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Design, synthesis and structure–activity relationships of 1,3,4-oxadiazole derivatives as novel inhibitors of glycogen synthase kinase-3β

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ABSTRACT

Glycogen synthase kinase-3β (GSK-3β) is implicated in abnormal hyperphosphorylation of tau protein and its inhibitors are expected to be a promising therapeutic agents for the treatment of Alzheimer's disease. Here we report design, synthesis and structure–activity relationships of a novel series of oxadiazole derivatives as GSK-3β inhibitors. Among these inhibitors, compound **20x** showed highly selective and potent GSK-3β inhibitory activity in vitro and its binding mode was determined by obtaining the X-ray co-crystal structure of **20x** and GSK-3β.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease having pathological features of neurofibrillary tangles (NFTs) and senile plaque. The temporal and spatial distribution of NFTs correlates well with the clinical disease severity. This suggests new strategies to inhibit factor(s) leading to NFT formation and neuronal cell death for the prevention of AD.[1–8] NFTs are composed of abnormally hyperphosphorylated microtubule-associated protein tau. Hyperphosphorylated tau loses its normal function of stabilizing microtubules, leading to a disruption in microtubule assembly and deficits in axonal transport. GSK-3β is a serine/threonine kinase and is thought to be a key factor for aberrant tau phosphorylation.[9] Activated GSK-3β coexists with progression of NFTs and neurodegeneration in the AD brain.[10–12] A conditional GSK-3β overexpressing transgenic mouse exhibits persistent tau hyperphosphorylation, pretangle-like somatodendritic localization of tau, neuronal death in hippocampus and cognitive deficits.[13,14] These studies suggest that GSK-3β is associated with AD progression, and GSK-3β inhibition is expected to be a promising therapeutic approach for AD.

Many GSK-3β inhibitors have been reported and they reviewed in the literature.[15–17] Maleimide derivatives have been reported from many groups.[18,19] In addition, CHIR98023,[20] SB-415286[21] and 2,5-diaminopyrimidines[22] with different chemical structure have been reported. Furthermore, natural product derived GSK-3β inhibitors such as indurubin,[23] paullones,[24] manzamines[25] have been described.

In this paper, we report the design and synthesis of novel small molecule GSK-3β inhibitors based on the 1,3,4-oxadiazole scaffold.

2. Inhibitor design

High-throughput screening of our proprietary compound collection identified hit compound **1** with an IC_{50} value of 65 nM in in vitro assay. The X-ray co-crystal structure of this compound with GSK-3β indicates that this compound binds to the ATP binding site and that the O1 oxygen atom and hydrogen atom on the C2-carbon of the benzodioxole made hydrogen bonds with the amide NH hydrogen and carbonyl oxygen of Val135 in the hinge region, respectively. The 4-methoxy-3-fluorobenzyl group fills the hydrophobic site and both the N3 and N4-nitrogen atoms of the oxadiazole are incorporated into the unique hydrogen bond network between Lys85-Glu97-Asp200 through two water molecules, as shown in Figure 1.

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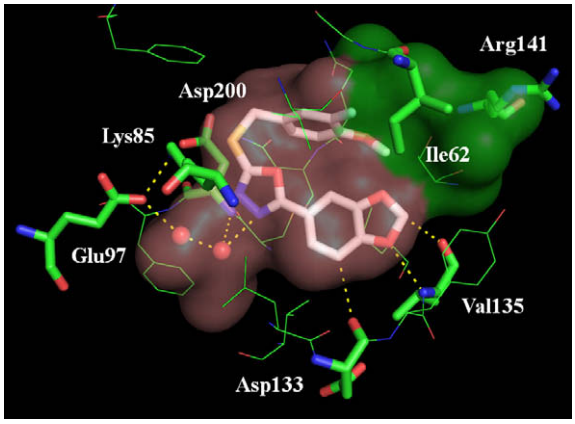


Figure 1. X-ray co-crystal structure of **1** in complex with GSK-3β. The figure was prepared with PyMOL.[26]

The X-ray co-crystal analysis suggested that the 1,3,4-oxadiazole was suitable for interaction with the Lys85-Glu97-Asp200 hydrogen bond network. Thus we fixed the 1,3,4-oxadiazole ring and designed novel compounds having 6,5 or 6,6-fused heterocyclic rings with heteroatom (Z = O, N) and hydrogen atom at appropriate positions for hydrogen bond interaction with the hinge region (Val135). In addition, hydrophobic amino acids were observed near the benzodioxole ring and Arg141 was found adjacent to the gatekeeper. To enhance potency, we also designed compounds incorporating a 4-methoxyphenyl group on the 6,5-fused heterocyclic core, aiming to fill in the hydrophobic site with a phenyl ring and interaction between Arg141 and methoxy group (Fig. 2).

3. Chemistry

The heterobicyclic esters and the hydrazide, the starting materials for oxadiazole derivatives **20a**–**y** were prepared as shown in Scheme 1. Reaction of commercially available aldehyde **4** with iodine and KOH gave ester **5**. Oxidation of dihydrobenzofuran ring of **5** with *N*-bromosuccinimide (NBS) yielded benzofuran **6**. Bromination of benzofuran **6** at the 3-position using bromine gave 3-bromobenzofuran **7** and subsequent coupling with 4-methoxyphenylboronic acid yielded 3-(4-methoxyphenyl)benzofuran **8**. Esterification of carboxylic acid **9** followed by reaction with *p*-anisidine or methylamine gave anilines **11a**,**b**. The nitro groups of **11a**,**b** were reduced with sodium hydrosulfite to provide anilines **12a**,**b**, which were cyclized with formic acid to form benzimidazoles **13a**,**b**. Condensation of the acid **14** with *tert*-butyl carbazate yielded *tert*-butyloxycarbonyl (Boc) hydrazide **15** and removal of the Boc group by exposure to trifluoroacetic acid (TFA) yielded hydrazide **16**.

The synthesis of oxadiazole derivatives **20a**–**y** was performed as outlined in Scheme 2. The heterobicyclic esters **5**,**6**,**8**,**13a**,**b**,**17a**–**c** were treated with hydrazine monohydrate to give hydrazides **18a**–**h**. Treatment of hydrazides **16**,**18a**–**g** in EtOH with carbon disulfide and potassium hydroxide or Et_3N yielded oxadiazolethiols **19a**–**h**. Oxadiazoles **20a**–**y** were prepared by benzylation of oxadiazolethiols **19b**–**d**,**f**–**h** or directly converted from the corresponding hydrazides **18a**,**d**,**h** without isolation of the intermediate oxadiazolethiols.

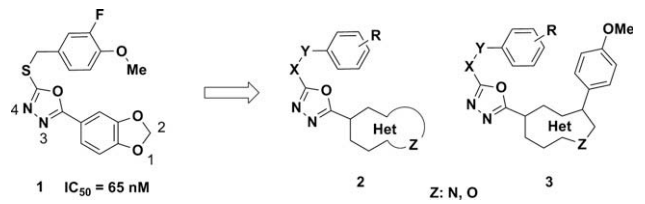


Figure 2. Structure of the hit compound **1** and design of 1,3,4-oxadiazole derivatives **2** and **3**.

Conversion of the linker (S–CH_2) between the oxadiazole and the phenyl ring to ethylene (CH_2CH_2), aminomethylene (NH–CH_2), oxymethylene (O–CH_2) and methylenethio (CH_2–S) is shown in Scheme 3. Condensation of hydrazide **18a** with 3-(3-fluorophenyl)propionic acid in phosphorus oxychloride provided phenethyl oxadiazole **21**. Reaction of **18a** with 3-fluorobenzyl isocyanate and subsequent cyclization using polystyrene-bound triphenylphosphine resin (PS–PPh_3) gave the benzylamino oxadiazole **23**. Methylation of thiol **19a** and subsequent oxidation of the sulfide yielded sulfone **25** followed by substitution of sulfone with 3-fluorobenzyl alcohol to give the benzyloxy oxadiazole **26**. Treatment of **18a** with 2-chloro-1,1,1-triethoxyethane yielded chloromethyl oxadiazole **27** and substitution of chloride with 3-fluorobenzenethiol provided **28**.

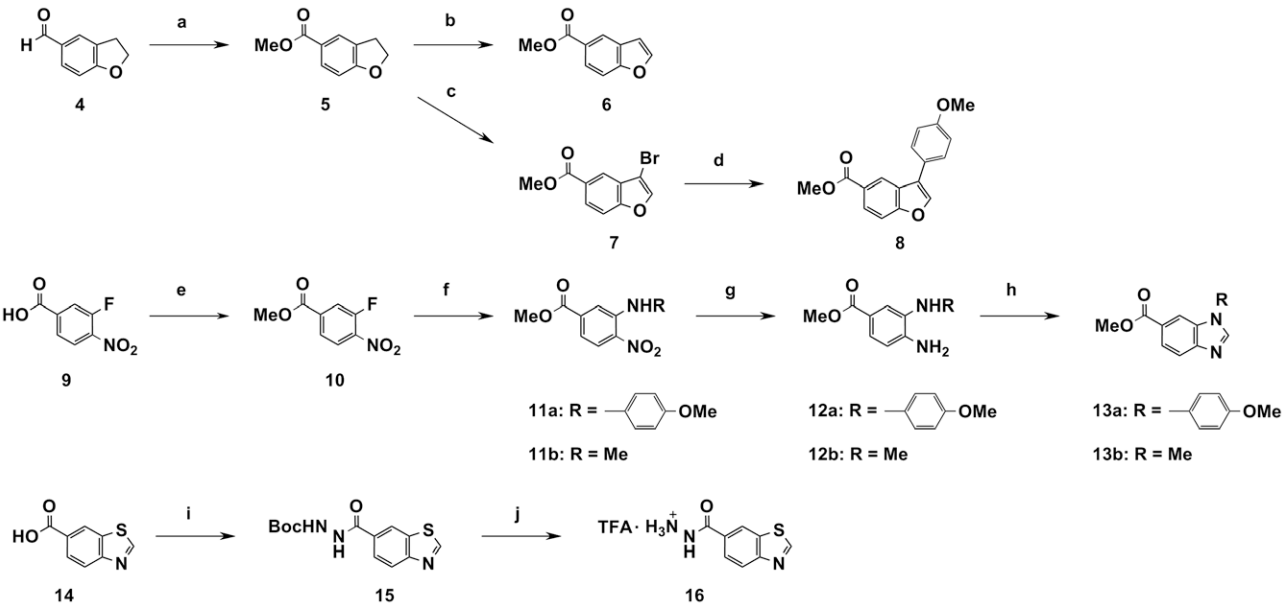
4. Results and discussion

The compounds were evaluated for GSK-3β inhibitory activity in a non-RI kinase assay using Kinase-Glo reagent (Promega, U.S.A.) and the results are shown in Table 1. Initially, we replaced the benzodioxole core as the hinge binding unit of compound **1** to dihydrobenzofuran. The dihydrobenzofuran derivative **20j** showed improved activity with an IC_{50} value of 44 nM compared to compound **1**.

Next, we examined the effect of substituents on the S-benzyl group of dihydrobenzofuran derivatives. Removal of the methoxy group and fluorine atom of **20j** led to unsubstituted phenyl derivative **20a**, which resulted in a 5-fold decrease in activity. The 3-fluorobenzyl derivative **20b** retained activity, however the 4-methoxybenzyl derivative **20c** showed less activity than **20j**. Introduction of a chlorine atom at the 3-position of the phenyl ring showed a 2-fold enhancement of activity compared to **20a**, while introduction at the 2- or 4-position showed no improvement of activity (**20d**,**f**). Compound **20g**, with an electron-withdrawing cyano group at the 3-position, showed similar activity to 3-chloro derivative **20e**, and introduction of a 3-trifluoromethyl group (**20i**) slightly increased activity compared to 3-chlorobenzyl derivative **20e**. However 3-methoxycarbonylbenzyl derivative **20h** was less potent than 3-chlorobenzyl derivative **20e**. It is speculated that introduction of an electron-withdrawing group of the appropriate size into the 3-position increased activity. Furthermore, introduction of a methoxy group at the 4-position of the phenyl ring in 3-chloro, 3-cyano and 3-trifluoromethyl benzyl derivatives (**20k**–**m**) resulted in high potency compared to compound **1** or benzyl derivatives monosubstituted at the 3-position (**20e**,**g**,**i**). Among these derivatives, 4-methoxy-3-(trifluoromethyl)benzyl derivative **20m** showed the most potent inhibitory activity with an IC_{50} of 5.7 nM. These results suggested that combination of an electron-withdrawing group at the 3-position and an electron-donating methoxy group at 4-position on the phenyl ring enhanced activity.

Next, we modified the linker between the oxadiazole and the phenyl ring. Replacement of the sulfur atom to carbon or nitrogen (**21**,**23**) resulted in a 6-fold less potency compared to **20b** and replacement of the sulfur atom with oxygen (**26**) markedly reduced potency. Phenylthiomethyl derivative **28** showed about 10-fold less potency than **20b**.

On the basis of these results, we further examined the effect of other heterocycles as potential hinge binders. Benzofuran derivative **20n** showed a slight increase in activity and benzothiazole derivative **20o** showed a 2-fold increase in activity compared to dihydrobenzofuran derivative **20m**. Indazole, imidazo[1,2-*a*]pyridine and quinoline derivatives **20p**–**r** showed decreased activity.



Scheme 1. [a]Synthesis of heterocyclic esters and benzothiazole-6-carbohydrazide. [a]Reagents and conditions: (a) KOH, I_2, MeOH, 0 °C, 86%; (b) NBS, AIBN, CCl_4, reflux, 83%; (c) (1) NBS, AIBN, chlorobenzene, reflux; (2) Br_2, CH_2Cl_2, then, KOH, MeOH–THF, rt, 86%; (d) 4-MeOPhB$(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3 aq, DME, reflux, 94%; (e) AcCl, MeOH, reflux, 99%; (f) *p*-anisidine, DMSO, 70 °C, 79%; (g) $Na_2S_2O_4$, THF–EtOH, 71%; (h) HCO_2H, 100 °C, 95%; (i) $BocNHNH_2$, WSC, HOBt, DMF, rt, 75%; (j) TFA, rt, 94%.

Finally, introduction of a 4-methoxyphenyl group at the 3-position of the benzofuran or the 1-position of benzimidazole derivatives was carried out. Although compound **20s** showed a 5-fold decrease in activity, interestingly, removal of the 4-methoxy functionality from the S-benzyl group resulted in increased activity (**20t**) and 3-cyanobenzyl derivative **20u** showed potent activity (IC_{50} = 3.5 nM). Conversion of the benzofuran to benzimidazole for these compounds showed similar SAR on the S-benzyl group, and 3-cyano derivative **20x** showed the most potent inhibitory activity with an IC_{50} value of 2.3 nM. Decreased activity was found for 1-methylbenzimidazole **20y** and this result suggests that the 4-methoxyphenyl group contributes to potent inhibitory activity. Moreover, potency of compound **20x** is comparable to the reported compounds such as CHIR98023 (GSK-3, IC_{50} = 10 nM)[20] and macrocyclic bis-maleimide (GSK-3β, IC_{50} = 17 nM).[27]

X-ray analysis of compound **20x** bound to GSK-3β was performed. The co-crystal structure could not be fully characterized due to cleavage of the S–C bond in the X-ray beam to form the de-benzylated compound as shown in Figure 3; however, the S-benzyl group was presumed to be located in a similar position to that observed for compound **1** (Fig. 4). The co-crystal structure of the de-benzylated compound indicated that nitrogen of the benzimidazole forms a hydrogen bond with the backbone NH of the hinge region at Val135, and the N3-nitrogen of the oxadiazole ring directly makes a hydrogen bond with the NH of Asp200. The N4-nitrogen of the oxadiazole ring makes a hydrogen bond with the conserved water molecule, as observed in the co-crystal structure of hit compound **1**. The 4-methoxy group on the phenyl ring interacts with Arg141 according to our drug design. In the series of the 4-methoxyphenyl derivatives **20s–x**, 3,4-disubstituted S-benzyl derivatives showed reduced inhibitory activity, while 3-substituted derivatives maintained potent activity. The results might be due to the steric hindrance between the 4-methoxyphenyl group and the substituent at the 4-position of the S-benzyl group.

We evaluated the kinase selectivity of compound **20x**, and the results are reported in Table 2. Compound **20x** notably showed more than 1000-fold selectivity against CDK1, CDK2 and CDK5, which have high homology in ATP binding site. Although compound **20x** exhibited weak inhibitory activity against MEKK (IC_{50} = 8.1 μM) and PKCθ (IC_{50} = 3.5 μM), selectivity for GSK-3β was also more than 1000-fold as well as other protein kinases. Compound **20x** was found to be a highly selective and potent GSK-3β inhibitor.

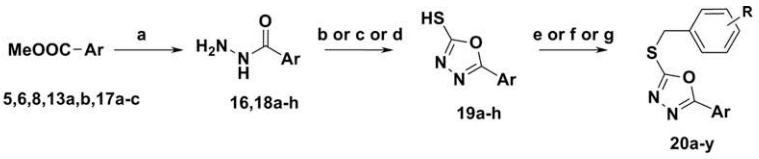
Rat cassette dosing experiments of compound **20x** were performed and the pharmacokinetic parameters are summarized in Table 3. The systemic clearance of compound **20x** after iv dosing averaged 2135 mL/h/kg with volume of distribution (V_{dss}) of 1453 mL/kg and the concentration–time curve (AUC) after po dosing of 7.9 ng h/mL. Compound **20x** showed low oral bioavailability (1.7%), probably due to poor intestinal absorption.

5. Conclusion

Based on the X-ray co-crystal structure of compound **1**, a novel series of 1,3,4-oxadiazole derivatives were designed and synthesized. Compound **20m** having the 4-methoxy-3-(trifluoromethyl)benzyl group and derivatives **20t,u,w,x** having the 4-methoxyphenyl group on the core heterocycles and electron-withdrawing groups such as 3-trifluoromethyl or 3-cyano groups on the S-benzyl group showed single-digit nanomolar potent inhibitory activity. Although the X-ray co-crystal structure of **20x** could not be fully characterized, the hydrogen bonding interaction of the benzimidazole core with the hinge region and the oxadiazole with Asp200 were observed. Additionally, interaction of 4-methoxyphenyl group with Arg141 was observed. Further optimization to improve pharmacokinetic profiles of oxadiazole series will be reported in due course.

6. Experimental

All microwave-assisted reactions were carried out using an Emrys Optimizer (Biotage AB). Melting points were determined on a Büchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Mercury 300 (300 MHz) or Bruker DPX300 (300 MHz) instruments. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multi-



Compd			Ar
5	18a	19a	
6	18b	19b	
-	16	19c	
17a	18c	19d	
17b	18d	19e	
17c	18e	19f	
8	18f	19g	
13a	18g	19h	
13b	18h	-	

Compd	Ar	R
20a		H
20b		3-F
20c		4-OMe
20d		2-Cl
20e		3-Cl
20f		4-Cl
20g		3-CN
20h		3-CO_2Me
20i		3-CF_3
20j		3-F-4-OMe
20k		3-Cl-4-OMe
20l		3-CN-4-OMe
20m		3-CF_3-4-OMe
20n		3-CF_3-4-OMe
20o		3-CF_3-4-OMe
20p		3-CF_3-4-OMe

Compd	Ar	R
20q		3-CF_3-4-OMe
20r		3-CF_3-4-OMe
20s		3-CF_3-4-OMe
20t		3-CF_3
20u		3-CN
20v		3-CF_3-4-OMe
20w		3-CF_3
20x		3-CN
20y		3-CF_3

Scheme 2. [a]Synthesis of 2-(benzylsulfanyl)-1,3,4-oxadiazole derivatives. [a]Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$, MeOH or EtOH, reflux, 30–96%; (b) CS_2, Et_3N, EtOH, reflux, 51–97%; (c) CS_2, KOH, EtOH, reflux, then DMF, microwave, 150 °C, 36%; (d) CS_2, KOH, EtOH, reflux, then DMF, 100 °C, 53%; (e) Benzyl halides, K_2CO_3, DMF, rt, 24–100% (f) Benzyl halides, KOH, DMF, 0 °C, 24–94%. (h) $BocNHNH_2$, WSC, HOBt, DMF, rt, 75%; (i) TFA, rt, 94%; (j) CS_2, Et_3N, EtOH, reflux, 83%.

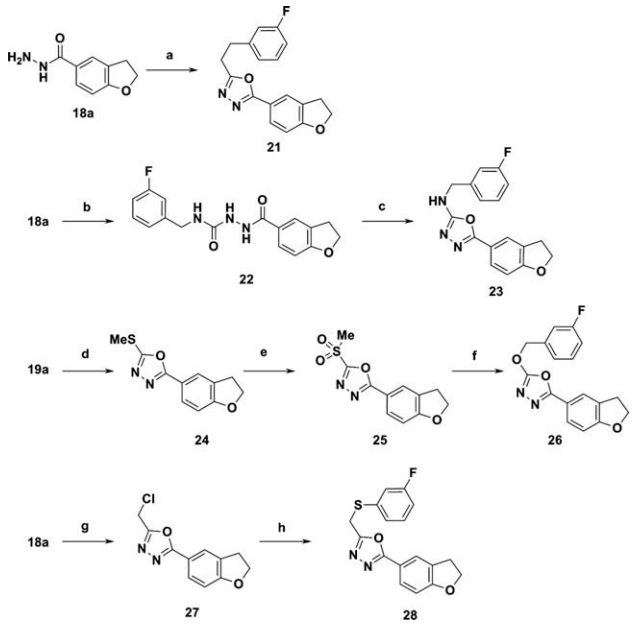
plicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; ddd, doublet of doublet of doublets; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Element analyses were carried out by Takeda Analytical Laboratories. LC–MS (ESI^+) was performed on a Waters ZQ-2000 apparatus with acetonitrile/water mobile phase. Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F_{254} plate (Merck) or NH TLC plates (Fuji Silysia chemical Ltd). Chromatographic purification was carried on silica gel columns 60 (0.063–0.200 mm or 0.040–0.063 mm, Merck), basic silica gel (ChromatorexNH, 100–200 mesh, Fuji silysia chemical Ltd) or Purif-Pack (SI 60 μM or NH 60 μM, Fuji Silysia, Ltd). Commercial reagents and solvents were used without additional purification. Abbreviations are used as follows: $CDCl_3$, deuterated chloroform; DMSO-d_6, dimethyl sulfoxide-d_6; EtOAc, ethyl acetate; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DMSO, dimethyl sulfoxide.

6.1. Methyl 2,3-dihydro-1-benzofuran-5-carboxylate (5)

To a solution of **4** (24.9 g, 0.17 mol) and KOH (85%) (27.7 g, 0.42 mol) in MeOH (200 ml) at 0 °C was added I_2 (53.3 g, 0.21 mol) in MeOH (250 ml) slowly, and the mixture was stirred at 0 °C for 4 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with saturated aqueous Na_2SO_3 solution, water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (THF) to give **5** (27.4 g, 91%) as a colorless solid: ^1H NMR ($CDCl_3$) δ 3.24 (2H, t, J = 8.9 Hz), 3.87 (3H, s), 4.65 (2H, t, J = 8.9 Hz), 6.79 (1H, d, J = 8.3 Hz), 7.85–7.89 (2H, m).

6.2. Methyl 1-benzofuran-5-carboxylate (6)

To a solution of **5** (25.0 g, 140.3 mmol) and 2,2-azobis(isobutyronitrile) (0.461 g, 2.81 mmol) in CCl_4 (500 ml) was added *N*-bromosuccinimide (26.2 g, 147.3 mmol), and the mixture was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **6** (20.6 g, 83%) as colorless crystals: mp 62–63 °C; ^1H NMR ($CDCl_3$) δ 3.94 (3H, s), 6.85 (1H, dd, J = 0.9, 2.3 Hz), 7.53 (1H, dt, J = 0.8, 8.7 Hz), 7.69 (1H, d, J = 2.3 Hz), 8.03 (1H, dd, J = 1.7, 8.7 Hz), 8.35 (1H, dd, J = 0.4, 1.7 Hz).



Scheme 3. [a]Synthesis of the compounds having various linkers between the oxadiazole and phenyl ring. [a]Reagents and conditions: (a) $POCl_3$, 3-(3-fluorophenyl)propionic acid, 100 °C, 59%; (b) 3-fluorobenzyl isocyanate, pyridine, rt, 65%; (c) PS–PPh_3, CCl_4, Et_3N, THF, 80 °C, 51%; (d) (1) CS_2, Et_3N, EtOH, reflux; (2) MeI, K_2CO_3, DMF, 45%; (e) *m*-CPBA, CH_3CN, 78%; (f) 3-fluorobenzyl alcohol, K_2CO_3, DMF, rt, 48%; (g) $(EtO)_3CCH_2Cl$, microwave, 160 °C, 75%; (h) 3-fluorobenzenethiol, K_2CO_3, DMF, rt, 14%.

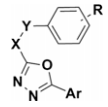
6.3. Methyl 3-bromo-1-benzofuran-5-carboxylate (7)

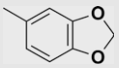
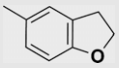
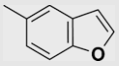
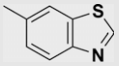
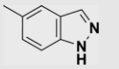
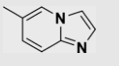
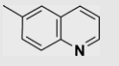
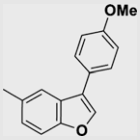
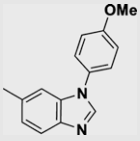
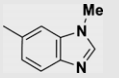
To a solution of **5** (35.0 g, 196.4 mmol) and 2,2-azobis(isobutyronitrile) (0.645 g, 3.93 mmol) in chlorobenzene was added *N*-bromosuccinimide (38.9 g, 216.0 mmol), and the mixture was stirred at 85 °C for 2 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane–EtOAc). The product was dissolved in CH_2Cl_2 (150 ml), cooled to 0 °C and Br_2 (10.1 ml, 196.4 mmol) slowly added. The mixture was stirred at room temperature for 1 h and the organic layer was washed with 1 M Na_2SO_3 in water and brine, dried over Na_2SO_4 and concentrated in vacuo. The obtained residue was suspended in THF (250 ml), cooled to 0 °C and slowly added a solution of KOH (85%) (13.0 g, 196.4 mmol) in MeOH (50 ml). The mixture was stirred at 0 °C for 15 min, EtOAc was added and the organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **7** (44.6 g, 89%) as colorless crystals: mp 119–120 °C; ^{1}H NMR ($CDCl_3$) δ 3.97 (3H, s), 7.53 (1H, dd, J = 0.8, 8.7 Hz), 7.72 (1H, s), 8.09 (1H, dd, J = 1.9, 8.7 Hz), 8.30 (1H, dd, J = 0.8, 1.9 Hz).

6.4. Methyl 3-(4-methoxyphenyl)-1-benzofuran-5-carboxylate (8)

A mixture of **7** (1.28 g, 5.00 mmol), 4-methoxyphenylboronic acid (0.836 g, 5.50 mmol), $Pd(PPh_3)_4$ (0.173 g, 0.150 mmol), Na_2CO_3 (1.17 g, 11.0 mmol), DME (15 ml) and H_2O (5 ml) was heated at reflux overnight under Ar atmosphere. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **8** (1.33 g, 94%) as colorless crystals: mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 3.95 (3H, s), 7.02–7.07

Table 1
GSK-3β inhibitory activity.



Compd	Ar	R	X	Y	GSK-3β IC_{50} (nM)
1		3-F-4-OMe	S	CH_2	65
20j		3-F-4-OMe	S	CH2	44
20a		H	S	CH_2	210
20b		3-F	S	CH_2	54
20c		4-OMe	S	CH_2	220
20e		3-Cl	S	CH_2	94
20d		2-Cl	S	CH_2	200
20f		4-Cl	S	CH_2	280
20g		3-CN	S	CH_2	91
20i		3-CF_3	S	CH_2	68
20h		3-CO_2Me	S	CH_2	630
20k		3-Cl-4-OMe	S	CH_2	13
20l		3-CN-4-OMe	S	CH_2	28
20m		3-CF_3-4-OMe	S	CH_2	5.7
21		3-F	CH_2	CH_2	340
23		3-F	NH	CH_2	330
26		3-F	O	CH_2	>10000
28		3-F	CH_2	S	680
20n		3-CF_3-4-OMe	5	CH_2	4.9
20o		3-CF_3-4-OMe	S	CH_2	3.1
20p		3-CF_3-4-OMe	S	CH_2	16
20q		3-CF_3-4-OMe	S	CH_2	6.5
20r		3-CF_3-4-OMe	S	CH_2	18
20s		3-CF_3-4-OMe	S	CH_2	25
20t		3-CF_3	S	CH_2	8.4
20u		3-CN	S	CH_2	3.5
20v		3-CF_3-4-OMe	S	CH_2	8.6
20w		3-CF_3	S	CH_2	2.5
20x		3-CN	S	CH_2	2.3
20y		3-CF_3	S	CH_2	9.4

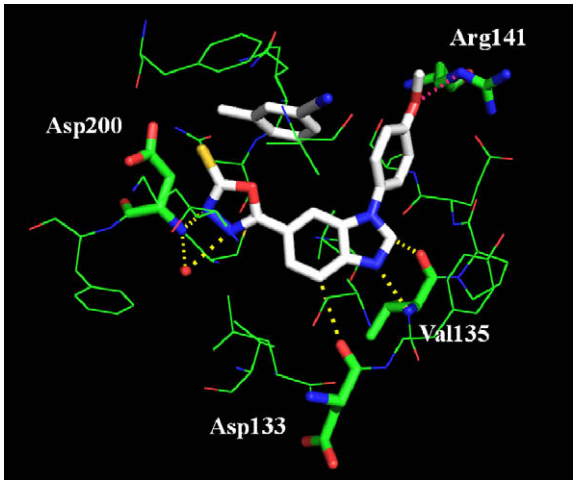


Figure 3. X-ray co-crystal structure of **20x** with GSK-3β. The figure was prepared with PyMOL.[26]

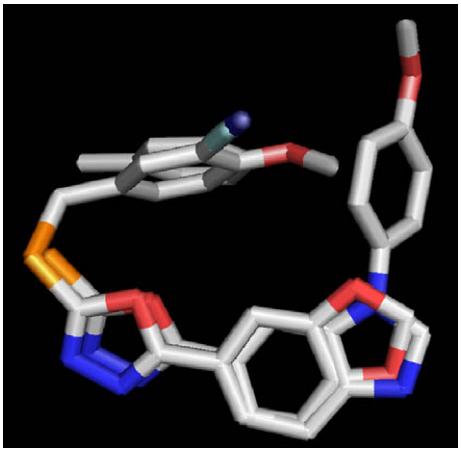


Figure 4. Overlay of **20x** and compound **1** in co-crystal. The figure was prepared with PyMOL.[26]

Table 2
Kinase selectivity of compound **20x**

Kinase	IC50 (μM)
EGFR	>10
HER2	>10
Src	>10
Lck	>10
Tie-2	>10
c-Kit	>10
c-Met	>10
VEGFR	>10
FGFR-3	>10
Aurora-B	>10
MEK1	>10
BRAF	>10
ERK1	>10
PKA	>10
CDK1	4.6
CDK2	>10
CDK5	>10
p38α	>10
JNK1	>10
IKKβ	>10
MEKK-1	8.1
PKCθ	3.5
CK1δ	>10

Table 3
Pharmacokinetic parameters of compounds **20x**[a]

Compd	CL_{total}, (mL/h/kg)	V_{dss} (mL/kg)	AUC_{po}(ng h/ml)	F[b] (%)
20x	2135	1453	7.9	1.7

[a] Rats were given intravenously ai 0.1 mg/kg, and orally at 1.0 mg/kg.
[b] Bioavailability.

(2H, m), 7.54–7.60 (3H, m), 7.78 (1H, s), 8.07 (1H, dd, J = 1.9, 8.7 Hz), 8.53 (1H, d, J = 1.9 Hz); Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.57; H, 5.19.

6.5. Methyl 3-fluoro-4-nitrobenzoate (10)

AcCl (7.66 ml, 108 mmol) was added dropwise to MeOH (150 ml) and **9** (5.01 g, 27.1 mmol) in MeOH (50 ml) was added to the solution, and the mixture was heated at reflux overnight. The reaction mixture was cooled to room temperature and diluted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo to give **10** (4.97 g, 99%) as a pale green solid: ^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s), 7.93–7.96 (1H, m), 7.97–7.99 (1H, m), 8.07–8.16 (1H, m).

6.6. Methyl 3-[(4-methoxyphenyl)amino]-4-nitrobenzoate (11a)

To a solution of **10** (10.0 g, 54.0 mmol) in dimethyl sulfoxide (300 ml) was added 4-methoxyaniline (13.3 g, 108 mmol) and the mixture was stirred at 70 °C for 3.5 h. The reaction mixture was diluted with EtOAc, and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from hexane–THF to give **11a** (12.9 g, 79%) as red crystals: ^{1}H NMR (DMSO-d_6) δ 3.80 (6H, s), 7.04 (2H, d, J = 8.9 Hz), 7.23–7.31 (3H, m), 7.49–7.53 (1H, m), 8.21 (1H, d, J = 8.9 Hz), 9.39 (1H, s).

6.7. Methyl 3-(methylamino)-4-nitrobenzoate (11b)

The compound **11b** was prepared in a manner similar to that described for **11a** to yield an orange solid (86%): ^{1}H NMR (DMSO-d_6) δ 3.00 (3H, d, J = 5.1 Hz), 3.89 (3H, s), 7.15 (1H, dd, J = 1.7, 8.9 Hz), 7.47 (1H, d, J = 1.7 Hz), 8.18 (1H, d, J = 8.9 Hz), 8.20–8.28 (1H, m).

6.8. Methyl 4-amino-3-[(4-methoxyphenyl)amino]benzoate (12a)

To a solution of $Na_2S_2O_4$ (40.7 g, 233.6 mmol) in water (190 ml) was cooled to 0 °C and added **11a** (3.39 g, 11.1 mmol) in THF–EtOH (150–75 ml), and the mixture was stirred at room temperature for 2.5 h. To the reaction mixture was added saturated aqueous $NaHCO_3$ solution and the mixture extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane–EtOAc) to give **12a** (2.14 g, 71%) as a pale brown solid: ^{1}H NMR (DMSO-d_6) δ 3.69 (3H, s), 3.70 (3H, s), 5.59 (2H, s), 6.70 (1H, d, J = 8.3 Hz), 6.76–6.86 (5H, m), 7.38 (1H, dd, J = 2.1, 8.3 Hz), 7.49 (1H, d, J = 2.1 Hz).

6.9. Methyl 4-amino-3-(methylamino)benzoate (12b)

The compound **12b** was prepared in a manner similar to that described for **12a** to yield a pale red solid (66%): ^{1}H NMR (DMSO-d_6) δ 2.74 (3H, s), 3.73 (3H, s), 4.82 (1H, s), 5.35 (2H, s), 6.54 (1H, d, J = 8.1 Hz), 6.92 (1H, d, J = 1.9 Hz), 7.16 (1H, dd, J = 1.9, 8.1 Hz).

6.10. Methyl 1-(4-methoxyphenyl)-1*H*-benzimidazole-6-carboxylate (13a)

A solution of **12a** (1.05 g, 3.84 mmol) in formic acid (10.7 ml) was stirred overnight at 100 °C. The solvent was concentrated in vacuo and the residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **13a** (1.03 g, 95%) as a pale red solid: ^{1}H NMR ($CDCl_3$) δ 3.91 (3H, s), 3.93 (3H, s), 7.10 (2H, d, J = 9.0 Hz), 7.43 (2H, d, J = 8.9 Hz), 7.88 (1H, d, J = 8.5 Hz), 8.04 (1H, dd, J = 1.6, 8.6 Hz), 8.16–8.18 (2H, m).

6.11. Methyl 1-methyl-1*H*-benzimidazole-6-carboxylate (13b)

The compound **13b** was prepared in a manner similar to that described for **13a** to yield a colorless solid (95%): ^{1}H NMR (DMSO-d_6) δ 3.89 (3H, s), 3.92 (3H, s), 7.72–7.76 (1H, m), 7.81–7.88 (1H, m), 8.19–8.25 (1H, m), 8.39 (1H, s).

6.12. *tert*-Butyl 2-(1,3-benzothiazol-6-ylcarbonyl)hydrazine carboxy late (15)

To a solution of **14** (4.48 g, 25.0 mmol), *tert*-butyl carbazate (3.63 g, 27.5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.75 g, 30.0 mmol) in DMF (50 ml) was added 1-hydroxybenzotriazole (4.05 g, 30.0 mmol) and the mixture stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc–THF) to give **15** (5.51 g, 75%) as a colorless solid: mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.52 (9H, s), 6.81 (1H, br s), 7.89 (1H, dd, J = 1.7, 8.7 Hz), 8.12 (1H, d, J = 8.7 Hz), 8.40 (1H, br s), 8.47 (1H, dd, J = 0.6, 1.7 Hz), 9.12 (1H, s); Anal. Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32. Found: C, 53.10; H, 5.13; N, 14.38.

6.13. 1,3-Benzothiazole-6-carbohydrazide trifluoroacetate (16)

A solution of **15** (5.28 g, 18.0 mmol) in trifluoroacetic acid (20 ml) was stirred at room temperature for 1 h. The solvent was concentrated and the residue was recrystallized from EtOH to give **16** (5.18 g, 94%) as colorless crystals: mp 154–155 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (1H, dd, J = 1.7, 8.7 Hz), 8.22 (1H, d, J = 0.6, 8.7 Hz), 8.74 (1H, dd, J = 0.6, 1.7 Hz), 9.60 (1H, s), 11.24 (1H, br s); Anal. Calcd for $C_{10}H_8F_3N_3O_3S$: C, 39.09; H, 2.62; N, 13.68. Found: C, 39.10; H, 2.50; N, 13.75.

6.14. 2,3-Dihydro-1-benzofuran-5-carbohydrazide (18a)

To a solution of **5** (3.56 g, 20.0 mmol) in MeOH (35 ml) was added hydrazine hydrate (4.85 ml, 100 mmol) and the mixture was heated at reflux for 2 days. After cooling to room temperature, the solvent was concentrated in vacuo, and the residue was washed with hexane–EtOH to give **18a** (3.03 g, 85%) as a colorless solid: mp 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 3.24 (2H, t, J = 8.7 Hz), 4.06 (2H, d, J = 4.1 Hz), 4.64 (2H, t, J = 8.7 Hz), 6.79 (1H, d, J = 8.3 Hz), 7.19 (1H, br s), 7.50–7.53 (1H, m), 7.64–7.65 (1H, m); Anal. Calcd for $C_9H_{10}N_2O_2$: C, 60.66; H, 5.66; N, 15.72. Found: C, 60.64; H, 5.71; N, 15.78.

Compounds **18b–h** were prepared in a manner similar to that described for **18a**.

6.15. 1-Benzofuran-5-carbohydrazide (18b)

Colorless solid (96%): ^{1}H NMR (DMSO-d_6) δ 4.47 (2H, br s), 7.05 (1H, dd, J = 1.1, 2.3 Hz), 7.65 (1H, d, J = 8.7 Hz), 7.80 (1H, dd, J = 1.5, 8.7 Hz), 8.07 (1H, d, J = 2.3 Hz), 8.15 (1H, d, J = 1.5 Hz), 9.76 (1H, br s).

6.16. 1*H*-Indazole-5-carbohydrazide (18c)

Colorless solid (93%): mp 251–252 °C; ^{1}H NMR (DMSO-d_6) δ 4.48 (2H, s), 7.56 (1H, ddd, J = 0.8, 0.9, 8.9 Hz), 7.83 (1H, dd, J = 1.5, 8.9 Hz), 8.19 (1H, d, J = 0.8 Hz), 8.29 (1H, dd, J = 0.9, 1.5 Hz), 9.74 (1H, s), 13.27 (1H, s); Anal. Calcd for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.50; H, 4.52; N, 31.85.

6.17. Imidazo[1,2-*a*]pyridine-6-carbohydrazide (18d)

Colorless solid (79%): 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 4.54 (2H, br s), 7.58–7.65 (3H, m), 8.07 (1H, s), 9.09 (1H, t, J = 1.5 Hz), 9.87 (1H, s).

6.18. Quinoline-6-carbohydrazide (18e)

Colorless solid (97%): mp 193–194 °C; ^{1}H NMR (DMSO-d_6) δ 4.60 (2H, br s), 7.61 (1H, dd, J = 4.1, 8.3 Hz), 8.06 (1H, d, J = 8.9 Hz), 8.15 (1H, dd, J = 1.9, 8.9 Hz), 8.45–8.49 (2H, m), 8.98 (1H, dd, J = 1.7, 4.1 Hz), 10.01 (1H, br s); Anal. Calcd for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.16; H, 4.93; N, 22.46.

6.19. 3-(4-Methoxyphenyl)-1-benzofuran-5-carbohydrazide (18f)

Colorless solid (92%): mp 184–185 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s), 4.51 (2H, s), 7.07–7.12 (2H, m), 7.69–7.75 (3H, m), 7.88 (1H, dd, J = 1.5, 8.7 Hz), 8.35 (1H, d, J = 1.5 Hz), 8.36 (1H, s), 9.90 (1H, br s); Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.22; H, 5.16; N, 9.86.

6.20. 1-(4-Methoxyphenyl)-1*H*-benzimidazole-6-carbohydrazide (18g)

Colorless solid (30%): mp 196–197 °C; ^{1}H NMR ($CDCl_3$) δ 3.91 (3H, s), 4.12 (2H, br s), 7.06–7.11 (2H, m), 7.38–7.44 (2H, m), 7.45 (1H, br s), 7.63 (1H, dd, J = 1.9, 8.7 Hz), 7.89 (1H, dd, J = 0.8, 8.7 Hz), 7.97 (1H, dd, J = 0.8, 1.9 Hz), 8.15 (1H, s); Anal. Calcd for $C_{15}H_{14}N_4O_2{\cdot}H_2O$: C, 59.99; H, 5.37; N, 18.66. Found: C, 59.96; H, 5.37; N, 18.61.

6.21. 1-Methyl-1*H*-benzimidazole-6-carbohydrazide (18h)

Colorless solid (65%): ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 4.52 (br s, 2H) 7.65–7.69 (m, 1H) 7.70–7.76 (m, 1H) 8.09–8.13 (m, 1H), 8.30 (s, 1H) 9.76 (s, 1H).

6.22. 5-(2,3-Dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole-2-thiol (19a)

To a solution of **18a** (535 mg, 3.00 mmol) in EtOH (5 ml) were added carbon disulfide (0.397 ml, 6.60 mmol) and triethyl amine (0.469 ml, 3.30 mmol) and the mixture was heated at reflux overnight. The reaction mixture was diluted with EtOAc and the organic layer was washed with 0.1 M HCl and brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the obtained residue was recrystallized from hexane–EtOAc to give **19a** (529 mg, 80%) as a pale yellow crystals: mp 219–221 °C; ^{1}H NMR ($CDCl_3$) δ 3.28 (2H, t, J = 8.9 Hz), 4.28 (2H, t, J = 8.9 Hz), 6.88 (1H, d, J = 8.3 Hz), 7.71–7.75 (1H, m), 7.76–7.77 (1H, m), 10.85 (1H, br s); Anal. Calcd for $C_{10}H_8N_2O_2S$: C, 54.53; H, 3.66; N, 12.72. Found: C, 54.62; H, 3.70; N, 12.73.

6.23. 5-(1-Benzofuran-5-yl)-1,3,4-oxadiazole-2-thiol (19b)

To a solution of **18b** (1.50 g, 8.51 mmol) in EtOH (60 ml) were added KOH (85%) (0.72 g, 12.8 mmol) and carbon disulfide

(15.3 ml, 25.5 mmol), and the mixture was stirred at room temperature for 5 h. After the solvent was concentrated in vacuo, the residue was dissolved in DMF (30 ml), poured into a microwave tube and heated by microwave irradiation for 1 min at 150 °C. The reaction mixture was cooled, 1 M HCl added and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc to give **19b** (0.66 g, 36%) as colorless crystals: ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, dd, J = 0.9, 2.3 Hz), 7.62 (1H, d, J = 8.7 Hz), 7.74 (1H, d, J = 2.3 Hz), 7.90 (1H, dd, J = 1.7, 8.7 Hz), 8.22 (1H, d, J = 1.7 Hz), 10.34 (1H, br s).

The compounds **19c–g** were prepared in a manner similar to that described for **19a**.

6.24. 5-(1,3-Benzothiazol-6-yl)-1,3,4-oxadiazole-2-thiol (19c)

Pale yellow solid (83%): mp 274–275 °C; ^{1}H NMR (DMSO-d_6) δ 8.03 (1H, dd, J = 1.7, 8.7 Hz), 8.26 (1H, d, J = 0.6, 8.7 Hz), 8.81 (1H, dd, J = 0.6, 1.7 Hz), 9.59 (1H, s), 14.78 (1H, br s); Anal. Calcd for $C_9H_5N_3OS$: C, 45.94; H, 2.14; N, 17.86. Found: C, 45.81; H, 2.13; N, 17.72.

6.25. 5-(1*H*-Indazol-5-yl)-1,3,4-oxadiazole-2-thiol (19d)

Colorless solid (97%): mp 283–284 °C; ^{1}H NMR (DMSO-d_6) δ 7.71 (1H, m), 7.84 (1H, dd, J = 1.5, 8.9 Hz), 8.27 (1H, s), 8.37 (1H, dd, J = 0.9, 1.5 Hz), 13.48 (1H, br s), 14.67 (1H, br s); Anal. Calcd for $C_9H_6N_4OS$: C, 49.53; H, 2.77; N, 25.67. Found: C, 49.43; H, 2.85; N, 25.49.

6.26. 5-Imidazo[1,2-*a*]pyridin-6-yl-1,3,4-oxadiazole-2-thiol (19e)

Colorless solid (80%): ^{1}H NMR ($CDCl_3$) δ 1.15–1.20 (3H, t, J = 7.2 Hz), 3.07–3.14 (2H, q, J = 7.2 Hz), 7.59–7.72 (3H, m), 8.10 (1H, s), 9.23 (1H, t, J = 0.6 Hz).

6.27. 5-Quinolin-6-yl-1,3,4-oxadiazole-2-thiol (19f)

Colorless solid (51%): ^{1}H NMR (DMSO-d_6) δ 7.67 (1H, dd, J = 4.1, 8.3 Hz), 8.18 (2H, d, J = 1.1 Hz), 8.60–8.63 (2H, m), 9.03 (1H, dd, J = 1.7, 4.1 Hz), 14.92 (1H, br s).

6.28. 5-[3-(4-Methoxyphenyl)-1-benzofuran-5-yl]-1,3,4-oxadiazole-2-thiol (19g)

Colorless solid (87%): mp 204–205 °C; ^{1}H NMR ($CDCl_3$) δ 3.89 (3H, s), 7.03–7.08 (2H, m), 7.53–7.58 (2H, m), 7.65 (1H, dd, J = 0.8, 8.7 Hz), 7.82 (1H, s), 8.95 (1H, dd, J = 1.7, 8.7 Hz), 8.37 (1H, dd, J = 0.4, 1.7 Hz), 10.63 (1H, br s); Anal. Calcd for $C_{17}H_{12}N_2O_3S$: C, 62.95; H, 3.73; N, 8.64. Found: C, 62.90; H, 3.82; N, 8.52.

6.29. 5-[1-(4-Methoxyphenyl)-1*H*-benzimidazol-6-yl]-1,3,4-oxadiazole-2-thiol (19h)

To a solution of **18g** (1.09 g, 3.86 mmol) in EtOH (20 ml) were added KOH (85%) (0.32 g, 5.79 mmol) and carbon disulfide (2.31 ml, 38.6 mmol), and the mixture was stirred overnight at room temperature. After the solvent was concentrated in vacuo, the residue was added to DMF (15 ml) and stirred overnight at 100 °C. The reaction mixture was cooled, added 1 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was washed with water to give **19h** (0.66 g, 53%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 3.87 (3H, s), 7.22 (2H, d, J = 8.9 Hz), 7.65 (2H, d, J = 8.9 Hz), 7.77–7.86 (2H, m), 7.89–7.95 (1H, m), 8.66 (1H, s).

6.30. 2-(Benzylsulfanyl)-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (20a)

To a solution of **19a** (0.20 g, 0.91 mmol) and benzyl bromide (0.11 ml, 0.91 mmol) in H_2O (4 ml) was added 1 M NaOH (1.00 ml, 1.00 mmol), and the mixture was stirred overnight at room temperature. Precipitated solids were collected and recrystallized from hexane–EtOAc to give **20a** (0.12 g, 51%) as colorless crystals: 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.8 Hz), 4.50 (2H, s), 4.66 (2H, t, J = 8.8 Hz), 6.85 (1H, d, J = 8.7 Hz), 7.28–7.38 (3H, m), 7.42–7.48 (2H, m), 7.75 (1H, dd, J = 1.7, 8.3 Hz), 7.84 (1H, d, J = 1.7 Hz); Anal. Calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 65.49; H, 4.44; N, 8.93.

6.31. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-[(3-fluorobenzyl)sulfanyl]-1,3,4-oxadiazole (20b)

The compound **20b** was prepared in a manner similar to that described for **20a**.

Colorless solid (74%): mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.8 Hz), 4.48 (2H, s), 4.66 (2H, t, J = 8.8 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.95–7.03 (1H, m), 7.15–7.35 (3H, m), 7.72–7.78 (1H, m), 7.82–7.85 (1H, m); Anal. Calcd for $C_{17}H_{13}FN_2O_2S$: C, 62.18; H, 3.99; N, 8.53. Found: C, 62.04; H, 3.96; N, 8.55.

6.32. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-[(4-methoxybenzyl)sulfanyl]-1,3,4-oxadiazole (20c)

To a solution of **19a** (0.20 g, 0.91 mmol) and K_2CO_3 (0.15 g, 1.09 mmol) in DMF (5 ml) was added 4-methoxybenzyl chloride (0.18 ml, 1.36 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **20c** (0.19 g, 61%) as colorless crystals: mp 121–124 °C; ^{1}H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.8 Hz), 3.79 (3H, s), 4.46 (2H, s), 4.66 (2H, t, J = 8.8 Hz), 6.83–6.90 (3H, m), 7.37 (2H, d, J = 8.7 Hz), 7.72–7.79 (1H, m), 7.81–7.85 (1H, m); Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.45; H, 4.71; N, 8.19.

6.33. 2-[(2-Chlorobenzyl)sulfanyl]-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (20d)

The compound **20d** was prepared in a manner similar to that described for **20a**.

Pale red solid (48%): mp 140–141 °C; ^{1}H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.9 Hz), 4.61 (2H, s), 4.66 (2H, t, J = 8.9 Hz), 6.85 (1H, d, J = 8.7 Hz), 7.19–7.29 (2H, m), 7.38–7.43 (1H, m), 7.59–7.64 (1H, m), 7.73–7.77 (1H, m), 7.82–7.85 (1H, m); Anal. Calcd for $C_{17}H_{13}ClN_2O_2S$: C, 59.21; H, 3.80; N, 8.12. Found: C, 59.09; H, 3.68; N, 8.12.

6.34. 2-[(3-Chlorobenzyl)sulfanyl]-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (20e)

To a solution of **19a** (50 μmol) in DMF (0.5 ml) was added 3-chlorobenzyl chloride (62.5 μmol) in DMF (0.5 ml), and the mixture was stirred at 60 °C for 24 h. The reaction mixture was filtered and the filtrate was purified by HPLC to give **20e** (13.4 mg, 78%) as a colorless amorphous solid: HPLC purity: >99%; LC–MS (ESI^+) 345.

The oxadiazoles **20f–h** were prepared in a manner similar to that described for **20e**.

6.35. 2-[(4-Chlorobenzyl)sulfanyl]-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (20f)

Colorless amorphous solid (74%): HPLC purity: >99%; LC–MS (ESI^+) 345.

6.36. 3-({[5-(2,3-Dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}methyl)benzo-nitrile (20g)

Colorless amorphous solid (82%): HPLC purity; >99%; LC–MS (ESI^+) 336.

6.37. Methyl 3-({[5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}methyl)benzoate (20h)

Colorless amorphous solid (79%): HPLC purity; >96%; LC–MS (ESI^+) 369.

6.38. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-{[3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazole (20i)

The compound **20i** was prepared in a manner similar to that described for **20a**.

Colorless solid (81%): mp 88–90 °C; ^1H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.8 Hz), 4.53 (2H, s), 4.66 (2H, t, J = 8.8 Hz), 6.85 (1H, d, J = 8.7 Hz), 7.42–7.50 (1H, m), 7.52–7.59 (1H, m), 7.66–7.77 (3H, m), 7.80–7.84 (1H, m); Anal. Calcd for $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40. Found: C, 57.17; H, 3.34; N, 7.41.

6.39. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-[(3-fluoro-4-methoxybenzyl)sulfanyl]-1,3,4-oxadiazole (20j)

To a solution of **18a** (356 mg, 2.00 mmol) in EtOH (15 ml) were added KOH (85%) (198 mg, 3.00 mmol) and carbon disulfide (0.18 ml, 3.00 mmol), and the mixture was stirred at room temperature for 1 h. After the solvent was concentrated in vacuo, the residue was dissolved in DMF (10 ml), poured into a microwave tube and heated under microwave irradiation for 0.5 min at 150 °C. The reaction mixture was cooled, added 3-fluoro-4-methoxybenzyl chloride (698 mg, 4.00 mmol) and the mixture was stirred at room temperature for 2 h. The organic layer was diluted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc to give **20j** (478 mg, 67%) as colorless crystals: mp 121–122 °C; ^1H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.9 Hz), 3.87 (3H, s), 4.43 (2H, s), 4.66 (2H, t, J = 8.9 Hz), 6.84–6.93 (2H, m), 7.15–7.22 (2H, m), 7.74–7.77 (1H, m), 7.83–7.84 (1H, m); Anal. Calcd for $C_{18}H_{15}FN_2O_3S$: C, 60.32; H, 4.22; N, 7.82. Found: C, 60.50; H, 4.47; N, 7.92.

The compounds **20k–o** were prepared in a manner similar to that described for **20c**.

6.40. 2-[(3-Chloro-4-methoxybenzyl)sulfanyl]-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (20k)

Colorless solid (87%): mp 136–137 °C; ^1H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.9 Hz), 3.89 (3H, s), 4.42 (2H, s), 4.66 (2H, t, J = 8.9 Hz), 6.84–6.89 (2H, m), 7.33 (1H, dd, J = 2.3, 8.5 Hz), 7.47 (1H, d, J = 2.3 Hz), 7.74–7.77 (1H, m), 7.82–7.84 (1H, m); Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47. Found: C, 57.66; H, 4.00; N, 7.44.

6.41. 5-({[5-(2,3-Dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazol-2-yl]sulfanyl}methyl)-2-methoxybenzonitrile (20l)

Colorless solid (72%): mp 176–177 °C; ^1H NMR ($CDCl_3$) δ 3.28 (2H, t, J = 8.8 Hz), 3.92 (3H, s), 4.43 (2H, s), 4.67 (2H, t, J = 8.8 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.93 (1H, d, J = 9.4 Hz), 7.66–7.70 (2H, m), 7.73–7.78 (1H, m), 7.80–7.85 (1H, m); Anal. Calcd for $C_{19}H_{15}N_3O_3S$: C, 62.45; H, 4.14; N, 11.50. Found: C, 62.45; H, 4.08; N, 11.71.

6.42. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-{[4-methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazole (20m)

Colorless solid (81%): ^1H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.9 Hz), 3.89 (3H, s), 4.46 (2H, s), 4.66 (2H, t, J = 8.9 Hz), 6.85 (1H, d, J = 8.3 Hz), 6.96 (1H, d, J = 8.3 Hz), 7.60–7.66 (2H, m), 7.72–7.78 (1H, m), 7.81–7.84 (1H, m); LC–MS (ESI^+) 409; Anal. Calcd for $C_{25}H_{19}F_3N_4O_3S$: C, 55.88; H, 3.70; N, 6.86. Found: C,55.73; H, 3.87; N, 6.57.

6.43. 2-(1-Benzofuran-5-yl)-5-{[4-methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazole (20n)

Colorless solid (100%): mp 135–136 °C; ^1H NMR ($CDCl_3$) δ 3.89 (3H, s), 4.49 (2H, s), 6.86 (1H, dd, J = 0.8, 2.2 Hz), 6.96 (1H, d, J = 8.3 Hz), 7.58–7.68 (3H, m), 7.71 (1H, d, J = 2.2 Hz), 7.96 (1H, dd, J = 1.6, 8.7 Hz), 8.24 (1H, d, J = 1.6 Hz); Anal. Calcd for $C_{19}H_{13}F_3N_2O_3S$: C, 56.16; H, 3.22; N, 6.89. Found: C, 56.12; H, 3.31; N, 6.78.

6.44. 6-(5-{[4-Methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)-1,3-benzothiazole (20o)

Colorless solid (82%): mp 145–146 °C; ^1H NMR ($CDCl_3$) δ 3.89 (3H, s), 4.52 (2H, s), 6.97 (1H, d, J = 8.5 Hz), 7.63–7.68 (2H, m), 8.16 (1H, dd, J = 1.7, 8.5 Hz), 8.24 (1H, dd, J = 0.6, 8.5 Hz), 8.62 (1H, dd, J = 0.6, 1.7 Hz), 9.13 (1H, s); Anal. Calcd for $C_{18}H_{12}F_3N_3O_2S_2$: C, 51.06; H, 2.86; N, 9.92. Found: C, 50.84; H, 2.89; N, 9.93.

6.45. 5-(5-{[4-Methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)-1*H*-indazole (20p)

To a solution of **19d** (218 mg, 1.00 mmol) and 1 M NaOH (1.00 ml, 1.00 mmol) in DMF (5 ml) was added 4-methoxy-3-(trifluoromethyl)benzyl bromide (269 mg, 1.00 mmol) at room temperature, and the mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (THF) and the product was recrystallized from MeOH/H_2O to give **20p** (310 mg, 76%) as colorless crystals: mp 136–137 °C; ^1H NMR ($CDCl_3$) δ 3.89 (3H, s), 4.50 (2H, s), 6.97 (1H, d, J = 8.3 Hz), 7.60–7.68 (3H, m), 8.07 (1H, dd, J = 1.5, 8.9 Hz), 8.19 (1H, d, J = 0.8 Hz), 8.40 (1H, dd, J = 0.8, 1.5 Hz), 10.32 (1H, br s); Anal. Calcd for $C_{18}H_{13}F_3N_4O_2S{\cdot}H_2O$: C, 52.05; H, 3.40; N, 13.49. Found: C, 52.13; H, 3.57; N, 13.46.

6.46. 6-(5-{[4-Methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)imidazo[1,2-*a*]pyridine (20q)

To a solution of **18d** (1.00 g, 5.67 mmol), carbon disulfide (0.85 ml, 14.2 mmol) and Et_3N (0.97 ml, 7.09 mmol) in EtOH (50 ml) was heated at reflux overnight. The reaction mixture was cooled to room temperature and the solvent was concentrated in vacuo. To the residue were added DMF (10 ml) and 4-methoxy-3-(trifluoromethyl)benzyl bromide (1.83 g, 6.00 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **20q** (1.67 g, 71%) as colorless crystals: mp 146–

147 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (3H, s), 4.62 (2H, s), 7.25 (2H, d, J = 8.4 Hz), 7.67–7.79 (4H, m), 8.11 (1H, s), 9.37 (1H, t, J = 1.2 Hz); Anal. Calcd for $C_{18}H_{13}F_3N_4O_2S{\cdot}0.1H_2O$: C, 52.96; H, 3.26; N, 13.72. Found: C, 52.94; H, 3.28; N, 13.42.

6.47. 6-(5-{[4-Methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)quinoline (20r)

The compound **20r** was prepared in a manner similar to that described for **20p**.

Colorless solid (74%): mp 130–131 °C; ^{1}H NMR ($CDCl_3$) δ 3.89 (3H, s), 4.53 (2H, s), 6.98 (1H, d, J = 8.5 Hz), 7.50 (1H, dd, J = 4.1, 8.3 Hz), 7.64–7.69 (2H, m), 8.20–8.27 (2H, m), 8.32 (1H, dd, J = 1.9, 8.9 Hz), 8.46 (1H, d, J = 1.9 Hz), 9.01 (1H, dd, J = 1.7, 4.1 Hz); Anal. Calcd for $C_{20}H_{14}F_3N_3O_2S$: C, 57.55; H, 3.38; N, 10.07. Found: C, 57.44; H, 3.35; N, 10.11.

The compounds **20s–x** were prepared in a manner similar to that described for **20c**.

6.48. 2-[3-(4-Methoxyphenyl)-1-benzofuran-5-yl]-5-{[4-methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazole (20s)

Colorless solid (91%): mp 162–163 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (6H, s), 4.50 (2H, s), 6.96 (1H, d, J = 8.1 Hz), 7.02–7.07 (2H, m), 7.55–7.60 (2H, m), 7.61–7.66 (3H, m), 7.80 (1H, s), 8.00 (1H, dd, J = 1.7, 8.7 Hz), 8.43 (1H, dd, J = 0.6, 1.7 Hz). Anal. Calcd for $C_{26}H_{19}F_3N_2O_4S$: C, 60.93; H, 3.74; N, 5.47. Found: C, 60.95; H, 3.95; N, 5.36.

6.49. 2-[3-(4-Methoxyphenyl)-1-benzofuran-5-yl]-5-{[3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazole (20t)

Colorless solid (94%); mp 119–120 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 4.56 (2H, s), 7.02–7.07 (2H, m), 7.46 (1H, t, J = 7.7 Hz), 7.54–7.59 (3H, m), 7.63 (1H, dd, J = 0.6, 8.7 Hz), 7.69–7.74 (2H, m), 7.80 (1H, s), 7.99 (1H, dd, J = 1.7, 8.7 Hz), 8.42 (1H, dd, J = 0.6, 1.7 Hz); Anal. Calcd for $C_{25}H_{17}F_3N_2O_3S$: C, 62.23; H, 3.55; N, 5.81. Found: C, 62.23; H, 3.66; N, 5.77.

6.50. 3-[({5-[3-(4-Methoxyphenyl)-1-benzofuran-5-yl]-1,3,4-oxadiazol-2-yl}sulfanyl)methyl]benzonitrile (20u)

Colorless solid (87%); 145–146 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 4.52 (2H, s), 7.03–7.07 (2H, m), 7.45 (1H, dt, J = 0.4, 7.7 Hz), 7.54–7.60 (3H, m), 7.63 (1H, dd, J = 0.6, 8.7 Hz), 7.74–7.81 (3H, m), 7.99 (1H, dd, J = 1.7, 8.7 Hz), 8.41 (1H, dd, J = 0.6, 1.7 Hz); Anal. Calcd for $C_{25}H_{17}N_3O_3S$: C, 68.32; H, 3.90; N, 9.56. Found: C, 68.36; H, 3.87; N, 9.56.

6.51. 1-(4-Methoxyphenyl)-6-(5-{[4-methoxy-3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)-1*H*-benzimidazole (20v)

Colorless solid (34%): mp 163–164 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s), 3.91 (3H, s), 4.49 (2H, s), 6.95 (1H, d, J = 8.3 Hz), 7.11 (2H, d, J = 9.0 Hz), 7.43 (2H, d, J = 9.0 Hz), 7.59–7.67 (2H, m), 7.95 (2H, d, J = 1.0 Hz), 8.09 (1H, t, J = 1.0 Hz), 8.16 (1H, s); Anal. Calcd for $C_{25}H_{19}F_3N_4O_3S$: C, 58.59; H, 3.74; N, 10.93. Found: C, 58.38; H, 3.87; N, 10.69.

6.52. 1-(4-Methoxyphenyl)-6-(5-{[3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)-1*H*-benzimidazole (20w)

Colorless solid (61%): mp 174–175 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (3H, s), 4.55 (2H, s), 7.11 (2H, d, J = 8.9 Hz), 7.40–7.49 (3H, m), 7.52–7.58 (1H, m), 7.66–7.74 (2H, m), 7.95 (2H, d, J = 0.9 Hz), 8.08 (1H, t, J = 0.9 Hz), 8.16 (1H, s); Anal. Calcd for $C_{24}H_{17}F_3N_4O_2S$: C, 59.75; H, 3.55; N, 11.61. Found: C, 59.73; H, 3.52; N, 11.54.

6.53. 3-[({5-[1-(4-Methoxyphenyl)-1*H*-benzimidazol-6-yl]-1,3,4-oxadiazol-2-yl}sulfanyl)methyl]benzonitrile (20x)

Colorless solid (24%): mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (3H, s), 4.51 (2H, s), 7.11 (2H, d, J = 8.9 Hz), 7.39–7.48 (3H, m), 7.55–7.61 (1H, m), 7.71–7.80 (2H, m), 7.95 (2H, s), 8.05–8.09 (1H, m), 8.16 (1H, s); Anal. Calcd for $C_{24}H_{17}N_5O_2S$: C, 65.59; H, 3.90; N, 15.94. Found: C, 65.34; H, 3.66; N, 16.04.

6.54. 1-Methyl-6-(5-{[3-(trifluoromethyl)benzyl]sulfanyl}-1,3,4-oxadiazol-2-yl)-1*H*-benzimidazole (20y)

To a solution of **18h** (0.20 g, 1.05 mmol) and Et_3N (0.18 ml, 1.26 mmol) in EtOH was added carbon disulfide (0.16 ml, 2.63 mmol), and the mixture was heated at reflux overnight. The mixture was cooled to 0 °C, added KOH (85%) (58.9 g, 1.05 mmol) and 3-(trifluoromethyl)benzyl chloride (0.16 ml, 1.03 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **20y** (49.9 mg, 12%) as a colorless solid: mp 126–127 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (3H, s), 4.57 (2H, s), 7.44–7.51 (1H, m), 7.54–7.60 (1H, m), 7.68–7.77 (2H, m), 7.85–7.93 (2H, m), 7.99 (1H, br s), 8.09 (1H, br s); Anal. Calcd for $C_{18}H_{13}F_3N_4OS$: C, 55.38; H, 3.86; N, 14.35. Found: C, 55.18; H, 3.45; N, 14.11.

6.55. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-[2-(3-fluorophenyl)ethyl]-1,3,4-oxadiazole (21)

To a solution of **18a** (0.50 g, 2.81 mmol) and 3-(3-fluorophenyl)propionic acid (0.47 g, 2.81 mmol) was added $POCl_3$ (3 ml), and the mixture was stirred overnight at 100 °C. The reaction mixture was cooled to room temperature, poured into a mixture of cold water and saturated aqueous $NaHCO_3$ solution, and the water layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **21** (0.51 g, 59%) as a colorless solid: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ *3.07–3.22* (4H, m), 3.28 (2H, t, J = 8.8 Hz), 4.66 (2H, t, J = 8.8 Hz), 6.86 (1H, d, J = 8.7 Hz), 6.89–7.06 (3H, m), 7.19–7.32 (1H, m), 7.77 (1H, dd, J = 1.7, 8.3 Hz), 7.86 (1H, d, J = 1.7 Hz); LC–MS (ESI$^+$) 311; Anal. Calcd for $C_{18}H_{15}FN_2O_2$: C, 69.67; H, 4.87; N, 9.03. Found: C, 69.66; H, 4.98; N, 9.01.

6.56. 2-(2,3-Dihydro-1-benzofuran-5-ylcarbonyl)-*N*-(3-fluorobenzyl)hydrazinecarboxamide (22)

To a solution of **18a** (0.50 g, 2.81 mmol) in pyridine (5 ml) was added 3-fluorobenzyl isocyanate (0.85 g, 5.61 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The product was washed with EtOAc to give **22** (0.60 g, 65%) as a colorless solid: mp 200–201 °C; ^{1}H NMR (DMSO-d_6) δ 3.21 (2H, t, J = 8.7 Hz), 4.25 (2H, d, J = 6.0 Hz), 4.60 (2H, t, J = 8.7 Hz), 6.83 (1H, d, J = 8.3 Hz), 6.96–7.17 (4H, m), 7.27–7.40 (1H, m), 7.68–7.76 (1H, m), 7.81 (1H, s), 7.96 (1H, s), 9.99 (1H, s). Anal. Calcd for $C_{17}H_{16}FN_3O_3$: C, 62.00; H, 4.90; N, 12.76. Found: C, 61.92; H, 4.84; N, 12.69.

6.57. 5-(2,3-Dihydro-1-benzofuran-5-yl)-*N*-(3-fluorobenzyl)-1,3,4-oxadiazol-2-amine (23)

To a solution of **22** (0.20 g, 2.81 mmol), PS–PPh_3 (2.15 mmol/g, 0.76 g, 1.64 mmol), Et_3N (0.17 ml, 1.22 mmol) in THF (5 ml) was added CCl_4 (0.12 ml, 1.22 mmol), and the mixture was stirred at 80 °C for 4 h. The reaction mixture was filtered and the filtrate was partitioned between water and EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc to give **23** (97 mg, 51%) as colorless crystals: mp 180–181 °C; ^{1}H NMR ($CDCl_3$) δ 3.25 (2H, t, J = 8.9 Hz), 4.56–4.68 (4H, m), 5.36 (1H, br s), 6.82 (1H, d, J = 8.3 Hz), 6.95–7.05 (1H, m), 7.07–7.22 (2H, m), 7.27–7.38 (1H, m), 7.59–7.66 (1H, m), 7.75 (1H, br s); Anal. Calcd for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.35; H, 4.56; N, 13.40.

6.58. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-(methylsulfanyl)-1,3,4-oxadiazole (24)

The compound **24** was prepared in a manner similar to that described for **20y**.

Colorless solid (45%): ^{1}H NMR ($CDCl_3$) δ 2.76 (3H, s), 3.27 (2H, t, J = 8.8 Hz), 4.66 (2H, t, J = 8.8 Hz), 6.86 (1H, d, J = 8.3 Hz), 7.77 (1H, m), 7.83–7.87 (1H, m).

6.59. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-(methylsulfonyl)-1,3,4-oxadiazole (25)

To an ice-cooled solution of **24** (0.50 g, 2.13 mmol) in CH_3CN (10 ml) was added *m*-chloroperbenzoic acid (69–75%) (1.51 g, 6.40 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous $Na_2S_2O_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **25** (0.44 g, 78%) as a colorless solid: mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 3.31 (2H, t, J = 8.7 Hz), 3.51 (3H, s), 4.71 (2H, t, J = 8.7 Hz), 6.91 (1H, d, J = 8.3 Hz), 7.90–7.95 (1H, m), 7.96–7.98 (1H, m).

6.60. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-[(3-fluorobenzyl) oxy]-1,3,4-oxadiazole (26)

To a solution of **25** (0.15 g, 0.56 mmol) and K_2CO_3 (0.11 g, 0.80 mmol) in DMF (5 ml) was added 3-fluorobenzyl alcohol (91 μl, 0.83 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (hexane–EtOAc) and the product was recrystallized from hexane–EtOAc to give **26** (84 mg, 48%) as colorless crystals: mp 91–92 °C; ^{1}H NMR ($CDCl_3$) δ 3.27 (2H, t, J = 8.9 Hz), 4.65 (2H, t, J = 8.9 Hz), 5.51 (2H, s), 6.84 (1H, d, J = 8.3 Hz), 7.04–7.13 (1H, m), 7.19–7.30 (2H, m), 7.34–7.44 (1H, m), 7.66–7.73 (1H, m), 7.76–7.81 (1H, m); Anal. Calcd for $C_{17}H_{13}FN_2O_3$: C, 59.29; H, 3.81; N, 8.13. Found: C, 59.24; H, 3.85; N, 8.15.

6.61. 2-(Chloromethyl)-5-(2,3-dihydro-1-benzofuran-5-yl)-1,3,4-oxadiazole (27)

A mixture of **26** (2.02 g, 11.3 mmol) and 2-chloro-1,1,1-trimethoxyethane (7.92 ml, 56.7 mmol) in a microwave tube was heated under microwave irradiation for 5 min at 160 °C. The residue was recrystallized from hexane to give **27** (2.01 g, 75%) as colorless crystals: ^{1}H NMR ($CDCl_3$) δ 3.29 (2H, t, J = 8.9 Hz), 4.68 (2H, t, J = 8.9 Hz), 4.75 (2H, s), 6.89 (1H, d, J = 8.7 Hz), 7.81–7.88 (1H, m), 7.90–7.94 (1H, m).

6.62. 2-(2,3-Dihydro-1-benzofuran-5-yl)-5-{[(3-fluoro phenyl)sulfanyl]methyl}-1,3,4-oxadiazole (28)

To a solution of **27** (0.16 g, 0.68 mmol) and 3-fluorothiophenol (57 μl, 0.68 mmol) in DMF (5 ml) was added K_2CO_3 (0.10 g, 0.75 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **28** (32 mg, 14%) as a colorless solid: mp 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 3.28 (2H, t, J = 8.7 Hz), 4.30 (2H, s), 4.67 (2H, t, J = 8.9 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.92−7.01 (1H, m), 7.16−7.33 (3H, m), 7.73−7.78 (1H, m), 7.83−7.86 (1H, m); Anal. Calcd for $C_{17}H_{13}FN_2O_2S$: C, 62.18; H, 3.99; N, 8.53. Found: C, 62.17; H, 3.82; N, 8.54.

6.63. GSK-3β or CDK5 kinase assay

Human GSK-3β was expressed as the N-terminal FLAG-tagged protein using a baculovirus expression system (Takeda Pharmaceutical Company Ltd., Osaka, Japan). Human p35/ cyclin dependent kinase 5 (CDK5) was purchased from Millipore Corp. (Bedford, MA), which was expressed as N-terminal GST fusion protein using baculovirus expression system. The kinase assay was performed in a reaction mixture that contained 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol, and 0.01% BSA and serially diluted test compounds. The assay was conducted in a 96-well plate assay format. The final amount of enzyme was 20 ng/well for CDK5 or 40 ng/well for GSK-3β. The final amount of CDK5 substrate was 1 ng/well (Calbiochem, La. Jolla, CA) for CDK5 or 100 ng/well of GSK-3β substrate peptide (Millipore Corp.) for GSK-3β.

The kinase reactions were initiated by addition of ATP-solution (final 500 nM), and were incubated for 90 min at 37 °C for GSK-3β or for 45 min at room temperature for CDK5. The reactions were terminated by the addition of Kinase-Glo reagent containing EDTA (50 μl/well, Promega Corp., Madison, WI). Ten minutes after addition of Kinase-Glo reagent, the luminescence was measured on a Wallac ARVO 1420 instrument (PerkinElmer, Shelton, CT). The reaction window was calculated from the difference of the average signals obtained from the control (5% DMSO) and the background wells. The inhibitory activity of compounds is expressed by the inhibitor concentration that produced 50% inhibition (IC_{50}) of the enzyme activity in the absence of inhibitor. The IC_{50} values were obtained by linear regression analysis with a GraphPad Prism (version 3.02 for Windows, GraphPad software, Inc. San Diego, CA). The best-fit lines were obtained by analyzing the logistic fitting equation.

6.64. Serine/threonine kinase profiling by IC_{50} measurement

Assays for 14 serine/threonine kinases using radio labeled [γ-^{33}P] ATP (GE Healthcare, Piscataway, NJ) were performed in 96 well plates. Mitogen-activated protein kinase p38α (p38α), extracellular signal-regulated kinase 1 (ERK1), protein kinase C θ (PKCθ), Jun N-terminal kinase 1 (JNK1) and B-raf were expressed as N-terminal FLAG-tagged protein using a baculovirus expression system. I κ B kinase β (IKKβ) and MEK kinase 1 (MEKK1) were expressed as C-terminal FLAG-tagged protein using a baculovirus expression system. Aurora-B was expressed as N-terminal 6xHis tagged protein using a baculovirus expression system. MEK1 was expressed as N-terminal GST fusion protein using a freestyle293 (Invitrogen Life Technologies, Carlsbad, CA) expression system.

Cyclic AMP-dependent protein kinase (PKA) was expressed using an *Escherichia coli (E. coli)* expression system. Casein kinase 1 δ *(CK1δ)* was expressed as N-terminal GST fusion protein using an *E. coli* expression system. Checkpoint kinase (CHK1) was expressed as N-terminal GST fusion protein using a baculovirus expression system. Cyclin-dependent kinase 1 (CDK1)/CycB and CDK2/CycA were expressed as C-terminal 6His-tagged CDK1 or CDK2, and N-terminal GST-tagged Cyclin B or Cyclin A protein using a baculovirus expression system.

The reaction conditions were optimized for each kinase: p38α (100 ng/well of enzyme, 1 μg/well of MBP (Wako Pure Chemical Ind., Osaka, Japan), 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); ERK1 (100 ng/well of enzyme, 2 μg/well of MBP, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); MEKK1 (25 ng/well of enzyme, 1 μg/well of MBP, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); PKCθ (25 ng/well of enzyme, 2 μg/well of MBP, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); JNK1 (10 ng/well of enzyme, 1 μg/well of c-Jun, 0.1 μCi/well of [γ-^{33}P] ATP, 60 min reaction at 30 °C); IKKβ (20 ng/well of enzyme, 1 μg/well of IκBα, 0.1 μCi/well of [γ-^{33}P] ATP, reaction at room temperature); B-raf (25 ng/well of enzyme, 1 μg/well of GST-MEK1(K96R), 0.1 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); MEK1 (100 ng/well of enzyme, 0.3 μg/well of GST-ERK1 (K71A) 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); Aurora-B (50 ng/well of enzyme, 30 μM of Aurora substrate peptide, 0.2 μCi/well of [γ-^{33}P] ATP, 60 min reaction at room temperature); PKA (3 nM of enzyme, 1 μM of PKA substrate peptide (Millipore, Corp.), 0.2 μCi/well of [γ-^{33}P] ATP, 10 min reaction at room temperature); CDK1/CycB (4.2 ng/well of enzyme, 1 μg/well of Histone H1 (Calbiochem), 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CDK2/CycA (1.8 mUnits/well of enzyme, 1 μg/well of Histone H1 (Calbiochem), 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CK1δ (120 ng/well of enzyme, 2.4 μmol/L of CK1tide (Millipore Corp.), 0.2 μCi/well of [γ-^{33}P] ATP, 20 min reaction at room temperature); CHK1 (30 ng/well of enzyme, 25 μmol/L of CHKtide (Millipore Corp.), 0.2 μCi/well of [γ-^{33}P] ATP, 10 min reaction at room temperature).

Except for PKCθ, enzyme reactions were performed in 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol and 0.5 μM ATP containing optimized concentration of enzyme, substrate and radiolabeled ATP as described above in a total volume of 50 μl. For PKCθ, enzyme reactions were performed in 25 mM HEPES, pH 7.5, 10 mM magnesium acetate, 1 mM dithiothreitol, lipid activator (Millipore Corp.).

Prior to the kinase reaction, compound and enzyme were incubated for 5 min at reaction temperature as described above. The kinase reactions were initiated by adding ATP. After the reaction period as described above, the reactions were terminated by the addition of 10% (final concentration) trichloroacetic acid. The [γ-^{33}P]-phosphorylated proteins were filtrated in Harvest Plate (Millipore Corp.) with a Cell Harvester (PerkinElmer) and then free of [γ-^{33}P] ATP was washed out with 3% phosphoric acid. The plates were dried, followed by the addition of 40 μl of MicroScint0 (PerkinElmer). The radioactivity was counted by a TopCount scintillation counter (PerkinElmer).

6.65. Tyrosine kinase profiling by IC_{50} measurement

The cytoplasmic domain of vascular endothelial growth factor receptor 2 (VEGFR2) was expressed as N-terminal FLAG-tagged proteins using a baculovirus expression system. The cytoplasmic domains of HER2 and epidermal growth factor receptor (EGFR) were expressed as N-terminal peptide (DYKDDDD)-tagged protein using a baculovirus expression system. These expressed kinase proteins were purified by using anti-FLAG M2 affinity gel (Sigma–Aldrich, St. Louis, MO, USA). Fibroblast growth factor receptor 3 (FGFR3), platelet-derived growth factor receptor α (PDGFRα), PDGFRβ, TIE2, Met, Kit, Src, insulin receptor (IR) and lymphocyte-specific protein tyrosine kinase (Lck) were purchased from Millipore Corp.

Assays for 10 tyrosine kinases except HER2 and EGFR using anti-phosphotyrosine antibody were performed in 384 well plates using the Alphascreen® system (PerkinElmer) at room temperature. Enzyme reactions were performed in 50 mM Tris–HCl, pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20, 2 mM dithiothreitol and 0.1 μg/ml biotinylated poly-GluTyr (4:1) containing optimized concentration of enzyme and ATP as described below.

Prior to the kinase reaction, compound and enzyme were incubated for 5 min at room temperature. The reactions were initiated by adding ATP. After the reaction period as described below at room temperature, the reactions were stopped by the addition of 25 μl of 100 mM EDTA, 10 μg/ml Alphascreen streptavidin donor beads and 10 μg/ml acceptor beads described below in 62.5 mM HEPES, pH 7.4, 250 mM NaCl, and 0.1% BSA. Plates were incubated in the dark for more than 12 h and then read by EnVision 2102 Multilabel Reader (PerkinElmer). The well containing substrate and enzyme without compound was used as total reaction control. The reaction conditions for these 10 kinases were optimized for each kinase: VEGFR2 (19 ng/ml of enzyme, 10 μM ATP, 10 min reaction, PY-100 conjugated acceptor beads (PY-100)); FGFR3 (20 ng/ml of enzyme, 20 μM ATP, 10 min reaction, PY-100); PDGFRα (50 ng/ml of enzyme, 10 μM ATP, 30 min reaction, PT66 conjugated acceptor beads (PT66)); PDGFRβ (50 ng/ml of enzyme, 20 μM ATP, 60 min reaction, PT66); TIE2 (20 ng/ml of enzyme, 2 μM ATP, 10 min reaction, PT66); Met (1 ng/ml of enzyme, 2 μM ATP, 10 min reaction, PT66); Kit (10 ng/ml of enzyme, 20 μM ATP, 20 min reaction, PT66); Src (0.33 ng/ml of enzyme, 2 μM ATP, 10 min reaction, PY-100); IR (100 ng/ml of enzyme, 10 μM ATP, 60 min reaction, PT66); Lck (100 ng/ml of enzyme, 2 μM ATP, 30 min reaction, PY-100).

Assays for HER2 and EGFR kinase using radio labeled [γ-^{32}P] ATP (GE Healthcare) were performed in 96 well plates. HER2 and EGFR kinase reactions were performed in 50 mM Tris–HCl, pH 7.5, 5 mM $MnCl_2$, 0.01% Tween-20 and 2 mM dithiothreitol containing 0.9 μCi of [γ-^{32}P] ATP per reaction, 50 μM ATP, 5 μg/ml poly-Glu-Tyr (4:1), 0.1% DMSO and 0.25 μg/ml of HER2 or EGFR cytoplasmic domains in a total volume of 50 μl. Prior to the kinase reaction, compound and enzyme were incubated for 5 min at room temperature. The kinase reactions were initiated by adding ATP. After the kinase reaction for 10 min (HER2) and 5 min (EGFR) at room temperature, the reactions were terminated by the addition of 10% (final concentration) trichloroacetic acid. The [γ-^{32}P]-phosphorylated proteins were filtered in Harvest plates (Millipore Corp.) with a cell harvester (PerkinElmer) and washed free of [γ-^{32}P] ATP with 3% phosphoric acid. The plate was dried, followed by the addition of 25 μl of MicroScint0 (PerkinElmer). The radioactivity was counted by a Topcount scintillation counter (PerkinElmer).

6.66. Expression, purification, crystallization and structure solution

The gene for human GSK-3β (residues 48–433) was cloned into a pSXB100 baculovirus expression vector with an n-terminal 6-Histidine tag containing an rTEV protease cleavage site. The protein was expressed in Hi5 insect cells and purified by immobilized metal-chelate affinity chromatography (IMAC). Protein bound to the IMAC column was eluted with 0.5 M imidazole, the tag removed by cleavage with rTEV protease, and reapplied to the IMAC column to remove misfolded protein with incomplete tag cleavage. The eluent from the second IMAC column was dialyzed versus buffer solution containing 25 mM HEPES, pH 7.4 and 150 mM NaCl. The dialyzed protein was diluted with equal volume of 10 mM

HEPES pH 7.4 then bound to a Resource 15S cation exchange column pre-equilibrated in 10 mM HEPES pH 7.4, 50 mM NaCl and eluted using a 30 min linear gradient to 10 mM HEPES pH 7.4, 1 M NaCl. Fractions containing the singly-phosphorylated protein species were pooled and concentrated to approximately 1 mg/ml. An inhibitor in DMSO solution was added to the protein slowly, with stirring to a final concentration of 1 mM, and the enzyme inhibitor complex further concentrated to 10–15 mg/ml.

Crystallization experiments were performed at 4 °C by mixing 50 nl of enzyme:inhibitor solution with 50 nl of precipitant solution containing 20% PEG 3350 and 0.15 M NaCl. Crystals were harvested by mixing precipitant solution with the cryoprotectant ethylene glycol to a final concentration of 22% and flash-frozen by direct immersion in liquid nitrogen. X-ray diffraction data were collected at the Advanced Light Source in Berkeley, CA, on Beamline 5.0.3 at a wavelength of 1.0 Å. Diffraction data for the co-crystal with compound **1** extended to 2.4 Å resolution with an R_{merge} of 0.056. For compound **20x** data extended to 2.6 Å with an R_{merge} of 0.447. Both crystals belong to the monoclinic space group $P2_1$ with approximate cell dimensions $67 \times 115 \times 67$ Å, $\beta = 102°$, with two molecules in the crystallographic asymmetric unit. The structures were solved by molecular replacement with AMoRe[28] using the published apo structure of GSK-3β (PDB entry 1H8F) and refined with REFMAC within the CCP4 suite of programs.[29] The final refined crystallographic statistics for the co-crystal structure with compound **1** are, $R = 20.3$ (R_{free}, 25.4), with root-mean-square deviations (RMSD) is the bond lengths of 0.009 Å, and in the bond lengths of 1.31°. For compound **20x**, these values are $R = 19.9$ (R_{free}, 28.1), 0.012 Å in bond lengths, and 1.58° in bond angles. The structures have been deposited with the RCSB structure database with accession codes 3F7Z (compound **1**) and 3F88 (compound **20x**).

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