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Natural products as novel scaffolds for the design of Glycogen Synthase Kinase 3β inhibitors

Article highlights

- The involvement of GSK-3β in many pathways makes it a very attractive target to be addressed in different diseases;
- Natural products represent an invaluable source of active compounds. In particular, many compounds of marine origin are potent and characterized GSK-3β inhibitors;
- Active compounds of natural origin endowed of pharmacological properties, may show some drawbacks related to their poor pharmacokinetic profile or limited efficacy, as well as the problems related to their supply;
- Structural modification of natural scaffolds is a strategy to be pursued in order to overcome issues related to natural products endowed with pharmacological activity;
- total or semisynthetic modification as well as SAR-based modification, represent proven strategies to obtain more selective and potent GSK-3β inhibitors from natural compounds belonging to alkaloids or flavonoids.

Abstract

Introduction. The different and relevant roles of GSK-3 are of critical importance since they deal with development, metabolic homeostasis, cell polarity and fate, neuronal growth and differentiation as well as modulation of apoptotic potential. Then, its involvement in different diseases is the reason why many investigations have been carried out with the aim of discovering new and promising inhibitors for this target.

Areas covered. Natural products represent an invaluable source of active molecules. In order to overcome issues as poor pharmacokinetics properties or efficacy, frequently associated to this kind of compounds, different GSK-3β inhibitors belonging to alkaloids or flavonoids classes were subjected to structural modifications in order to obtain more potent and safer compounds. Here we report results obtained by using natural compounds as leads, thus providing new kinase inhibitors endowed with better inhibitory profile.

Expert opinion. The strategy based on the structural modification of natural scaffolds is a proven approach as the role of natural products in drug discovery is undoubtedly extremely important due to their pharmacological properties. Whatever the strategy adopted and despite the limitations associated to the structural complexity of natural products this approach is to be pursued for the discovery of novel and potent GSK-3β inhibitors.

Key words
GSK-3β; natural scaffolds; SAR; inhibitors; semisynthetic modification

1. Introduction

1.1 Glycogen Synthase Kinase 3β: structure and regulation.

Most aspects related to cell life are regulated by protein kinases. That's why some mutations or changes in their genes are closely related to cancer and other diseases. In the twenty-first century, GSK-3β has emerged as one of the most attractive therapeutic targets involved in unmet severe pathologies. Although the first role assigned to this kinase concerns its involvement in the regulation of glycogen metabolism, many other roles in pathologies such as Alzheimer's Disease (AD), frontotemporal dementia, type 2 diabetes, bipolar disorders, stroke, other tau pathologies as well as cancer and inflammation have been attributed to this kinase [1]. Undeniably, it controls many cellular processes such as gene transcription and neuronal cell functions, as well as embryonic development, where it plays a fundamental role in the Wnt signaling pathway [2]. For all these roles, it can be considered as a cellular nexus connecting several signaling systems, including a wide range of second messengers and cellular stimulants.

GSK-3 is a highly conserved protein kinase identified in all investigated eukaryotic genomes. GSK-3A and GSK-3B are the two genes that encode two proteins with 51 and 47 kDa, termed GSK-3α and GSK-3β respectively. They share an almost complete sequence identity (98%) between their protein kinase domains. Differences are detectable in their N- and C-terminal regions. In fact, the 4 kDa difference between the two isoforms is due to the presence of glycine-rich region at the amino-terminus, specific for the GSK-3α isoform [3]. The first crystal structure of GSK-3β was published in 2001 showing a typical serine/threonine kinase fold endowed with a small N-terminal domain (residues 25 to 134) consisting of seven antiparallel β-strands and the alpha-C helix, and a larger C-terminal domain (residues 135-380) [4][5]. The ATP binding site is located at N- and C-terminal domains interface and is surrounded by the glycine-rich loop (residues 60-70) and the hinge region (residues 134-139) Fig. 1. The DFG (Asp200 to Gly202) sequence is the starting motif of the activation loop that ends with the APE motif (Ala224 to Glu226). The activation loop forms one of the edges of the substrate-binding groove, the other one is then constituted by a loop that connects β-strand five with the alpha-C helix. The residues from 330 to 384 form a cluster of helices and loops that pack against the C-terminal domain [6].

In particular, the ATP-binding pocket can be visualized as five distinct regions devoted to specific interactions with the ATP-Mg^{2+} complex. These regions are classified as follow: the phosphate transfer area (highly conserved in kinases), the glycine-rich loop (a flexible lid covering the ATP binding site), the upper and lower hinge regions (the first one is involved in H-bond formation with ATP or other small molecules, while the second forms the edge of the solvent opening) and the hydrophobic pocket (presents a series of sequence variations that can be targeted by newly designed inhibitors to increase selectivity). A large network of hydrogen bonds connects ATP with the phosphorylation substrate facilitating the transfer of the ATP γ-phosphate group. In fact, the backbone atoms of residues Asp133, Val 135, Thr138 and Gln185, located in the upper hinge region of GSK-3β, establish H-bonds with the adenine moiety of ATP.

Regarding the regulation mechanism, firstly it must be specified that GSK-3 is ubiquitously expressed and constitutively active in unstimulated tissues. Both isoforms are post-translationally regulated or controlled by protein-protein interactions. The catalytic activity is actually regulated through a plethora of signaling pathways. The activity of this kinase is functionally expressed through the inactivation of specific substrates by phosphorylation. A strong preference is exhibited in phosphorylating “primed” substrates, that are previously phosphorylated protein substrates [7]. As consequence, the signals that act to suppress the activity of GSK-3 induce the activity of these substrates. In particular, the regulation in both proteins occurs by phosphorylation at their amino-terminal domain. Ser9 and Tyr216 represent the two phosphorylation sites that control the catalytic activity of GSK-3β. Tyr216 is conserved in many kinases and it is located in the GSK-3β activation loop where it plays the role of gatekeeper of the substrate-binding groove. pTyr216 is held in place by Arg220 and Arg223 and the phosphorylation of this residue in the activation loop actually controls the catalytic activity of GSK-3 which depends on the correct alignment of the N- and C-terminal domains [8] and greatly increases the enzyme’s catalytic activity [9][10].

Many protein kinases are involved in the inactivating and phosphorylation process at the N-terminal domain site. Among them, the most studied is protein kinase B (PKB/Akt) due to the major role in the insulin response [11][12][13]. Other protein kinases that act on the N-terminal domain of GSK-3 are AMP-dependent protein kinase (PKA) [14][15], atypical protein kinase C’s (PKC) [16] and p90Rsk [17]. Alternative theories have established that GSK-3β is activated by phosphorylation of Tyr216 when cells undergo apoptosis [18] or may undergo an autophosphorylation process [19].

Regarding substrates phosphorylated by GSK-3β, more than 40 substrates were recognized [13]. It was not possible to establish a common phosphorylation mechanism for all of them. Many of them are recognized by the canonical phosphorylation motif SXXXpS containing the phospho accepting Ser or Thr separated by three residues from a phosphoserine or phosphothreonine. In fact, a different kinase must first phosphorylate the substrate at the P+4 position before it can be phosphorylated by GSK-3β at P0 residue. For example, the tau protein is phosphorylated by CDK5 at Ser235 and is then phosphorylated by GSK-3β at Thr231 [20][21].

Such an intricate regulation of this enzyme gives rise of the many pathologies in which the regulation of GSK-3β is implicated. The most studied treatments based on GSK-3-targeting are obesity/diabetes [22][23] and bipolar disordes (BD) [24]. So many other diseases involve this target: schizophrenia [25]; Alzheimer's Disease (AD) [26]; Parkinson's Disease (PD) [27]; cancer [28] and developmental disorders [29]. In light of this the discovery of GSK-3β inhibitors represents an extremely stimulating goal.

1.2 GSK-3 inhibitors

All the different but connected functions covered by GSK-3 make the regulation of this enzyme by inhibitors really a challenge and a lot of effort has been put into the discovery and development of new GSK-3 inhibitors during the last few years. Currently, only few compounds such as TDZD-8, Tideglusib, AZD2858, Cazpaullone and Alsterpaullone, TWS119, CHIR-99021, and SB216763, are undergoing clinical trials as GSK-3β inhibitors (Fig. 2).

Research by both academic centers and pharmaceutical companies has contributed to the discovery of many inhibitors belonging to different chemical families, including organic compounds as well as small cations, showing great chemical diversity [30][31]. In this context, Lithium represented the first GSK-3 inhibitor applied in clinical practice to the treatment of bipolar disorders and depression. Regarding organic GSK-3 inhibitors they may be of synthetic origin or were derived directly or indirectly from small molecules of natural origin [32]. In light of this, it is almost easy to understand that all these entities have very different structures capable of covering a wide range of chemical spaces. The most common classification for GSK-3 inhibitors is based on their different inhibition mechanisms being divided into two main groups: ATP-competitive and non-ATP-competitive inhibitors. Most of the molecules studied act as ATP-competitive inhibitors, so that the blockage of the enzyme

occurs thanks to the molecule's competition with ATP for its binding site. Small molecule acting as inhibitors bind to this site showing very high affinity (concentrations in the nanomolar range). The opportunity to obtain selective ATP-competitive inhibitors endowed with very high affinity may be achieved using structure-based methodologies. Indeed, plenty of ATP-competitive inhibitors have been co-crystallized with the enzyme, giving the opportunity of solving the complex structures by X-ray crystallography. In the meantime, due to the extremely high degree of conservation around this binding site, the opportunity to obtain isoform-selective inhibitors remains remote. Indeed, one of the many issues related to this class of compound is the lack of selectivity [33]. This very important feature can be achieved by molecules that act as non-ATP-competitive inhibitors, capable of interacting with specific kinase sites, such as allosteric sites or substrate-binding domain (Fig. 1). The advantage of non-ATP-competitive GSK-3 inhibitors does not relay only in the selectivity, but also in the possibility of avoiding toxic effects. Indeed, the higher selectivity gives the opportunity of using lower doses of inhibitors and as consequence of reducing side effects. The concerns about the toxic effects related to GSK-3 inhibition are based on the important role of GSK for life, then its inhibition could affect the normal cellular activity. On the other hand, the necessity of inhibiting GSK-3 appears in pathological condition, that is when its activity is elevated. In this case a mild inhibition only restores the kinase activity. In diabetic models its inhibition restored glucose homeostasis, without causing hypoglycemia or hyperinsulinemia. At the same time no evidence has indicated *in vivo* tumorogenesis as consequence of GSK-3 inhibition. This could be ascribed to the activation of the proto-oncogene β-catenin by GSK inhibition. In addition to this it's worth mentioning that the inhibition of GSK-3 reduces cells proliferation and enhance cell death in certain cancers. All this evidence are complemented by the fact that treatment of bipolar disorder with Litium, as GSK-3 inhibitor, has never been associated with tumorogenesis or cancer deaths. Despite GSK-3 inhibition being demonstrated to be safe, a weak to moderate inhibition is suggested for both classes.[34]

Here we provide the interactions established by some of the most important GSK-3 inhibitors belonging to both categories. For in-depth discussion, readers are referred to some excellent reviews of GSK-3 inhibitors and their structure-activity relationship [30][31]. Starting with ATP-competitive inhibitors, it is worth mentioning the maleimide-based compounds. Enzyme interactions for these compounds are based on the creation of H-bonds with the carbonyl oxygen of Asp133, located within the hinge region, and the nitrogen atom of the scaffold. Another interaction occurs between one of the two carbonyl oxygens of maleimide group

with the backbone nitrogen of Val135 [31]. Another compound, representing one of the most relevant ATP-competitive inhibitor is the compound AR-A014418 whose structure is based on the one of thiazolylureas. This compound shows very high affinity in the nanomolar range, it is highly selective and acts by blocking tau phosphorylation. The interactions established by this compound occur in the hinge region where three H-bonds are formed: two with Val135 and one with Pro136 [35]. The Val135 residue, together with Lys85, is also involved in H-bonds formation with two potent GSK-3 inhibitors namely paullones and alsterpaullone, [36].

Regarding non-ATP-competitive inhibitors, the most relevant compounds are represented by Tideglusib [37], Palinurin [38] and halomethyl ketone derivatives which form a covalent bond with the Cys199 residue located at the entrance of ATP binding site [39]. Compared to ATP-competitive inhibitors, these compounds establish weaker interactions with the enzyme and, as consequence, a lower inhibition rate is reached. This condition, as previously reported, is favorable since the therapeutic effect can be achieved by a weak inhibition capable of bringing enzyme activity back to physiological levels and preventing toxic effects [40].

Along with the discussed inhibitors belonging to malemides, thiadiazolidindiones, pyridyl-oxadiazoles and pyrazolopyrimidines, a prominent role in the generation of novel GSK-3 inhibitors is played by marine invertebrates. In this context, compounds such as hymenialdesine, meridianines and indirubins were isolated from several sponges, ascidians and gastropod mollusks, respectively. Some marine natural products have been used as lead compounds, providing a rich source for the discovery of next-generation kinase inhibitors capable of targeting the enzyme in allosteric regions or stabilizing inactive conformations in order to prevent the function of certain kinases [41].

2. Drug Design areas covered

Natural compounds represent a rich source of active entities. Many optimization strategies can be applied to obtain new derivatives with better pharmacokinetic profile or efficacy [42]. A large number of natural compounds were found active towards GSK-3β [41][32][43]. Then some of them were considered as hit compounds and were explored in order to obtain new GSK-3β inhibitors and are reported in table 1. The selection of such molecules not endowed with very high inhibitory potency as hit compounds, meets the necessity of obtaining a moderate activity towards this target. The aim of this paper is to provide an overview of the main available literature reporting the natural product-inspired molecules obtained as GSK-

3β inhibitors. In addition, some structure activity relationships are discussed in order to provide useful information for those who attempt to design new GSK-3β inhibitors starting from a natural scaffold.

2.1 Alkaloids Derivatives

Indirubin derivatives. The bis-indole alkaloid indirubin (1), reported in Fig. 3, was first described in the 1980s as the main active constituent of Danggui Longhui Wan, a mixture of 11 herbal medicines used in traditional Chinese medicine for cancer treatment. More specifically, indirubin is the red colored isomer of indigo found in different indigo plant species and marine organisms (gastropod mollusks). Indirubin acts as a potent ATP competitive inhibitor of CDKs (IC_{50} values in the range of 50–100 nM) and GSK-3β (IC_{50} values in the 5–50 nM range). Structure-activity relationship studies confirmed that indirubins binds to GSK-3β ATP binding pocket in a way similar to their binding to CDKs [44]. Subsequent preclinical studies confirmed indirubin as endowed with antileukemic properties and low toxicity, inspiring the synthesis of new derivatives with better chemical and pharmacological properties.

Hence, alkylated, halogenated and N- and O-substituted indirubins where the first synthesized indirubins with higher antitumoral activity than that of natural indirubin [44][45][46][47]. Halogenated indirubins embody an interesting scaffold for exploring specific kinase inhibition. The naturally halogenated indirubin, 6-bromoindirubin (6BI) (), has a selective and strong inhibitory activity for the mammal GSK-3β (0.01 – 0.1 μM) while other 6-substituted synthetic analogues carrying Cl, F and I showed similar inhibitory potency, but with loss of selectivity [45]. Crystallographic data of GSK-3β in complex with various indirubins confirmed that bromine substitution at position 6 is crucial for selectivity while substitutions at 3' are important for the binding activity. The larger biding pocket of GSK-3β compared to CDKs provides enough space for the bromine atom to be inserted into the back of the cavity, explaining the selectivity of 6BI towards GSK-3β. Halogen substitution at position 5 increases protein kinase inhibition potential, but slightly impairs selectivity [44], whereas, in general, substitution at position 7 decreases the inhibitory activity and brings no benefit in terms of selectivity. Later, 2D-and 3D QSAR studies confirmed that selectivity has a direct correlation with the presence of atoms that electron-withdraw electrons, such as halogens in position 5/6 rather than in position 7/4. Any substitution at position 4, except for hydrogen, causes distortion and loss of the molecular planarity of the structure which is

necessary for the binding, while substitution at position 7 causes unfavorable contacts with the protein backbone [46].

Indirubins show hydrophobic properties, like most inhibitors that bind through hydrophobic interactions within the enzyme's ATP-binding pocket. The conversion of the 3' carbonyl group into an oxime group was an important modification for the modulation of solubility and selectivity properties. The oxime moiety is an ideal site for the introduction of hydrophilic substituents also providing stabilization of these structures. Once again, 6 halogenated substituted indirubins containing the oxime moiety showed greater GSK-3 selectivity and inhibitory potential when compared to other substituted indirubin-3'-oximes. 6-bromoindirubin-3′-oxime (6BIO) (3) is a semi-synthetic cell-permeable analogue of 6BI with a remarkable low nanomolar inhibition range (< 10 nM) currently marketed under the name "BIO" and "GSK-3 inhibitor IX". It is considered a prototype inhibitor for the development of selective and potent pharmacological inhibitors of GSK-3 [45]. A series of bisubstituted 3'-oxime indirubins derivates at positions 5 and 6 were synthesized and the best inhibitory activity and selectivity was observed for 5,6-bisubstituted 6-bromoindirubin-3′-oxime analogues bearing CH_3 and NO_2 and 6-cloroindirubin-3'oxime bearing a chloride atom at position 5 [46]. Nevertheless, despite the presence of the oxime group, simple indirubin analogues still suffer from low hydrophilicity (6BIO LogD 2.59 vs indirubin LogD 2.5) [47]. Therefore, several 6-bromo-indirubin analogues with different substitutions at the 3' position were synthesized. The introduction of amino-aliphatic chains on the 3' oxime group was essential to obtain a series of compounds with increased solubility with logD values varying from 1.90 to -0.87 [47]. Sugar moieties were also introduced to the basic indirubin core at positions 1 and 1′ increasing the solubility of bioactive indirubins [48].

Indirubins consistently proved to be an important molecular scaffold from which very selective and active molecules against GSK-3β emerged. Due to the incredible potential of these compounds as selective GSK-3 inhibitors and possible numerous biological applications, there is still scope and rationale for the synthesis of new indirubins aiming at optimal water solubility without losing their remarkable bioactivity and selectivity properties.

Manzamines derivatives. Manzamines (Fig. 4) are complex alkaloids isolated from Indo-Pacific sponges and characterized as having an intricate and novel 5-, 6-, 6-, 8-,13-membered heterocyclic ring system coupled to a β-carboline moiety. Manzamine A (4) was first isolated in the late 1980s from the Okinawan sponge of the genus *Haliclona*, and latter from other marine sponge families that also produces manzamine-related alkaloids [49][50].

Manzamine A not only inhibits human GSK-3 *in vitro* (IC_{50} value of 10.2 μM) acting as a non-competitive inhibitor of ATP binding, but also inhibits CDK-5, reduces tau hyperphosphorylation in human neuroblastoma cell lines and crosses the blood-brain barrier (BBB) demonstrating its potential to interfere with tau pathologies [51][52].

Hamman et al. (2007) used natural and semi-synthetic analogues to assess the structure-activity relationship in GSK-3 inhibition of some substituents in the carboline moiety and aliphatic heterocyclic system. Replacement of hydrogen on the carboline moiety at position 8 with OH and tosylate groups (OTs) generated slightly more active compounds than manzamine A, while other substitutions with hydroxy derivates at position 6 yielded equipotent analogues. Meanwhile, nitrogen substitution at position 9 of the carboline heterocycle with larger groups such as *i*-But, or *t*-BuOCOMe led to inactive derivatives. Regarding the aliphatic heterocyclic system, the double bond between positions 15 and 16 was essential to promote GSK-3 inhibition. Analogues with a carbonyl group on the cyclooctane ring (5) are less potent than manzamine A while an epoxy function restores GSK-3 inhibition (6). In agreement with the previous statement, an increase in GSK-3 inhibition is observed when bulkier moieties at position 9 are combined with a carbonyl group on the cyclooctane ring, indicating the possibility of a pocket in the GSK-3 enzyme where the phenyl ring of the carboline heterocycle should be allocated. In addition, enzyme inhibition is favored by increasing conformational restriction in the aliphatic part of manzamines (4) [51].

Manzamine-derived alkaloids are considered an intriguing group of marine alkaloids with remarkable therapeutic potential and the subject of a series of studies on their biological activities. However, the vast majority of those studies were carried out with isolated derivatives of marine natural products. In summary, the unique structure together with the extraordinary bioactivity makes manzamine A an interesting scaffold to initiate rational drug design efforts in search of more potent and selective GSK-3 inhibitors that could be designed as potential therapeutic agents for the treatment of diseases mediated by GSK-3, like AD.

Hymenialdisine derivatives. The alkaloid hymenialdisine (HMD) (7) (Fig. 5), is a known metabolite that was isolated for the first time in 1980s from *Hymeniacidon aldis, Axinella verrucosa, and Acanthella aurantiaca* [53]. Chemically, it is a member of the family of tricyclic pyrrole compounds consisting of a brominated pyrrolo[2,3-c]azepine skeleton and a 5-membered glycocyamidine ring system [54].

It was characterized in terms of kinase inhibition and it was found to be not only a potent inhibitor of several related CDKs [55] but also of the nuclear transcription factor NF-kB [46-48]. In this context our interest in hymenialdisine is related to its activity as competitive nanomolar inhibitor towards GSK-3β (IC_{50}: 10 nM), CK1(IC_{50}: 35 nM), CDK1/cyclin B (IC_{50}: 22 nM), CDK2/cyclin E (IC_{50}: 40 nM) and CDK5/p25 (IC_{50}: 28 nM) [46] Crystallographic data revealed for this compound its binding in the ATP binding pocket (Fig. 6) [56].

It is easy to deduce that the principal issue related to this very potent natural compound concerns the low selectivity. Indeed, with the intent of improving selectivity some hymenialdisine derivatives were synthetized in 2004 by Gray and coworkers. The structural modifications were intended to retain activity towards CDKs or GSK-3β and at the same time enhance selectivity. The design of the new derivatives involved the substitution on the pyrrole ring as well as the replacement of the same one with an indole group with various substituents (Fig. 7).

The aim was to optimize the interactions in hydrophobic pocket and to retain important and conserved hydrogen bonds. The selectivity of these compounds was then evaluated towards a panel of kinases. Indeed, the inhibitory activity of the new derivatives has been investigated towards GSK-3β, CDK5/p25 and CDK1/cyclin B. Regarding the inhibitory activity towards GSK-3β, the compounds showing a chloride or bromide substituent in R_1, R_2 or both the positions maintained the inhibitory activity in the lower nanomolar range (IC_{50} from 0.05 to 1.39 μM). Except for the compound reporting a bromide substituent in R_1 position, these derivatives were found to be selective towards CDK1/cyclin B. On the other hands they showed similar activity towards both CDK5/p25 and GSK-3β. Indeed, a decrease in inhibitory activity towards both these enzymes was detected for those compounds reporting a methyl group in R_3 (IC_{50}: 0.27 μM) as well as an ethyl or acetyl group in R_4 position (IC_{50} 0.36 μM and IC_{50}: 1.39 μM respectively). Regarding the inhibitory activity of indole derivatives, it was found to be comparable to that of pyrrole derivatives (IC_{50} from 0.47 to 2.70 μM). Even then, the acylation or alkylation of glycocyamiadine amide reduces activity. Based on these results it is then possible to deduce that R_3 and R_4 substituents are more influent than R_1 and R_2 in the binding activity. Moreover, for the 7-bromoindole analogue a 10-fold improvement of selectivity for GSK-3β (IC_{50}: 0.05 μM) rather than for CDKs (IC_{50}: 1.00 μM) was detected.

The substitution of glycocyamidine ring by arylhydrazones was also investigated (Fig. 8).

For compound showing a bromide group in both R_1 and R_2 positions the activity was not improved (IC_{50}: 4.28 μM). The same results were also obtained for analogues reporting a

fluoride or chloride substituent in R_1 position (IC_{50}: 0.17 μM and IC_{50}: 0.40 μM respectively). This aspect is not surprising since the glycocyamidine ring contributes to multiple hydrogen bonds to the kinase in the ATP binding pocket [56]. Once fixed the hydrazone moiety the differences among pyrrolo- or indolo-azepinones was investigated. Compared to pyrrole analogues the indole derivatives showed at least a 10-fold improvement in activity. Moreover, the introduction of halogen as R_2 substituent increases the inhibitory activity of derivatives against GSK-3β and CDK5/p25. In general, compared to the close structural analogues of HMD, the hydrazones obtained show much reduced activity towards GSK-3β (IC_{50} > 0.09 μM).

Amaryllidaceae Alkaloids Derivatives. Amaryllidaceae alkaloids are produced exclusively by plants of the Amaryllidaceae family and are characterized by polycyclic nitrogen-containing structures grouped in 12 types of rings. These species grow wild in the tropical and subtropical regions of the world, but are widespread mainly in Andean South America, South Africa and the Mediterranean coasts. In traditional medicine, the use of Amaryllidaceae dates to the 4th century B.C., when Hippocrates of Cos used daffodil oil (i.e., *Narcissus poeticus* L.) for the treatment of uterine tumours. Today, Amaryllidaceae alkaloids are being studied for the treatment of drug resistant cancers. For instance, Pancratistatine, Narciclasine, Lycorine, Haemantamine, Distichamine and their derivatives are known for their cytotoxic activity on specific cancer cell lines. Some of them are even involved in different stages of drug development. Moreover, its acetylcholinesterase (AChE) inhibitory property has been exploited in research for the treatment of AD. Galantamine is actually one of the few drugs currently approved by the FDA for the symptomatic treatment of cognitive decline in AD patients [57].

In light of this, Cahlikova research group from Charles University focused its studies on the isolation, characterization and ability to inhibit GSK-3β of several Amaryllidaceae alkaloids. The first work published in 2018 was based on the characterization of twenty-eight Amaryllidaceae alkaloids in terms of GSK-3β inhibitory activity. Three of them were selected on the basis of the percentage of inhibition obtained at 50 μM concentration. Then they were characterized in terms of IC_{50}. For three of them, whose structure are reported in Fig. 9, an IC_{50} value of approximately 30 μM was determined.

In 2019, the same research group published a study about the characterization of twenty-one Amaryllidaceae alkaloids of the narcimatuline type isolated from Narcissus pseudornarcissus L. cv. Dutch Master [58].

The structure of narcimatuline (11) was elucidated by combining MS, HRMS, 1D and 2D NMR spectroscopic techniques and is reported in Fig 10.

This compound, which presents merged in its structure both galanthamine and galanthindole basic scaffold, showed the most interesting biological profile. Indeed, this new isolated compound was found able to *in vitro* inhibit not only GSK-3β (67% of inhibition at 10uM), but also acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and propyl oligopeptidase (POP). Despite the multipotent profile showed by this very interesting natural compound, the structure of haemanthamine (12) (52,4% inhibition at 10uM concentration toward GSK-3β) was selected as starting point for the preparation of some semisynthetic derivatives. The choice of haemanthamine as molecule to be modified was also related to the possibility of isolating a large quantity of this compound from its natural source. Indeed, some derivatives of hemanthamine alkaloid were developed [59].

All the compounds were tested in order to evaluate their antiproliferative activity. Due to the low cytotoxicity values obtained, the interaction of haemanthemine derivatives (13) towards cholinesterases and GSK-3β was investigated. Only three of the tested compounds showed some inhibitory activity towards GSK-3β. Their structures are reported in Fig 11. The percentage of inhibition obtained at 10 μM concentration was found to be lower than that obtained for haemanthemine thus revealing the decrease in inhibitory potency obtained after structural modification. On the other hand, the activity towards cholinesterases was ameliorated for some of them. For compound 13c that showed the most promising profile, a high selectivity towards AchE was detected together with a moderate inhibitory potency (IC_{50} equal to 34,8 uM). Moreover, the assessed BBB permeability makes this compound a promising lead compound.

Staurosporine derivatives. In 1977, Omura's group [60] discovered the world's first indolocarbazole compound, isolated from a microorganism known as *Streptomyces staurosporeus*. This new compound, which was later called "staurosporine" (14), had its structure determined in 1994 by X-ray crystallography (Fig. 12). With regard to its biological activity, antifungal and hypotensive properties were initially reported, however, the most interesting thing is that this compound can be considered the pioneer of a new class of molecules that led to a breakthrough in cancer treatment. In fact, in the decade after its isolation it was discovered that staurosporine was a highly potent inhibitor of protein kinases, particularly tyrosine kinases, thus inducing a significant cytotoxic effect on cancer cells [61]. Staurosporine is highly promiscuous ATP-competitive kinase inhibitor with high activity

toward a wide range of kinases (GSK-3β, IC_{50}: 7.988 ± 1.550 nM) and a variety of staurosporine analogues have been synthesized and optimized for the inhibition of several different kinase targets, including GSK-3β [62].

Kozikowski's group screened a series of staurosporine-based analogues with two indole rings, one indazole ring, and one indole ring or two indazole rings against a family of 30 kinases identifying the compound indolyl-indazolylmaleimide (15) as being capable to inhibit GSK -3β activity by 98% at 10 μM [63]. A series of 3-indolyl-4-indazolylmaleimide derivatives (16) were synthesized to better understand the SARs of this compound in order to identify more potent analogues against GSK-3β. A particularly interesting effect on GSK-3β activity was observed in relation to the presence or absence of the N-methyl group (R_1) in 3-indolyl-4-indazolylmaleimide with bromine (IC_{50}: 0.0035 μM) and fluorine (IC_{50}: 0.05 μM) at position 5. The N-methyl group resulted in a 250-fold increased activity in the brominated compound (IC_{50}: 0.850 μM and IC_{50}: 0.0035 μM, unmethylated and methylated compound respectively), but, on the other hand, it has a negative effect on the fluorinated ligand with 4.3-fold decrease in activity (IC_{50}: 0.0114 μM and IC_{50}: 0.049 μM, unmethylated and methylated compound respectively) [63]. This rather intriguing effect was explained by coupling studies and the well-known crystal structure of the GSK-3β enzyme which showed that the 5-fluoro substituent on the indole ring occupies a position close to the positively charged amino group of Lys85 at the same time as the ring indazole interacts with Tyr134 and Ile62. In contrast, the larger 5-bromo substituent cannot occupy the same position due to steric obstruction of the Leu132 and Lys85 side chains. Thus, bromine-substituted compounds shift position slightly to avoid this steric shock, interacting within the lipophilic pouch formed by the lipophilic moieties of Glu97, Leu130, Leu132, Val110, Met101, Phe201, Cys199 and Lys85. This results in greater exposure of the 5-fluoro-substituted ligands to the solvent, while the substituted bromine is inserted deeper into the binding pocket. As far as the respective NH and N-methyl groups of the in 5-brominated ligands are concerned, both are less exposed to solvent establishing a tighter contact with the binding site [63].

In a recent study, Liu and colleagues (2020) have modified the staurosporine structure into disubstituted amino pyrazole derivatives (Fig. 13) to develop GSK-3β inactivation-centric agents with multitarget profiles as a new therapeutic strategy against the multifactorial etiopathology of AD. Their strategy included changing the planar conformation of staurosporine, replacing the y-lactam ring with other heterocyclic rings maintaining the necessary hydrogen bonding capability to obtain a) monoindole substituted aminopyrazole derivatives having an indole-substitution at the 4-position and a phenyl group at the 5-

position; b) monoindole-substituted aminopyrazole derivatives with indole-substitution at 5-position; c) bisindole-substituted aminopyrazole derivatives, obtained introducing different R1 and R2 groups to the starting indole scaffold; d) bisindole-substituted aminopyrazole derivatives consisting of both the indole unit and the 1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole unit [64].

Among the aminopyrazole derivatives, the 5-indolyl-aminopyrazole (17) with the indoyl group oriented in opposite direction to the amino group (Fig. 14) showed the highest activity with an IC_{50} in the micromolar range (IC_{50}: 7.46 ± 1.07 μM). However, the introduction of different substituents on the phenyl or the indole ring gave rise to compounds with less than 50% inhibitory rate at 10 μM. Interestingly, 4-indolyl-based compound with the indolyl group oriented towards the amino group were inactive against GSK-3β at 10 μM. The bisindolyl-substituted aminopyrazole group, represented here by compound 18 showed a good inhibitory activity against GSK-3β with (IC_{50}: 4.20 ± 0.87 μM). Furthermore, bisindolyl-substituted aminopyrazole derivatives carrying a halogen or a methoxy group in the 5-position of the indole ring (IC_{50}: 1.93 ± 0.22 μM and IC_{50}: 1.76 ± 0.19 μM respectively) showed an activity on average 2.61 times higher than the non-substituted precursor resulting in compounds with IC_{50} in the lower micromolar range. Substituents located at positions 4-, 6- and 7- of the of the indole ring led to an unfavourable inhibition on GSK-3β with an inhibitory rate of less than 50% at a maximum concentration of 10 μM. An aminoethyl substitution on the indole nitrogen (IC_{50}: 8.81 ÷ 0.90 μM) decreased GSK-3β inhibition by approximately 2-fold compared with compound 18, while the piperidinyl substitution (IC_{50}: 3.92 ÷ 0.09 μM) maintained almost the same activity seen in compound 18. In contrast, the presence of aminoethyl or piperidinyl N-Boc substituents caused a significant decrease in GSK-3β inhibition. Bisindole-substituted aminopyrazole derivatives consisting of both the indole unit and the 1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-hi]indole unit containing a diazepinoindole scaffold resulted in a ~3-fold increase in potency compared with compound 19 (IC_{50}: 1.48 ± 0.30 μM) [64].

Together, these new classes of compounds derived from the core of the natural compound staurosporine showed incredible potential not only against the GSK-3β enzyme but also against other AD-related targets consolidating its role as an interesting precursor to new, more potent and more selective compounds.

2.2 Flavonoids derivatives.

Flavonoids are pigments that constitute a group of natural compounds biosynthesized from phenylalanine and found in dietary plants and herbal remedies [65].

From a long time, due to their diversity and easy availability, flavonoids represent a fascinating and rich class of compounds well known for their medicinal use. So far, more than 7,000 flavonoids have been isolated from natural sources such as wine, fruits, plants and vegetables. Pharmacological studies have demonstrated the relevance of the dietary consumption of flavonoids and flavonoids-rich foods such as blueberry, green tea, cocoa, and others.[65]

Since flavonoids can bind to many proteins and interfere with hormones, DNA, transporters, enzymes and scavenge free radicals, an important role of these compounds in the management of diabetes mellitus, cancer, cardiovascular diseases, microbial diseases are reported. In addition, it is proven that flavonoids can significantly ameliorate cognitive abilities and delay the aging process in related neurodegenerative disorders such as AD [65][66].

Many mechanisms of action have been identified, including angiogenesis inhibition, antioxidant effect, apoptosis induction and inhibition of some functional enzymes like aromatases, topoisomerases, tyrosine kinases and glycogen phosphorylases cyclin-dependent kinases. It is reported that flavonoids can potentially influence tau phosphorylation interacting with mitogen-activated protein kinase (MAPK) and other protein kinase signaling pathways. In addition, another reported mechanism for the inhibition of tau phosphorylation by flavonoids is the activation of the PI3K/Akt pathway that inhibits the tau kinase GSK-3 by phosphorylation at Ser9.[67]

Considering that flavonoids can play a pivotal role in the drug discovery using the main molecule as starting scaffold for the synthesis of various drugs, many subclasses among them have been modified in order to increase their activity. In particular, molecules obtained from flavopiridol and C-glycosylflavones core demonstrated an interesting interaction with GSK-3β [68].

Flavopiridol derivatives. The natural anti-rheumatic flavonoid “Rohitukine” (20) isan anti-cancer chromone alkaloid from an Indian tree while Flavopiridol (alvocidib) (GSK-3β IC_{50}: 1.184 μM) (21) is its semi-synthetic flavone analogue. Flavopiridol is under clinical trial for the treatment of chronic lymphocytic leukaemia due to its cytotoxic activity on human cell lines and its potent inhibition of cyclin-dependent kinases (CDKs) CDK2/cyclin E1 and CDK4/cyclinD1 as well as GSK-3β [69][70].

New synthetic flavopiridol-based compounds were synthesized in order to identify precursors that meet the structural requirements necessary for the inhibition of kinases such as CDK9, CDK10 and GSK-3β with greater potency. Within this context, a new series of compounds with the flavopiridol core containing olefin D ring was synthesized with a focus on exploring the chemical space of the thiol substituted flavopiridol C ring bearing different azoles moieties (22) (Fig. 15) [71].

All tested compounds were shown to more efficiently inhibit the human isoform of GSK-3β than flavopiridol. In particular compound 23, with the introduction of the methyl-benzimidazole on the flavopiridol C ring and the substitution of R1 with a chlorine atom, demonstrated to be optimal for the activity, proving to be 20 folds more active than flavopiridol with an IC_{50} of 0.059 μM.

Molecular docking experiments showed the benzimidazole moiety located in the solvent area of the crystal structures and proved that this compound acts as an ATP-competitive inhibitor for GSK-3β [71]. These results can be considered an important starting point for the development of more efficient and selective inhibitors of GSK-3β in the pathogenesis of AD.

C-glycosylflavones derivatives. C-Glycosylflavones and their aglycones are ubiquitous in plants and it has been shown that extracts from Corn silks (CS), from the maize food crop (*Zea mays* L.), contain many phytotherapeutic agents with anti-inflammatory, antioxidative, antibiotic, antidiabetic and anticancer activities. Furthermore, CS has been an alternative medicine widely adopted by Asia-Pacific populations for many treatments such as cystitis, prostate disorder, hyperlipidemia, urinary infections and hyperglycemia. Thanks to phytochemical and metabolomic studies, it was proved that metabolites from CS are rich in flavonoids, polysaccharides, terpenoids, and steroids. In particular, flavones are more abundant, and these molecules are likely to be responsible for the unique biological properties attributed to CS extracts. CS extract was submitted to a bioassay-guided fractionation and two already-known 6-C-glycosylflavones were found: isoorientin and 3′-methoxymaysin. Enzyme kinetics and molecular docking studies demonstrated that isoorientin reversibly inhibited GSK-3β (IC_{50} values of 185 μM) via an ATP non-competitive mechanism, acting as competitive inhibitor of GSK-3β substrate. Thus, due to its considerable promise in preventing and managing many disorders isoorientin has gained attention [72][73]. Inspired by isoorientin a new structural class of GSK-3β competitive inhibitors containing a 6-C-glycosylflavone scaffold was semi synthesized (Fig. 16) [74].

The obtained enzymatic activity indicated that the phenolic hydroxyl groups have a smaller contribution in the inhibition of GSK-3β considering the weak decreased potency of the compounds bearing tetramethylated alcohol (IC_{50}: 239.2 ± 1.2 μM) and tetramethylated carboxylic acid (IC_{50}: 237.3 ± 1.4 μM) compared to isoorientin (IC_{50}: 184.9 ± 1.4 μM). The compound with methyl esters at R_1 positions, increased the activity (IC_{50}:135.0 ± 1.3μM), proposing that hydrophobic groups at the primary hydroxyl position are better for GSK-3β inhibition. Furthermore, the conversion of the primary alcohol into amide at X_2, demonstrated the essential role of this residue in increasing potency, generating compounds with IC_{50} values lower than 20 μM. Probably due to the size of the hydrophobic pocket at the substrate site in GSK-3β, cyclopentyl and cyclohexyl analogues as R_2 substituents (IC_{50}:13.1 ± 1.1μM; IC_{50}: 9.0 ± 1.3μM, respectively) demonstrated higher inhibitory activity over alicyclic rings [74].

In addition, at R_2 the isopropyl group showed better inhibition than the linear propyl group; aliphatic and alicyclic amides displayed greater inhibitory activity for GSK-3β than aromatic and monofluorination did not increased affinity. Finally, the substitution in R_2 with the trifluoromethyl group proved to be essential in increasing the inhibition of GSK-3β resulting in a compound with a group a (S)-CF_3 with IC_{50}: 0.59 μM and to its epimer with a (R)-CF_3 group with IC_{50}: 2.3 μM. [74]. It was demonstrated by SAR analyses and in silico mechanistic investigations that both the presence and chemical position of C-glycone in the flavone core are pivotal to bind the substrate site on GSK-3β. Compounds with the isopropyl moiety have IC_{50} values of 5.4 ± 0.1, 0.59 ± 0.04, and 2.3 ± 0.5 μM suggesting that they have an appropriate carbon chain length and topological size within the hydrophobic cleft of the substrate site of GSK-3β. Moreover, the hydrophobic, π-cation and orthogonal multipolar interactions of compound bearing the (S)-CF_3 with the substrate site produce selective inhibition against GSK-3β. Taking into account the very high increase in activity, the new synthesized inhibitors can be considered as drug leads with therapeutic activity in GSK-3β dependent diseases [74].

2.3 Curcumin derivatives.

Natural products in the diet have shown significant effects on human health and longevity with few side effects. The diferuloylmethane molecule is the dimeric derivative of ferulic acid better known as curcumin and isolated from the rhizomes of *Curcuma longa*. It is a food component used as a seasoning for a long time with proven efficacy and safety for the prevention and treatment of various disorders [75].

In fact, curcumin has proven pleiotropic properties acting as free radical scavenger with a wide range of effects such as anti-inflammatory, antineoplastic, hypoglycaemic, antioxidant, antimicrobial ones. In addition, a neuroprotective effect linked to several pathways related to AD is proven, such as aggregation of Aβ and tau proteins, oxidative stress, and neuroinflammation [76].

At the molecular level these properties are due to the impact on several cell signalling pathways, such as Nrf-2, NF-kappa B, Akt. The various pharmacological properties of curcumin suggest a potent direct inhibitory action on GSK-3β (IC_{50}: 17.95±1.03 μM). However, curcumin had several limitations as a consequence of its low solubility, limited absorption, incomplete tissue distribution, metabolic and chemical instability mainly attributed to the 3- methoxy-4-hydroxy aryl moieties. Taking these assumptions into account, some synthetic analogues were designed, synthesized and tested in order to preserve the activity against GSK-3β [77].

Chemically, curcumin in solution exists in two interconvertible tautomeric forms: ketone (1E,6E-1,7-bis-4-hydroxy-3-methoxyphenylhepta-1,6-diene-3,5- dione) and enol (1E,4Z,6E5-hydroxy-1,7-bis-4-hydroxy-3-methoxyphenylhepta-1,4,6-trien3-one), which is the most abundant species according to the stabilizing effect of the intramolecular H-bond in the α,γ-unsaturated-keto-enol moiety (Fig. 17).

Computational studies have revealed that curcumin bears a highly electrophilic α,β-unsaturated carbonyl system, which probably covalently interacts with the crucial Cys199 residue of GSK-3β. In particular, in GSK-3β binding pocket, the central β-keto-enol function is involved in an H-bond with Tyr134 and Val135 while the two aryl functions interact with Lys85, Glu97, and Arg141, and Glu173, respectively.

To explore the chemical space of the target and considering that an essential pharmacokinetic property for CNS-targeted drugs is crossing the blood−brain barrier (BBB), different substitutions were evaluated on the aryl functions of the molecule and some others were performed modifying the heptadienone fragment as well. The potency of the synthesized compounds was evaluated with a luminescent assay using a human recombinant enzyme.[78] The new synthetic molecules showed a good to moderate GSK-3β inhibitory activities in the micromolar range, although only slightly increased compared to the reference natural compound curcumin.

Concerning ring A, the structure−activity relationship revealed that in R_1 the presence of hydrogen or methoxy group improves the activity as well as the presence of electro-donating hydroxyl and methoxyl in R. In fact, the comparison between compound with hydroxyl

residue (IC_{50}: 8.39 ± 1.59 μM) in R and compound with methoxyl in R too, proved the efficacy of the para-methoxy substitution, giving compound 24 (Fig. 18), the most active in the series, with an IC_{50} value of 0.53 ± 0.27 μM. On ring B the R_2 substitution allowed to a compound with para-benzyloxyphenyl (IC_{50}: 0.90 ± 0.38 μM) and to a compound with the para-tolyl residue (IC_{50}: 2.09 ± 0.51 μM) demonstrating a good GSK-3β inhibition.

On the other hand, the insertion of the benzyloxyphenyl and the fluoro-benzyloxyphenyl moieties in the A ring and the fluoro-benzyloxyphenyl also in the B ring causes a decrease in activity. Furthermore, the introduction of a diethyl fumarate fragment at position R_3 of the heptadienone backbone resulted in a loss of potency (IC_{50}> 6.09 ± 0.53 μM).

A docking study of compound 24 showed the presence of a thia-Michael reaction due to a nucleophilic attack of GSK-3β Cys199 residue on the reactive α,β-unsaturated carbonyl function of the synthetic molecule. Also, the conformation of compound 24 stabilizes interactions within the hinge region of GSK-3β. Probably, the non-covalent interactions of compound 24 with the enzyme binding pocket are essential for the optimal orientation of the electrophile pharmacophore toward a specific protein nucleophile residue [77].

Finally, a small series of compounds was synthesized in order to evaluate the role of the β-keto-enol tautomer in comparison to the diketo one.

Concerning the diketo tautomer (Fig.19), compound with methoxyl residues in R_4 and R_5 (IC_{50}: 15.30 ± 3.64 μM) showed a significant loss of potency compared to the β-ketoenol analogue. On the other hand, the variously fluorine substituted benzyloxyphenyl groups (IC_{50} range between 5.56 ± 0.01 and 9.66 ± 1.02 μM) showed only a moderate decrease in activity.

In conclusion, a low-micromolar inhibition activity was observed giving good promises in the development of curcumin-based candidates for GSK-3β inhibition.

2.4 Trans-cinnamoyl derivatives

Polyphenols are secondary metabolites widely present in vegetables, fruits, seeds, cereals and oils. Recently, interest in polyphenols has increased due to their high antioxidant properties. Furthermore, polyphenols have been reported to be useful in preventing multiple diseases including type II diabetes, cancer, atherosclerosis, cardiovascular and neurodegenerative diseases. The structure of polyphenols consists of two phenyl rings with hydroxyl groups in ortho or para positions whose number is directly proportional to the antioxidant capacity of the molecules. Phenolic acids are classified into two other main

categories: hydroxybenzoic and hydroxycinnamic acid derivatives as Caffeic, Coumaric, and Ferulic Acids.[79][80][81]

Ferulic acid (FA) showed an anti-inflammatory effect, through the inhibition of cyclooxygenases, good metal chelating activity and ability to interfere in the β-amyloid aggregation process. For all these reasons, FA may represent a potential multi-target AD drug candidate that could be evaluated against other crucial AD targets such as GSK-3β.[82] In fact, other phenolic acids have already been proven to decrease tau phosphorylation and inhibit GSK-3β such as caffeic acid (IC_{50}: 425.01 ± 7.61 μM) and rosmarinic acid (IC_{50}: 135.35 ± 4.69 μM) that share with ferulic acid the trans cinnamoyl scaffold [83].

Starting from the molecular structure of the ferulic acid ethyl ester (ethyl 4-hydroxy-3-methoxycinnamate) (25) which share the same biological properties as FA, but has increased blood-brain barrier (BBB) permeability, Milelli and co-workers (2017) synthetized five trans-cinnamoyl derivatives (Fig. 20) designed to act as antioxidants, thanks to the substituted aromatic portion derived from phenolic acid (Ferulic Acid and Caffeic Acid); to interact with GSK-3β through the α-β unsaturated system of the same trans cinnamoyl scaffold and finally to interfere with β-amyloid aggregation through the substituted aromatic moiety and the extended planar conjugate system. The introduction of 3-pyridyl ring indeed increased the interaction with $A\beta_{42}$ growing fibrils. However, even though the resulting inhibitory activity of these derivatives was increased compared to the ethyl ferulate, most showed poor inhibition range between 25.18 to 43.55% at 50 μM. Only the derivative characterized by a 3,4-dihydroxy substitution on the aromatic ring (26) emerged as a remarkable GSK-3β inhibitor with an IC_{50} value of 24.36 ± 0.01 μM. SARs studies suggested that the presence of the two hydroxy groups on the aromatic ring is a critical requirement for the activity. In fact, their replacement with methoxy or ethoxy functions or the presence of a single hydroxy group dramatically affects the inhibitory response [58][84].

3. Conclusion

Structural modification of natural products is to be considered a very interesting approach in drug discovery [85]. The development of new and complementary strategies to modify natural scaffold has found its application also in the design and discovery of GSK-3β inhibitors. Starting from the marine environment, all of nature has offered many interesting compounds active toward GSK-3β. The idea of subjecting some of those scaffolds to structural modifications has proved to be a successful approach since all the case study reported in this review show the opportunity to obtain inhibitors with higher potency and

selectivity or endowed with a better pharmacokinetic profile. The extraordinary structural diversity of natural products, together with the opportunity of exploring the mode of action of the studied compounds[85], and thanks to all information related to GSK-3β structures and regulating mechanism, were useful in disclosing the structure-activity relationships of different chemical classes of kinase inhibitors thus helping in the design of new potent inhibitors.

4. Expert Opinion

The interest in GSK-3β has its foundation in the role played in different and severe diseases. That's why more than one thousand papers have been published on the design and synthesis of GSK-3β inhibitors in the last year. Many approaches can be adopted to reach the final goal of obtaining novel GSK-3β inhibitors. Beside some classical approaches as the rational design, as well as the Multi Target Direct Ligands (MTDLs) strategy [86], the modification of natural scaffolds can be considered a strategy to be pursued. This last strategy represents a proven approach as the role of natural products in drug discovery is undoubtedly extremely important due to their special features. Let's just consider their major roles in cancer, cardiovascular and infectious diseases, or multiple sclerosis [87][88]. Small molecules of natural origin interact with proteins in their natural environments acting as signaling molecules between different forms of life, with great potential for applications in human health. All these aspects take on particular importance if we consider that many organic GSK-3β inhibitors have a natural origin. Marine invertebrates have played a prominent role in the generation of novel GSK-3β inhibitors. The marine environment indeed represents an enormous source for the discovery of potential therapeutic agents, including metabolites derived from microorganisms, and has offered many successful scaffolds in the areas for GSK-3β drug discovery program. Some of the most significant examples of natural products active as GSK-3β inhibitors are represented by molecules such as manzamine A, isoflavone, genisteine (metabolite of *Micromonospore sp*), furanosesquiterpene and palinurin. On the other hand, some naturally occurring marine compounds have also been used as lead compounds, providing an abundant source for the discovery of next-generation kinase inhibitors endowed with higher selectivity. Selectivity probably represents one of the most common issues related to GSK-3β inhibitors. Hence, one of the goals to be pursued in the modification of natural scaffold, is to obtain compounds endowed with higher selectivity than the starting compound. For example, by obtaining compounds able of targeting allosteric regions away from ATP binding sites [41]. The development and

optimization of already known classes of natural products aims to obtain new entities with a better pharmacokinetic profile and/or efficacy [42]. Thus, together with the study of new active natural compounds, research needs to focus on the development and optimization of already known classes of natural products. In fact, molecules of natural origin may show suboptimal pharmacokinetics properties or efficacy that do not suit the desired profile. Furthermore, the development of botanical drugs has always been accompanied by some challenges related to the variability in the composition of the plant material. This strategy was largely adopted in the last decade and has also been applied to the design of many GSK-3β inhibitors giving rise to more potent inhibitors, as discussed in this review. We are confident that many other compounds from plants and microorganism will be isolated and characterized as active towards this target. The opportunity to use such complex structures as starting point for a semisynthetic modification program represents a great opportunity both in terms of costs and efforts in the drug discovery process. This approach proves to be effective and highly recommended when one observes that a large part of the small molecule drugs that have been approved by regulatory agencies in the last decades are pure natural products or their derivates [42]. For all those reasons, a successful drug design strategy could be the development of natural product hits into good leads and ultimately into successful drugs. In light of this, structural modifications can occur based on total or semi-synthetic strategies as well as on biosynthetic engineering. This last one represents, together with the chemical semisynthesis, the way to be pursued to obtain NP analogues with superior pharmacological properties. Indeed, behind the opportunity given by this strategy to obtain higher amount of substance, it represents a strategy to optimize natural products lead compounds as well as to obtain structural diversification of natural-product libraries. [89] Moreover, it could be intended also as an approach to overcome difficulties endowed with chemical synthesis.[90]

Recent advances in these fields are reinforcing the discovery of natural product-based drugs giving the opportunity to overcome some drawbacks related to natural products and making their complex scaffolds accessible with the ultimate goal of obtaining natural products analogues with superior properties [91].

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**This review highlights the importance of structural modification of NPs in drug development.

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**This manuscript is very inspiring since it shows all the advantages that natural compounds can give to drug discovery process and how reach the goal of obtaining a drug starting from

a NP.

Captions

Fig. 1. GSK-3β bound to ADP (PDB ID: 1J1C) is represented as cartoon, with labelled N-lobe (lilac) and C-lobe (light orange). Relevant structural elements cited in the text are highlighted in different colors: the glycine-rich loop (yellow), the αC helix (red), the activation loop (green), the hinge region (magenta) and the APE motif (blue), and the DFG motif (light blue, sticks are also shown). The ADP molecule is represented as capped grey sticks, with CPK coloring for heteroatoms, and the magnesium ion is represented as a sphere. In the insets on the right, a close-up of the ATP site (top panel) and of the substrate binding site (bottom panel) are reported: binding site residues are labelled and reported as lines, hydrogen bonds are shown as black dashed lines.

Fig. 2. Structure of GSK-3 inhibitors and the IC_{50} values towards GSK-3.

Fig. 3. Structures of Indirubin and its derivatives and the IC_{50} values towards GSK-3.

Fig. 4. Manzamines structures and the IC_{50} values towards GSK-3.

Fig. 5. Hymenialdisine structure and the IC_{50} value towards GSK-3.

Fig. 6. Hymenialdisine interactions in the ATP binding pocket.

Fig. 7. Hymenialdisine Pyrrol (A) and Indole (B) analogues.

Fig. 8. Hymenialdisine arylhydrazones derivatives.

Fig. 9. Amaryllidaceae Alkaloids selected and the IC_{50} values towards GSK-3.

Fig. 10. Narcimatuline structure and the IC_{50} value towards GSK-3.

Fig. 11. Selected haemanthemine derivatives and the percentages of inhibition obtained at 10 μM. The IC_{50} value towards GSK-3β is reported for the most active compound.

Fig. 12. Staurosporine-based analogues.

Fig. 13. Staurosporine disubstituted aminopyrazole derivatives.

Fig. 14. The most active staurosporine derivatives and the IC_{50} values towards GSK-3.

Fig. 15. Flavopiridol derivatives and the IC_{50} values towards GSK-3.

Fig. 16. General chemical structure of the modified 6-C-Glycosylflavone.

Fig. 17. General chemical structure of β-keto-enol curcumin tautomer.

Fig. 18. Highest GSK-3β inhibitory activity of the curcumin derivatives and the IC_{50} values towards GSK-3.

Fig. 19. General chemical structure of diketo curcumin tautomer

Fig. 20. Trans cinnamoyl derivatives.

Table 1. Biological activity, inhibitory potency and source of the most relevant natural products whose scaffolds have been modified to optimize their active towards GSK-3.