 1,3-thiazole counterpart **9ab**. All these results are in agreement with our structural hypothesis of a section of the binding pocket without stringent electronic and steric requirements that can tolerate a broad series of terminal groups, while also identifying specific polar interactions with Arg141 or Arg144 as proposed for compound **9z** depicted in Figure 2.

Figure 2. Docking study of compound **9z** in the GSK-3β ATP site

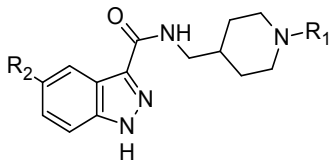


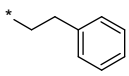
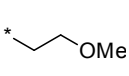
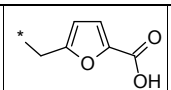
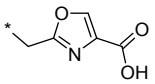
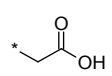
Interesting is the loss of potency observed for the 4-substituted 1,3-thiazole-2-carboxylic ethyl ester **9ad**, in comparison to its methyl ester isomer **9ac**, indicating a preference for the position of the hydrogen-bond acceptor heteroatoms. Alkylmorpholine substitution, such as in **9ag**, was poorly tolerated.

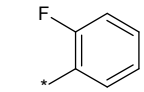
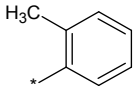
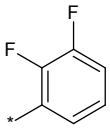
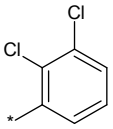
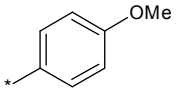
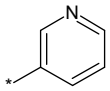
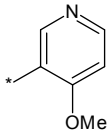
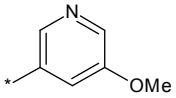
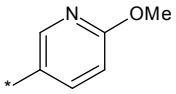
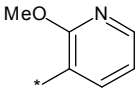
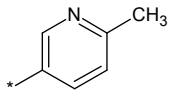
Following the investigation of the effect of substituent's extending from the piperidine ring we turned our attention to the 5-position of the indazole, replacing the initial methoxy group with bromine, alkyl, aryl or heteroaryl groups, with the aim of identifying additional H-bond or van der Waals interactions. The effect of these changes on the GSK-3β activity was studied using a range of piperidine N-substituent's to cover potential mismatches in the structure-activity relationship (SAR) between the two positions (Table 2).

Based on the results obtained with the previous 5-methoxy-indazole-3-carboxamide derivatives, five different piperidine-nitrogen substituent's (selected on the basis of potency and physicochemical properties) were selected for this SAR analysis: phenethyl group (**13**), methoxyethyl chain (**14**), 5-methylen-furan-2-carboxylic acid (**15**), 2-methylen-1,3-oxazole-4-carboxylic acid (**16**) and acetic acid (**17**).

Table 2. Substituent combinations used to study the GSK-3β inhibitory activity of an indazole 5-substitieunt - R_2



Inhibitory Potencies for Human GSK-3β; IC_{50}[a], nM (**compound ID**)					
R_2	**R_1**				
					
MeO		870 (**9e**)	400 (**9x**)	70 (**9z**)	670 (**9b**)
MeO			330 (**9y**)[c]	210 (**9aa**)[b]	
Br	n.d. (**13c**)	n.d. (**14c**)	210 (**15c**)[d]		
Br			260 (**15d**)[c]	360 (**16d**)[b]	530 (**17d**)[c]
Et					541 (**17e**)
Ph		200 (**14f**)			

	112 (**13g**)	72 (**14g**)	17 (**15g**)		45 (**17g**)
		580 (**14h**)			
	67 (**13i**)	18 (**14i**)	12 (**15i**)	6.0 (**16i**)	27 (**17i**)
					40 (**17j**)
		560 (**14k**)	300 (**15k**)		
	39 (**13l**)	31 (**14l**)			
	20 (**13m**)	45 (**14m**)	9.6 (**15m**)		
		26 (**14n**)			18 (**17n**)
		53 (**14o**)	21 (**15o**)		
		1400 (**14p**)	360 (**15p**)		
	40 (**13q**)	64 (**14q**)			

[a] IC_{50} values were calculated from data points obtained as averages of duplicate wells. Table with complete list of IC_{50} and 95% confidence intervals is showed in supplementary information file (Table S. I. 1). [b] Methyl ester; [c] ethylester; [d] sodium salt. Blank cells indicate compounds not prepared.

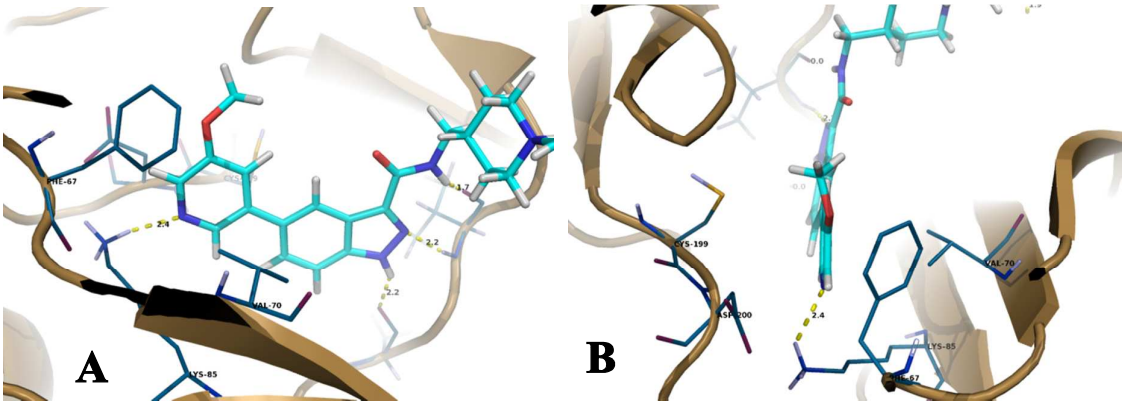
A marked increase in inhibitory potency was obtained with indazole 5-aryl or 5-heteroaryl substituent's compared with 5-methoxy or 5-bromo substituted analogues. For instance the 5-phenyl derivative **14f** in comparison with its 5-methoxy counterpart **9e** showed a 4-fold

increase in activity (IC_{50} of 200 nM and 870 nM respectively). A further 3-fold increase in potency was noted when switching to the 2-fluorophenyl derivative **14g** (IC_{50} = 72 nM) with further improvements in activity with the 2,3-difluorophenyl derivative **14i** (IC_{50}=18 nM).

Replacing the two fluorine atoms of **17i** with two chlorine atoms (**17j**) gave comparable potency (IC_{50} of 27 and 40 nM respectively) but no advantage to compensate for the increased lipophilicity. In contrast 2-methyl derivative **14h** or 4-methoxy phenyl derivatives **14k** and **15k**, showed a marked loss in potency being comparable to the corresponding 5-methoxy indazole derivatives.

The introduction of a (pyridine-3-yl) indazole 5-substituent gave potent GSK-3β inhibitors **13l** and **14l**, with activity comparable to the 2,3-difluorophenyl analogues **13i** and **14i**. The incorporation of a methoxy group at the 4-, 5- or 6-position of the pyridine ring generally was well tolerated providing two of the most potent kinase inhibitors (**15m** and **17n**). Interestingly, the methoxypyridine **14o** is ten-fold more potent than the corresponding 4-methoxyphenyl analogue **14k**, suggesting a direct effect of the pyridine nitrogen.

Figure 3. Docking study of compound **14n** in the GSK-3β ATP site: (A) view of the hinge region interactions, (B) view from the inner cavity toward the hinge region and solvent accessible area



The significant increase in potency produced by 5-(hetero)aryl indazole derivatives, could be rationalized through docking studies. Compound **14n**, for example, (Figure 3A and 3B), with its 5-methoxy-3-pyridine moiety, is able to form a new H-bond interaction with Lys85 (Figure 3A) and hydrophobic interactions with the residues lining the deep part of the binding pocket (e.g., Cys199, Val70, Phe67) (Figure 3B). The trends in activity seen with the indazole 5-substituent were independent of the indazole 3-substituent, consistent with conserved indazole-hinge region interactions regardless of the piperidine substituent proximal to the solvent accesible area of the binding site.

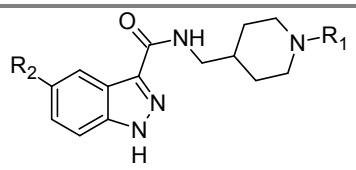
Finally, 2-methoxy substitution (**14p** and **15p**) had at least a ten-fold drop in potency compared with the other methoxypyridyl regioisomers (also **14m**) suggesting a steric clash between the methoxy group and the nearby amino acid residues. No significant difference in potency was found replacing the 6-methoxy group of **14o** with a methyl group (**14q**).

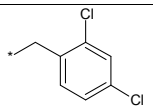
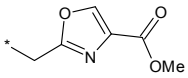
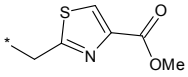
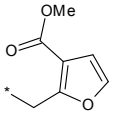
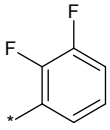
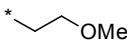
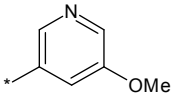
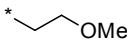
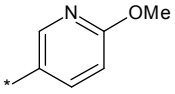
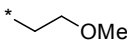
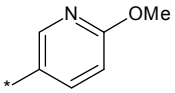
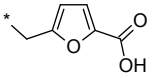
Mode of action. Two compounds (**14i** and **9c**) were selected to characterize in more detail their enzymatic mode of action using microfluidic technology. Their mechanism of inhibition was determined by performing phosphorylation assays while varying inhibitor and substrate concentrations around their IC_{50} and Michaelis constant (K_M), respectively. Results were fitted to 3 equations for competitive, noncompetitive, and uncompetitive mechanisms. The inhibition for both compounds was found to be reversible, linear and ATP competitive (**14i** Ki=15nM; **9c** Ki=600nM), consistent with the ATP site binding mode used for *in silico* screening and confirmed with crystallographic studies with compound **5** .

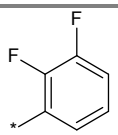
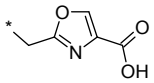
Functional Tau phosphorylation assay. A series of the most potent GSK-3β inhibitors were also evaluated, in duplicate, for their activity in a whole cell Tau phosphorylation assay using recombinant CHO pTau expressing cells. The cells were incubated with the selected compounds for 2 hours at 37 °C, 5% CO_2 lysed and processed for the detection of

phosphorylated tau protein (Luminex). The comparison between IC_{50}'s obtained in the enzyme inhibition and cell-based assays is shown in Table 3.

Table 3. Cellular Tau phosphorylation inhibition results



Compd	R_2	R_1	GSK-3β IC_{50} (μM)	CHO pTau IC_{50} [a] (μM)	cLogP[b]
9p	MeO-		0.310	1.39	4.3
9aa	MeO-		0.210	5.17	1.7
9ac	MeO-		0.130	2.13	2.0
9af	MeO-		0.230	0.82	2.4
14i			0.018	0.24	3.3
14n			0.026	0.80	2.0
14o			0.053	0.79	2.6
15o			0.021	70.8	0.34

16i			0.006	>100	0.43

[a] IC_{50} values were calculated from data points obtained as averages of duplicate wells. Table with complete list of IC_{50} and 95% confidence intervals is showed in supplementary information file (Table S. I. 3). [b] cLogP calculated by ACD/Percepta.

Selected compounds showed potent inhibitory effect in the Tau protein phosphorylation cellular assay with IC_{50} in the low micromolar range reflecting a loss in potency of 10 – 40 fold between the isolated enzyme and whole cell pTau assays. Compounds **15o** and **16i** showed a major loss of activity probably due to their zwitterionic nature (both cLogP < 0.5; cLogD7.4 respectively of -0.08 and -0.01) that limits their membrane permeability.

Pharmacokinetic characterization. Compounds **9ac**, **9af**, **14i** and **14n** were assessed for mouse plasma stability at the concentration of 5 μM. We measured the time related percentage of remaining compound and noticed that ester **9ac** was rapidly hydrolyzed to the corresponding carboxylic acid, with elimination half-life less than 10 minutes. Other compounds including the vinylogous carbonate **9af** were stable, with percent of remaining compounds ≥ 80% after 2h.

On the basis of their plasma stability compounds **9af**, **14i** and **14n** were progressed to *in vivo* PK studies. Plasma and brain levels were evaluated after intraperitoneal (i.p.) administration of 10 mg/kg of compounds. The PK parameters obtained for the selected compounds are shown in table 4.

Table 4. In vivo PK parameters in mice (10 mg/kg, ip)

Entry	Plasma						Brain
	$AUC_{0,inf}$ (μg*min/ml)	**Cmax** (μg/ml)	**T_{max}** (min)	**$t_{1/2}$** (min)	**Vd** (L/kg)	**Cl** (L/min/kg)	**Cmax** (ng/mg prot.)
9af	21±4	0.7±0.1	15	68±13	47±9	0.5±0.1	1.5±0.1
14i	370±96	2.1±0.7	15	203±53	6±2	0.02±0.01	5.5±0.4

14n	34±11	1.3±0.2	15	48±15	20±6	0.3±0.1	0.7±0.5

Among the tested compounds, **14i** displayed the highest exposures, both in plasma and in brain tissue, reaching the maximum concentration in plasma in 15 minutes with a favorable elimination half-life ($t_{1/2}$) of 203 min and moderate clearance (Cl) of 22 ml/min/kg. Consistent with its good PK parameters, **14i** was selected for evaluation in *in vivo* efficacy model of mood disorders.

Amphetamine model. This model is believed to mimic the hyperactivity component of bipolar disorders. Both reference GSK-3β inhibitors tested, i.e. LiCl and TDZD-8 given intraperitonelly, were very effective in blocking amphetamine hyperactivity with LiCl being slightly more active than TDZD-8 (Figure 4A and 4B). At the highest dose tested both compounds also showed a trend to inhibit spontaneous locomotor activity, suggesting the appearance of some sedative effects. Compound **14i** was tested in the range of doses between 1 and 30 mg/kg and a dose-dependent inhibition of amphetamine induced hypermotility was observed (Figure 4C). The first significant dose was 1 mg/kg and the effect was maximal at 3 and 10 mg/kg. The efficacy of **14i** at 10 mg/kg was similar to lithium at the highest non sedative dose and higher than TDZD-8 (compare Figures 4A, B and C). Also **14i**, when dosed at 30 mg/kg, showed some sedative activity on spontaneous locomotor activity.

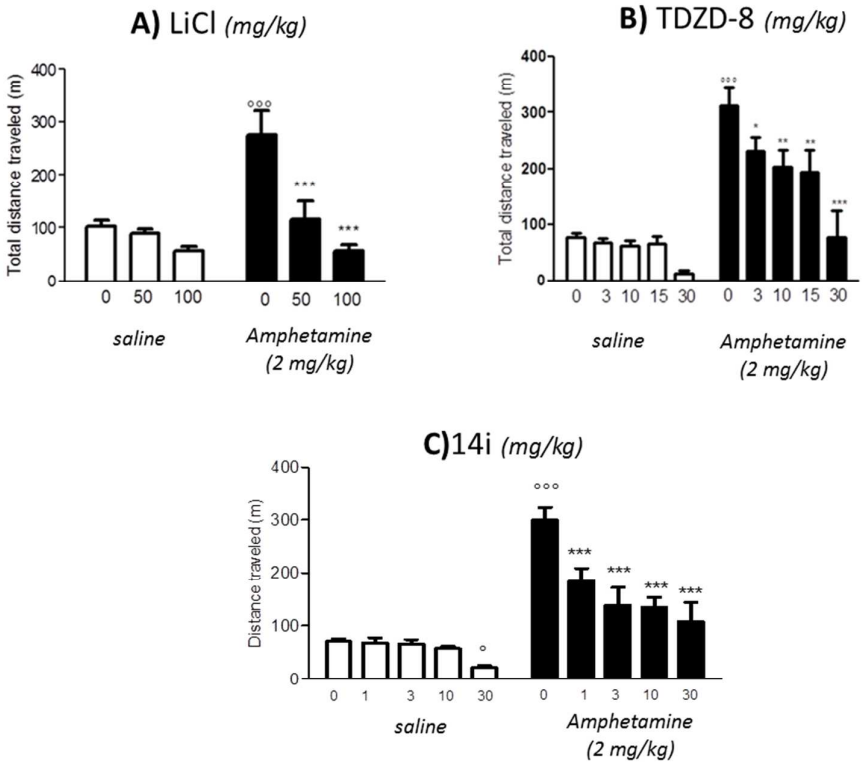


Figure 4. Effect of Lithium Chloride (A), TDZD-8 (B) and **14i** (C) on spontaneous motility (saline) and on amphetamine hyperactivity (amphetamine). Each bar is the average ± S.E.M. (n = 8 each group). °°° $p<0.001$ vs saline; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs amphetamine. 2 way ANOVA, Bonferroni post test.

Kinases selectivity assays. The kinase profile of **14i** was firstly assessed in a 216 kinase assay platform performed by CEREP, at 10 μM in duplicate. Briefly, human recombinant kinases were incubated in the presence of specific peptide substrates plus ATP for different times at 22 °C. The results are expressed as a percent of inhibition of control specific activity obtained in the presence of the test compounds (See supplementary information Table S. I. 3, for the complete list of results). Only for 34 kinases **14i** showed more than 50% inhibition at the screening concentration. For these selected kinases the IC_{50} determination was performed (See supplementary information Table S. I. 4, for the complete list of results).

These experiments showed that compound **14i** has a IC_{50} selectivity ratio versus GSK-3β, below 30 fold, only for seven additional human recombinant kinases: DYRK1a and DYRK2, CLK1, LynB Kinase, ERK5, GRK2 (ADRBK1) and GSK-3α (the isoform of GSK-3β). respectively. (Table 5).

Table 5. GSK3 kinase selectivity of compound 14i over many other kinases

Assay	**IC_{50}** *(μM)*	**Selectivity ratio** *(vs GSK-3β)*
h-GSK-3β	0.018	1
h-DYRK1a	0.040	2.2
h-GSK-3α	0.040	2.2
h-CLK1	0.071	3.9
h-LynB Kinase	0.170	9.4
h-ERK5	0.220	12.2
h-DYRK2 kinase	0.530	29.4
h-GRK2 (ADRBK1)	0.530	29.4

Based on available literature, none of the above seven kinases have been specifically linked to mood disorders and they are not expected to affect **14i** efficacy in major depression or bipolar disorder. In fact, DYRK1A and DYRK2, have been linked to cell proliferation and neurocognitive deficits associated to Down syndrome[23,24] while ERK5 has been also linked to cell proliferation, differentiation, and death.[29] CLK1 has been involved in neurodegeneration and Parkinsonism.[25,26] Lyn kinase participates to the regulation of myeloid lineage proliferation,[27,28] and finally, GRK2, a member of the G protein-coupled receptor kinases (GRKs) family, participates, together with arrestins, to the regulation of G protein-coupled receptors (GPCR).[30]

CONCLUSION

The hit to lead program described in this work for the novel class of 5-substituted-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamides identified a series of novel and potent GSK-3β inhibitors. Key interventions leading to an increase GSK-3β inhibition potency were the identification of additional interactions with the target from both the indazole 3-position (e.g. Arg141) and from the indazole 5-position (probing to Lys85) while improving hydrophobic interactions. Activity against the isolated enzyme translated well to cells, which is indicative of good permeability. Using two compounds (**9c**, **14i**), we demonstrated that their mechanism of action was ATP-competitive and reversible, consistent with the binding hypothesis studied *in silico* and results of X-ray co-crystallization studies. Some indazole-3-carboxamide derivatives showed the necessary cellular potency and plasma stability to be progressed to an *in vivo* assessment. Compound **14i**, showing good preliminary pharmacokinetic profiles after i.p. administration was tested in an *in vivo* efficacy animal model: the low dose amphetamine test, believed to mimic mania, a key symptom of mood disorders. In this model, the selected compound showed excellent efficacy and maximal effect (i.e. complete reversal of amphetamine hypermotility). Some sedative effects, at the highest doses tested, were noticed with the positive controls (TDZD-8 and LiCl) and with **14i**. As known selectivity versus other kinases is a critical factor for the potential clinic uses of kinase inhibitors. To address this point compound **14i** was tested on a panel of 216 kinases and we found potency ratio below 30 fold only on six assays (in addition to the expected isoform GSK-3α) indicating an excellent degree of kinase selectivity.

In summary, this paper describes the synthesis and the initial pharmacological characterization of a novel class of GSK-3β inhibitors. The encouraging results of our work, suggest that the present class of selective GSK-3β inhibitor may lead to molecules providing a significant improvement over existing mood disorders therapy.

MATERIALS AND METHODS.

Chemistry. Reagents were purchased from Sigma-Aldrich and were used as received. Reaction progress was monitored by TLC or UPLC-qtof chromatography. For TLC was used Merck silica gel 60 F254 (0.04-0.063 mm) with detection by UV (214 or 254 nm). Merck silica gel 60 or aluminum oxide 90 (active neutral) were used for column chromatography. Melting points (uncorrected) were determined in open Pyrex capillary tubes using a Buchi 510 melting point apparatus. Final compound purity, always >95%, was determined both by high pressure liquid chromatography (HPLC) and Ultra Performance Liquid Chromatography (UPLC), using the area percentage of all peaks detected. HPLC analysis were carried out with a pump/autosampler Waters (2695 – Alliance model), a UV photo diode array detector Waters (2996 model) and a Waters system data management (Empower 2). The column used was generally Suplex pkb-100 (250x4.6 mm, 5μm). UPLC/QToF Cromatography (exact mass data) were obtained by means of a SYNAPT MS - ACQUITY UPLC system, Waters. The system was operated in positive ion mode in the "V-Optics" configuration. Leucine-enkephalin (200 pg/μl) was employed as the lock mass in order to provide authenticated exact mass measurement in MS and MS/MS modes within 5 ppm RMS mass accuracy. The column was an Acquity BEH C18 (2.1x50 mm, 1.7μm). Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) were obtained using a Bruker Avance system, operating at 300 MHz. All resonance bands were referenced to tetramethylsilane (internal standard). For ^{1}H-NMR spectroscopy: (s) = singlet; (d) = doublet; (t) = triplet; (br) = broad; (dd) = double doublet; (dt) = double triplet; (ddd) = double double doublet ;(dtd) = double triple doublet; (m) = multiplet; J = coupling constant; δ = chemical shift (in ppm). Preparative HPLC/MS system consisted of a Waters 2767 Sample manager, a Waters 2478 dual λ absorbance detector and a Waters Micromass ZQ single quadrupole mass spectrometer with an electrospray ionization (ESI) source. The column used was a X-Bridge Prep C18 5 μm with 19x10mm (Waters) pre-column. Fraction collection was available from the system software

MassLynx™ v. 4.1. Detection wavelength was set to 230 nm and temperature to 25°C. Elemental Analysis was conducted by means of a CHNS-O EA1108 elemental analyzer, Carlo Erba Instruments, and the results were within ±0.3% of the theoretical values, unless otherwise noted. Compounds were purified with one of the following techniques: flash chromatography on silica gel (Grace Reveleris flash chromatography system with 40 μM silica cartridge (generally flow was 60 ml/min); with an appropriate gradient of mixtures of $CHCl_3$ and CH_3OH as eluents); preparative HPLC/MS system (sample was dissolved (50 mg/ml) in DMSO/CH_3CN in 1:1 ratio; using an appropriate gradient of the two phases CH_3CN + 0.1% formic acid and H_2O + 0.1% formic acid; flow = 40 ml/min). The starting material 1-(1-methylpiperidin-4-yl)methanamine (**6a**), ethyl 3-[4-(aminomethyl)piperidin-1-yl]propanoate (**6c**), *N*-{2-[4-(aminomethyl)piperidin-1-yl]ethyl}methanesulfonamide (**6f**), 3-[4-(aminomethyl)piperidin-1-yl]-*N*,*N*-dimethylpropan-1-amine (**6g**), 1-{1-[2-(pyridin-4-yl)ethyl]piperidin-4-yl}methanamine (**6v**), tert-butyl 4-(aminomethyl)piperidine-1-carboxylate (**6z**) were purchased from commercial sources.

General method for the preparation of intermediates 7, 9a,c,f-g,n-q,u-v

1-Hydroxybenzotriazole (HOBt, 7.40 g, 54.8 mmoles) and *N*,*N*'-dicyclohexylcarbodiimide (DCC, 11 g, 53.3 mmoles) were added to a solution of an appropriately substituted 1*H*-indazole-3-carboxylic acid **10** (49.8 mmoles) in DMF (200 ml) at 0°C. After 1 hour, a solution of an appropriate 1-substituted [piperidin-4-yl]methanamine (**6a**,**c**,**f-g**,**o-q**,**u-z**) (58.1 mmoles) in DMF (100 ml) was added at the same temperature. The mixture was stirred at 0 °C for 2 hours then it was left to reach room temperature overnight. The mixture was diluted with AcOEt then the solid was removed by filtration. The solution was extracted three times with hydrochloric acid (HCl) 2N. The pH of the acid phase was increased (about 13) with 5N NaOH and solution was extracted three times with dichloromethane (DCM). The organic

phase was dried with anhydrous Na_2SO_4. The solution was filtered, evaporated under reduced pressure and the residue was adequately purified.

5-Methoxy-*N*-[(1-methylpiperidin-4-yl)methyl]-1*H*-indazole-3-carboxamide (9a) hydrochloride. (61%) ^{1}H NMR (300MHz, DMSO-d_6) δ = 13.55 (s, 1H), 10.27 (br. s., 1H), 8.47 (t, *J*=5.8 Hz, 1H), 7.56 (d, *J*=2.4 Hz, 1H), 7.53 (dd, *J*=0.5, 9.0 Hz, 1H), 7.06 (dd, *J*=2.5, 9.1 Hz, 1H), 3.81 (s, 3H), 3.38 (d, *J*=11.0 Hz, 2H), 3.32 (s, 2H), 2.88 (t, *J*=11.5 Hz, 2H), 2.69 (s, 3H), 2.18 - 1.72 (m, 3H), 1.70 - 1.26 (m, 2H). [M+H$^+$] exact mass found 303.1816 for $C_{16}H_{23}N_4O_2$.

[4-({[(5-Methoxy-1*H*-indazol-3-yl)-carbonyl]amino}methyl)piperidin-1-yl]propionic acid (9c) hydrochloride. Compound **9c** was obtained by hydrolysis of the crude ethyl [4-({[(5-methoxy-1*H*-indazol-3-yl)-carbonyl]amino}methyl)piperidin-1-yl]propionate during the reaction alkalyne work-up. (42%) ^{1}H-NMR spectra of the HCl salt. ^{1}H NMR (300MHz, DMSO-d_6) δ = 8.24 (t, *J*=6.0 Hz, 1H), 7.55 (s, 1H), 7.53 (dd, *J*=0.5, 7.1 Hz, 1H), 7.03 (dd, *J*=2.6, 8.7 Hz, 1H), 3.81 (s, 3H), 3.18 (t, *J*=6.3 Hz, 2H), 2.83 (d, *J*=11.3 Hz, 2H), 2.49 – 2.43 (m, 2H), 2.17 – 2.05 (m, 2H), 1.89 (d, *J*=12.6 Hz, 2H), 1.76 – 1.46 (m, 4H), 1.30 – 1.10 (m, 3H). [M+H$^+$] exact mass found 361.1870 for C18H25N4O4.

5-Methoxy-*N*-[(1-{2-[(methylsulfonyl)amino]ethyl}piperidin-4-yl)methyl]-1*H*-indazole-3-carboxamide (9f). (46%) ^{1}H NMR (300MHz, DMSO-d_6) δ = 13.43 (br. s., 1H), 8.27 (t, *J*=6.1 Hz, 1H), 7.56 (d, *J*=2.0 Hz, 1H), 7.51 (dd, *J*=0.5, 9.0 Hz, 1H), 7.06 (dd, *J*=2.5, 9.1 Hz, 1H), 6.81 (t, *J*=6.2 Hz, 1H), 3.81 (s, 3H), 3.19 (t, *J*=6.2 Hz, 2H), 3.04 (q, *J*=5.1 Hz, 2H), 2.93 (s, 3H), 2.85 (d, *J*=11.3 Hz, 2H), 2.38 (t, *J*=6.8 Hz, 2H), 1.91 (t, *J*=10.6 Hz, 2H), 1.72 – 1.45 (m, 3H), 1.34 – 1.04 (m, 2H). [M+H$^+$] exact mass found 410.1856 for $C_{18}H_{28}N_5O_4S$.

***N*-({1-[3-(dimethylamino)propyl]piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9g) di-hydrochloride.** (67%) ^{1}H NMR (300MHz, DMSO-d_6) δ = 8.62 (t, *J* =

5.9 Hz, 1 H), 8.0 (br. s., 5 H), 7.56 (d, *J* = 2.6 Hz, 1 H), 7.49 (d, *J* = 8.9 Hz, 1 H), 7.06 (dd, *J* = 2.4, 9.1 Hz, 1 H), 3.80 (s, 3 H), 3.55 - 3.34 (m, 4 H), 3.16 - 3.02 (m, 4 H), 2.75 (s, 6 H), 2.15 (quin, *J* = 7.5 Hz, 2 H), 2.04 - 1.81 (m, 3 H), 1.61 (dtd, *J* = 4.3, 12.2, 12.4 Hz, 2 H). [M+H$^+$] exact mass found 374.2550 for $C_{20}H_{32}N_5O_2$.

5-Methoxy-*N*-({1-[4-(trifluoromethyl)benzyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide (9o). (53%) ^{1}H NMR (300MHz, DMSO-d$_6$) δ = 13.40 (s, 1H), 8.27 (t, *J* = 6.10 Hz, 1H), 7.67 (d, *J* = 8.01 Hz, 2H), 7.47-7.57 (m, 4H), 7.05 (dd, *J* = 2.44, 9.06 Hz, 1H), 3.80 (s, 3H), 3.53 (s, 2H), 3.20 (t, *J* = 6.27 Hz, 2H), 2.78 (d, *J* = 11.50 Hz, 2H), 1.94 (t, *J* = 10.80 Hz, 2H), 1.47-1.77 (m, 3H), 1.12-1.36 (m, 2H). HRMS, Q-TOF found for $C_{23}H_{26}F_3N_4O_2$: 447.2003.

***N*-{[1-(2,4-Dichlorobenzyl)piperidin-4-yl]methyl}-5-methoxy-1*H*-indazole-3-carboxamide (9p).** (Yield 68%) ^{1}H NMR (DMSO-d$_6$) δ 13.38 (br. s., 1H), 8.28 (t, *J* = 6.22 Hz, 1H), 7.56 (t, *J* = 2.38 Hz, 2H), 7.52 (d, *J* = 2.56 Hz, 1H), 7.50 (d, *J* = 1.83 Hz, 1H), 7.41 (dd, *J* = 2.20, 8.20 Hz, 1H), 7.06 (dd, *J* = 2.56 and 9.15 Hz, 1H), 3.81 (s, 3H), 3.51 (s, 2H), 3.21 (t, *J* = 6.22 Hz, 2H), 2.80 (d, *J* = 11.34 Hz, 2H), 2.01 (t, *J* = 10.79 Hz, 2H), 1.48-1.78 (m, 3H), 1.07-1.35 ppm (m, 2H). HRMS, Q-TOF found $C_{22}H_{25}Cl_2N_4O_2$: 447.1357.

***N*-({1-[4-(Benzyloxy)benzyl]piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9q).** (Yield 77%) ^{1}H NMR (DMSO-d$_6$) δ 13.44 (br. s., 1H), 8.25 (t, *J* = 6.22 Hz, 1H), 7.55 (d, *J* = 1.83 Hz, 1H), 7.50 (d, *J* = 9.15 Hz, 1H), 7.28-7.46 (m, 5H), 7.14-7.21 (m, 2H), 7.04 (dd, *J* = 2.38 and 8.96 Hz, 1H), 6.88-6.98 (m, 2H), 5.07 (s, 2H), 3.79 (s, 3H), 3.35 (s, 2H), 3.18 (t, *J* = 6.22 Hz, 2H), 2.77 (d, *J* = 11.34 Hz, 2H), 1.85 (t, *J* = 10.79 Hz, 2H), 1.45-1.71 (m, 3H), 1.00-1.32 ppm (m, 2H). HRMS, Q-TOF found for $C_{29}H_{33}N_4O_3$: 485.2548.

***N*-{[1-(4-Hydroxybenzyl)piperidin-4-yl]methyl}-5-methoxy-1*H*-indazole-3-carboxamide (9n).** Compound **9q** (0.6 mmol) was dissolved in THF and hydrogenated in a micro reactor continuous flow system (H-Cube) using CartCart Pd/C 10% as cartridge. Key

parameters of H-Cube were set as follows: temperature 80 °C; pressure 1 bar; flow 1 ml/minute. The solvent was removed by evaporating under reduced pressure, and the compound **9n** was purified by preparative HPLC-MS. (Yield 47%) ^{1}H NMR (DMSO-d_6) δ 13.50 (s, 1H), 9.70 (br. s., 1H), 8.35 (d, *J* = 17.17 Hz, 1H), 7.55 (d, *J* = 2.31 Hz, 1H), 7.51 (d, *J* = 8.92 Hz, 1H), 7.27 (br. s., 2H), 7.05 (dd, *J* = 2.31 and 8.92 Hz, 1H), 6.78 (d, *J* = 7.60 Hz, 2H), 3.80 (s, 3H), 2.60-3.59 (m, 8H), 0.99-2.15 ppm (m, 5H). HRMS, Q-TOF found for $C_{22}H_{27}N_4O_3$: 395.2083.

5-Methoxy-*N*-({1-[2-(4-methoxyphenyl)ethyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide (9u). (Yield 55%) ^{1}H NMR (DMSO-d_6) δ 13.62 (br. s., 1H), 8.24 (t, *J* = 6.06 Hz, 1H), 7.55 (d, *J* = 2.42 Hz, 1H), 7.52 (dd, *J* = 0.61 and 8.88 Hz, 1H), 7.07 – 7.16 (m, 2H), 7.03 (dd, *J* = 2.42 and 8.88 Hz, 1H), 6.75 – 6.87 (m, 2H), 3.80 (s, 3H), 3.71 (s, 3H), 3.19 (t, *J* = 6.26 Hz, 2H), 2.90 (d, *J* = 11.10 Hz, 2H), 2.57 – 2.74 (m, 2H), 2.34 – 2.47 (m, 2H), 1.80 – 1.98 (m, 2H), 1.46 – 1.76 (m, 3H), 1.08 – 1.30 ppm (m, 2H). HRMS, Q-TOF found for $C_{24}H_{31}N_4O_3$: 423.2402.

5-Methoxy-*N*-({1-[2-(pyridine-4-yl)ethyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide (9v). (Yield 39%) ^{1}H NMR (DMSO-d_6) δ 13.43 (br. s., 1H), 8.51 - 8.36 (m, 2H), 8.26 (t, *J* = 6.2 Hz, 1H), 7.56 (d, *J* = 1.8 Hz, 1H), 7.51 (d, *J* = 9.1 Hz, 1H), 7.29 - 7.21 (m, 2H), 7.06 (dd, *J* = 2.6 and 9.1 Hz, 1H), 3.81 (s, 3H), 3.19 (t, *J* = 6.4 Hz, 2H), 2.90 (d, *J* = 11.3 Hz, 2H), 2.80 - 2.67 (m, 2H), 2.6 – 2.4 (m, 2H), 2.02 - 1.82 (m, 2H), 1.72 - 1.48 (m, 3H), 1.32 - 1.03 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{28}N_5O_2$: 394.2238.

Tert-butyl 4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidine-1-carboxylate (7a). (Yield 96%) ^{1}H NMR (DMSO-d_6) δ 13.47 (br. s., 1H), 8.40 (t, *J* = 6.1 Hz, 1H), 7.55 (d, *J* = 2.6 Hz, 1H), 7.52 (d, *J* = 9.2 Hz, 1H), 7.05 (dd, *J* = 2.6 and 8.9 Hz, 1H), 3.99 - 3.87 (m, 2H), 3.80 (s, 3H), 3.17 (t, *J* = 6.4 Hz, 2H), 2.78 - 2.60 (m, 2H), 1.81 - 1.61 (m, 3H), 1.39 (s, 9H), 1.14 - 0.92 ppm (m, 2H). HRMS, Q-TOF found for $C_{20}H_{29}N_4O_4$: 389.2183.

Tert-butyl 4-({[(5-bromo-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidine-1-carboxylate (7b). (Yield 87%) ^{1}H NMR (DMSO-d_6) δ 13.76 (br. s., 1H), 8.49 (t, *J* = 6.2 Hz, 1H), 8.32 (dd, *J* = 0.8 and 1.9 Hz, 1H), 7.61 (dd, *J* = 0.8 and 8.9 Hz, 1H), 7.53 (dd, *J* = 1.9 and 8.9 Hz, 1H), 3.92 (d, *J* = 12.7 Hz, 2H), 3.20 (t, *J* = 6.4 Hz, 2H), 2.79 - 2.59 (m, 2H), 1.93 - 1.56 (m, 3H), 1.39 (s, 9H), 1.11 - 0.95 (m, 2H). HRMS, Q-TOF found for $C_{19}H_{26}BrN_4O_3$: 437.1182.

5-Methoxy-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide hydrochloride (8a). 2M HCl in Et_2O (1.8 L) was added to a solution of compound **7a** (92.8 g, 0.24 moles) in MeOH (500 mL). The mixture was stirred for 3 hours at room temperature then the resulting solid was filtered and dried to give 5-methoxy-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide hydrochloride **(8a)** (61.1 g, 89% yield). ^{1}H NMR (DMSO-d_6) δ 13.43 (br. s., 1H), 8.27 (t, *J* = 6.13 Hz, 1H), 7.56 (d, *J* = 2.01 Hz, 1H), 7.51 (dd, *J* = 0.55, 8.96 Hz, 1H), 7.06 (dd, *J* = 2.47, 9.06 Hz, 1H), 6.81 (br. s., 1H), 3.81 (s, 3H), 3.19 (t, *J* = 6.22 Hz, 2H), 2.85 (d, *J* = 11.34 Hz, 2H), 1.91 (t, *J* = 10.61 Hz, 2H), 1.45 – 1.72 (m, 3H), 1.04 – 1.34 ppm (m, 2H). HRMS, Q-TOF found for $C_{15}H_{21}N_4O_2$: 289.1648.

5-Bromo-*N*-(piperidin-4-ylmethyl)-1*H*-indazole-3-carboxamide hydrochloride (8b). (Yield 76%) ^{1}H NMR (DMSO-d_6) δ 14.00 (br. s., 1H), 9.12 (br. s., 1H), 8.99 - 8.69 (m, 1H), 8.60 (t, *J* = 6.2 Hz, 1H), 8.32 (d, *J* = 2.2 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.53 (dd, *J* = 2.2 and 8.8 Hz, 1H), 3.23 (t, *J* = 6.4 Hz, 4H), 2.93 - 2.69 (m, 2H), 1.98 - 1.73 (m, 3H), 1.56 - 1.32 ppm (m, 2H). HRMS, Q-TOF found for $C_{15}H_{18}BrN_4O_3$: 381.0557.

General method for the preparation of compounds 9 and 20.

A mixture of compound **8a** or **8b** (5.4 mmol) and potassium carbonate (16.6 mmol) in DMF (45 mL) was stirred for 1 hour at 80 °C. A solution of the proper alkyl halide (8 mmol) in DMF (5 mL) was added drop-wise. After 3 hours at 70 °C the reaction mixture was cooled,

diluted with water and extracted with EtOAc. The collected organic phases were dried over Na_2SO_4, concentrated under vacuum and then properly purified.

Ethyl [4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]acetate (20b). (Yield 42%) ^{1}H NMR (DMSO-d_6) δ 13.47 (br. s., 1 H), 8.36 (t, *J* = 5.9 Hz, 1H), 7.57 - 7.49 (m, 2H), 7.07 (dd, *J* = 2.6 and 8.9 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 3.80 (s, 3H), 3.29 - 3.12 (m, 4H), 2.88 - 2.76 (m, 2H), 2.19 - 2.05 (m, 2H), 1.69 - 1.48 (m, 3H), 1.34 - 1.07 ppm (m, 5H). HRMS, Q-TOF found for $C_{19}H_{27}N_4O_4$: 375.2027.

Ethyl 4-[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]butanoate hydrochloride (20d). (Yield 63%) ^{1}H NMR (DMSO-d_6) δ 8.12 (br. s., 1H), 7.71 - 7.43 (m, 2H), 7.06 (d, *J* = 8.6 Hz, 1H), 6.98 - 5.82 (m, 2H), 4.05 (q, *J* = 7.3 Hz, 2H), 3.81 (s, 3H), 3.25 (br. s., 2H), 3.04 (d, *J* = 10.6 Hz, 2H), 2.55 (t, *J* = 6.9 Hz, 2H), 2.40 - 2.11 (m, 4H), 2.00 - 1.53 (m, 5H), 1.48 - 1.26 (m, 2H), 1.17 ppm (t, *J* = 7.1 Hz, 3H). HRMS, Q-TOF found for $C_{21}H_{31}N_4O_4$: 403.233982.

5-Methoxy-*N*-{[1-(2-methoxyethyl)-piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (9e). (Yield 38%) ^{1}H NMR (DMSO-d_6); δ 13.41 (br. s., 1H), 8.25 (t, *J* = 6.07 Hz, 1H), 7.56 (d, *J* = 2.50 Hz, 1H), 7.51 (d, *J* = 9.06 Hz, 1H), 7.06 (dd, *J* = 2.50 and 9.05 Hz, 1H), 3.81 (s, 3H), 3.41 (t, *J* = 5.97 Hz, 2H), 3.23 (s, 3H), 3.19 (t, *J* = 6.26 Hz, 2H), 2.85 (d, *J* = 11.56 Hz, 2H), 2.43 (t, *J* = 5.97 Hz, 2H), 1.79 – 2.06 (m, 2H), 1.48 – 1.73 (m, 3H), 0.99 – 1.39 ppm (m, 2H). HRMS, Q-TOF found for $C_{18}H_{27}N_4O_3$: 347.2080.

***N*-[(1-Benzylpiperidin-4-yl)methyl]-5-methoxy-1*H*-indazole-3-carboxamide (9m).** (Yield 43%) ^{1}H NMR (DMSO-d_6) δ 13.39 (br. s., 1H), 8.25 (t, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.50 (dd, *J* = 0.5 and 9.0 Hz, 1H), 7.36 - 7.17 (m, 5H), 7.05 (dd, *J* = 2.5 and 9.1 Hz, 1H), 3.80 (s, 3H), 3.43 (s, 2H), 3.20 (t, *J* = 6.1 Hz, 2H), 2.79 (d, *J* = 11.2 Hz, 2H), 1.89 (t, J = 10.6 Hz, 2H), 1.74 - 1.46 (m, 3H), 1.34 - 1.07 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{27}N_4O_2$: 379.2124.

***N*-({1-[2-(4-Hydroxyphenyl)ethyl]-piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9t).** (Yield 41%) ^{1}H NMR (DMSO-d_6); δ 13.40 (br. s., 1H), 9.07 (s, 1H), 8.26 (t, *J* = 6.16 Hz, 1H), 7.55 (d, *J* = 2.42 Hz, 1H), 7.50 (dd, *J* = 0.61 and 9.08 Hz, 1H), 7.05 (dd, *J* = 2.50 and 9.10 Hz, 1H), 6.94 - 7.02 (m, 2H), 6.60 - 6.68 (m, 2H), 3.80 (s, 3H), 3.19 (t, *J* = 6.36 Hz, 2H), 2.89 (d, *J* = 11.30 Hz, 2H), 2.53 - 2.65 (m, 2H), 2.30 - 2.46 (m, 2H), 1.79 - 2.00 (m, 2H), 1.47 - 1.74 (m, 3H), 1.06 - 1.31 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{29}N_4O_3$: 409.2226.

5-Methoxy-*N*-({1-[2-(3-methylcyclohexyl)ethyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide (9w). A solution of compound **8a** (1.46 mmol) in DMF (45 ml) and triethylamine (0.61 ml, 4.4 mmol) was stirred at 80 °C for 1h and then was treated with 1-(2-bromoethyl)-3-methylcyclohexane (300 mg, 1.46 mmol). The mixture was stirred overnight at the same temperature. The reaction was then cooled to room temperature and the solvent was removed by evaporation at reduced pressure. The residue was poured in water and extracted with AcOEt. The organic phases were dried with Na_2SO_4 then evaporated under reduced pressure. 5-Methoxy-*N*-({1-[2-(3-methylcyclohexyl)ethyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide was purified by flash chromatography using a mixture of $CHCl_3$/MeOH (9:1) as eluent. (Yield = 45 mg, 18.0 %). ^{1}H NMR (DMSO-d_6) δ 13.41 (s, 1H), 8.30-8.20 (t, *J* = 6.11 Hz, 1H), 7.58-7.53 (d, *J* = 2.31 Hz, 1H), 7.53-7.47 (d, *J* = 8.59 Hz, 1H), 7.08-7.02 (dd, *J* = 8.92 and 2.32 Hz, 1H), 3.80 (s, 3H), 3.23-3.13 (t, *J* = 6.28 Hz, 2H), 2.90-2.78 (d, *J* = 10.57 Hz, 2H), 2.35-2.20 (m, 2H), 1.97-1.05 (m, 17H), 0.90-0.45 ppm (m, 5H). HRMS, Q-TOF found for $C_{24}H_{37}N_4O_2$: 413.2910.

Ethyl 5-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}furan-2-carboxylate (9y). (Yield 71%.) ^{1}H NMR (DMSO-d_6) δ 13.37 (br. s., 1H), 8.25 (t, *J* = 6.04 Hz, 1H), 7.42-7.60 (m, 2H), 7.21 (d, *J* = 3.29 Hz, 1H), 6.97-7.12 (m, 1H), 6.48 (d, *J* = 3.29 Hz, 1H), 4.26 (q, *J* = 7.32 Hz, 2H), 3.80 (s, 3H), 3.53 (s, 2H), 3.18 (t, *J* =

6.22 Hz, 2H), 2.81 (d, *J* = 11.34 Hz, 2H), 1.87-2.05 (m, 2H), 1.46-1.73 (m, 3H), 1.28 (t, *J* = 6.95 Hz, 3H), 1.01-1.41 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{29}N_4O_5$: 441.2134.

Methyl 2-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl] methyl}-1,3-oxazole-4-carboxylate (9aa). (Yield 45%) ^{1}H NMR (DMSO-d_6) δ 13.40 (br. s., 1H), 8.80 (s, 1H), 8.26 (t, J = 6.2 Hz, 1H), 7.55 (d, *J* = 2.6 Hz, 1H), 7.51 (d, *J* = 9.1 Hz, 1H), 7.05 (dd, *J* = 2.4 and 9.0 Hz, 1H), 3.80 (s, 6H), 3.67 (s, 2H), 3.18 (t, *J* = 6.2 Hz, 2H), 2.82 (d, *J* = 11.3 Hz, 2H), 2.14 - 1.93 (m, 2H), 1.74 - 1.45 (m, 3H), 1.29 - 1.10 ppm (m, 2H). HRMS, Q-TOF found for $C_{21}H_{26}N_5O_5$: 428.1926.

Methyl 2-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl] methyl}-1,3-thiazole-4-carboxylate (9ac). (Yield 32%) ^{1}H NMR (DMSO-d_6) δ 13.41 (s, 1H), 8.46 (s, 1H), 8.29 (t, *J* = 6.0 Hz, 1H), 7.55 (d, *J* = 2.2 Hz, 1H), 7.50 (d, *J* = 9.1 Hz, 1H), 7.05 (dd, *J* = 2.4 and 9.0 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 5H), 3.21 (t, *J* = 6.2 Hz, 2H), 2.89 (d, *J* = 11.3 Hz, 2H), 2.13 (t, *J* = 10.8 Hz, 2H), 1.78 - 1.54 (m, 3H), 1.37 - 1.14 ppm (m, 2H). HRMS, Q-TOF found for $C_{21}H_{26}N_5O_4S$: 444.1700.

Ethyl 4-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl] methyl}-1,3-thiazole-2-carboxylate (9ad). (Yield 11%). ^{1}H NMR (DMSO-d_6) δ 13.39 (s, 1H), 8.26 (t, *J* = 6.0 Hz, 1H), 7.86 (s, 1H), 7.55 (d, *J* = 2.2 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.04 (dd, J = 2.2, 8.8 Hz, 1H), 4.37 (q, J = 7.0 Hz, 2H), 3.80 (s, 3H), 3.64 (s, 2H), 3.19 (t, J = 6.2 Hz, 2H), 2.85 (d, *J* = 11.3 Hz, 2H), 1.98 (t, *J* = 10.6 Hz, 2H), 1.79 - 1.45 (m, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.29 - 0.96 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{28}N_5O_4S$: 458.1855.

***N*-({1-[(5-Carbamoyl-1,2,4-oxadiazol-3-yl)methyl]piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9ae).** (Yield 14 %). ^{1}H NMR (DMSO-d_6) δ 13.18 (br. s., 1H), 8.70 (br. s., 1H), 8.32 (br. s., 1H), 8.26 (t, *J* = 6.2 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.50 (dd, *J* = 0.7 and 9.1 Hz, 1H), 7.05 (dd, *J* = 1.8, 9.1 Hz, 1H), 3.80 (s, 3H), 3.69 (s, 2H), 3.19 (t, *J* =

6.2 Hz, 2H), 2.86 (d, *J* = 11.0 Hz, 2H), 2.19 - 1.93 (m, 2H), 1.82 - 1.39 (m, 3H), 1.33 - 1.07 ppm (m, 2H). HRMS, Q-TOF found for $C_{19}H_{24}N_7O_4$: 414.1881.

Methyl 2-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl] methyl}furan-3-carboxylate (9af). (Yield 13%). ^{1}H NMR (DMSO-d_6) δ 13.39 (s, 1H), 8.23 (t, *J* = 6.0 Hz, 1H), 7.70 (d, *J* = 2.2 Hz, 1H), 7.54 (d, *J* = 2.6 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 2.4 and 9.0 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 1H), 3.83 (s, 2H), 3.80 (s, 3H), 3.76 (s, 3H), 3.17 (t, *J* = 6.4 Hz, 2H), 2.80 (d, *J* = 11.3 Hz, 2H), 2.10 - 1.88 (m, 2H), 1.70 - 1.42 (m, 3H), 1.31 - 1.02 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{27}N_4O_5$: 427.1977.

***N*-[(1-{2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]ethyl}piperidin-4-yl)methyl]-5-methoxy-1*H*-indazole-3-carboxamide (9ag).** (Yield 48%). ^{1}H NMR (DMSO-d_6) δ 13.40 (s, 1H), 8.30-8.14 (t, *J* = 6.11 Hz, 1H), 7.58-7.53 (d, *J* = 1.98 Hz, 1H), 7.53-7.46 (dd, *J* = 8.92, 0.66 Hz, 1H), 7.11-6.96 (dd, *J* = 8.92, 2.31 Hz, 1H), 3.80 (s, 3H), 3.57-3.43 (m, 2H), 3.21-3.11 (t, *J* = 6.28 Hz, 2H), 2.92-2.77 (d, *J* = 11.23 Hz, 2H), 2.76-2.63 (d, *J* = 10.24 Hz, 2H), 2.44-2.26 (m, 4H), 1.97-1.77 (t, *J* = 10,90 Hz, 2H), 1.71-1.46 (t, *J* = 10.73 Hz, 4H), 1.27-1.07 (m, 3H), 1.06-0.94 ppm (d, *J* = 6.28 Hz, 6H). HRMS, Q-TOF found for $C_{23}H_{36}N_5O_3$: 430.2816.

***N*-[(1-{3-[(2R,6S)-2,6-dimethylmorpholin-4-yl]propyl}piperidin-4-yl)methyl]-5-methoxy-1*H*-indazole-3-carboxamide (9ah).** (Yield 59%). ^{1}H NMR (DMSO-d_6) δ 12.12 (s, 1H), 7.80-7.62 (d, *J* = 2.20 Hz, 1H), 7.40-7.32 (d, *J* = 9.15, 1H), 7.27-7.18 (t, *J* = 6.04 Hz, 1H), 7.07-6.99 (dd, *J* = 9.15 and 2.20 Hz, 1H), 3.89-3.78 (s, 3H), 3.76-3.53 (m, 2H), 3.47-3.30 (t, *J* = 6.22 Hz, 2H), 3.07-2.93 (m, 2H), 2.75-2.68 (d, *J* = 10.98 Hz, 2H), 2.45-2.24 (m, 4H), 2.07-1.88 (t, *J* = 10.79 Hz, 2H), 1.83-1.59 (m, 7H), 1.53-1.35 (m, 2H), 1.18-1.05 ppm (d, *J* = 6.22 Hz, 6H). HRMS, Q-TOF found for $C_{24}H_{38}N_5O_3$: 444.2971.

General method for the alkaline hydrolysis of ester derivatives 20b,d and 9y,aa,ac.

To a solution of an ester derivative (**9y**, **9aa**, **9ac**, **20b** or **20d**, 1.85 mmol) in MeOH (10 mL), aqueous NaOH (1 M, 3.7 mL) was added. The solution was heated to reflux overnight then the organic solvent was removed under vacuum, the residue was diluted with H_2O and the pH was adjusted to 5-6 by adding 1M HCl. The mixture was kept at 4 °C overnight then the resulting solid was filtered, washed with water, dried under vacuum and then properly purified.

[4-({[(5-Methoxy-1*H*-indazol-3-yl)-carbonyl]amino}methyl)piperidin-1-yl]acetic acid (9b). (Yield 87%) ^{1}H NMR (DMSO-d_6) δ 13.95 (br. s., 2H), 8.24 (t, *J* = 6.06 Hz, 1H), 7.38 – 7.62 (m, 2H), 6.86 – 7.13 (m, 1H), 3.81 (s, 3H), 3.18 (t, *J* = 6.16 Hz, 2H), 2.94 (d, *J* = 11.10 Hz, 2H), 2.74 (s, 2H), 1.99 (t, *J* = 10.90 Hz, 2H), 1.45 – 1.66 (m, 3H), 1.11 – 1.35 ppm (m, 2H). HRMS, Q-TOF found for $C_{17}H_{23}N_4O_4$: 347.1677.

4-[4-({[(5-Methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]butanoic acid (9d). (Yield 75%) ^{1}H NMR (DMSO-d6) δ 13.54 (br. s., 1H), 11.25 (br. s., 1H), 8.46 (t, *J* = 6.1 Hz, 1H), 7.55 (d, *J* = 2.3 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 1H), 7.05 (dd, *J* = 2.3, 8.9 Hz, 1H), 3.80 (s, 3H), 3.37 (d, *J* = 12.2 Hz, 2H), 3.23 (t, *J* = 6.1 Hz, 2H), 3.00 - 2.89 (m, 2H), 2.81 (t, *J* = 11.4 Hz, 2H), 2.32 (t, *J* = 7.1 Hz, 2H), 2.01 - 1.70 (m, 5H), 1.64 - 1.41 ppm (m, 2H). HRMS, Q-TOF found for $C_{19}H_{27}N_4O_4$: 375.2029.

Sodium 5-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}pyridine-2-carboxylate (9r). Compound **9r** was obtained after the alkaline work-up of the crude methyl 5-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)-piperidin-1-yl]methyl}pyridine-2-carboxylate. (Yield 91%) ^{1}H NMR (DMSO-d_6) δ 13.86 (br. s., 1H), 8.37 (d, *J* = 1.2 Hz, 1H), 8.24 (t, *J* = 6.1 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.65 (dd, *J* = 2.0, 8.1 Hz, 1H), 7.61 - 7.48 (m, 2H), 7.01 (dd, *J* = 2.5 and 8.8 Hz, 1H), 3.79 (s, 3H), 3.47 (s, 2H), 3.19 (t, *J* = 6.1 Hz, 2H), 2.77 (d, *J* = 10.9 Hz, 2H), 1.91 (t, *J* = 10.9 Hz, 2H), 1.65 (s, 3H), 1.35 - 1.07 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{26}N_5O_4$: 424.1977.

4-{[4-({[(5-Methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl} pyridine-2-carboxylic acid (9s). Compound **9s** was obtained after the alkaline work-up of the crude methyl 4-{[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}pyridine-2-carboxylate (Yield 16%). ^{1}H NMR (DMSO-d_6) δ 13.25 (br. s, 2H), 8.54 (d, *J* = 4.8 Hz, 1H), 8.27 (t, *J* = 6.0 Hz, 1H), 7.93 (s, 1H), 7.56 (d, *J* = 2.2 Hz, 1H), 7.51 (d, *J* = 9.5 Hz, 1H), 7.43 (d, *J* = 4.0 Hz, 1H), 7.04 (dd, *J* = 2.2, 9.5 Hz, 1H), 3.80 (s, 3H), 3.53 (s, 2H), 3.20 (t, *J* = 6.0 Hz, 2H), 2.78 (d, *J* = 11.0 Hz, 2H), 1.96 (t, *J* = 10.6 Hz, 2H), 1.75 - 1.45 (m, 3H), 1.35 - 1.16 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{26}N_5O_4$: 424.1981.

5-{[4-({[(5-Methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl} furan-2-carboxylic acid (9x). (Yield 84%). ^{1}H NMR (DMSO-d_6) δ 12.78-14.43 (m, 1H), 8.26 (t, *J* = 6.04 Hz, 1H), 7.55 (d, *J* = 2.56 Hz, 1H), 7.52 (d, *J* = 9.15 Hz, 1H), 7.04 (dd, *J* = 2.60 and 9.10 Hz, 1H), 6.91 (d, *J* = 3.29 Hz, 1H), 6.35 (d, *J* = 3.29 Hz, 1H), 4.04 (br. s., 1H), 3.80 (s, 3H), 3.50 (s, 2H), 3.18 (t, *J* = 6.22 Hz, 2H), 2.83 (d, *J* = 11.34 Hz, 2H), 1.97 (t, *J* = 10.79 Hz, 2H), 1.47-1.73 (m, 3H), 1.04-1.33 ppm (m, 2H). HRMS, Q-TOF found for $C_{21}H_{25}N_4O_5$: 413.181946.

2-{[4-({[(5-Methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}-1,3-oxazole-4-carboxylic acid (9z). (Yield 82%). ^{1}H NMR (DMSO-d_6) δ 13.40 (s, 1H), 12.99 (br. s., 1H), 8.67 (s, 1H), 8.26 (t, *J* = 6.0 Hz, 1H), 7.55 (d, *J* = 2.2 Hz, 1H), 7.50 (d, *J* = 9.5 Hz, 1H), 7.04 (dd, *J* = 2.6 and 9.1 Hz, 1H), 3.80 (s, 3H), 3.66 (s, 2H), 3.18 (t, *J* = 6.4 Hz, 2H), 2.82 (d, *J* = 11.0 Hz, 2H), 2.05 (t, *J* = 10.4 Hz, 2H), 1.76 - 1.44 (m, 3H), 1.33 - 1.05 (m, 2H). HRMS, Q-TOF found for $C_{20}H_{24}N_5O_5$: 414.177195.

2-{[4-({[(5-Methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}-1,3-thiazole-4-carboxylic acid (9ab). (Yield 43%). ^{1}H NMR (DMSO-d_6) δ 13.42 (br. s., 1H), 12.91 (br. s., 1H), 8.34 (s, 1H), 8.29 (t, *J* = 6.0 Hz, 1H), 7.56 (d, *J* = 2.2 Hz, 1H), 7.51 (d, *J* = 9.1 Hz, 1H), 7.05 (dd, *J* = 2.6 and 9.1 Hz, 1H), 3.87 - 3.69 (m, 5H), 3.22 (t, *J* = 6.2 Hz,

2H), 2.89 (d, *J* = 11.3 Hz, 2H), 2.12 (t, *J* = 10.6 Hz, 2H), 1.81 - 1.50 (m, 3H), 1.37 - 1.11 ppm (m, 2H). HRMS, Q-TOF found for $C_{20}H_{24}N_5O_4S$: 430.1545.

Tert-butyl {3-[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]propyl}carbamate (20l). A solution of compound **8a** (1.37g, 4.36mmol) in DMF (45 ml) and triethylamine (1.3ml, 9.5mmol) was stirred at 80 °C for 1h and treated with tert-butyl (3-bromopropyl)carbamate (1.7g, 7.1 mmol). The mixture was stirred overnight at the same temperature. The reaction was cooled to room temperature and the solvent was removed by evaporation at reduced pressure. The crude tert-butyl {3-[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]propyl}carbamate **20l** was used for the following reaction without further purification. HRMS, Q-TOF found for $C_{23}H_{36}N_5O_4$: 446.2763.

***N*-{[1-(3-Aminopropyl)piperidin-4-yl]methyl}-5-methoxy-1*H*-indazole-3-carboxamide (21l).** A solution of crude tert-butyl {3-[4-({[(5-methoxy-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]propyl}carbamate **20l** (approx. 1.8 g) in CH_2Cl_2 (15 ml) was treated with trifluoroacetic acid (7 ml) at room temperature overnight. The solution was poured into water (50 ml) and washed with CH_2Cl_2. The acid phase was basified and concentrated at reduced pressure. The solid residue was extracted with a mixture of CH_3Cl/CH_3OH in 8/2 ratio and the solvent evaporated at reduced pressure. The crude *N*-{[1-(3-aminopropyl)piperidin-4-yl]methyl}-5-methoxy-1*H*-indazole-3-carboxamide **21l** was used for the next step without further purifications.

General procedure for the synthesis of compounds 9h-l.

To a solution of crude *N*-{[1-(3-aminopropyl)piperidin-4-yl]methyl}-5-methoxy-1*H*-indazole-3-carboxamide **21l** (approx. 350 mg, 1 mmol) in DMSO (1.5 mL) and CH_2Cl_2 (10 mL) was added the appropriate acyl chloride (0.61 mmol). The solution was then stirred at room temperature for 2h. The mixture was added to water and extracted with CH_2Cl_2. The

combined organic phases was concentrated at reduced pressure and the crude product was purify by preparative HPLC-MS.

5-Methoxy-*N*-({1-[3-(propanoylamino)propyl]piperidin-4-yl}methyl)-1*H*-indazole-3-carboxamide (9h). (Yield 69%). ^{1}H NMR (DMSO-d_6) δ 13.28 (s, 1H), 8.17-8.00 (m, 1H), 7.65-7.55 (m, 1H), 7.58-7.54 (d, *J* = 2.20 Hz, 1H), 7.52-7.45 (d, *J* = 9.20 Hz, 1H), 7.10-7.00 (dd, *J* = 9.15 and 2.60 Hz, 1H), 3.81 (s, 3H), 3.30-2.85 (m, 8H), 2.11-2.00 (q, *J* = 7.70 Hz, 2H), 1.80-1.52 (m, 6H), 1.40-1.15 (m, 3H), 1.03-0.95 ppm (t, *J* = 7.70 Hz, 3H). HRMS, Q-TOF found for $C_{21}H_{32}N_5O_3$: 402.2499.

***N*-({1-[3-(Butanoylamino)propyl]piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9i).** (Yield 36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.44 (s,1H), 8.41-8.26 (t, *J* = 6.11 Hz, 1H), 7.89-7.69 (t, *J* = 5.12 Hz, 1H), 7.58-7.54 (d, *J* = 2.31, 1H), 7.53-7.47 (dd, *J* = 8.92 and 0.66 Hz, 1H), 7.11-6.96 (dd, *J* = 9.08, 2.48 Hz, 1H), 3.80 (s, 3H), 3.52-2.77 (m, 10H), 2.10-1.92 (t, *J* = 7.27 Hz, 2H), 1.81-1.12 (m, 9H), 0.91-0.77 ppm (t, *J* = 7.27, 3H). HRMS, Q-TOF found for $C_{22}H_{34}N_5O_3$: 416.265616.

***N*-[(1-{3-[(2E)-but-2-enoylamino]propyl}piperidin-4-yl)methyl]-5-methoxy-1*H*-indazole-3-carboxamide (9j).** (Yield 51%). ^{1}H NMR (DMSO-d_6) δ 13.44 (s, 1H), 8.45-8.25 (m, 1H), 8.00-7.75 (m, 1H), 7.60-7.53 (d, *J* = 2.40 Hz, 1H), 7.53-7.47 (d, *J* = 8.90 Hz, 1H), 7.09-7.01 (dd, *J* = 2.70 and 2.30 Hz, 1H), 6.67-6.50 (m, 1H), 5.75-6.00 (m, 1H), 3.80 (s, 3H), 3.50-1.00 ppm (m, 20H). HRMS, Q-TOF found for $C_{22}H_{32}N_5O_3$: 414.2501.

***N*-({1-[3-(But-2-ynoylamino)propyl]piperidin-4-yl}methyl)-5-methoxy-1*H*-indazole-3-carboxamide (9k).** (Yield 17%). ^{1}H NMR (DMSO-d_6) δ 13.34 (s, 1H), 8.52-8.42 (t, *J* = 5.31 Hz, 1H), 8.27-8.18 (t, *J* = 6.04 Hz, 1H), 7.56-7.52 (d, *J* = 2.20 Hz, 1H), 7.52-7.47 (d, *J* = 8.78 Hz, 1H), 7.05-6.99 (dd, *J* = 8.96, 2.38 Hz, 1H), 3.80 (s, 3H), 3.21-3.13 (t, *J* = 6.40 Hz, 2H), 3.11-3.01 (q, *J* = 6.59 Hz, 2H), 2.87-2.75 (d, *J* = 11.34 Hz, 2H), 2.30-2.18 (t, *J* = 6.95 Hz,

2H), 1.94 (s, 3H), 1.88-1.73 (t, *J* = 10.61 Hz, 2H), 1.70-1.45 (m, 5H), 1.30-1.10 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{30}N_5O_3$: 412.2346.

5-Methoxy-*N*-[(1-{3-[(phenylcarbonyl)amino]propyl}piperidin-4-yl)methyl]-1*H*-indazole-3-carboxamide (9l). (Yield 23%) ^{1}H NMR (DMSO-d_6) δ 13.43 (s,1H), 8.59-8.47 (t, *J* = 5.31 Hz, 1H), 8.38-8.24 (t, *J* = 6.04 Hz, 1H), 7.90-7.74 (m, 2H), 7.61-7.35 (m, 5H), 7.10-6.99 (dd, *J* = 9.15 and 2.56 Hz, 1H), 3.89-3.69 (s, 3H), 3.39-3.12 (m, 6H), 3.11-2.94 (m, 2H), 2.25-1.89 (m, 2H), 1.83-1.53 (m, 5H), 1.36-1.12 ppm (d, *J* = 11.34 Hz, 2H). HRMS, Q-TOF found for $C_{25}H_{32}N_5O_3$: 450.2498.

Synthesis of 5-bromoindazole intermediates 13c, 14c, 15d, 16d and 17d.

Compound **13c**, **14c**, **15d**, **16d** and **17d** was prepared using the same procedure described for compound **9a**, starting from the 5-bromoindazole intermediate **8b**.

5-Bromo-*N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (13c). (Yield 53%). ^{1}H NMR (DMSO-d_6) δ = 13.73 (br. s., 1H), 8.42 (t, *J* = 6.0 Hz, 1H), 8.32 (dd, *J* = 0.7, 1.8 Hz, 1H), 7.61 (dd, *J* = 0.7 and 8.8 Hz, 1H), 7.53 (dd, *J* = 1.8 and 8.8 Hz, 1H), 7.32 - 7.10 (m, 5H), 3.20 (t, *J* = 6.3 Hz, 2H), 2.93 (d, *J* = 10.2 Hz, 2H), 2.79 - 2.64 (m, *J* = 8.4 Hz, 2H), 2.50 - 2.41 (m, 2H), 1.93 (t, *J* = 9.9 Hz, 2H), 1.64 (br. s., 3H), 1.39 - 1.04 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{26}BrN_4O$: 441.1284.

5-Bromo-*N*-{[1-(2-methoxyethyl)piperidine-4-yl]methyl}-1*H*-indazole-3-carboxamide (14c). (Yield 42%) ^{1}H NMR (DMSO-d_6) δ 13.4 (br. s., 1H), 8.42 (t, *J* = 6.07 Hz, 1H), 8.31 (dd, *J* = 1.83 and 0.67 Hz, 1H), 7.61 (dd, *J* = 8.70 and 0.70 Hz, 1H), 7.53 (dd, *J* = 8.70 and 1.70 Hz, 1H), 3.41 (t, *J* = 5.97 Hz, 2H), 3.22 (s, 3H), 3.13-3.21 (m, 2H), 2.78-2.94 (m, 2H), 2.30-2.47 (m, 2H), 1.82-2.09 (m, 2H), 1.39-1.77 (m, 3H), 1.08-1.30 ppm (m, 2H). HRMS, Q-TOF found for $C_{17}H_{24}BrN_4O_2$: 395.1077.

Ethyl 5-{[4-({[(5-bromo-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}furan-2-carboxylate (15d). (Yield 62%) ^{1}H NMR (DMSO-d_6) δ 13.73 (br. s., 1H),

8.41 (t, *J* = 6.04 Hz, 1H), 8.32 (dd, *J* = 0.73 and 1.83 Hz, 1H), 7.57-7.65 (m, 1H), 7.45-7.56 (m, 1H), 7.21 (d, *J* = 3.66 Hz, 1H), 6.48 (d, *J* = 3.66 Hz, 1H), 4.27 (q, *J* = 7.32 Hz, 2H), 3.53 (s, 2H), 3.19 (t, *J* = 6.40 Hz, 2H), 2.81 (d, *J* = 11.34 Hz, 2H), 1.82-2.09 (m, 2H), 1.64 (d, *J* = 12.44 Hz, 3H), 1.02-1.36 ppm (m, 5H). HRMS, Q-TOF found for $C_{22}H_{26}BrN_4O_4$: 489.1132.

Methyl 2-{[4-({[(5-bromo-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}-1,3-oxazole-4-carboxylate (16d). (Yield 16%). ^{1}H NMR (DMSO-d_6) δ 13.73 (br. s., 1H), 8.80 (s, 1H), 8.42 (t, *J* = 6.0 Hz, 1H), 8.31 (dd, *J* = 0.8, 1.8 Hz, 1H), 7.60 (dd, *J* = 0.8, 8.8 Hz, 1H), 7.52 (dd, *J* = 1.8, 8.8 Hz, 1H), 3.80 (s, 3H), 3.67 (s, 2H), 3.18 (t, *J* = 6.4 Hz, 2H), 2.81 (d, J = 11.3 Hz, 2H), 2.13 - 1.95 (m, 2H), 1.74 - 1.44 (m, 3H), 1.32 - 1.06 ppm (m, 2H). HRMS, Q-TOF found for $C_{20}H_{23}BrN_5O_4$: 476.0928.

Ethyl [4-({[(5-bromo-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]acetate (17d). (Yield 31%). ^{1}H NMR (DMSO-d_6) δ 13.74 (s, 1H), 8.43 (t, *J* = 6.0 Hz, 1H), 8.32 (dd, *J* = 0.6 and 1.9 Hz, 1H), 7.61 (dd, *J* = 0.6 and 8.9 Hz, 1H), 7.53 (dd, *J* = 1.9 and 8.9 Hz, 1H), 4.07 (q, *J* = 7.1 Hz, 2H), 3.20 (t, *J* = 6.4 Hz, 2H), 3.17 (s, 2H), 2.81 (d, *J* = 11.2 Hz, 2H), 2.22 - 2.03 (m, 2H), 1.72 - 1.46 (m, 3H), 1.31 - 1.08 ppm (m, 5H). HRMS, Q-TOF found for $C_{18}H_{24}BrN_4O_3$: 423.1026.

Sodium 5-{[4-({[(5-bromo-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}furan-2-carboxylate (15c). Compound **15c** was prepared with the same procedure described for compound **9b**, starting from the 5-bromoindazole intermediate **15d**. (Yield 98%). ^{1}H NMR (DMSO-d_6) δ 13.78 (br. s., 1H), 8.43 (t, *J* = 5.85 Hz, 1H), 8.32 (d, *J* = 1.21 Hz, 1H), 7.61 (d, *J* = 8.80 Hz, 1H), 7.53 (dd, *J* = 2.00 and 8.80 Hz, 1H), 7.11 (d, *J* = 3.63 Hz, 1H), 6.45 (d, *J* = 3.23 Hz, 1H), 3.57 (s, 2H), 3.19 (t, *J* = 6.26 Hz, 2H), 2.85 (d, *J* = 11.30 Hz, 2H), 2.02 (t, *J* = 10.90 Hz, 2H), 1.45-1.77 (m, 3H), 1.08-1.37 ppm (m, 2H). HRMS, Q-TOF found for $C_{20}H_{22}BrN_4O_4$: 461.081887.

General method for the preparation of compounds 13-17.

A mixture of the starting 5-bromoindazole derivative (**13c**, **14c**, **15d**, **16d** or **17d**) (0.44 mmol), the appropriately substituted arylboronic acid (1.77 mmol), [1,1′-bis(diphenylphosphino)ferrocene]-dichloro-palladium(II) [Pd(dppf)Cl_2] (81 mg, 0.11 mmol) and caesium carbonate (575 mg, 1.76 mmol) in 1,4-dioxane and water (ratio 3/1; 8 mL) was subjected to microwave irradiation in a Milestone oven: (45' @ T=160 °C, max power 300W). After microwave irradiation, solvents were removed by evaporating under reduced pressure and the reaction mixture was diluted with a mixture of chloroform and methanol in a 2:1 ratio and filtered. Final compounds were purified by preparative HPLC-MS method.

5-(2-Fluorophenyl)-*N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (13g). (Yield 45%) ^{1}H NMR (DMSO-d_6) δ 15.02 - 11.90 (m, 1H), 8.38 (t, *J* = 6.0 Hz, 1H), 8.33 (d, *J* = 0.7 Hz, 1H), 7.72 (dd, *J* = 0.7 and 8.6 Hz, 1H), 7.64 - 7.52 (m, 2H), 7.49 - 7.06 (m, 8H), 3.22 (t, *J* = 6.3 Hz, 2H), 2.92 (d, *J* = 11.2 Hz, 2H), 2.78 - 2.65 (m, 2H), 2.50 - 2.41 (m, 2H), 1.93 (t, *J* = 10.6 Hz, 2H), 1.78 - 1.47 (m, 3H), 1.33 - 1.07 ppm (m, 2H). HRMS, Q-TOF found for $C_{28}H_{30}FN_4O$: 457.2398.

5-(2,3-Difluorophenyl)-*N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (13i). (Yield 28%) ^{1}H NMR (DMSO-d_6) δ 13.71 (s, 1H), 8.48 - 8.20 (m, 2H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.51 - 7.03 (m, 8H), 3.22 (t, *J* = 6.2 Hz, 2H), 2.92 (d, *J* = 11.3 Hz, 2H), 2.81 - 2.61 (m, 2H), 2.49 - 2.41 (m, 2H), 1.93 (t, *J* = 10.7 Hz, 2H), 1.77 - 1.49 (m, 3H), 1.34 - 1.08 ppm (m, 2H). HRMS, Q-TOF found for $C_{28}H_{29}F_2N_4O$: 475.2305.

***N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-5-(pyridin-3-yl)-1*H*-indazole-3-carboxamide (13l).** (Yield 19%) ^{1}H NMR (DMSO-d_6) δ 13.70 (br. s., 1H), 8.91 (dd, *J* = 0.6 and 2.5 Hz, 1H), 8.59 (dd, *J* = 1.6 and 4.8 Hz, 1H), 8.49 - 8.31 (m, 2H), 8.10 (ddd, *J* = 1.6, 2.3 and 7.9 Hz, 1H), 7.76 (d, *J* = 1.5 Hz, 2H), 7.51 (ddd, *J* = 0.8, 4.8 and 7.9 Hz, 1H), 7.33 - 7.09 (m, 5H), 3.23 (t, *J* = 6.3 Hz, 2H), 2.92 (d, *J* = 11.3 Hz, 2H), 2.79 - 2.64 (m, 2H), 2.52 -

2.41 (m, 2H), 1.93 (t, *J* = 10.6 Hz, 2H), 1.75 - 1.51 (m, 3H), 1.32 - 1.09 ppm (m, 2H). HRMS, Q-TOF found for $C_{27}H_{30}N_5O$: 440.2446.

5-(4-Methoxypyridin-3-yl)-*N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (13m). (Yield 17%) ^{1}H NMR (DMSO-d_6) δ 13.32 (s, 1H), 8.47 (d, *J* = 5.7 Hz, 1H), 8.39 (s, 1H), 8.34 (t, *J* = 6.0 Hz, 1H), 8.24 (dd, *J* = 0.9 and 1.6 Hz, 1H), 7.67 (dd, *J* = 0.8 and 8.7 Hz, 1H), 7.50 (dd, *J* = 1.6 and 8.6 Hz, 1H), 7.32 - 7.10 (m, 6H), 3.87 (s, 3H), 3.21 (t, *J* = 6.4 Hz, 2H), 2.92 (d, *J* = 11.9 Hz, 2H), 2.77 - 2.67 (m, 2H), 2.50 - 2.42 (m, 2H), 2.02 - 1.83 (m, 2H), 1.74 - 1.50 (m, 3H), 1.33 - 1.08 (m, 2H). HRMS, Q-TOF found for $C_{28}H_{32}N_5O_2$: 470.2552.

5-(6-Methylpyridin-3-yl)-*N*-{[1-(2-phenylethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (13q). (Yield 23%) ^{1}H NMR (DMSO-d_6) δ 13.68 (br. s., 1H), 8.77 (d, *J* = 2.6 Hz, 1H), 8.46 - 8.34 (m, 2H), 7.99 (dd, *J* = 2.6 and 8.1 Hz, 1H), 7.74 (d, *J* = 0.9 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.31 - 7.11 (m, 5H), 3.23 (t, *J* = 6.2 Hz, 2H), 2.92 (d, *J* = 11.3 Hz, 2H), 2.72 (dd, *J* = 6.2 and 9.1 Hz, 2H), 2.53 (s, 3H), 2.50 - 2.43 (m, 2H), 1.93 (t, *J* = 10.6 Hz, 2H), 1.76 - 1.49 (m, 3H), 1.34 - 1.10 ppm (m, 2H). HRMS, Q-TOF found for $C_{28}H_{32}N_5O$: 454.2600.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-phenyl-1*H*-indazole-3-carboxamide (14f).** (Yield 15%). ^{1}H NMR (DMSO-d_6) δ 13.65 (br. s., 1H), 8.40 (t, *J* = 1.28 Hz, 1H), 8.36 (t, *J* = 6.13 Hz, 1H), 7.65-7.75 (m, 4H), 7.44-7.53 (m, 2H), 7.32-7.41 (m, 1H), 3.40 (t, *J* = 6.04 Hz, 2H), 3.12-3.27 (m, *J* = 6.00 and 6.00 Hz, 5H), 2.84 (d, *J* = 11.34 Hz, 2H), 2.42 (t, *J* = 6.04 Hz, 2H), 1.83-1.97 (m, 2H), 1.49-1.71 (m, 3H), 1.09-1.31 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{29}N_4O_2$: 393.2290.

5-(2-Fluorophenyl)-*N*-{[1-(2-methoxyethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (14g). (Yield 39%). ^{1}H NMR (DMSO-d_6) δ 13.55 (s, 1H), 8.25-8.40 (m, 2H), 7.70 (dd, *J* = 0.73 and 8.78 Hz, 1H), 7.50-7.63 (m, 2H), 7.38-7.49 (m, 1H), 7.28-7.38 (m,

2H), 3.40 (t, *J* = 5.95 Hz, 2H), 3.12-3.25 (m, 5H), 2.84 (d, *J* = 11.34 Hz, 2H), 2.42 (t, *J* = 6.04 Hz, 2H), 1.82-1.99 (m, 2H), 1.46-1.72 (m, 3H), 1.06-1.28 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{28}FN_4O_2$: 411.2196.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(2-methylphenyl)-1*H*-indazole-3-carboxamide (14h).** (Yield 35%). ^{1}H NMR (DMSO-d_6) δ 13.59 (s, 1H), 8.34 (t, *J* = 6.13 Hz, 1H), 8.06 (dd, *J* = 1.56 and 0.82 Hz, 1H), 7.65 (dd, *J* = 0.73 and 8.60 Hz, 1H), 7.39 (dd, *J* = 1.65 and 8.60 Hz, 1H), 7.35-7.20 (m, 4H), 3.40 (t, *J* = 5.95 Hz, 2H), 3.27 – 3.13 (m, 5H), 2.84 (d, *J* = 11.53 Hz, 2H), 2.42 (t *J* = 6.04 Hz, 2H), 2.23 (s, 3H), 1.90 (t, *J* = 10.61 Hz, 2H), 1.50-1.70 (m, 3H), 1.10-1.30 ppm (m, 2H). HRMS, Q-TOF found for $C_{24}H_{31}N_4O_2$: 407.2453.

5-(2,3-Difluorophenyl)-*N*-{[1-(2-methoxyethyl)piperidin-4-yl]methyl}-1*H*-indazole-3-carboxamide (14i). (Yield 19%). ^{1}H NMR (DMSO-d_6) δ 13.09 (s, 1H), 8.23-8.42 (m, 2H), 7.72 (dd, *J* = 0.82 and 8.69 Hz, 1H), 7.55 (td, *J* = 1.76 and 8.74 Hz, 1H), 7.24-7.49 (m, 3H), 3.40 (t, *J* = 6.04 Hz, 2H), 3.22 (s, 3H), 3.18 (d, *J* = 6.40 Hz, 2H), 2.84 (d, *J* = 11.53 Hz, 2H), 2.42 (t, *J* = 5.95 Hz, 2H), 1.82-2.02 (m, 2H), 1.41-1.71 (m, 3H), 1.06-1.31 (m, 2H). HRMS, Q-TOF found for $C_{23}H_{27}F_2N_4O_2$: 429.2105.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(4-methoxyphenyl)-1*H*-indazole-3-carboxamide (14k).** (17%). ^{1}H NMR (DMSO-d_6) δ 13.20 (s, 1H), 8.18-8.40 (m, 2H), 7.49-7.73 (m, 4H), 6.88-7.10 (m, 2H), 3.81 (s, 3H), 3.40 (t, *J* = 5.95 Hz, 2H), 3.22 (s, 5H), 2.84 (d, *J* = 11.34 Hz, 2H), 2.42 (t, *J* = 5.95 Hz, 2H), 1.79-2.01 (m, 2H), 1.43-1.74 (m, 3H), 1.09-1.29 ppm (m, 2H). HRMS, Q-TOF found for $C_{24}H_{31}N_4O_3$: 423.2400.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(pyridin-3-yl)-1*H*-indazole-3-carboxamide (14l).** (Yield 31%). ^{1}H NMR (DMSO-d_6) δ 13.71 (br. s., 1H), 8.90 (dd, *J* = 0.82 and 2.47 Hz, 1H), 8.58 (dd, *J* = 1.56 and 4.67 Hz, 1H), 8.42-8.44 (m, 1H), 8.40 (t, *J* = 6.00 Hz, 1H), 8.09 (ddd, *J* = 1.65, 2.42 and 8.00 Hz, 1H), 7.70-7.81 (m, 2H), 7.51 (ddd, *J* = 0.82, 4.76 and 7.96 Hz, 1H), 3.37-3.44 (m, 2H), 3.14-3.24 (m, 5H), 2.84 (d, *J* = 11.53 Hz,

2H), 2.43 (t, *J* = 6.04 Hz, 2H), 1.82-1.99 (m, 2H), 1.47-1.74 (m, 3H), 1.09-1.29 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{28}N_5O_2$: 394.2241.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(4-methoxypyridin-3-yl)-1*H*-indazole-3-carboxamide (14m).** (Yield 38%). ^{1}H NMR (DMSO-d_6) δ 13.66 (br. s., 1H), 8.47 (d, *J* = 5.85 Hz, 1H), 8.33-8.42 (m, 2H), 8.24 (dd, *J* = 0.91, 1.65 Hz, 1H), 7.66 (dd, *J* = 0.91, 8.60 Hz, 1H), 7.53 (dd, *J* = 1.65, 8.60 Hz, 1H), 7.19 (d, *J* = 5.67 Hz, 1H), 3.48 (s, 3H), 3.38-3.45 (m, 2H), 3.15-3.25 (m, 5H), 2.88 (d, *J* = 11.34 Hz, 2H), 2.48 (t, *J* = 6.00 Hz, 2H), 1.98 (t, *J* = 10.89 Hz, 2H), 1.47-1.73 (m, 3H), 1.09-1.31 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{30}N_5O_3$: 424.2350.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(5-methylpyridin-3-yl)-1*H*-indazole-3-carboxamide (14n).** (Yield 43%). ^{1}H NMR (DMSO-d_6) δ 13.71 (br. s., 1H), 8.49 (d, *J* = 1.65 Hz, 1H), 8.43 (dd, *J* = 0.91 and 1.65 Hz, 1H), 8.40 (t, *J* = 6.13 Hz, 1H), 8.30 (d, *J* = 2.74 Hz, 1H), 7.78 (dd, *J* = 1.60 and 8.70 Hz, 1H), 7.73 (dd, *J* = 0.90 and 8.70 Hz, 1H), 7.62 (dd, *J* = 1.83 and 2.74 Hz, 1H), 3.94 (s, 3H), 3.40 (t, *J* = 6.04 Hz, 2H), 3.10-3.26 (m, 5H), 2.84 (d, *J* = 11.53 Hz, 2H), 2.43 (t, *J* = 5.95 Hz, 2H), 1.82-2.00 (m, 2H), 1.44-1.73 (m, 3H), 1.05-1.31 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{30}N_5O_3$: 424.2350.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(6-methoxypyridin-3-yl)-1*H*-indazole-3-carboxamide (14o).** (Yield 25%). ^{1}H NMR (DMSO-d_6) δ 13.36 (s, 1H), 8.47 (dd, *J* = 0.73 and 2.56 Hz, 1H), 8.26-8.37 (m, 2H), 8.01 (dd, *J* = 2.60 and 8.60 Hz, 1H), 7.70 (dd, *J* = 1.00 and 8.80 Hz, 1H), 7.65 (dd, *J* = 1.80 and 8.80 Hz, 1H), 6.93 (dd, *J* = 0.73 and 8.60 Hz, 1H), 3.91 (s, 3H), 3.40 (t, *J* = 6.04 Hz, 2H), 3.22 (s, 5H), 2.84 (d, *J* = 11.34 Hz, 2H), 2.42 (t, *J* = 6.04 Hz, 2H), 1.80-2.01 (m, 2H), 1.47-1.74 (m, 3H), 1.02-1.35 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{30}N_5O_3$: 424.2343.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(2-methoxypyridin-3-yl)-1*H*-indazole-3-carboxamide (14p).** (Yield 35%). ^{1}H NMR (DMSO-d_6) δ 13.6 (br. s, 1H), 8.24-

8.38 (m, 2H), 8.18 (dd, *J* = 1.83 and 4.94 Hz, 1H), 7.76 (dd, *J* = 1.83 and 7.32 Hz, 1H), 7.62-7.69 (m, 1H), 7.46-7.58 (m, 1H), 7.11 (dd, *J* = 4.94 and 7.14 Hz, 1H), 3.89 (s, 3H), 3.40 (t, *J* = 5.95 Hz, 2H), 3.22 (s, 5H), 2.84 (d, *J* = 11.53 Hz, 2H), 2.42 (t, *J* = 6.04 Hz, 2H), 1.82-1.97 (m, 2H), 1.47- 1.72 (m, 3H), 1.06-1.29 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{30}N_5O_3$: 424.2340.

***N*-{[1-(2-Methoxyethyl)piperidin-4-yl]methyl}-5-(6-methylpyridin-3-yl)-1*H*-indazole-3-carboxamide (14q).** (Yield 18%). ^{1}H NMR (DMSO-d_6) δ 13.68 (br. s., 1H), 8.77 (d, *J* = 1.83 Hz, 1H), 8.32-8.43 (m, 2H), 7.98 (dd, *J* = 2.47 and 7.96 Hz, 1H), 7.73 (d, *J* = 1.28 Hz, 2H), 7.37 (d, *J* = 8.05 Hz, 1H), 3.41 (t, *J* = 5.95 Hz, 2H), 3.23 (s, 5H), 2.85 (d, *J* = 11.34 Hz, 2H), 2.53 (s, 3H), 2.43 (t, *J* = 5.95 Hz, 2H), 1.79-2.01 (m, 2H), 1.44-1.74 (m, 3H), 1.07- 1.33 ppm (m, 2H). HRMS, Q-TOF found for $C_{23}H_{30}N_5O_2$: 408.2397.

5-({4-[({[5-(2-Fluorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}methyl)furan-2-carboxylic acid (15g). (Yield 27%). ^{1}H NMR (DMSO-d_6) δ 13.7 (br. s., 2H), 8.39 (t, *J* = 6.04 Hz, 1H), 8.32 (s, 1H), 7.65-7.76 (m, 1H), 7.50-7.63 (m, 2H), 7.38-7.50 (m, 1H), 7.23-7.38 (m, 2H), 7.08 (d, *J* = 3.29 Hz, 1H), 6.43 (d, *J* = 3.29 Hz, 1H), 3.52 (s, 2H), 3.20 (t, *J* = 6.22 Hz, 2H), 2.82 (d, *J* = 10.98 Hz, 2H), 1.98 (t, *J* = 10.79 Hz, 2H), 1.44-1.79 (m, 3H), 1.02-1.38 ppm (m, 2H). HRMS, Q-TOF found for $C_{26}H_{26}FN_4O_4$: 477.1934.

5-({4-[({[5-(2,3-Difluorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}methyl)furan-2-carboxylic acid (15i). (Yield 21%). ^{1}H NMR (DMSO-d_6) δ 13.7 (br. s., 2H), 8.42 (t, *J* = 5.65 Hz, 1H), 8.35 (s, 1H), 7.69-7.80 (m, 1H), 7.55-7.67 (m, 1H), 7.21-7.54 (m, 3H), 7.05 (d, *J* = 3.23 Hz, 1H), 6.41 (d, *J* = 3.23 Hz, 1H), 3.52 (s, 2H), 3.20 (t, *J* = 6.06 Hz, 2H), 2.83 (d, *J* = 10.50 Hz, 2H), 1.98 (t, *J* = 10.70 Hz, 2H), 1.42-1.79 (m, 3H), 1.04-1.35 ppm (m, 2H). HRMS, Q-TOF found for $C_{26}H_{25}F_2N_4O_4$: 495.1837.

5-({4-[({[5-(4-Methoxyphenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}methyl)furan-2-carboxylic acid (15k). (Yield 15%). ^{1}H NMR (DMSO-d_6) δ 13.5 (br. s,

2H), 8.27-8.41 (m, 2H), 7.64-7.72 (m, 2H), 7.61 (d, *J* = 8.88 Hz, 2H), 7.05 (d, *J* = 8.88 Hz, 2H), 6.96 (br. s., 1H), 6.37 (d, *J* = 3.23 Hz, 1H), 3.81 (s, 3H), 3.49 (s, 2H), 3.20 (t, *J* = 6.26 Hz, 2H), 2.82 (d, *J* = 10.90 Hz, 2H), 1.86-2.05 (m, 2H), 1.66 (d, *J* = 12.11 Hz, 3H), 1.09-1.33 ppm (m, 2H). HRMS, Q-TOF found for $C_{27}H_{29}N_4O_5$: 489.2131.

5-({4-[({[5-(4-Methoxypyridin-3-yl)-1*H*-indazol-3-yl]carbonyl}amino)methyl] piperidin-1-yl}methyl)furan-2-carboxylic acid (15m). (Yield 24%). ^{1}H NMR (DMSO-d_6) δ 13.6 (br. s., 2H), 8.47 (d, *J* = 5.85 Hz, 1H), 8.31-8.43 (m, 2H), 8.24 (s, 1H), 7.66 (d, *J* = 8.78 Hz, 1H), 7.53 (dd, *J* = 1.46 and 8.42 Hz, 1H), 7.18 (d, *J* = 5.49 Hz, 1H), 7.09 (d, *J* = 3.29 Hz, 1H), 6.43 (d, *J* = 3.29 Hz, 1H), 3.86 (s, 3H), 3.54 (s, 2H), 3.19 (t, *J* = 6.04 Hz, 2H), 2.83 (d, *J* = 10.98 Hz, 2H), 1.99 (t, *J* = 10.79 Hz, 2H), 1.44-1.79 (m, 3H), 0.98-1.36 ppm (m, 2H). HRMS, Q-TOF found for $C_{26}H_{28}N_5O_5$: 490.2085.

5-({4-[({[5-(6-Methoxypyridin-3-yl)-1*H*-indazol-3-yl]carbonyl}amino)methyl] piperidin-1-yl}methyl)furan-2-carboxylic acid (15o). (Yield 18%). ^{1}H NMR (DMSO-d_6) δ 13.6 (br. s., 2H), 8.48 (d, *J* = 2.02 Hz, 1H), 8.39 (t, *J* = 6.06 Hz, 1H), 8.34 (s, 1H), 7.96-8.07 (m, 1H), 7.70 (d, *J* = 1.21 Hz, 2H), 7.03 (d, *J* = 3.23 Hz, 1H), 6.94 (d, *J* = 8.07 Hz, 1H), 6.40 (d, *J* = 3.23 Hz, 1H), 3.92 (s, 3H), 3.51 (s, 2H), 3.21 (t, *J* = 6.26 Hz, 2H), 2.83 (d, *J* = 10.90 Hz, 2H), 1.98 (t, *J* = 10.90 Hz, 2H), 1.48-1.78 (m, 3H), 1.07-1.34 ppm (m, 2H). HRMS, Q-TOF found for $C_{26}H_{28}N_5O_5$: 490.2085.

5-({4-[({[5-(2-Methoxypyridin-3-yl)-1*H*-indazol-3-yl]carbonyl}amino)methyl] piperidin-1-yl}methyl)furan-2-carboxylic acid (15p). (Yield 15%). ^{1}H NMR (DMSO-d_6) δ 13.62 (br. s., 1H), 8.36 (t, *J* = 6.04 Hz, 1H), 8.28 (s, 1H), 8.19 (dd, *J* = 1.83 and 5.12 Hz, 1H), 7.77 (dd, *J* = 2.20, 7.32 Hz, 1H), 7.65 (dd, *J* = 0.80 and 8.80 Hz, 1H), 7.58 (dd, *J* = 1.80 and 8.80 Hz, 1H), 7.11 (dd, *J* = 5.12 and 7.32 Hz, 1H), 6.84 (br. s., 1H), 6.31 (d, *J* = 2.93 Hz, 1H), 3.89 (s, 3H), 3.47 (s, 2H), 3.19 (t, *J* = 6.22 Hz, 2H), 2.99 (s, 1H), 2.82 (d, *J* = 10.98 Hz,

2H), 1.83-2.04 (m, 2H), 1.41-1.75 (m, 3H), 1.06-1.34 ppm (m, 2H). HRMS, Q-TOF found for $C_{26}H_{28}N_5O_5$: 490.2082.

2-({4-[({[5-(2,3-Difluorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}methyl)-1,3-oxazole-4-carboxylic acid (16i). (Yield 26%). ^{1}H NMR (DMSO-d_6) δ 13.70 (s, 1H), 12.99 (br. s., 1H), 8.57 (s, 1H), 8.42 (t, *J* = 6.0 Hz, 1H), 8.34 (d, *J* = 0.7 Hz, 1H), 7.73 (dd, *J* = 0.8 and 8.8 Hz, 1H), 7.61 (td, *J* = 1.8 and 8.7 Hz, 1H), 7.52 - 7.21 (m, 3H), 3.64 (s, 2H), 3.20 (t, *J* = 6.2 Hz, 2H), 2.82 (d, *J* = 11.0 Hz, 2H), 2.04 (t, *J* = 10.6 Hz, 2H), 1.73 - 1.45 (m, 3H), 1.33 - 1.09 ppm (m, 2H). HRMS, Q-TOF found for $C_{25}H_{24}F_2N_5O_4$: 496.1793.

[4-({[(5-Ethyl-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]acetic acid (17e). A mixture of product **17d** (170 mg, 0.4 mmol), vinyl-boronic acid pinacol ester (0.53 mmol), [1,1′-bis(diphenylphosphino)ferrocene]-dichloro-palladium(II) (50 mg, 0.06 mmol), saturated sodium carbonate solution (1.7 mL) in toluene/ethanol (ratio 1:1, 10 mL) was heated in a Milestone microwave oven @ 150 °C, 500W for 2 h. After filtration through celite, solvents were removed under reduce pressure and the crude product was eluted through a silica gel cartridge with a mixture of chloroform/methanol 1:1 ratio. Solvents were removed under reduced pressure and the resulting crude intermediate was dissolved in ethanol (20 mg/mL) and hydrogenated over a 10% Pd/C cartridge at 30 °C, 1 mL/min in a Thales Nano H-CUBE hydrogenator to obtain [4-({[(5-ethyl-1*H*-indazol-3-yl)carbonyl]amino}methyl)piperidin-1-yl]acetic acid **17e**, purified using preparative HPLC-MS. (170 mg, yield 41%). ^{1}H NMR (DMSO-d_6) δ 13.48 (br. s., 1H), 8.38 (t, *J* = 6.1 Hz, 1H), 7.97 (s, 1H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.28 (dd, *J* = 1.6 and 8.6 Hz, 1H), 4.38 (br. s., 1H), 3.33 - 3.09 (m, 6H), 2.73 (q, *J* = 7.5 Hz, 2H), 2.65 - 2.53 (m, 2H), 1.83 - 1.60 (m, 3H), 1.54 - 1.31 (m, 2H), 1.23 ppm (t, *J* = 7.5 Hz, 3H). HRMS, Q-TOF found for $C_{18}H_{25}N_4O_3$: 345.1921.

{4-[({[5-(2-Fluorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}acetic acid (17g). (Yield 36%). ^{1}H NMR (DMSO-d_6) δ 13.71 (br. s., 1H), 8.45 (t, *J* = 6.0

Hz, 1H), 8.41 (s, 1H), 7.86 - 7.66 (m, 2H), 7.63 - 7.41 (m, 3H), 7.19 (dddd, *J* = 2.4, 2.6, 6.5 and 9.0 Hz, 1H), 4.75 (br. s., 1H), 3.34 - 3.07 (m, 6H), 2.64 - 2.53 (m, 2H), 1.75 (d, *J* = 11.0 Hz, 3H), 1.42 ppm (q, *J* = 11.5 Hz, 2H). HRMS, Q-TOF found for $C_{22}H_{24}FN_4O_3$: 411.1825.

{4-[({[5-(2,3-Difluorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}acetic acid (17i). (Yield 17%). ^{1}H NMR (DMSO-d_6) δ 13.68 (br. s., 1H), 8.51 (t, *J* = 6.1 Hz, 1H), 8.35 (d, *J* = 0.6 Hz, 1H), 7.74 (dd, *J* = 0.7 and 8.8 Hz, 1H), 7.61 (td, *J* = 1.7 and 8.7 Hz, 1H), 7.51 - 7.25 (m, 3H), 4.0 (br. s., 1H), 3.33 - 3.10 (m, 6H), 2.64 - 2.53 (m, 2H), 1.74 (d, *J* = 10.5 Hz, 3H), 1.54 - 1.29 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{23}F_2N_4O_3$: 429.1733.

{4-[({[5-(2,3-Dichlorophenyl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}acetic acid (17j). (Yield 15%). ^{1}H NMR (DMSO-d_6) δ 13.81 (br. s., 1H), 8.50 (t, *J* = 6.0 Hz, 1H), 8.17 (dd, *J* = 0.9 and 1.6 Hz, 1H), 7.78 - 7.60 (m, 2H), 7.54 - 7.34 (m, 3H), 4.1 (br. s., 1H), 3.38 - 2.96 (m, 6H), 2.58 (t, *J* = 11.0 Hz, 2H), 1.74 (d, *J* = 11.0 Hz, 3H), 1.42 ppm (q, J = 11.6 Hz, 2H). HRMS, Q-TOF found for $C_{22}H_{23}Cl_2N_4O_3$: 461.1142.

{4-[({[5-(5-Methoxypyridin-3-yl)-1*H*-indazol-3-yl]carbonyl}amino)methyl]piperidin-1-yl}acetic acid (17n). (Yield 28%). ^{1}H NMR (DMSO-d_6) δ 13.7 (br. s., 2H), 8.54 - 8.49 (m, 1H), 8.48 (d, *J* = 1.6 Hz, 1H), 8.44 - 8.39 (m, 1H), 8.30 (d, *J* = 2.7 Hz, 1H), 7.81 - 7.76 (m, 1H), 7.76 - 7.69 (m, *J* = 0.7 Hz, 1H), 7.61 (dd, *J* = 1.8 and 2.7 Hz, 1H), 3.93 (s, 3H), 3.29 - 3.12 (m, 6H), 2.69 - 2.55 (m, 2H), 1.75 (d, *J* = 11.0 Hz, 3H), 1.58 - 1.27 ppm (m, 2H). HRMS, Q-TOF found for $C_{22}H_{26}N_5O_4$: 424.1979.

Enzymatic GSK-3β Assay. Using a described methods,[22] compounds were assayed on human GSK-3β at several concentrations ranging from 1 nM to 100 μM with ten-fold dilutions in duplicate. Human recombinant enzyme GSK-3β was incubated for 90 minutes at 22 °C in the presence of compounds or vehicle in a reaction buffer containing ATP plus 100 nM unphosphorylated specific substrate peptide (Ulight-CFFKNIVTPRTPPPSQGK-amide).

Substrate phosphorylation was measured by LANCE technology (PerkinElmer, CT, USA). IC_{50} curves were generated using GraphPad 5 and a standard 4-parameter nonlinear regression algorithm (log (inhibitor) versus response – variable slope). Data points are the averages of duplicate wells. See supplementary information file for the full list of results expressed as IC_{50} plus 95% confidence intervals.

Cellular GSK-3β Assay. Recombinant Chinese Hamster Ovary (CHO) pTau over-expressing cells were suspended in medium at the appropriate concentration and then plated in a 96-well plate at 15,000 cells per well. The cells were incubated with the selected compounds (each compound was tested at 7 concentrations; 1:5 dilutions starting from 30 μM in duplicate) for 2 hours at 37 °C, 5% CO2 and then lysed and processed for the detection of phosphorylated tau protein (Luminex). *N*-[(4-Methoxyphenyl)methyl]-*N*'-(5-nitro-2-thiazolyl) urea (AR-A014418), a selective GSK-3 inhibitor, was used as reference compound. The percent values of activity with respect to control (untreated cells) were calculated. IC_{50} curves were generated using GraphPad 5 and a standard 4-parameter nonlinear regression algorithm (log (inhibitor) versus response – variable slope). Data points are the averages of duplicate wells. See supplementary information file for the full list of results expressed as IC_{50} plus 95% confidence intervals.

Mode of action method. The mode of action of compounds **14i** and **9c** was investigated using a microfluidic technology where the phosphorylated product and substrate are separated by electrophoresis and detected via laser-induced fluorescence. The standard screening reaction is carried out in 100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 0.015% Brij-35, 1.5 μM Na_2VO_4, 1.5 μM peptide substrate, 14 μM ATP. The reaction is started by addition of ATP and incubated for 60 minutes at RT. The reaction is terminated by the addition of stop buffer containing 100mM HEPES (pH 7.5), 30 mM EDTA, 0.015% Brij-35, 5% DMSO. Phosphorylated and unphosphorylated substrates are separated by charge

using electrophoretic mobility shift. Product formed is compared to control wells to determine inhibition or enhancement of enzyme activity. Results were analyzed with GraphPad Prism v5.0, fitting to non-linear regression with variable slope, constraints of 0 and 100 for bottom and top, respectively. [23]

***In vitro* plasma stability assessment.** Compound was added to blank mouse plasma pre-incubated at 37 °C. Final molecule concentration was 5µM. Final DMSO concentration was 2.5%. The mixture was shaken at 37 °C for three hours. Aliquots (50µl) were taken at various time points and crashed with 150µl of acetonitrile spiked with 500nM internal standard. After mixing and centrifugation, 3µl of supernatant are analyzed by LC-MS/MS by multiple reactions monitoring (MRM).

Amphetamine model.

Animal preparation. Male C57BL/6J mice, 8 weeks old (Charles River, Calco, Italy), were used for in-vivo studies. Animals were group-housed in ventilated cages and had free access to food and water. They were maintained under a 12-hour light/dark cycle (lights on at 8:00 am) at a controlled temperature of (21 ± 1 °C) and relative humidity of (55 ± 10 %). All experiments were carried out in accordance with the guidelines established by the European Communities Council Directive (Directive 2010/63/EU of 22 September 2010) and approved by the National Council on Animal Care of the Italian Ministry of Health. All efforts were made to minimize animal suffering and to use the minimal number of animals required to produce reliable results.

Behavioral assay. 8 week old male C57BL/6J mice were used. Drugs were administered intraperitoneally by dissolving in PEG400 / Tween 80 / saline solution at 10 / 10 / 80 % (v/v) respectively. Experiments were started by placing the mouse in an open field apparatus. At this stage motility was not recorded and the mouse could freely explore the box for 20 min (habituation). The mouse was then treated with the test molecule, placed back in the box and

motility was recorded for the subsequent 15 min (effect on spontaneous motility). Finally amphetamine was administered (2 mg/kg i.p.) and motility was recorded for the subsequent 90 min, to measure the inhibition of amphetamine hyperactivity. Two way ANOVA was used to evaluate statistical significance, followed by Bonferroni's post-hoc test. GraphPad Prism 5 was used for all statistical analysis (GraphPad Software Inc. San Diego, CA, USA). P values less than 0.05 were considered significant.

Kinases selectivity assays. The kinase profile of **14i** was firstly assessed in a 216 kinase assay platform performed by CEREP, at 10 μM in duplicate. Briefly, human recombinant kinases were incubated in the presence of specific peptide substrates plus ATP for different times at 22 °C. Phosphorylated substrate was detected by LANCE or HTRF technology. In each experiment, a relevant reference compound was tested concurrently with the test compound. The results are expressed as a percent of inhibition of control specific activity obtained in the presence of the test compounds (mean of two values ± standard deviation). For 34 selected kinases the IC_{50} determination was performed. **14i** was tested in duplicate at five concentrations in the range from 10 nM to 100 μM. IC_{50} curves were generated using GraphPad 5 and a standard 4-parameter nonlinear regression algorithm (log (inhibitor) versus response – variable slope). See supplementary information file for the full list of results expressed as IC_{50} plus 95% confidence intervals.

ASSOCIATED CONTENT

Supporting_Information. Experimental description of the synthetic preparation of (piperidin-4-yl)methan-amines **6o-q,u**, with their analytical characterizations, *In vivo* pharmacokinetic assessment procedures, full lists of enzymatic and cellular GSK-3β assays results, full list of the kinase selectivity results for **14i** and crystallization conditions and X-

ray data collection. "This material is available free of charge via the Internet at http://pubs.acs.org."

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

The authors would like to thank Prof. Giovanni Zappia and Dr. Rod Porter for the excellent support provided during the preparation of the manuscript. GF would also thank the Angelini's Physical Chemistry Laboratory for the analytical supports provided for the characterization of the compounds.

ABBREVIATIONS

GSK-3, glycogen synthase kinases 3; ATP, Adenosine triphosphate; HOBt, hydroxybenzotriazole; DCC, N,N'-dicyclohexylcarbodiimide; IC_{50}, half maximal inhibitory concentration; SAR, structure-activity relationship; K_M, Michaelis constant ; Ki, dissociation constant, $t_{1/2}$, elimination half-life; PK, Pharmacokinetic; i.p., intraperitoneal; $AUC_{0,inf}$, area under the curve; C_{max}, peak plasma or brain concentration; T_{max}, time to reach C_{max}; Vd, volume of distribution; Cl, clearance; mpk, mg/kg.

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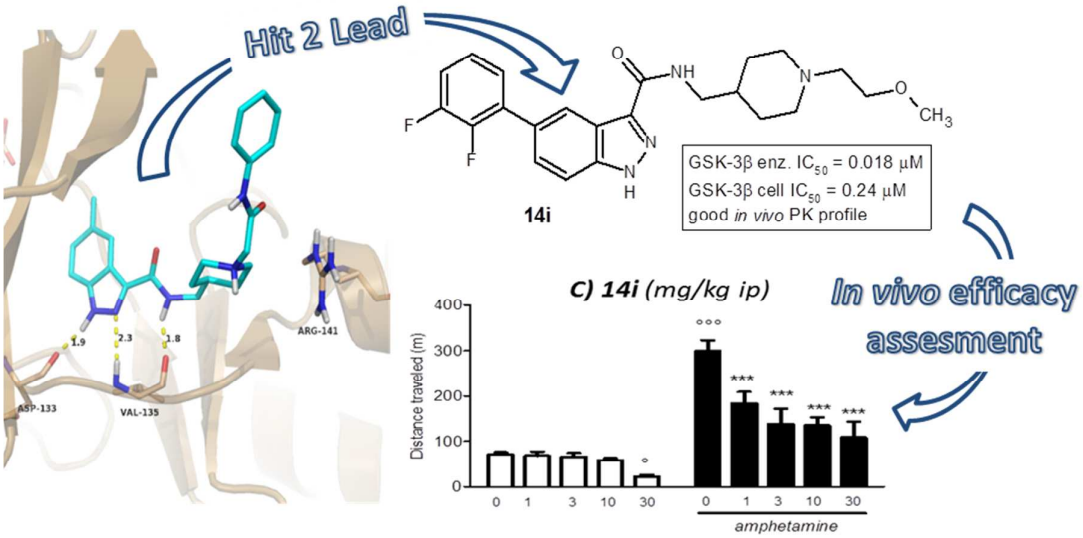
TABLE OF CONTENTS GRAPHIC


Hit 2 Lead
14i
GSK-3β enz. IC50 = 0.018 μM
GSK-3β cell IC50 = 0.24 μM
good in vivo PK profile
In vivo efficacy assesment
C) 14i (mg/kg ip)
Distance traveled (m)
amphetamine
ASP-133
VAL-135
ARG-141