Laine, Aino E.; Lood, Christopher; Koskinen, Ari M.P.

Pharmacological Importance of Optically Active Tetrahydro-beta-carbolines and Synthetic Approaches to Create the C1 Stereocenter

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Abstract: 1,2,3,4-Tetrahydro-β-carbolines (THβCs) are a pharmacologically important group of compounds belonging to the indole alkaloids. C1-Substituted optically active THβCs have been the target of extensive synthetic efforts due to the presence of the scaffold in numerous natural products and synthetic targets. This review briefly summarizes the methods to obtain the C1 stereocenter and concentrates on evaluating the pharmacological importance of optically active C1-substituted THβCs, including their PDE5-inhibitory, antimalarial, antiviral and antitumor activities.

Keywords: tetrahydro-β-carboline; THβC; pharmacological importance; biological activity; C1-substituted THβC

1. Introduction

1,2,3,4-Tetrahydro-β-carbolines (THβCs), a compound class within the indole alkaloids, consist of a variety of both simple and complex natural and synthetic compounds [1]. These compounds possess a vast spectrum of biological activities and their use in novel pharmacological applications is under constant study, as the THβC structure is present in drugs currently available on the market, drug candidates under development and many other pharmacologically interesting compounds [2–17].
One synthetically interesting subgroup among the THβCs is the optically active THβCs with C1-substitution. A stereocenter at C1 is a typical feature in natural THβCs and establishment of this stereocenter has received plenty of attention. C1-Substituted THβCs have a wide variety of pharmacological properties, including PDE5-inhibitory [2], antimalarial [3–9], antiviral [10–13] and antitumor [14–17] activities. This review summarizes the methods to create the C1-stereocenter and describes the pharmacological activity of simple C1 substituted THβCs. This review offers a welcome update to a previous review discussing β-carbolines [18]. Furthermore, this is the only review focusing on C1-substituted THβCs and this focus allows covering these compounds in more detail.

2. Structure and Occurrence

β-Carboline alkaloids are an important group of natural and synthetic indole alkaloids which all bear the common feature of a tricyclic pyrido[3,4-b]indole ring structure [19]. The first β-carboline alkaloid recognized was harmalin, originally isolated in 1841 from Peganum harmala [20], also known as Syrian rue. The occurrence of β-carbolines in Nature is widespread, presumably due to their simple biogenesis from tryptamine (or tryptophan), and today β-carbolines have been isolated from various plant families, fungi, animal tissues and marine sources [1]. The fully aromatic members of this group are named β-carbolines (βCs) 1, whereas the members with partially saturated C-rings are known as 3,4-dihydro-β-carbolines (DHβCs) 2 and 1,2,3,4-tetrahydro-β-carbolines (THβCs) 3 (Figure 1). The three rings are referred to as A, B and C-ring, as labeled in structure 1.

![Figure 1](image-url) The basic structural units of βC (1), DHβC (2) and THβC (3).

The best known natural THβCs have been isolated from Peganum harmala and Pausinystalia yohimbe (formerly Corynathe yohimbe). Yohimbe alkaloids encompass such pharmacologically interesting natural products as yohimbine and its isomers, reserpine and ajmalicine (Figure 2) the latter two being currently used as antihypertensive drugs. Harmala alkaloids include various β-carbolines including the THβCs tetrahydroharmine (an active ingredient in yaje, or ayahuasca, a hallucinogenic brew prepared from the Amazonian plant Banisteriopsis caapi), tryptoline, harmicine and pinoline (a melatonin metabolite produced in the pineal gland) [21]. Today, the most important synthetic compound encompassing the THβC structure is tadalafil, which has reached almost $2 billion annual sales in the treatment of erectile dysfunction under the brand name Cialis [2]. Tadalafil is also used for pulmonary arterial hypertension treatment under the brand name Adcirca.
3. Biosynthesis

The biosynthetic route from tryptamine (4) or tryptophan and a carbonyl compound to THβC 5 is simple and the starting materials and their derivatives are widely available in Nature. The reaction from tryptamine to THβC is an enzymatic Pictet-Spengler cyclization and several “Pictet-Spenglerases” have been isolated. The Pictet-Spengler reaction is essentially a two-part reaction (Scheme 1). First, the amine and an aldehyde condense to form an iminium ion. Second, the indole attacks the iminium species from the 3-position, forming a spirocycle that rearranges to a positively charged intermediate which then finally undergoes aromatization via deprotonation to yield the THβC 5 [1,22].

Scheme 1. Biosynthesis of THβCs.

In the biosynthesis of indole alkaloids, the carbonyl species is often the iridoid glucoside secologanin. The condensation reaction between secologanin and tryptamine is catalyzed by the enzyme strictosidine synthase (STR). The resultant THβC strictosidine is a common precursor for a
number of β-carbolines as well as other alkaloids, such as ajmalicine, strychnine, reserpine, quinine, catharanthine and vindoline (Figure 3) [22,23].

**Figure 3.** Alkaloids formed from strictosidine.

4. Synthetic Methods to Create the C1 Stereocenter

The THβC skeleton is found in numerous pharmacologically interesting compounds and hence these alkaloids have been in the focus of synthetic efforts for a long time. The most popular synthetic routes utilize the Pictet-Spengler cyclization [24] (extensively reviewed in 1995 by James Cook [25] and more recently by Joachim Stöckigt in 2011 [23]) that could be considered as a biomimetic approach. Alternatively, a rather similar Bischler-Napieralski cyclization [26] can be used. In a Bischler-Napieralski reaction, a tryptamide 6 is cyclized. Usually dehydration reagents, such as PCl₅, POCl₃, SOCl₂ or ZnCl₂, are needed to promote the loss of the carbonyl oxygen. The product of the Bischler-Napieralski reaction is a DHβC 7 which can then be further reduced to form the corresponding THβC 5 (Scheme 2).

**Scheme 2.** A general Bischler-Napieralski cyclization and reduction to THβC.
Chirality can be introduced to the DHβC product by using asymmetric reduction protocols. Asymmetric transfer hydrogenation (ATH) using Noyori–type catalysts [27] offers a powerful method of accessing a chiral THβC skeleton. Due to the highly stereoselective nature of the reaction in question, this remains one of the most commonly employed procedures. Classical Noyori conditions use an azeotropic mixture of Et₃N and HCOOH as the hydrogen source to reduce compound 8 to the corresponding chiral THβC 9 (Scheme 3).

Scheme 3. Classical Noyori ATH conditions [27].

In addition to ATH, the stereochemistry of the reduction product can be controlled also by preexisting directing moieties in a diastereoselective fashion. In Woodward’s classic total synthesis of reserpine [28], published in 1958 (Scheme 4), a Bischler-Napieralski reaction from amide 10 to DHβC 11 was followed by a NaBH₄ reduction selectively forming THβC 12. Interestingly but not very surprisingly, this reduction selectively yielded the wrong diastereomer. However, in this case the configuration at C1 could be inverted at a later stage of the synthesis.

Scheme 4. Bischler-Napieralski reaction and diastereoselective reduction in the total synthesis of reserpine [28].
The stereochemistry in the THβCs can also be controlled by using chiral inductors in the Pictet-Spengler reaction. Internal induction as a means to control the stereochemistry at C1 uses chiral starting materials that are often derived from tryptophan. The existing stereochemistry guides the formation of the second chiral center in cases when C1 is substituted [29,30]. The diastereoselectivity of Pictet-Spengler reaction has been studied and discussed in detail by Bailey and Cook [25,31]. The conformation of the spiroindolenine intermediate determines whether a trans- or a cis-product is formed (Scheme 5). The trans-product is predominantly formed under thermodynamic control and under kinetic control the selectivity is turned towards the cis-product. However, the overall control of the cis/trans-selectivity is very complicated; in addition to the reaction temperature, the substitution pattern together with the size and electronic properties of the substituents have a considerable impact on the selectivity.

Scheme 5. Formation of cis- and trans-products from the spiroindolenine intermediate.

\( \text{a = axial, e = equatorial.} \)

Despite the complicated nature of this type of internal chiral induction, the reaction outcome has the potential of being highly stereoselective. It has been used extensively in indole alkaloid synthesis to control the stereochemistry at C1 [30,32]. An early example of successful use of internal induction is found in the ajmaline synthesis by Cook (Scheme 6) [33]. In this work, tryptophan benzyl ester 13 was used for the Pictet-Spengler reaction. The yield of the trans-product 14 was enhanced by acid induced epimerization that was conducted simultaneously with the Pictet-Spengler reaction.
The key in the epimerization is a reversible ring opening that favors the thermodynamically more stable trans-product (Scheme 7). Hence, a reliable protocol exists to yield trans-product in very high selectivity from N2 benzyl substituted tryptophan derivatives. The same strategy to reach intermediate 15 has been successfully used to synthesize other related alkaloids such as 11-methoxymacroline and alstophylline [34].

Scheme 7. Epimerization of 1,2,3-substituted THβCs favor trans-product.

Bailey et al. have studied kinetically controlled Pictet-Spengler reactions and found that in addition to trans-selectivity, under suitable reaction conditions and substitution pattern, the Pictet-Spengler reaction can become highly cis-selective [31]. In a representative example (Scheme 8), the cyano substituent in the tryptophan derivative 16 is necessary for the reaction outcome to achieve good cis-selectivity, to form product 17. The kinetically controlled reaction has been subsequently used e.g., in (−)-raumacline synthesis [35] and the conditions leading to the cis-selectivity have been studied thereafter [36,37].

Scheme 8. The Kinetically controlled Pictet-Spengler reaction in (−)-raumacline synthesis [35].
In addition to a directing group at C3, also chiral auxiliaries on N2 have been studied as an alternative. A benefit of an auxiliary on the nitrogen would be the easy attachment and removal of the chiral auxiliary. However, simple benzyl- or naphthyl-derived chiral groups provide only moderate diastereoselectivity and only 30%–80% de [38,39]. Yet, good diastereoselectivities have been obtained using N,N-phthaloylamino acids (Scheme 9) [40]. In this example the pre-formed imine 18 is protected with a phthaloylamino acid derivative and the N-protected THβC 19 is formed diastereoselectively.

**Scheme 9.** Asymmetric Pictet-Spengler using chiral N2-auxiliary [40].

Moreover, the source of stereochemical information in Pictet-Spengler reactions can be from chiral carbonyl compounds. Ducrot et al. condensed tryptamine 4 with a chiral aldehyde 20 derived from L-glutamic acid (Scheme 10) [41]. The preferred cis-compound 21 was formed exclusively when a carboxybenzyl (Cbz) protecting group was used (R = Cbz) and the selectivity was turned towards the trans-product 22 when the amine was protected with a pyrrole. Ducrot et al. speculated that the size of the protecting group is an important factor, but since pyrrole and Cbz–protecting groups are rather similar in size it seems more likely that this selectivity is guided by other factors.

**Scheme 10.** Pictet-Spengler reaction with chiral carbonyl species [41].

External asymmetric induction can also be used in the Pictet-Spengler reaction. The first enantioselective Pictet-Spengler reactions using external asymmetric induction were conducted in 1996 by Kawate et al. using diisopinocampheylchloroboranes and reaching 90% ee [42]. Today, various asymmetric reagents have been used for Pictet-Spengler reactions providing moderate to high ee:s. In recent publications, popular catalysts in asymmetric Pictet-Spengler reactions includes thiourea based catalysts [43,44] and chiral phosphoric acid diesters [45,46] (Scheme 11).
Despite the amount of publications related to asymmetric Pictet-Spengler reaction with external asymmetric induction, these methods have several limitations: the C1 substituent usually has to be rather bulky in order to achieve >80% ee’s; reaction times can increase to several days and the catalyst loading is often rather high, >10%.

While Pictet-Spengler and Bichler-Napieralski reactions are the most common methods to build the THβC scaffold, domino reactions incorporating the Heck reaction have also been suggested as a possible approach [47, 48]. Recently, Pfeffer et al. reported domino Heck-aza-Michael reactions with asymmetric induction [49]. The method provided related N-heterocycles such as tetrahydroisoquinolones with good de, however, THβCs were obtained with a modest 60% de only (Scheme 12).

Another example of establishing the C1-stereocenter has been demonstrated by Meyers et al. in their total syntheses of (+)-deplancheine and (−)-yohimbine [50, 51]. In their work, C1-substitution was introduced at a later stage using the N2-auxiliary as a directing group (Scheme 13). With this method high ee’s were obtained.

5. Pharmacological Importance

This chapter concentrates on the pharmacological importance of C1-substituted THβCs. As the skeleton is a common feature in many natural and synthetic compounds, both the multitude of compounds belonging to this group as well as their corresponding biological activities is vast. The
review emphasizes recent studies rather than more traditional applications of THβCs. The biochemical and pharmacological functions of β-carbolines (including THβCs) has been reviewed in 2007 [18] as well as the pharmacological importance of indole alkaloid marine natural products in 2005 [52].

5.1. Antiprotozoal Activity

Several THβCs have been reported to exhibit antiprotozoal, most notably antimalarial, activity (Figure 4). Malaria is one of the most important infectious diseases in the world. According to the World Health Organization (WHO) 200-300 million people are infected and 1.5–2.5 million people die of malaria annually. Some 90% of malaria deaths occur in Africa and 85% of the deceased are younger than 5 years-old [53]. Malaria is caused by red blood cell infecting protozoan parasites belonging to the Plasmodium genus, mainly Plasmodium falciparum [54]. Traditionally, malaria has been treated with quinine type drugs such as chloroquine. However, the emergence of drug resistant strains has created new challenges for efficient treatments [55]. Several recent studies have focused on the use of different THβC type compounds in the treatment of malaria [3–9].

(+)-7-Bromotrypargine (29) is a marine natural product that was recently isolated from a sponge, Ancorina sp. Davis et al. reported the isolation and the structural elucidation of the compound together with tests towards antimalarial activity [3]. The compound was tested against both chloroquine-resistant (Dd2) and chloroquine-sensitive (3D7) strains of P. falciparum and (+)-7-bromotrypargine was shown to display IC50 values of 5.4 μM (Dd2) and 3.5 μM (3D7). Similar compounds were also studied by Chan et al. and moderate antimalarial activity was reported [4].

Figure 4. THβCs with antiprotozoan properties.
In 2012, Gellis et al. synthesized a series of simple 1-substituted THβC derivatives with the general structure 30 with one or more substituents on the phenyl moiety. They tested a series of 20 compounds against the W2 culture adapted strain of *P. falciparum* resistant to chloroquine, pyrimethamine and proguanil and nine compounds showed antiplasmodial activity. The most active compound was a para-methoxy-substituted one with IC$_{50}$ of 0.7 µM (W2 IC$_{50}$ of chloroquine 0.7 µM) [5].

In 2008, Gupta et al. synthesized a series of chloroquine-THβC hybrid molecules with the general structure 31. Altogether 23 compounds were screened against chloroquine sensitive *P. falciparum* strain and the most active compounds had R = i-Pr, R = Me and R = Et and showed minimum inhibitory concentrations (MIC) of 0.05, 0.06, and 0.11 µM, respectively, thus showing significantly greater activity than the standard drug chloroquine (MIC = 0.391 µM) [6].

A new class of potent antimalarials that has recently gained attention are spiroindolones with a THβC structure. In 2010, these types of compounds were recognized as antimalarials in high-throughput screenings by the Novartis Institute of Tropical Diseases [7,8]. These compounds act against *P. falciparum* with a mechanism distinct from that of the existing antimalarial drugs [7] and the optimized lead compound NITD609 (32) has a very high activity of IC$_{50}$ = 0.2 nM [8]. In 2012, NITD609 entered phase 2 clinical trials [9].

In addition to antimalarial studies, THβCs have recently also gained attention as potential antileishmanial and trypanocidal compounds. Leishmaniasis is a tropical infectious disease and the number of people infected with leishmaniasis is ~12 million. The annual incidence of leishmaniasis is ~2 million cases and the numbers are increasing. Leishmaniasis is caused by the protozoan flagellate *Leishmania* spp., most notably *L. donovani*, which is spread by sand flies (*Phlebotomus* and *Lutzomyia* spp.). About 90% of leishmaniasis cases occur on the Indian Peninsula, in Brazil and in Sudan [54].

Trypanosoma spp. cause trypanosomiasis that can either be manifested as African trypanosomiasis (sleeping sickness) caused by *T. brucei* or as Chagas disease caused by *T. cruzi*. The incidence of African trypanosomiasis is 50,000–70,000 cases annually and it is endemic to the tropical Africa, while Chagas disease occurs in the Middle and Southern America. The approximated number of people with Chagas disease is 8–11 million [54].

It has been known for a long time that such complicated THβC alkaloids as α-yohimbine, corynanthine and buchtienine exhibit antileishmanial activity [56,57]. However, during the last 5 years a new interest has arisen towards smaller, synthetic THβC derivatives and several publications have reported antileishmanial activity. In 2010, Chauhan et al. synthesized a series of indolyglyoxylamides with the general structure 33 and reported good antileishmanial activities with IC$_{50}$ values of 3.79 µM and 5.17 µM for the ortho-bromosubstituted and para-ethylated compounds, respectively [58]. These values were several folds better than the standard drug activities (IC$_{50}$ of pentamidine: 20.43 µM). Kumar et al. have reported triazine derivatives 34 as well as other similar derivatives 35 as leishmanicidals [59,60]. The triazino derivatives have also been tested in vivo. Gellis et al. have tested their antimalarial compounds with the general structure 30 for antileishmanial activity. A p-bromosubstituted compound showed the most promising inhibitory activity towards *L. Donovani*, with IC$_{50}$ value of 6.1 µM (IC$_{50}$ of pentamidine: 6.3 µM) [5].

Some THβC derivatives have also been studied for trypanocidal activity. In 2010, Tonin and Valdez published studies on similar THβC derivatives (36 and 37) [61]. These compounds showed promising
activity and compound 37 has been further studied for synergistic activity with other medication [62] but these publications remain the only publications so far on trypanocidal activity of THβC derivatives.

5.2. Antiviral Activity

THβCs have been recognized as antiviral compounds since 1984 when Rinehart et al. first studied eudistomins against herpes simplex virus-1 (HSV-1). Eudistomins are marine alkaloids isolated from the colonial tunicate *Eudistoma olivaceum*, and four eudistomins contain the THβC scaffold (38–41, Figure 5) [10,11].

*Figure 5. THβCs with antiviral properties.*

In addition to the basic THβC structure, eudistomins C, E, K, and L have a condensed oxathiazepine ring system, only reported in these compounds. It has been reported that these four eudistomins have *in vitro* activities against Herpes simplex virus-1 (HSV-1) ranging from 25–250 ng/12.5 mm disc [10]. Later it was also reported that eudistomin K showed activity against the polio vaccine type-1 virus [63]. Eudistomins C and E are also known to possess activities against RNA viruses such as Coxsachie A-21 virus and equine rhinovirus [11]. In 1992, (−)-debromoeudistomin K (42) and its structural analogues were tested against a number of viruses and significant antiviral activities were reported against influenza A and B in Madin-Darby canine kidney (MDCK) cells. Activities have been reported also against respiratory cyncytial virus, vesicular stomatitis virus, Coxsachie virus B4 and polio vaccine type-1 virus [12].

The antiviral activities of these eudistomins have never been further studied or developed, but a different series of THβCs have been more recently studied against the human papilloma virus (HPV). In a study at GlaxoSmithKline, a series of 1-substituted THβC derivatives were optimized and resulted in compound 43 possessing nanomolar activity against HPV. The optimized compound had an activity of IC_{50} = 23 nm [13]. GlaxoSmithKline has patented the use of this type of THβCs for the treatment of HPV [64].

5.3. Anticancer

Since the 1980s, THβC derivatives have been tested against cancer cell lines. During the last decade, the interest has increased tremendously as traditional THβC targets such as the mitotic kinase Eg5 and phosphodiesterase 5 have been recognized as cancer targets.
The first reports on the cytotoxicity of compounds with THβC structure came in 1990 when the newly isolated eudistomins were studied for antileukemic properties. Eudistomin B (44, Figure 6) showed antitumor activity against leukemic cell lines L1210 and L5178Y [65]. Later also eudistomin K (40, Figure 5) was described as an antitumor lead against the murine leukemia cell line P-388, the human leukemia cell line L-1210 and human adenocarcinoma cell lines A-549 and HCT-8 [12]. Eudistomin E (39) is also active against the human mouth epidermal carcinoma KB cell line [66]. Apart from eudistomins, few THβCs derivatives had been studied for antitumor properties until recent years. However, the group of THβCs that are today recognized as antitumor compounds is growing.

Figure 6. THβCs with cytotoxicity activity.

In 2005, Shen et al. synthesized a series of simple THβC and DHβC derivatives with the general structure 45. The compounds were examined against the murine cell line P-388 and the human cell lines KB-16 and A-549, and the human colon adenocarcinoma cell line HT-29. All synthesized compounds exhibited moderate cytotoxicity [67]. In 2011, Shen et al. published a new study in which they had increased the size of the substituent in C1 and had a series of THβCs and DHβCs with general structure 46. The series was evaluated for antitumor activity against human tumor cells including KB, DLD, NCI-H661, Hepa, and HepG2/A2 cell lines. In this study, the DHβC derivatives gave generally better results though also the THβCs showed significant cytotoxicity [14].

In 2009, Santos et al., inspired by arborescidine alkaloids, synthesized tetracyclic compounds resembling arborescides and tested them for antitumor activity towards human lung fibroblasts (MRC-5), human gastric adenocarcinoma (AGS), human lung cancer (SK-MES-1), human bladder carcinoma (J82) and human leukemia (HL-60) cells [68]. From the arborescidine resembling compounds, compound 47 showed most activity having IC50 values in micromolar range. The research group also tested all the intermediate compounds they had synthesized and found that the non-cyclic compound 48 actually gave better response to almost all tested cell lines with IC50 values ranging from 8.8 to 18.1 µM for lung fibroblasts, gastric adenocarcinoma, lung cancer and bladder carcinoma (IC50
of standard etoposide: 0.36–3.93 µM). Kumar et al. have also tested their leishmanicidal triazine THβC hybrids (34, Figure 4) for cytotoxicity and found that they display nanomolar cytotoxic activity. Their best hit was compound 49, which had an IC₅₀ value of 122 nM [69]. In 2012, Skouta et al. synthesized a series of 1,2-disubstituted THβCs and found that compound 50 showed a unique selectivity towards tumorigenic versus non-tumorigenic cells and induced cell death without the activation of caspases, hence inducing a non-apoptotic cell death [15]. Simple 1,3-disubstituted THβCs have also been tested for cytotoxic activity against the insect origin Spodoptera frugiperda Sf9 cell line and the most promising compound was 1-phenyl-THβC-3-carboxylic acid (51). Furthermore, these compounds experienced substantial insecticidal activity against mosquito larvae of Culex pipiens quinquefasciatus species and mustard aphid (Lipaphis erysimi) [70].

Today, one very interesting feature in THβCs is their recognition as mitotic kinesin spindle protein (KSP, also referred to as Eg5) inhibitors. Mitosis is the part of cell division in which the chromosomes condense and divide into two identical sets. The mitotic kinesins are intimately involved in the formation of the mitotic spindle, chromosome segregation, checkpoint control and cytokinesis (Figure 7). The kinesin spindle proteins are highly expressed in breast, ovary, colon, lung, uterine and retinoblastoma tumors [71].

Figure 7. Mitosis and the mitotic kinesins involved in the five steps [72].

KSPs became an important cancer target when monastrol, the first KSP inhibitor, was discovered in 1999. During the last decade, the development of KSP inhibitors has been rapid and many pharmaceutical companies now have KSP inhibitor drugs in clinical trials [71]. During the last ten
years, several papers have been published on the KSP inhibitory properties of THβC derivatives (Figure 8) [16,73–78]. The mitotic kinesin spindle proteins as cancer targets have been the subject of several recent reviews such as the extensive reviews by Schmidt and Bastians in 2007 [79] and Chan et al. in 2012 [80].

Figure 8. Mitotic kinesin spindle protein (KSP) inhibitors.

In 2003 Hotha et al. published the results of an extensive screening that revealed a THβC derivative HR22C16 (52) as a potential lead compound for KSP inhibition. They reported that HR22C16 had an IC$_{50}$ value of 800 nM against KSP [16]. After the discovery of HR22C16, several related derivatives and their inhibitory actions have been reported. In 2005, Sunder-Plassmann et al. published a series of HR22C16 derivatives and reported that replacing the N-butyl side chain with an N-benzyl side chain increases inhibitory activity to IC$_{50} = 650$ nM [73]. These type of indolopyridines were patented in 2009 as KSP inhibitors by a German pharmaceutical company, 4SC [74]. The company has now one KSP inhibitor in clinical Phase I trials (SC4-205) [75] and although its structure is not yet revealed, it was speculated in a recent review that it is based on the indolopyridine scaffold [76].

HR22C16 inspired compounds have also been further studied by Liu et al. who reported that the metabolically liable phenol group can be replaced with indolyl without losing inhibitory activity [77]. The research group also replaced the fourth ring in the HR22C16 structure with a simple acyl group on N2 giving compounds of general structure 53, thus returning to the original THβC three-ring system. Barsanti et al. also published a paper in which 1,2-disubstituted THβCs 54 were evaluated as KSP inhibitors [78]. The structures of Barsanti’s compounds 54 and Liu’s compounds 53 are highly similar. Barsanti’s most promising lead had an IC$_{50}$ value of 58 nM. The group was also able to co-crystallize the inhibitor with KSP making it possible to observe the major interactions in the binding site of KSP with their ligand (Figure 9).

A novel application for THβCs arose when phosphodiesterase 5 (PDE5) became a promising cancer treatment target. Phosphodiesterases are enzymes that catalyze the breakdown of cyclic guanosine monophosphate (cGMP) to guanosine monophosphate (GMP). PDE5 inhibition is one of the common targets for compounds with the THβC structure. It has traditionally been a target for treating erectile dysfunction and pulmonary arterial hypertension. Increased concentration of cGMP in vascular smooth muscle cells leads to vasodilation and subsequently erection. In 2006, Serafini et al. first recognized PDE5 inhibitors as antitumor agents [17]. The use of PDE5 inhibitors in the treatment of cancer was reviewed in 2009 [81].

As tadalafil (Figure 2) was one of the PDE5 inhibitors Serafini et al. used when testing antitumor properties, it is not surprising that many papers discuss the cytotoxicity of tadalafil-inspired compounds. Tadalafil acts as a PDE5 inhibitor in low nanomolar range and analogues with similar IC$_{50}$
values have been synthesized [82–85]. The generalized Markush structure 55 (Figure 10) and its use as a PDE5 inhibitor was patented in 2011 [86].

**Figure 9.** Schematic presentation of interactions between the ATP binding pocket of KSP and inhibitor [78].

![Figure 9](image_url)

**Figure 10.** PDE5 inhibitor.

![Figure 10](image_url)

5.4. Other Pharmacological Uses

Complex natural alkaloids that contain the THβC structure such as yohimbine or reserpine have a wide range of pharmacological activities. The extracts from *Rauwolfia* spp. has been a part of traditional medicine in tropical and subtropical areas. Some known mechanisms of action of this type of molecules are serotonin receptor (5HT) antagonism and α-adrenergic receptor antagonism. Hence these molecules have a profound effect on the CNS, being hallucinogens, vasodilators and analgesics. However, as the range of activities is broad and these compounds lack inherent selectivity, they haven’t been very useful in modern medicine [87].

Serotonin receptor antagonism has been studied with simple THβCs. An example of such a study was done in the Lilly research laboratories in 1996 by Audia *et al.* who synthetized a series of 1-substituted THβCs in which the substituent consisted of various benzyl or naphthyl groups, as in compound 56 (Figure 11) [88]. The compounds showed moderate selective antagonism towards the 5HT2B-receptor. Similar studies were conducted by Giorgioni *et al.* in 2005 [89]. No recent studies have been published on THβCs as 5HT antagonists.

During the last decade, several novel receptor interactions and possible applications of THβC derived compounds have been suggested. Glennon *et al.* has reported the binding of simple
C1-unsubstituted THβCs 57 to imidazole receptors I₂ and I₃ [90]. In 2001, Poitout et al. first described 1,3-substituted THβC derivatives 58 as selective somatostatin receptor type 3 (SSTR3) antagonists [91,92]. Somatostatin receptors are G-protein coupled receptors inhibiting adenylyl cyclase, thus exerting various other effects on intracellular messenger systems. SSTRs are known to mediate cognitive effects, growth hormone inhibition and insulin secretion inhibition [93]. Merck and Co have been granted a patent in the use of THβC based compounds similar to 58 as SSTR3 antagonists in the treatment of type 2 diabetes mellitus [94].

**Figure 11.** THβC derivatives with miscellaneous pharmacological activities.

THβC derivatives have also been patented for several other uses: protein tyrosine phosphatase (PTP) inhibition [95], growth hormone secretagogue receptor (GHSR) antagonism [96] and histamine receptor modulation [97]. In 2013, THβC derivatives were reported to target fatty acid amide hydrolase (FAAH) and transient receptor potential (TRP) channels [98]. Furthermore, two publications have suggested that THβC RGD peptidomimetic conjugate 59 acts as an antithrombotic agents and have free radical scavenging properties [99,100].

6. Conclusions

This review illustrates the pharmacological importance of C1-substituted optically active THβCs featuring numerous recent studies. Development has been rapid especially in antitumor applications as well as in antimalarial applications. Moreover, several novel targets have been recently recognized. Although methods to establish the C1 stereocenter exist, there is room for development and additional contributions.

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Author Contributions

A.E.L designed and conducted the study. A.E.L. wrote the manuscript and C.L. and A.M.P.K. critically revised it and gave scientific advisory.

Conflicts of Interest

The authors declare no conflict of interest.

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