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REVIEW

An updated research of glycogen synthase kinase-3β inhibitors: a review

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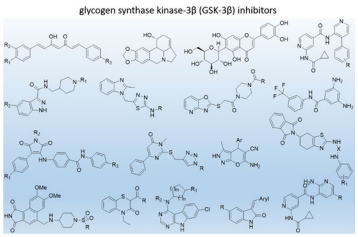
Abstract

Glycogen synthase kinase-3β (GSK-3β) is a highly conserved multifunctional serine/threonine (Ser/Thr) protein kinase widely expressed in many tissues. GSK-3β inhibitors could be used in the treatment of human key diseases, such as cancer, Alzheimer's disease, Parkinson's disease, inflammation, type-II diabetes, and so on, due to the multi-role of GSK-3β in the hepatic glycolysis regulation, cell signaling pathways, and phosphorylation of various proteins. Recently, sets of diverse GSK-3β inhibitors have been prepared, and biologically evaluated in vitro and in vivo in different screening models. This review summarizes the latest developments in GSK-3β inhibitors unclosed from 2015 to 2019, including their structure–activity relationship and bioactivity studies.

Graphic abstract



Keywords GSK-3β inhibitor · Rational design · Multi-target · Selectivity · Structure–activity relationship

Introduction

Glycogen synthase kinase-3β (GSK-3β) is a highly conserved multifunctional serine/threonine (Ser/Thr) protein kinase widely expressed in many tissues, which was first identified over 30 years ago [1]. GSK-3β was originally thought to regulate hepatic glycolysis by phosphorylating and inhibiting hepatic glycogen synthase, but now more studies have shown that GSK-3β was involved in multiple key cell-signaling pathways and had various phosphorylation targets [2].

Due to its versatility, GSK-3β plays important roles in the occurrence and development of human key diseases (Fig. 1), such as cancer, Alzheimer's disease (AD), Parkinson's disease, stroke, inflammation, type-II diabetes, and so on [3–6], therefore, GSK-3β inhibitors could be employed to target these kinds of diseases. For example, many small-molecule inhibitors of GSK-3β were explored for their effects in cancer treatment [7]. Recently, Sahin et al. outlined the prospects of GSK-3β in cancer therapy and summarized the preclinical and early clinical results of these potential anticancer GSK-3β inhibitors [8]. As a key regulator of cognitive function, GSK-3β has been involved in the process of neurodegenerative diseases, such as AD [9, 10]. Studies have shown that inhibiting GSK-3β can improve memory deficits and cognitive function of the aging mice model

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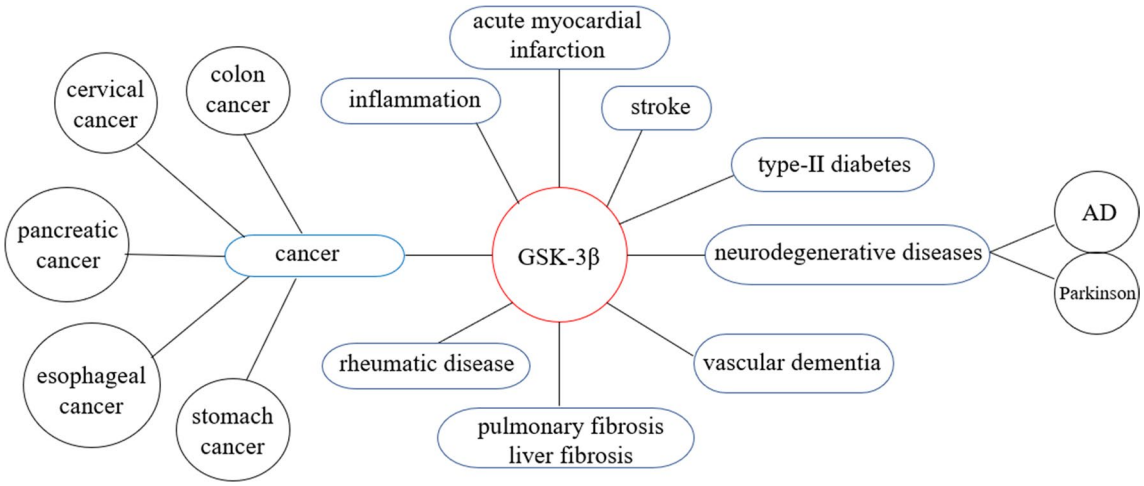


Fig. 1 The correlations of GSK-3β with various diseases

[11]. Besides, inhibition of GSK-3β is thought to reduce acute myocardial infarction [12], suppress inflammation [13], protect the lung from acute injury [14], delay pulmonary fibrosis [15], and improve severe cardiac dysfunction [16]. Moreover, some recent studies indicated that inhibition of GSK-3β could prevent liver fibrosis [17], treat vascular dementia [18], and target rheumatic disease [19].

The discovery of GSK-3β inhibitors in pharmaceutical companies and academic institutes has been carried out over the years, and its advances in the research of GSK-3β inhibitors were reviewed by several articles [20–23]. Despite massive efforts in the research and development of GSK-3β inhibitors in the past decades, only an irreversible and time-dependent GSK-3β inhibitor, tideglusib (a TDZD compound, $IC_{50} = 100$ nM), has entered the phase II clinical trials for the treatment of AD and progressive supranuclear palsy [24, 25]. The current dilemma pushes pharmaceutical chemists to look for new selective and reversible GSK-3β inhibitors, which might be derived from virtual screening, rational drug design, or the bioassay of natural products database. Herein, the present view aims to summarize the recent development of new GSK-3β inhibitors reported over the past five years (2015–2019) and to provide information for the future discovery and design of GSK-3β inhibitors.

The recent development of GSK-3β inhibitors

Curcumin-derived GSK-3β inhibitors

Curcumin is a *β*-diketone natural product isolated from the rhizomes of *Curcuma longa* L [26], and it has been investigated as an effective and safe versatile molecule for preventing and treating various disorders [27, 28]. Based on the analysis of structural features and biological profile of curcumin, Belluti et al. envisioned that the *α*,*β*-unsaturated carbonyl moiety of curcumin could covalently interact with the key residue (Cys199) of GSK-3β, and thus prepared a small library of curcumin-based analogs [29]. The bioassay results indicated that some of the analogs, such as **1a**–**1f** shown in Table 1, were potent dual-target inhibitors, which not only target GSK-3β (IC_{50} values of 0.04 ± 0.01–2.69 ± 1.01) but also BACE-1 (IC_{50} values of 0.53 ± 0.27–2.78 ± 0.44) in a potent and balanced way, making curcumin a promising scaffold for developing anti-AD drug candidates by dual targeting GSK-3β and BACE-1. The structure–activity relationship (SAR) study proved that the tautomeric enol form is an important feature to achieve good chemical stability and metabolic stability. Among them, the double-sided *p*-methoxy-substituted compound **1a** showed the best inhibitory activity with an IC_{50} value of 0.53 μM. However, the activity was found to decrease slightly when *p*-methoxy group in **1a** was replaced by other substituents.

Amaryllidaceae alkaloids

Amaryllidaceae alkaloids are a group of nitrogen-containing polycyclic compounds produced exclusively by the plants of the Amaryllidaceae family. As one of the best known amaryllidaceae alkaloids, galanthamine is a potent acetylcholinesterase (AChE) inhibitor clinically used in the treatment of AD. Recently, Cahlikova and coworker explored the potency of amaryllidaceae alkaloids on GSK-3β inhibition [30]. Consequently, twenty-eight amaryllidaceae alkaloids of seven structural types obtained from different Amaryllidaceae plants were tested for their inhibitory activity against GSK-3β, and three alkaloids including caranine (**2a**), 9-*O*-desmethylhomolycorine (**2b**), and masonin (**2c**) were found to be the most active with IC_{50} values around

Table 1 Curcumin-based analogs **1a**–**1f**

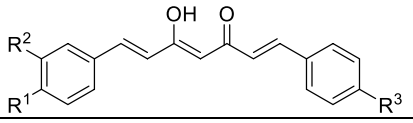
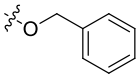
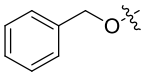
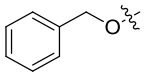
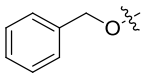
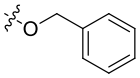
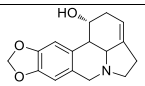
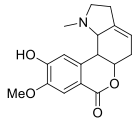
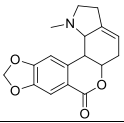
No.					
	R^1	R^2	R^3	IC_{50} /μM	
				BACE-1	GSK-3β
1a	OCH_3	H	OCH_3	1.65±0.01	0.53±0.27
1b	OH	OCH_3		0.97±0.43	0.90±0.38
1c	OH	OCH_3	CH_3	0.14±0.03	2.09±0.51
1d		H	OCH_3	2.28±0.64	2.78±0.44
1e		H	OH	2.69±1.01	2.01±0.71
1f		H		0.04±0.01	2.49±0.82

Table 2 Amaryllidaceae alkaloids GSK-3β inhibitors **2a**–**2c**

No.	Structure	IC_{50} /μM
Caranine (**2a**)		30.75±0.04
9-*O*-Demethylhomolycorine (**2b**)		30.00±0.71
Masonin (**2c**)		27.81±0.05

30 μM (Table 2). Since amaryllidaceae alkaloids can be easily obtained in large quantities from natural resources, they could serve as parent compounds used in further structural modification and biological optimization.

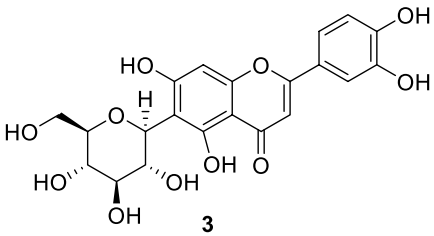
6-*C*-glycosyl flavonoids

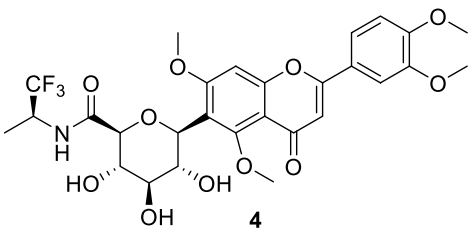
Isoorientin (**3**, Fig. 2) as known 6-*C*-glycosoflavone isolated Corn silks based on bioassay-guided method, was recently found to be a new non-ATP competitive GSK-3β inhibitors with IC_{50} values of 185 μM [31]. This compound could in vitro inhibit GSK-3β-mediated tau hyperphosphorylation and prevent β-amyloid (Aβ)-induced neuronal cytotoxicity in SH-SY5Y cells at the tested concentration of 112 μM, suggesting that compound **3** represents a promising lead candidate for novel anti-AD drug development. To increase the druggable potency of isoorientin, the bioactive improvement including structure–activity relationship (SAR), in vitro and in vivo study of isoorientin analogs was performed by Li's group [24]. Finally, a new class of selective substrate-competitive GSK-3β inhibitors was developed. In cells, these new inhibitors exhibited membrane-passive permeability, and attenuated GSK-3β-mediated tau protein hyperphosphorylation and Aβ-induced neurotoxicity. SAR analysis and computer simulation proved that the hydrophobicity, π-cation and orthogonal multipolar interaction of compound **4** (IC_{50} of 0.59 μM) with substrate sites of the enzyme were the key factors for GSK-3β inhibition and selectivity.

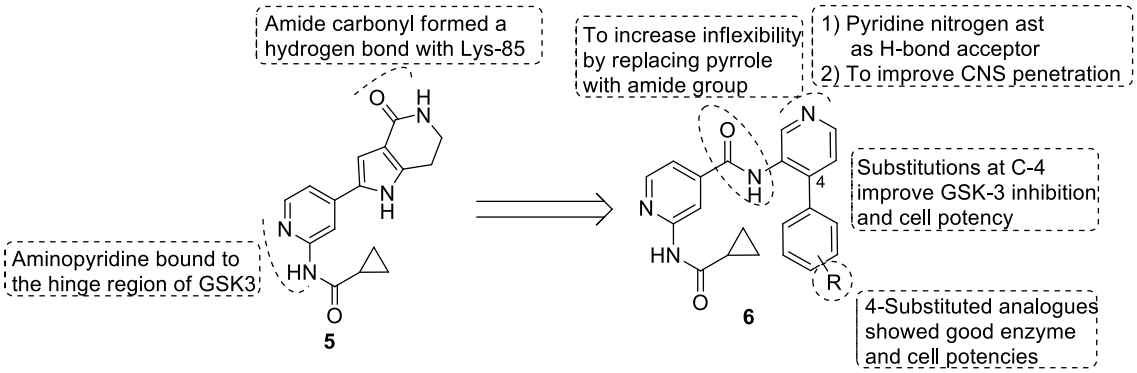
Isonicotinamides

A set of isonicotinamides **6** [32] shown in Fig. 3 were derived from GSK-3β inhibitor **5** by processing pyrrolopyridinone core [33]. Replacement of the lactam part with the

Fig. 2 Structures of isoorientin (**3**) and its derivative **4**







No.	R	IC_{50} /nM	No.	R	IC_{50} /nM
6a	H	9.8 ± 3.6	**6e**	4-Cl	5.9 ± 2.7
6b	2-F	13.0 ± 4.2	**6f**	4-OMe	2.1 ± 0.95
6c	3-F	16.0 ± 11.0	**6g**	4-OCF_3	11 ± 3.5
6d	4-F	5.2 ± 2.2	**6h**	4-CN	4.7 ± 1.9

Fig. 3 Design and structures of the 3-pyridinyl isonicotinamides **6**

pyridine ring was expected to keep the hydrogen-bond interaction with Lys-85 of GSK-3β and improve CNS penetration. In addition, the pyrrole moiety was further replaced with an amide group to make the rigid tricyclic scaffold more flexible. These new synthetic derivatives showed good GSK-3β and cell potency. However, most of the potent compounds had poor metabolic stability in rodent liver microsomes or had a high KBV efflux ratio, except compounds **6a**–**6h**. These isonicotinamides had good Caco-2 permeability and excellent selectivity towards GSK-3β over almost the other 400 kinases. Although these compounds had a high in vivo clearance rate in the triple-transgenic AD mouse model, they exhibited good in vivo efficacy to lower tau protein phosphorylation levels.

GSK-3β inhibitors with indazole core

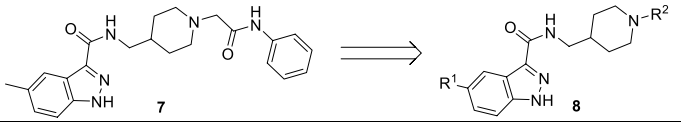
To discover GSK-3β inhibitors acting as mood stabilizers, Reggiani and coworkers identified a novel 1*H*-indazole-3-carboxamide GSK-3β inhibitor **7** through virtual screening [34, 35]. Based on the analysis of the X-ray co-crystal structure of **7** with GSK-3β, the hit **7** was structurally optimized by changing the substituents on C-5 of indazole and piperidine ring [34], which led to the production of a series of more potent *N*-[(1-alkylpiperidin-4-yl)methyl]-1*H*-indazole-carboxamide ATP-competitive GSK-3β inhibitors **8** (Fig. 4).

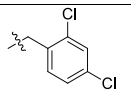
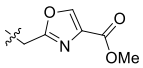
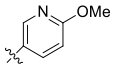
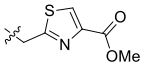
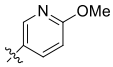
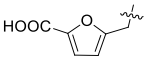
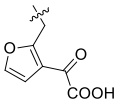
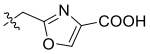
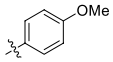
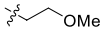
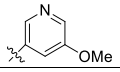
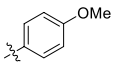
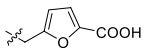
The analysis of the available SAR information suggested that the benzyl- or pyridine-substituted piperidine were favorable for the kinase inhibitory activity (IC_{50} values ranging from 0.64 to 0.25 μM). Besides, 5-methoxy, 5-aryl, or 5-heteroaryl in indole ring could significantly increase the inhibitory effect, while 5-aryl or 5-heteroaryl substituents are more preferable compared with 5-methoxy group. In contrast, the compounds having methyl or and 4-methoxyphenyl in piperidine ring showed a significant loss of efficacy. A total of nine most potent compounds **8a**–**8f** and **8h**–**8j** were later selected to be evaluated for their inhibitory effect against Tau protein phosphorylation in cells. The results showed that all of them, except for **8i** and **8j**, showed potent inhibitory effect with IC_{50} in the low micromolar range.

Benzimidazole core- and oxazolo[4,5-*b*]pyridine core-based GSK-3β inhibitors

The combination of benzimidazole core with different functional groups has led to various potent GSK-3β inhibitors. Recently, Hamid' group made a fruitful contribution to the development of benzimidazole-derived GSK-3β inhibitors. In 2016, they reported two types of benzimidazole GSK-3β inhibitors based thiadiazole (**9**) and carbohydrazide (**10**) conjugates (Fig. 5), and evaluated their anti-depressant activity. Benzimidazoles **9a**, **9b**, and **10a**–**10c** were found to be the most potent inhibitors with IC_{50} values ranging from 72 to 107 nM, of which **10c** was shown to have significant in vivo antidepressant activity in tail suspension and forced swim tests. In the same year, this group prepared a series of benzimidazole-based 1,3,4-oxadiazole-1,2,3-triazole conjugates as GSK-3β inhibitors (**11**) with in vivo anti-depressant activity [36]. The comparison of bioassay results of compounds **11a**–**11f** indicated that most of the substituents (R) on triazole were beneficial for inhibitory activity, but

Fig. 4 1*H*-indazole-3-carboxamide GSK-3β inhibitors **7** and **8**



No.	R^1	R^2	IC_{50} /μM	No.	R^1	R^2	IC_{50} /μM
8a	MeO-		0.310	**8g**	MeO-	Me	1.20
8b	MeO-		0.210	**8h**			0.053
8c	MeO-		0.130	**8i**			0.021
8d	MeO-		0.230	**8j**			0.006
8e			0.018	**8k**			0.56
8f			0.026	**8l**			0.3

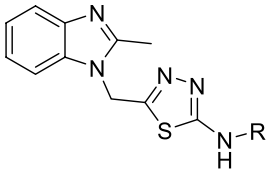
when R was 2-bromophenyl or 2-methylphenyl, the activity decreased significantly. In 2018, this research group synthesized another set of benzimidazole-based inhibitors (**12**) with 1,3,4-oxadiazole carboxamides moiety as a side chain [37]. Among 19 synthesized analogs, compounds **12a**–**12d** showed significant inhibitory effects with IC_{50} values range of 0.13, 0.22, 0.20, and 0.14 μM, respectively, while compound **12g** has the worst inhibitory activity (IC_{50} value of 2.04 μM).

In addition, the same research group also reported a novel type of GSK-3β inhibitors with oxazolo[4,5-*b*]pyridine-2-one core [38], which were designed based on their previous studies on anti-inflammatory benzoxazolinone and 2-mercaptobenzoxazole-based 1,2,3-triazole conjugates [39–41]. Among these conjugates, compounds **13a**–**13d** were found to be the strongest inhibitors with IC_{50} values of 0.19, 0.23, 0.31, and 0.37 μM, respectively. SAR analysis shows that pyridyl or 2-halophenyl has positive effect on the activity than phenyl or 2-halophenyl group. Further bioactivity study showed that compounds **13a**–**13d** had significant in vivo anti-inflammatory activity by inhibiting the pro-inflammatory mediators, such as NO, TNF-α, IL-1β, and IL-6.

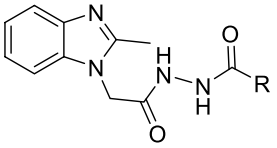
In 2017, the same group further optimized oxazolo[4,5-*b*]pyridine-2-one scaffold, and synthesized seventeen novel oxazolo[4,5-*b*]pyridine-based piperazinamides as GSK-3β inhibitors [42]. Of these synthesized analogs, compounds **14a**–**14d** had the best inhibitory activity against GSK-3β with IC_{50} values ranging from 0.53, 0.34, 0.39, and 0.47 μM, respectively, while compounds **14e**–**14h** showed poor inhibitory activity, indicating that the type and position of substituents had a greater impact on their activities. Amongst them, **14b** was found to inhibit the pro-inflammatory mediators, such as TNF-α, IL-1β, and IL-6, indicating the great values of these oxazolopyridine-based GSK-3β inhibitors in the development of anti-inflammatory agents.

3, 5-Diamino-*N*-substituted benzamide-based GSK-3β inhibitors

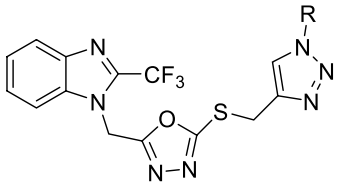
In 2019, Zhong and coworkers [43] reported the virtual screening, design and synthesis of seventy 3,5-diamino-*N*-substituted benzamides as GSK-3β inhibitors **15** (Fig. 6). All these compounds reserve the 3,5-diaminobenzamide fragment but having different substituents to the amide group. One of them, compound **15a** containing *N*-3-(trifluoromethyl)phenyl substituent has good selectivity and inhibitory effect towards GSK-3β (IC_{50} = 5.2 μM). Also this compound has the highest cytotoxic effect on human colon cancer cells (HCT-116). Further in vivo experiments demonstrated that **15a** has an acceptable selectivity and ADME characters, suggesting its significant guiding value for novel GSK-3β inhibitors used in anticancer candidates discovery. SAR study on this type of inhibitors can be summarized as follows: (1) When the substituent R in the amide group of **15** is a benzene ring, *meta*-substituent on benzene is the most optimal, followed by *para*-substituent, and then *ortho*-substituent; (2) electron-withdrawing group on benzene instead of electron-donating group lead to the increase of activity; (3) the type and number of substituents on the benzene ring



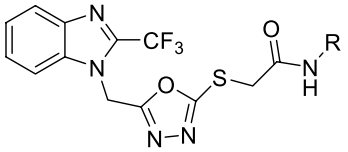
9a, R = 2-fluorophenyl, IC_{50} = 76 nM
9b, R = 4-nitrophenyl, IC_{50} = 107 nM



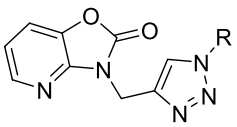
10a, R = 2-methylbenzyl, IC_{50} = 92 nM
10b, R = 4-phenoxyphenyl, IC_{50} = 80 nM
10c, R = 3-indoleacetaldehyde, IC_{50} = 72 nM



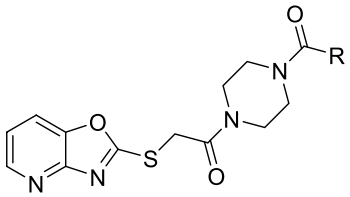
11a, R = 2-fluorophenyl, IC_{50} = 0.27±0.03 mM
11b, R = phenyl, IC_{50} = 0.32±0.02 mM
11c, R = 4-ethylphenyl, IC_{50} = 0.39±0.03 mM
11d, R = 3-pyridyl, IC_{50} = 0.15±0.02 mM
11e, R = 2-methylphenyl , IC_{50} = 17.38±1.06 mM
11f, R = 2-bromophenyl, IC_{50} = 20.39±1.34 mM



12a, R = phenyl, IC_{50} = 0.13±0.01 mM
12b, R = 4-methoxyphenyl, IC_{50} = 0.22±0.01 mM
12c, R = 4-fluorophenyl, IC_{50} = 0.20±0.02 mM
12d, R = 3-(trifluoromethyl)phenyl, IC_{50} = 0.14±0.01 mM
12e, R = 2-fluorophenyl, IC_{50} = 1.34±0.11 mM
12f, R = 2-bromophenyl, IC_{50} = 1.67±0.13 mM
12g, R = 3,4-dimethylphenyl, IC_{50} = 2.04±0.16 mM

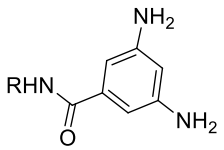


13a, R = 2-chlorophenyl, IC_{50} = 0.19 mM
13b, R = pyridin-3-yl, IC_{50} = 0.23 mM
13c, R = 2-bromophenyl, IC_{50} = 0.31 mM
13d, R = 2-fluorophenyl, IC_{50} = 0.37 mM
13e, R = 4-chlorophenyl, IC_{50} = 5.37 mM
13f, R = phenyl, IC_{50} = 10.07 mM
13g, R = 4-bromophenyl, IC_{50} = 8.64 mM
13h, R = 4-fluorophenyl, IC_{50} = 2.49 mM

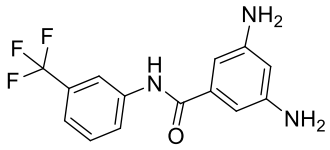


14a, R = 4-nitrophenyl, IC_{50} = 0.53±0.03 mM
14b, R = 4-chlorophenyl, IC_{50} = 0.34±0.02 mM
14c, R = 2-methylphenyl, IC_{50} = 0.39±0.03 mM
14d, R = 4-methylphenyl, IC_{50} = 0.47±0.05 mM
14e, R = 2-nitrophenyl, IC_{50} = 15.32±0.16 mM
14f, R = 2-methoxyphenyl, IC_{50} = 18.12±0.18 mM
14g, R = 4-nitrobenzyl, IC_{50} = 19.31±1.03 mM
14h, R = benzyl, IC_{50} = 18.9±0.87 mM

Fig. 5 GSK-3β inhibitors **9**–**14** reported by Hamid' and workers



15



15a

Fig. 6 Structures of 3,5-diamino-*N*-substituted benzamides **15**

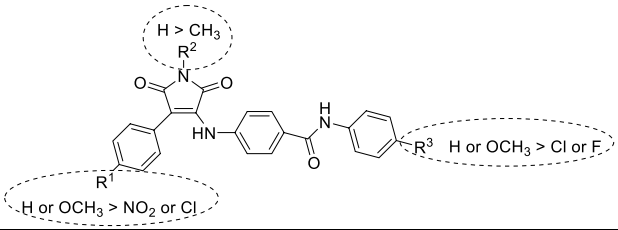
have a more significant effect on the compound's cytotoxicity, for example, the bulky substituent could increase the cytotoxicity towards tumor cells.

Maleimide-derived GSK-3β inhibitors

Among various reported GSK-3β inhibitors, maleimide-based analogs, such as SB415286 (anilinomaleimide) and SB216763 (arylindolemaleimide), are well-known representative potent, ATP-competitive GSK-3β inhibitors. These types of inhibitors have been shown to have potential applications in the treatment of many diseases, such as cancer, diabetes, and AD [44]. Up to date, lots of maleimide-derived GSK-3β inhibitors have been revealed and structurally modified to develop chemotherapeutical drugs.

Kalam and coworkers [45] synthesized a series of anilinomaleimide-based GSK-3β inhibitors (Fig. 7). Most of these synthetic compounds showed moderate to strong inhibitory activity against GSK-3β, with 1/3 of them having IC_{50}

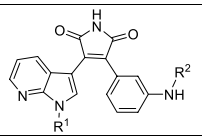
Fig. 7 Structures of anilinomaleimide-based GSK-3β inhibitors **16**



No.	R^1	R^2	R^3	IC_{50} /μM	No.	R^1	R^2	R^3	IC_{50} /μM
16a	H	H	H	0.21	**16i**	H	Me	H	16.49
16b	MeO	H	MeO	0.09	**16j**	H	Me	F	23.82
16c	H	H	MeO	0.09	**16k**	MeO	Me	MeO	15.37
16d	H	H	F	0.51	**16l**	MeO	Me	Cl	16.34
16e	Cl	H	MeO	0.62	**16m**	Cl	Me	H	20.71
16f	NO_2	H	H	0.026	**16n**	Cl	Me	F	>50
16g	NO_2	H	OCH_3	0.23	**16o**	NO_2	Me	H	>50
16h	NO_2	H	Cl	6.86	**16p**	NO_2	Me	F	>50

Table 3 The structures of (aza)indolyl maleimide-based GSK-3β inhibitors

Compd



	R^1	R^2	IC_{50} /nM
17a	Me_2CH	FCH_2CO	17
17b	Me_2CH	CH_2=CHCO	34
17c	Me	FCH_2CO	102
17d	Me	CH_2=CHCO	285

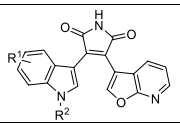
values less than 1 μM. The preliminary SAR study indicated that replacement of H (R^2) on the maleimide core by methyl led to the obvious loss of activity. In addition, the substituents (R^1 and R^3) on the benzene ring also have influence on their activity, for example, the compounds with an electron-donating group (MeO–) showing much better GSK-3β inhibitory activity than those with electron-withdrawing groups (–NO_2, –F, –Cl). Among these derivatives, compound **16b** exhibited the highest activity with an IC_{50} value of 0.09 μM. The further functional study proved that compounds **16b** and **16c** exhibited significant antidepressant activity by 1.4-fold than the positive control fluoxetine.

Pan et al. discovered a series of (aza)indolyl maleimide-based GSK-3β covalent inhibitors, which were achieved by optimizing non-covalent interactions and reactive groups [46]. Among these inhibitors, compounds **17a**–**17d** in Table 3 showed good selectivity towards GSK-3β, of which compound **17a** (IC_{50} = 6 nM) containing a mild *α*-fluoromethyl amide reactive group could effectively inhibit the phosphorylation of tau protein and update the expression of β-catenin in living cells.

A series of new 3-(furo[2,3-*b*]pyridin-3-yl)-4-(1*H*-indol-3-yl)maleimide GSK-3β inhibitors (Table 4) was designed

Table 4 GSK-3β inhibitory activity of 3-(furo[2,3-*b*]pyridin-3-yl)-4-(1*H*-indol-3-yl)maleimides **18**

No.



	R^1	R^2	IC_{50} /μM
18a	H	H	1.78±0.12
18b	H	Me	0.28±0.01
18c	7-Me	Me	0.29±0.03
18d	5-Cl	Me	0.17±0.02
18e	5-Br	Me	0.07±0.02
18f	H	isopropyl	0.19±0.02
18g	H	3-(1*H*-imidazol-1-yl)propyl	0.21±0.02
18h	5-Br	3-(1*H*-imidazol-1-yl)propyl	0.19±0.01
18i	5-Cl	3-(1*H*-imidazol-1-yl)propyl	0.24±0.02
18j	5-OMe	Me	1.31±0.11
18k	6-Cl	Me	1.84±0.10
18l	6-Br	Me	1.94±0.10

and synthesized by the same group [47]. Most compounds showed favorable inhibitory activities against GSK-3β. Based on bioassay results of these compounds, introduction of an alkyl at N^1-position of the indole ring made the activity of **18b**–**18j** increase by several folds compared with **18a**. With respect to the effects of halogen substitution on activity, it was clearly revealed that the compounds with halogen on 5-position of indole ring obviously showed better activity than those with halogen on the 6-position. In addition, compounds **18g**–**18i** with 3-(1*H*-imidazol-1-yl)propyl group at N^1-position also exhibited potent inhibition on GSK-3β and could inhibit the cellular GSK-3β activity in primary neurons to reduce GSK-3β substrate tau phosphorylation at Ser396. Among them, inhibitor **18i** could reduce infarct size and improve the neurological deficit in the cerebral ischemia animal model, suggesting its potential neuroprotective activity against brain ischemic stroke.

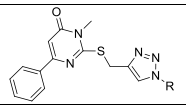
To find more potent and selective GSK-3β inhibitors, Pang et al. designed a series of novel 4,5-bisindolyl-1,2,4-triazolones based on the analysis of the interaction mode of bishetero-maleimide with the hinge residues (Asp133 and Val135) of GSK-3β (Fig. 8) [48]. This series of inhibitors could significantly reduce GSK-3β substrate Tau phosphorylation at Ser396 in primary neurons by inhibiting cellular GSK-3β. In this series of thirteen synthetic compounds in total, compounds **19c**, **19e**, and **19g** showed the most potent GSK-3β inhibitory activity. By the comparison of the activity of **19a**–**19h**, it was concluded that the halogen substitution on 5-position is the most optimal for increasing inhibitory activity towards GSK-3β. In addition, compounds **19c**, **19e**, and **19g** showed promising neuroprotective activity by preventing neuronal cells from glutamate-induced death.

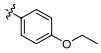
GSK-3β inhibitors with pyrimidin-4-one core

A set of pyrimidin-4-one-1,2,3-triazole conjugates as GSK-3β inhibitors have been synthesized by Hamid et al. using the click-chemistry approach [49]. The target compounds **20** (Table 5) were constructed from the linkage of pyrimidine-4-one core with triazole ring through a sulfur bond. Among these novel conjugates, four of them, **20a**–**20d**, showed improved inhibitory activity towards GSK-3β. The result of SAR analysis clearly indicates that when the substituent attached on triazole ring is methoxyphenyl, ethoxyphenyl, or pyridine, the compound's inhibitory activity will significantly decrease.

Later, the same research group reported another study on the GSK-3β inhibitors derived from the connection of substituted piperazine ring to pyrimidin-4-one backbone (Fig. 9) [50]. From the result of GSK-3β inhibition bioassay, the activity was increased in accordance with the following changes made in the SAR analysis shown in Fig. 9. Among these pyrimidin-4-one analogs, compounds **21a**–**21d** showed the most potent inhibition on GSK-3β with IC_{50} values ranging from 83 to 127 nM.

Table 5 Structures of pyrimidin-4-one-1,2,3-triazole GSK-3β inhibitors



No.	R =	IC_{50} /nM	No.	R =	IC_{50} /nM
20a		94	**20e**		1144
20b		122	**20f**		1443
20v		82	**20g**		1742
20d		54	**20h**		577

Watanabe et al. prepared and evaluated a similar series of promising pyrimidin-4-one derivatives containing piperazine moiety as GSK-3β inhibitors (Fig. 10) [51]. The SAR analysis shown in Fig. 10 indicated that (1) the analogs with (*R*)-methyl group had more potent activity and (2) the analogs with pyrimidinyl ring (**22a**) seemed to have more preferable GSK-3β inhibitory activity than those with pyridyl ring (**22b**). The further replacement of methyl by isopropyl, phenyl, benzyl, acyl, alkoxycarbonyl, and sulfonyl on the nitrogen atom of the piperazine resulted into a more potent compound **22c** (IC_{50} = 1.4 nM) with moderate metabolic activity and CYP2D6 inhibition. Then, compound **22c** was chosen as a hit compound for further optimization, which led to the generation of more potent GSK-3β inhibitors. From the bioassay results, it was shown that the substituents (such as –F, –Cl, –MeO) on the phenyl group generally increased the activity, while the nitrile group made the activity decrease, however, the position effect of substituents on the activity was unclear. Among them, analog **22j** showed the most potent activity towards GSK-3β (IC_{50} = 0.1 nM) with good in vitro and in vivo pharmacokinetic profiles, and could significantly decrease Tau phosphorylation in mice model. Later, the same Watanabe's group reported another set of GSK-3β inhibitors possessing phenylpiperazinyl-pyrimidin-4-one framework [52], of which **23a** and **23b**

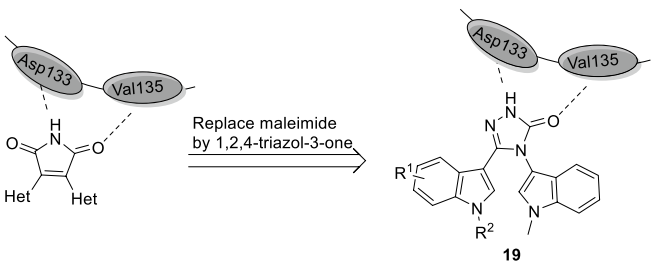


Fig. 8 Design strategy and structures of 4,5-bisindolyl-1,2,4-triazol-3-one GSK-3β inhibitors **19**

No.	R^1	R^2	IC_{50}/μM	No.	R^1	R^2	IC_{50}/μM
19a	H	H	18.15	**19e**	5-Cl	CH_3	0.61±0.08
19b	5-OMe	CH_3	6.20.88	**19f**	6-Cl	CH_3	25.39
19c	5-F	CH_3	1.88±0.33	**19g**	5-Br	CH_3	0.35±0.02
19d	6-F	CH_3	7.50	**19h**	6-Br	CH_3	22.07

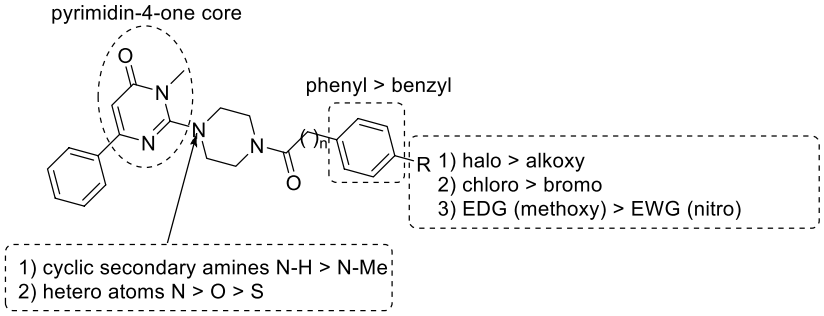
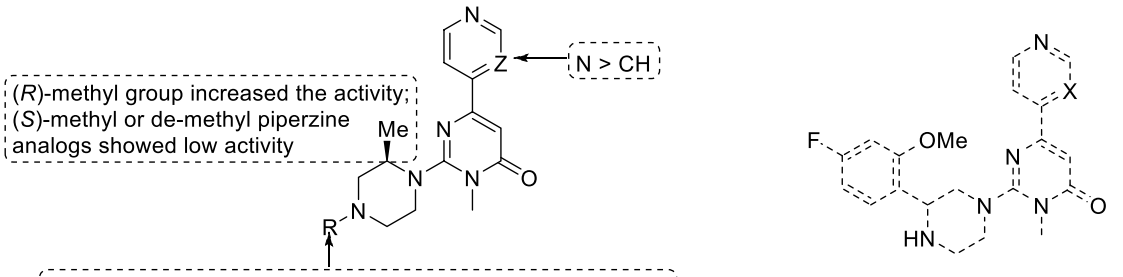


Fig. 9 Structures of GSK-3β inhibitors **21** and SAR analysis

No.	n =	R =	IC_{50} /nM	No.	n =	R =	IC_{50} /nM
21a	1	H	127	**21e**	1	4-nitro	921
21b	1	4-Cl	116	**21f**	1	2-methyl	1708
21c	0	H	83	**21g**	0	4-nitro	1503
21d	0	4-Cl	89	**21h**	0	4-methyl	289



No.	Z =	R =	IC_{50} /nM	Compd	Z =	R =	IC_{50} /nM
22a	CH	Me	80	**22h**	N	2-MeO-Ph	0.8
22b	N	Me	22	**22i**	N	3-MeO-Ph	0.2
22c	N	Ph	1.4	**22j**	N	4-MeO-Ph	0.1
22d	N	2-F-Ph	0.2	**22k**	N	2-CN-Ph	4.2
22e	N	3-F-Ph	0.5	**22l**	N	3-CN-Ph	2.2
22f	N	4-F-Ph	0.4	**22m**	N	4-CN-Ph	2.7
22g	N	4-Cl-Ph	1.2				

Fig. 10 Structures of GSK-3β inhibitors **22** and **23**

with 4-fluoro-2-methoxy group displayed the best inhibitory activity towards GSK-3β.

Benzoxazinone- and indole-based AK/GSK-3β dual inhibitors

Since adenosine kinase (AK) and GSK-3β are both involved in neurodegenerative disorders, in 2017 Brindisi et al. developed the first class of AK/GSK-3β dual inhibitors with benzoxazinone (**24**) or indole (**25**) scaffolds. Their preliminary SAR analysis is displayed in Fig. 11 [53].

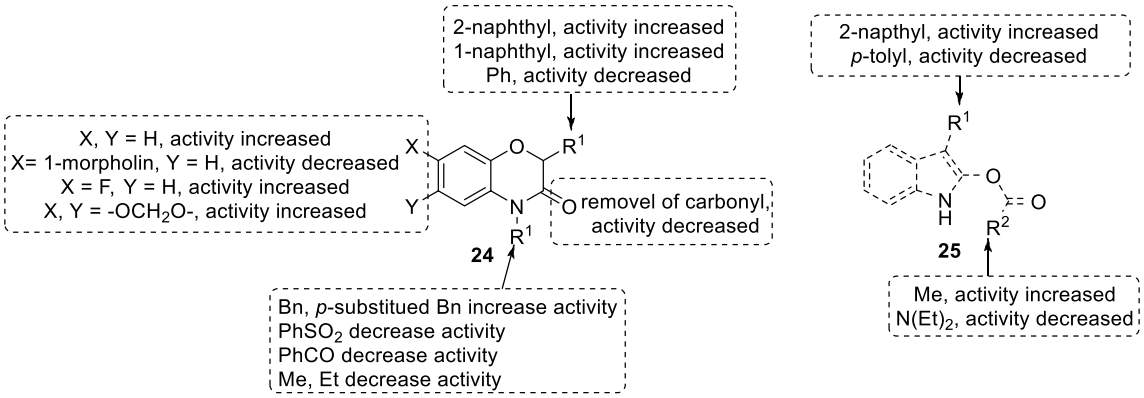


Fig. 11 SAR analysis of benzoxazinone- and indole-based AK/GSK-3β dual inhibitors **24** and **25**

2,4-Dihydropyrano[2,3-*c*]pyrazole-based GSK-3β inhibitor/Nrf2 inducer

The dual-target treatment strategy for AD has attracted more and more attention due to the complex pathogenesis of AD. In 2017, León et al. [54] reported the design of the first dual GSK-3β inhibitor/Nrf2 inducer, 2,4-dihydropyrano[2,3-*c*]pyrazoles **28** (Fig. 12), through analyzing the chemical features of the known GSK-3β inhibitors **26** and **27**. Among the twenty-one synthetic analogs, compounds **28a**–**28l** showed an inhibitory effect on GSK-3β. The SAR study shows that methoxyphenyl- and nitrophenyl-derivatives have better inhibitory activity ($IC_{50} < 10$ μM) than those having phenyl or methylphenyl substituents. In addition, the substituting position of halogen groups on phenyl also has an effect on the activity of compounds, which can be clearly demonstrated by the comparison of the activity of compounds **28c**–**28k**. Furthermore, some of these compounds were found to induce the Nrf2 phase II antioxidant and anti-inflammatory pathway at micromolar concentrations. Thus, this series of 2,4-dihydropyrano[2,3-*c*]pyrazole compounds were considered as multi-targeted leads for the development of anti-AD drugs.

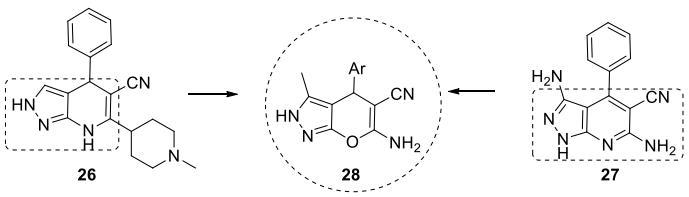
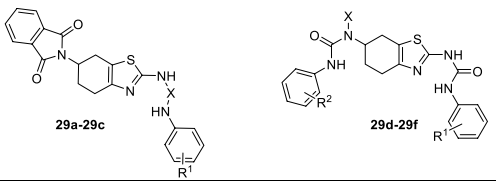


Fig. 12 Structures of 2,4-dihydropyrano[2,3-*c*]-pyrazoles **28** as the first dual GSK-3β inhibitors/Nrf2 inducers

No.	Ar	GSK-3β (IC_{50} /μM)	Nrf2 induction capability (CD /μM)
28a	3-OCH_3Ph	7.20±0.7	11.3±1.3
28b	4-OCH_3Ph	6.60±1.7	25.3±2.4
28c	2-CH_3Ph	>30	>30
28d	3-CH_3Ph	21.4±4.6	14.1±2.1
28e	4-CH_3Ph	17.5±1.9	25.4±2.5
28f	2-ClPh	>30	23.5±3.2
28g	4-ClPh	6.29±2.6	8.31±1.5
28h	2-BrPh	>30	>30
28i	4-BrPh	3.77±2.0	9.37±1.4
28j	2-NO_2Ph	3.89±1.9	11.3±5.5
28k	3-NO_2Ph	7.20±1.6	18.9±2.2
28l	Ph	>30	20.5±3.4

2,4-Dihydropyrano[2,3-*c*]pyrazole-based GSK-3β inhibitor/Nrf2 inducer

Due to that GSK-3β and casein kinase 2 (CK2) are both responsible for the phosphorylation of the tumor suppressor protein (PTEN), Vasu et al. designed and prepared a set of novel dual kinase CK2/GSK-3β inhibitors with 4,5,6,7-tetrahydrobenzo[*d*]thiazole core (Fig. 13) to maximize the effect the kinase inhibitors [55]. The bioassay results showed that compound **29a** had the highest dual kinase inhibitory activity, with IC_{50} values of 1.9 μM against CK2 and 0.67 μM against GSK-3β. Other analogs **29b**–**29f** also showed good dual CK2/GSK-3β inhibition. The SAR analysis indicated that the presence of a carboxyl group at the meta-position of the phenyl ring played a vital role in dual kinase inhibition.



No.	X	R^1	R^2	IC_{50} /μM	
				GSK-3β	CK2
29a		m-COOH	-	0.67±0.27	1.9±0.05
29b		m-COOEt	-	5.3±0.31	7.9±0.12
29c		p-COOH	-	4.6±0.22	4.9±0.09
29d	*n*-propyl	m-COOH	m-COOH	1.5±0.04	2.8±0.05
29e	H	m-COOEt	m-COOEt	2.5±0.23	20.4±0.35
29f	H	m-COOH	m-COOH	6.0±0.15	12.4±0.19

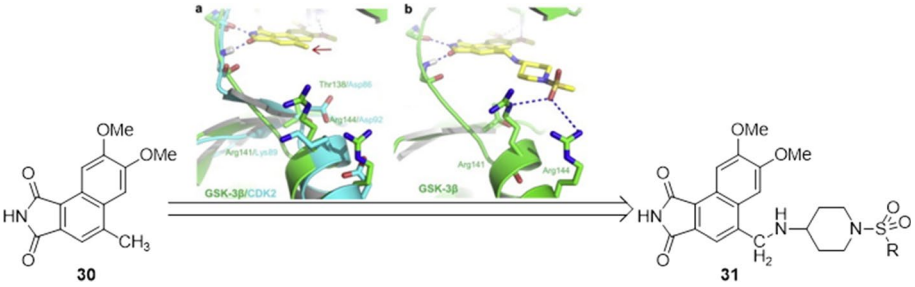
Fig. 13 Dual kinase GSK-3β/CK2 inhibitors **29**

Benzo[*e*]isoindole-1,3-dione-based selective GSK-3β inhibitors

Quan et al. reported a series of benzo[*e*]isoindole-1,3-dione derivatives (Fig. 14) as selective GSK-3β inhibitors through rational drug design [56]. By analyzing the detailed structure inhibitors of GSK-3β and cyclin-dependent kinase 2 (CDK2), they used compound **30** as a starting point to derive a series of inhibitors **31** by introducing a sulfonyl group, which was considered to interact with the positively charged residues Arg141 and Arg144 at the helix D of GSK-3β. Thereinto, five of them (**31a**–**31e**) showed high selectivity against GSK-3β over CDK2, and could significantly activate the cellular Wnt/β-catenin pathway.

Benzothiazinone-based GSK-3β inhibitors

Most of the known GSK-3β inhibitors are reported to be bound to the ATP-binding pocket of GSK-3β. To avoid the adverse effects induced by ATP-competitive GSK-3β inhibitors, Ye and coworkers synthesized a bunch of novel non-ATP competitive GSK-3β inhibitors with benzothiazinones



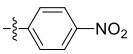
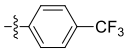
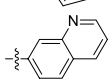
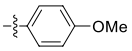
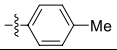
No.	R	GSK-3β IC_{50} /μM	Inhibition of CDK2 /% at 100 μM
30		1.01±0.14	
31a		0.31±0.03	12.3±6.3
31b	-Me	0.36±0.06	3.4±1.5
31c		0.53±0.11	23.7±3.4
31d	$-NO_2$	0.53±0.03	16.9±3.0
31e		0.54±0.09	2.7±1.2
31f		1.53±0.35	12.3±4.1
31g		2.61±0.22	8.7±0.9
31h		3.73±0.23	35.4±9.5

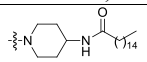
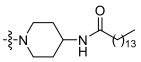
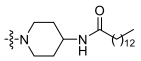
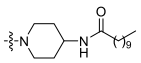
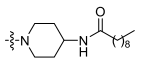
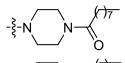
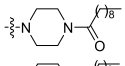
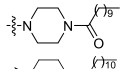
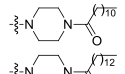
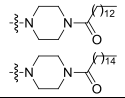
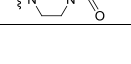
Fig. 14 Benzo[*e*]isoindole-1,3-dione selective GSK-3β inhibitors **30** and **31**

core (Table 6) based on their previous research [57]. These compounds displayed potent inhibition on GSK-3β with low micromolar activities, of which analogs **32a**–**32d** and **32h**–**32k** showed good potency toward GSK-3β. The poor performance of the remaining compounds may be due to the extension of the alkyl carbon chain. The antitumor bioassay indicated that most of these compounds had moderate in vitro anti-proliferative against A2780 and OVCA433 cancer cell lines.

7-Chloro-9*H*-pyrimido[4,5-*b*]indole-based GSK-3β inhibitors

Recently, Koch et al. reported the optimization of tofacitinib-derived 3-[(3*R*,4*R*)-3-[(7-chloro-9*H*-pyrimido[4,5-*b*]indol-4-yl)(methyl)-amino]-4-methylpiperidin-1-yl]-3-oxopropanenitrile (**33**) resulting into a novel class of GSK-3β inhibitors (**34**) by the rigidization of cyanoacetyl piperidine moiety (Fig. 15) [58]. The SAR study showed that when these compounds had a methyl group as a substituent (R^2), their inhibitory activities were decreased, while the formation of the pyrrolidine ring will lead the activity significantly increase. For example, compound **34a** showed the strongest inhibitory effect on GSK-3β with an IC_{50} value of 0.130 ± 0.008 μM, about 19-fold over the hit **33**. Molecular docking analysis indicated the importance of nitrile side chain for the activity of this series of inhibitors.

Table 6 In vitro inhibitory activities of **32** against GSK-3β kinase, and A2780, and OVCA433 cancer cell lines

No.	, R =	IC_{50}/μM		
		GSK-3β	A2780	OVCA433
32a		11.7	3.5	69.8
32b		22.4	4.6	8.3
32c		33.4	9.1	26.2
32d		45.9	23.4	34.4
32e		68.3	27.2	77.4
32f		>100	34.1	81.8
32g		51.7	36.0	89.5
32h		27.1	21.8	22.9
32i		23.2	16.6	19.5
32j		18.9	16.7	16.9
32k		8.7	13.2	15.4

3-Substituted 2-oxoindole GSK-3β inhibitors

A series of 3-substituted 2-oxoindole derivatives **35a**–**35×** and **36a**–**36g** (Fig. 16) as novel GSK-3β were disclosed by Lozinskaya and coworkers [59]. Abundant substituent groups intuitively show the increase or decrease effect between substituents and inhibitory activity, of them, compound **32a** (R = H; aryl = pyridin-2-yl) was the most potent GSK-3β inhibitor with an IC_{50} value of 4.19 nM. This compound exhibited no significant cytotoxicity towards leucocyte at 10 μM, and it further showed a high in vivo antidiabetic efficacy in streptozotocin-treated obese rat model.

2,3-Diaminopyridine moiety-containing GSK-3β inhibitors

To find multifunctional anti-AD molecules, a series of 2,3-diaminopyridine moiety-containing novel GSK-3β inhibitors (Fig. 17) with metal chelation activity were designed and synthesized by Liu et al. [60]. The bioassay results revealed that the amide derivatives **37a**–**37f** showed moderate potency against GSK-3β and weak chelating ability towards Cu^{2+}, Zn^{2+}, and Al^{3+}, while the imine derivatives **38a**–**38c** were potent dual GSK-3β inhibitors (IC_{50} = 38–72 nM)/selective Cu^{2+} and Al^{3+} chelates. In addition, imines **38a**–**38c** could inhibit the phosphorylation of tau protein and have neuroprotective activity by preventing

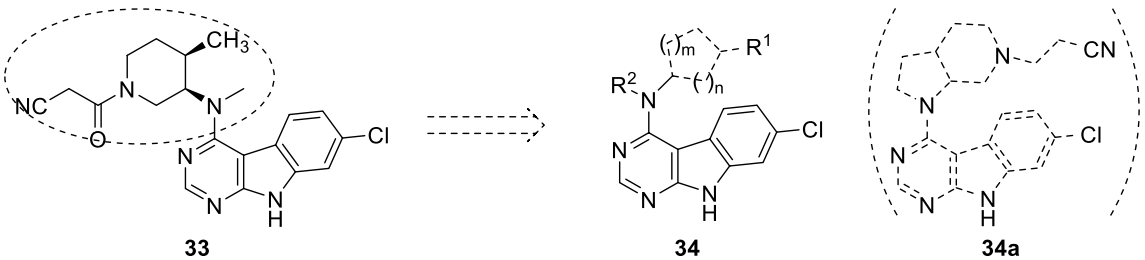


Fig. 15 Development of new type of GSK-3β inhibitors **34** derived from hit **33**

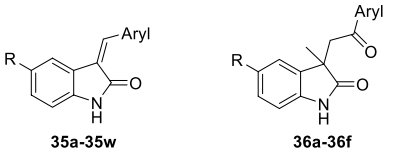
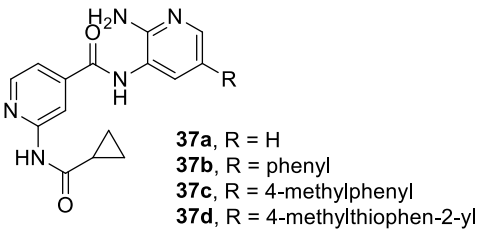


Fig. 16 Series of 3-substituted 2-oxoindole-derived GSK-3β inhibitors **35** and **36**

No.	R	Ar	IC_{50} /μM
35a	H	2-pyridyl	0.00419
35b	Br	4-Br-Ph	>10
35c	BzNH	4-OH-Ph	4.343
35d	(2-furoyl)NH	3,4,5-tri-MeO-Ph	>10
35e	(2-furoyl)NH	4-OH-Ph	>10
35f	$CH_3C(O)NH$	3-OH-Ph	>10
35g	$CH_3C(O)NH$	3,4,5-tri-MeO-Ph	0.2329
35h	MeOC(O)NH	4-OH-Ph	0.1554
35i	MeOC(O)NH	4-Br-Ph	>10
35j	MeOC(O)NH	4-NO_2-Ph	0.3479
35k	H	3-pyridyl	>10
35l	H	4-pyridyl	>10
35m	H	2-thienyl	>10
35n	H	2-furyl	>10
35o	Br	2-pyridyl	>10
35p	Br	3-pyridyl	>10
35q	Br	4-pyridyl	>10
35r	Br	2-thienyl	>10
35s	Br	2-furyl	>10
35t	NO_2	2-pyridyl	20.00
35u	NO_2	3-pyridyl	>10
35v	NO_2	4-pyridyl	>10
35w	NO_2	2-furyl	12.75
36a	OMe	4-Me-Ph	>10
36b	Br	4-Me-Ph	>10
36c	Br	4-MeO-Ph	>10
36d	NH-(2-furoyl)	4-Me-Ph	1.717
36e	H	4-Me-Ph	>10
36f	H	4-MeO-Ph	>10



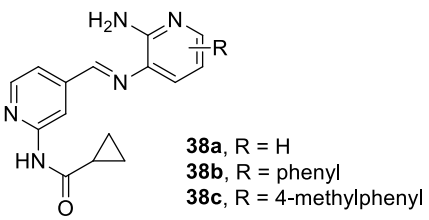


Fig. 17 2,3-Diaminopyridine moiety-containing GSK-3β inhibitors/metal chelates **37** and **38**

nerve SH-SY5Y and PC12 cells from Cu^{2+}-$A\beta_{1-42}$- and H_2O_2-induced cell injury.

Conclusion

GSK-3β was involved in the occurrence and development of various human diseases, such as cancer, AD, inflammation, type-II diabetes, mood disorders, etc. Thus, the development of GSK-3β inhibitors attracted more and more interest from the academic and industrial areas. The present review provided the recent development of GSK-3β inhibitors, including their design strategy, SAR, and in vitro or in vivo biological functional study targeting different diseases, especially cancer and AD. The information summarized herein is helpful for the design of new GSK-3β inhibitors.

As yet, many GSK-3β inhibitors have been discovered, however, only one GSK-3β inhibitor, tideglusib, has entered the phase II clinical trials, meaning that these available promising GSK-3β inhibitors still have great room for structural/biological improvement and more new types of inhibitors are waiting for exploration. Currently, the reversible non-ATP competitive GSK-3β inhibitors, such as allosteric modulators and substrate competitive inhibitors, were more attractive to medicinal chemists due to the advantages, such as higher selectivity and better in vivo efficacy, less adverse effects, and potential therapeutical effects. Besides, the dual/or multi-target GSK-3β inhibitors were also pursued by medicinal chemists with the understanding of both GSK-3β and other targets in multifactorial diseases, especially for AD and cancer associated with complicated pathogenesis. With the ongoing effect and interests towards GSK-3β inhibitors development, more GSK-3β inhibitors with great value in clinical application, either derived from natural product optimization, rational drug design, or drug virtual screening, will be disclosed.

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