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Review article

Research progress in the biological activities of 3,4,5-trimethoxycinnamic acid (TMCA) derivatives

Zefeng Zhao a, Huanhuan Song a, c, Jing Xie a, Tian Liu a, Xue Zhao a, Xufei Chen a, Xirui He b, Shaoping Wu a, c, e, Yongmin Zhang a, c, d, Xiaohui Zheng a, *

a Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, 229 Taibai Road, Xi'an, 710069, China
b Honghui Hospital, Xi'an Jiaotong University, Xi'an, 710054, China
c Biomedicine Key Laboratory of Shaanxi Province, Northwest University, Xi'an, Shaanxi, 710069, China
d Sorbonne Université, Institut Parisien de Chimie Moléculaire, CNRS UMR 8232, 4 place Jussieu, 75005, Paris, France

corresponding author. Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, 229 Taibai Road, Xi'an, 710069, China.
E-mail addresses: wushaoping@nwu.edu.cn (S. Wu), zhengxh@nwu.edu.cn (X. Zheng).

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A B S T R A C T

TMCA (3,4,5-trimethoxycinnamic acid) ester and amide are privileged structural scaffolds in drug discovery which are widely distributed in natural products and consequently produced diverse therapeutically relevant pharmacological functions. Owing to the potential of TMCA ester and amide analogues as therapeutic agents, researches on chemical syntheses and modifications have been carried out to drug-like candidates with broad range of medicinal properties such as antitumor, antiviral, CNS (central nervous system) agents, antimicrobial, anti-inflammatory and hematologic agents for a long time. At the same time, SAR (structure-activity relationship) studies have drawn greater attention among medicinal chemists, and many of the lead compounds were derived for various disease targets. However, there is an urgent need for the medicinal chemists to further exploit the precursor in developing chemical entities with promising bioactivity and druggability. This review concisely summarizes the synthesis and biological activity for TMCA ester and amide analogues. It also comprehensively reveals the relationship of significant biological activities along with SAR studies.

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* Corresponding author.
** Corresponding author. Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, 229 Taibai Road, Xi'an, 710069, China.
E-mail addresses: wushaoping@nwu.edu.cn (S. Wu), zhengxh@nwu.edu.cn (X. Zheng).

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1. Introduction

TMCA is a cinnamic acid substituted by multi-methoxy groups (Fig. 1). It is considered as an active metabolite of the root of Polygala tenuifolia Wild. (Polygalaceae), which have been used as traditional medicine in China for treating insomnia, headache and epilepsy [1,2]. TMCA has been reported to show anticonvulsant and sedative activity in former studies, and mechanism research reveal that it act as a GABAA/BZ receptor agonist for anti-seizure and insomnia therapy [3,4]. The appealing structural scaffold and pharmacological importance of TMCA has encouraged researches to synthesize its drivatives as novel drug candidates. Up to now, TMCA derivatives have attracted great attention and interest from researchers in the field of medicinal chemistry [5].

TMCA acts as a precursor to construct a large number of structural frameworks for diverse applications. The carboxyl group of TMCA is the most frequently concerned group for modification. Additionally, ester and amide derivatives are the most important analogues to report in this review. The substitution point at C=C bond and methoxy groups are also briefly mentioned in this article [6–9]. The modifications in these frameworks lead to the broadening of activity continuum of TMCA analogues [10,11].

As for the pharmacology researches, this review is focus on the properties of TMCA esters and amides as antitumor, antiviral, CNS agents, antimicrobial, anti-inflammatory and hemagglutinating agents. We summarized advances in natural products of TMCA analogues (N1–9) and synthetic derivatives (S1–74). As for the synthetic derivatives, both the investigations that TMCA as core nucleus or as active substituent are reviewed. When using TMCA as substituent, the analogues are compared to the most potent compound according to SAR. For these points of view, this review is dedicated to accomplish an urgent need of compilation and summarization of natural products, biological activities and SAR that could be helpful for researchers to design some new potentially active of TMCA analogues.

With the development of society, health problems arouse more and more attention worldwide. Side effects and multidrug-resistant exist in clinical utilizations of modern drugs have forced researchers to set their sights to natural products to seek precursors with more safety and efficiency. TCM (Traditional Chinese Medicine), a cluster of time-honored herb medicine, has attracted much more safety and efficiency. TCM has been absorbed into the investigation [12,13]. Combination of Traditional Chinese Medicine Chemistry, CTCMC, is our main drug design strategy (Fig. 2), which means to integrate active constituents based on TCM theory. Accordint to the strategy CTCMC, we have developed potential lead compounds including DBZ (tanshinol borneol ester) [14,15], 2-hydroxypropylbenzodiazepine-5,11-dione analogues [16] and TMCA-α-asarone ester [17,18]. We believe that this strategy is benefit to the innovation of new drugs from natural products. Moreover, we consider that this strategy can be helpful to decrease the costs during screening the test compounds and the blindness existing in the structural modification of natural products.

2. Biological activities of natural TMCA ester and amide derivatives

2.1. TMCA esters and amide isolated from natural products

TMCA esters are widely distributed in several types of medicinal plants. The genus Polygala, containing spieces P. tenuifolia, is the richest resource for TMCA ester, is recorded as the standards for quality control of P. tenuifolia using the HPLC (high performance liquid chromatography) determination method according to the 2015 edition of Chinese pharmacopoeia (Fig. 3) [22]. TMCA has been demonstrated to be the metabolite of N1 [23]. The latter showed antidepressant activity mediating via the inhibiting of MAO (monoamine oxidase)-A and MAO-B activity, reducing plasma cortisol and MDA levels, increasing SOD (superoxide dismutase) activity [24]. Tenuifoliside A (N2), another active TMCA ester isolated from P. tenuifolia, possessed antidepressant-like, cognitive enhancement and cerebral protective effects [25,26]. In addition, N2 was proved to promote the viability of rat glioma cells C6 through BDNF (brain derived neurotrophic factor)/TrkB-ERK (extracellular signal-regulated kinase)/PI3KCREB signaling pathway [27].

Bioactivity-guided fractionation of root extract of P. tenuifolia yielded some constituents with soluble epoxide hydrolase inhibitory activity (Table 1), including esters N2, N3, N4 and NS [28]. Thereinto, ester NS displayed the most potent inhibition of soluble epoxide hydrolase with the IC50 (half maximal inhibitory concentration) value of 6.4 μM. Ester N2, which was structurally similar to ester NS, performed best interaction between the soluble epoxide hydrolase in molecular docking (RCSB Protein Data Bank ID: 3ANS).
As for the SAR, research results demonstrated that the substituted benzoic acid esterification on the pyranose was helpful for the compounds to interact with active site of soluble epoxide hydrolase.

In 2013, Zhao et al. [29] presented that one of the major metabolite of *P. tenuifolia* in rat, 3,4,5-trimethoxycinnamate (N6), at the dosage of 15–30 μM markedly shortened APD50 (action potential duration at 50% repolarization) and APD90 (action potential duration at 90% repolarization) in cardiomyocytes in a concentration-dependent and a reversible manner (Fig. 4). Moreover, ester N6 suppressed L-type calcium current, but showed effect on neither Ito (transient outward potassium current) nor I_{K,SS} (steady-state potassium current). Furthermore, N6 abolished isoprenaline and BayK8644-induced EADs (early afterdepolarizations), suppressed

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**Table 1**

Inhibitory activity of soluble epoxide hydrolase and interaction for ester N2, N3, N4 and N5.

| Com. | Inhibitory activity | IC_{50} (μM) | Type | Interaction and Autodock score | Binding energyb |
|------|---------------------|--------------|------|-------------------------------|----------------|
| N2   | >100                | 9.1          | a    | Ty343(2.68), Gln384(2.79), Asn378(2.62), Met503(3.22) | −7.36          |
| N3   | >100                | 18.0         | a    | Thr360(2.71), Gln384(3.06)    | −6.79          |
| N4   | 97.4                | 27.2         | a    | Gln384(2.91)                  | −8.27          |
| N5   | >100                | 6.4          | a    | Asp333(3.30), Gln384(3.14)    | −7.87          |
| AUDAc |            | 4.4          |      |                                |                |

a: competitive; b: kcal/mol; c: positive control.
DADs (delayed afterdepolarizations) and Tas (triggered activities). The phenomenon revealed that N6 protected heart from arrhythmias via its inhibitory effect on calcium channel. Ester N6 was also described to probe the active site of esterase named FAE-III, with the Km (substrate concentration at which the reaction rate is half of V_max) value of 1.63, and Kcat (limiting rate of any enzyme-catalyzed reaction at saturation) value of 1063 [30].

Pervilleine A (N7) was isolated and characterized from Erythroxylum pervillei (Fig. 4) [20]. Cholinergic and adrenergic effects of N7 were investigated. Ester N7 (30 μM) non-competitively inhibited cholinergic response in the guinea-pig ileum and did not affect the carbachol-induced contraction of the rat anococcygeous smooth muscle. Further research indicated that N7 exhibited weak vascular antiadrenergic and nonspecific anticholinergic effects. Subsequently, compounds structurally similar to N7 were obtained from Erythroxylum pervillei. The cytotoxicity of isolated components as MDR (multi-drug resistant) inhibitors were speculated according to the SAR as well, suggesting that TMCA group at C-6 was necessary for cytotoxicity [31].

Rescinnamine (N8) isolated from Rauwolfia, known as moderil or anaprel, was considered as an angiotensin-converting enzyme inhibitor used as an antihypertensive drug clinically (Fig. 4) [21]. This ester exhibited significant inhibition against SARS (severe acute respiratory syndrome) as well. The minimal concentration of inhibition toward SARS-CoV (SARS coronavirus) was approached to be 10 μM [32]. As the analogue of reserpine, ester N8, which beared a substituted cannabinamine in place of a substituted benzene, was reported to modulate MDR [33]. Ester N8 enhanced the cytotoxic activity of natural product antitumor drugs in CEM/VLB100 cells on different dosages. Structure-function relationship revealed that compounds that retained the pendant benzoyl function in an appropriate spatial orientation all modulated MDR.

2.2. TMCA amides isolated from natural products

Pipilartine (N9), also known as piperlongumine, is the most frequently reported TMCA amide isolated from Piper plants (Fig. 5). Pipilartine has shown effective against various ailments including cancer, neurogenerative disease, arthritis, melanogenesis, lupus nephritis, and hyperlipidemic [34]. Several related molecular targets have been disclosed such as NF-κB (nuclear transcription factor-κB), MAPK (mitogen-activated protein kinase), IL-6 (interleukin-6), JAK (janus kinase) etc.

3. Bioactivities of synthetic TMCA ester and amide derivatives

The structure of TMCA could be prepared by several kinds of reactions for the synthesis cinnamic acid including Perkin and Knoevenagel reaction. For the synthesis of TMCA ester and amide derivatives, coupling reactions were utilized widely. Catalysts including DCC (dicyclohexylcarbodiimide)/DMAP (4-dimethylaminopyridine), DMAP/EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) were commonly chosen. Sometimes TMCA was converted into cinnamoyl chloride to increase the activity of reaction.

To date, numerous TMCA ester and amide analogues have been synthesized and evaluated the bioactivities. The largely unexplored derivatives possess a variety of pharmacological activities, ranging from antitumor, antiviral, CNS agents, antimicrobial, anti-inflammatory and hematologic agents. Next, these derivatives were discussed one by one in the following paragraphs.

3.1. Antitumor activity of synthetic TMCA derivatives

Nowadays, cancer is one of the leading causes of death and unremitting efforts being made by researchers to develop antitumor agents with more efficiency and safety [35]. So far, various TMCA ester and amide derivatives with antitumor effect have been reported. We summarized the progress of these active compounds as follow.

3.1.1. Synthetic TMCA esters as antitumor agents

The antitumor evaluation of a series of olive secoiridoids derivatives was carried out by Busnena and coworkers [36]. TMCA was introduced to give esterification product with tyrosol, which was the e major olive phenolic in olive oil. The result of in vitro activity demonstrated that ester S1 (Fig. 6) showed moderate antitumor activity against the MDA-MB231 human breast cancer cells (IC₅₀: 46.7 μM) with the c-MET (tyrosine-protein kinase Met) inhibition as the possible mechanism. The SAR studies indicated that function groups positioned with more hydrogen bond donor binding role of the hydroxyl groups were more conducive to the inhibitory activity compared with 3,4,5-trimethoxyl group on aromatic ring.

MDR is a major obstacle to successful cancer chemotherapy. Ester S2, inspired by the lead compound quercetin, processed antitumor activity against MDR by modulating activity of P-gp (P-glycoprotein 1 (Fig. 6) [37]. At 1.0 μM, ester S2 showed high P-gp and BCRP- modulating activities. When ester S2 was used along to evaluate the cytotoxicity for the mentioned cell lines, no significant cytotoxicity was observed (IC₅₀ > 100 μM). According to SAR investigation, TMCA moiety showed stronger P-gp-modulating and BCRP- modulating activities than substituted benzoic acid esters, suggesting that the extra C=C bond on cinnamic acid group was important for the activity.

Ester S3 was also explored as MDR modulator. A series of analogues were synthesized and split as cis-trans isomer (Fig. 6) [38]. Among them, the ester S3 with the configuration for trans/cis displayed most potent MDR modulating activity, with the [I]₀.₅ value of 0.01 μM.

Ester S4, which was a ester derivative of methylated epigallocatechin, possessed most promising P-gp inhibition among the synthetic methylated epigallocatechin analogues (Fig. 6) [39]. Noncytotoxic ester S4 reversed drug resistance of P-gp transfected breast cancer cell line LCC5MDR (EC₅₀: 123–195 nM). Further study demonstrated that ester S4 inhibited the active drug efflux of P-gp transporter. The SAR investigation revealed that the derivatives substituted with TMCA group exhibited favourable activity in both cis-methylated epigallocatechin derivatives and trans-methylated...
gallocatechin derivatives, suggesting the potential of TMCA group promoting the activity of lead compounds.

The structure of TMCA ester was widely used for the modify of natural products. Ester S5, which was the structure of dihydroartemisinin esterified with TMCA, exhibited significant anti-tumor activity (Fig. 6) [40], with the IC₅₀ values of ester S5 against cell lines: PC-3, SGC-7901, A549 and MDA-MB-435s were 17.22, 11.82, 0.50 and 5.33 μM, respectively. The cytotoxicities of ester S5 on normal hepatic L-02 cells was weak (IC₅₀: 58.65 μM). Meanwhile, the SI (selective index) (IC₅₀ normal/IC₅₀ cancer) value was 117.30. As for the SAR, on the one hand, multi-methoxyl group substituted cinnamic acid esters performed better than other substituted cinnamic acid esters. On the other hand, diester derivatives exhibited stronger inhibitory activity than compounds with bare hydroxy on the C-9 of dihydroartemisinin in general according to the pharmacological results.

Nam et al. [41] executed the esterification of 4-senecioyloxy methyl-6, 7-dimethoxycoumarin, which was a metabolite of the plant plant Crinum latifolium. Ester S6 showed moderate cytotoxicity on B16 and HCT116 cell lines with the IC₅₀ values of 6.74 and 8.31 μg/mL (Fig. 6) [30]. The most promising ester was the 4-methoxycinnamoyl substituted derivative S7, which was structurally similar to ester S6.

Based on the structure of the precursor 2',5'-dimethoxychalcone, a series of ester derivatives were synthesized [42]. Among them, ester S8 possessed broad spectrum antitumor activity in different cell lines (Fig. 6). When ester S8 was added in cell lines including A549, Hep 3B, HT-29 and MCF-7, the IC₅₀ values were 36.7, 23.2, 23.8 and 6.4 μM, respectively. Further research suggested that this cluster of esters could mediate cancer cell apoptosis via G2/M arrest.

Ester S9 was designed based on a cytotoxic natural ester isolated from Piper sinnense (Fig. 6) [43]. In several cell models including PC-3, Hela, A549 and BEL7404, ester S9 possessed cytotoxicity with the IC₅₀ values of 80, 64, 172 and 212 μM, respectively.

Han et al. [44] synthesized fumagillin analogues according to molecular modeling with MetAP2 (human methionine aminopeptidase-2). Among them, ester S10 exhibited the strongest anti-tumor activity in EL-4 and CPAE cells, with the IC₅₀ values of 0.15 and 0.03 μg/mL (Fig. 6), respectively. Ester S10 interacted well with MetAP2 according to the docking study. In subsequent study, ester S10 was demonstrated to inhibit MetAP2 significantly with the IC₅₀ value of 0.96 nM [45]. The SAR of the synthetic fumagillin analogues could be summarized as that the aromatic ring of the derivatives should be positioned to contact with the Leu447 of human MetAP-2 for maximizing hydrophobic interaction, and the activity of cis-cinnamic acid ester derivative was much less than trans-cinnamic acid ester derivatives.

Fig. 6. Structure of synthetic TMCA ester derivatives as antitumor agents (S1-S10).
3.1.2. Synthetic TMCA amides as antitumor agents

Piplartine was modified on heterocyclic ring via Baylis-Hillman reaction. The derivatives were determined antitumor activity [46]. In HeLa and IMR-32 cells, amides S11–S13 were demonstrated to enforced cell cycle inhibition arresting cells in G2-M phase of the cell cycle (Fig. 7). The enhanced ERK1/2, MAPK activation was significant when the potent compounds were used along or combined with chemotherapeutic drugs. In the combination treatment with colcemid and hydroxyurea, the enhanced elongation and inhibition of cell adhesion in both the cells were observed.

Multiple piplartine analogues with alkyl or halogen substituents at C-7 and morpholine substituents at C-2 were prepared by Wu et al. [7]. All the compounds showed modest selectivity for WI38 human fetal lung normal cells and MRC-5 human lung normal cells. Among the synthetic compounds, amide S14 (Fig. 7) displayed most potent inhibitory against four cancer cells in vitro (Table 2). Subsequently, amide S14 exerted significant antitumor potency in ROS (reactive oxygen species) elevation and excellent. SAR suggested that 2-Halo-7-alkylpiperlongumines retained in vitro anticancer activity, while analogue with morpholine substituents at position 7 of piplartine exhibited diminished cytotoxicity.

Based on the structure of piplartine and cenocladamide, a series of analogues were synthesized by Santos et al. [47]. Amide S15 was identified as the most promising compound against MDA-MB-231 cells (IC₅₀: 6.6 μM) (Fig. 7). Additionally, amide S15 also induced apoptosis on several tested cell lines (efficacy: 15%–80% of apoptosis), which was superior to the positive control doxorubicin. The proliferating inhibition also showed selectivity, because amide S15 was proved to be un conspicuous cytotoxicity to the non-tumorigenic cells including HMEC and MCF10A.

Zeng et al. [48] synthesized multiple N,N-disubstituted thiourea analogues as the inhibitors of HSP70 (heat shock protein 70). Amides S16 and S17 (Fig. 7), which were the molecules contained TMCA amide group, exhibited favourable inhibition against HSP70 in vitro.

| Amides | IC₅₀ (μM) |
|--------|----------|
|       | A549 | HCT116 | MDA-MB-231 | Hep3B | WI38 |
| S14   | 3.94 | 9.85  | 6.07       | 16.69 | 19.60 |
| Piplartine | 5.90 | 21.80 | 19.53      | 69.46 | 26.78 |

Table 2: Antitumor activity of S14 in vitro.

Fig. 7. Structure of synthetic TMCA amide derivatives as antitumor agents (S11-S29).
with the ratio of 50.42% and 50.45% in 200 μM. Amide S18 was the most potent compound with the percentages of inhibition of 65.36. Furthermore, amide S18 induced M-phase arrest in HL-60/VCR cells and was proved that it was not the substrate of P-glycoprotein drug transporters.

A series of phenylcinnamides were synthesized and assessed the antitumor activity [49]. Among them, amide S19 showed moderate antitumor effect against U-937 and HeLa cells with IC50 values of 9.7 and 38.9 μM (Fig. 7), respectively. The most potent amide S20, C-4 position on benzene etherification analogy, exerted apoptosis effect with IC50 values of 1.8 and 2.1 μM for the mentioned two kinds of cell lines, respectively.

According to the structure of Combretastatin A-4, a precursor exhibited cytotoxic effect against murine lymphocytic leukemia, a series of derivatives with higher aqueous solubility were designed and synthesized [50]. Among them, amide S21 (Fig. 7), a TMCA amide, showed favourable antitumor activity against several kinds of cell lines (Table 3). The most potent amides S22 and S23 were structurally similar to S21. SAR indicated that 3,4-substituted on benzene of the cinnamaldehyde was helpful to promote apoptosis activity.

Inspired by the structure of pipilarte and suberylanilide hydroxamic acid, which was a kind of HDACi (histone deacetylase inhibitor), active antileukemic amides S24 and S25 were synthesized (Fig. 7) [8]. Amide S24 were reported to mediate DNA damage and apoptosis. Subsequently, amide S25, which was an analogue introduced C2-chloro substituent to S25, improved apoptosis activity but reduced selectivity in noncancerous MCF-10A cell lines, amides S24 and S25 also performed antileukemic activity through mediating pro-apoptotic proteins expression, inhibiting DNA repair and pro-survival proteins expression, and interfering cellular GSH (glutathione) defense.

Zhang et al. developed a Namidine scaffold framework as MDR modulator [51]. Amides S26, which was an analogue with two units of 3-(3,4,5-trimethoxyphenyl)acryloyl in the molecule structure, was proved to be the most potential compound among the derivatives (Fig. 7). Amide S26 (1 mM) sensitized LLC6MDR cells toward Taxol 24.5 folds (EC50: 210.5 μM), which was more potent than verapamil.

TMCA amides were considered to be bestatin-based inhibitor of MetAP2 and inhibitor against HUVECs (human umbilical vein endothelial cells) [52]. A series of α-hydroxy-β-amino amide analogues were synthesized and evaluated inhibition against MetAP2 and HUVEC growth. Amides S27-29 (Fig. 7), which were TMCA amide analogues, exhibited significant inhibitory effect (Table 4). When the R group was substituted with benzyl group, both MetAP2 and HUVEC growth inhibition were favourable. When TMCA moiety were replaced with other group, the inhibition against MetAP2 and HUVEC growth can not be ensured at same time.

### 3.2. Antiviral activity of synthetic TMCA derivative

A variety of virus widely exist in the nature and threaten public health. TMCA is using to design antiviral agents, involving to anti-HBV (hepatitis B virus), anti-SARS and anti-influenza A agents. To date, nearly all the reported effective antiviral analogues are TMCA esters.

| Com. | MCF7 | DU145 | HOP62 | HeLa | K562 | SK-OV-3 | Colo205 | MIA PaCa-2 |
|------|------|-------|-------|------|------|---------|---------|-----------|
| S21  | 0.079| 0.095 | 24.8  | 14.9 | 28.0 | 20.9    | 76.0    | 64.5      |
| S22  | 0.056| 0.060 | 0.090 | 7.5  | 0.094| 0.099   | 0.099   | 25.9      |
| S23  | 0.031| 0.045 | 43.6  | 29.2 | 0.099| 29.8    | 74.9    | 74.0      |
| CA-4 | 0.033| 0.046 | 0.15  | 0.008| 0.031| 31.6    | 0.025   | —         |

Sixteen phenylpropionic acid analogues were prepared and screened for the anti-HBV effect [53]. Ester S30 (Fig. 8) displayed the outstanding HBV inhibitory, with the CC50 (half cytotoxicity concentration) value of 506.99 μM in HepG2 2.2.15 cells, and IC50 values of HBsAg (hepatitis B surface antigen) and HBeAg (hepatitis Be Antigen) were 107.19 and 74.80 μM, respectively.

Research group of Jijun Chen synthesized numerous of natural-based compounds and measured their anti-HBV activity, several TMCA esters exhibited anti-HBV activity in the studies (Table 5). Caudatin, an effective anti-HBV precursor tetracyclic triterpenoid separated from Cynanchum burke, was modified with cinnamic acid and measured for anti-HBV activity. Ester S31 (Fig. 8) exhibited most potent for anti-HBV activity, with the CC50 value of 1821.75 μM in HepG2 2.2.15 cells, and both the IC50 values of HBsAg and HBeAg were 5.52 μM. With the highest SI value of 330. The IC50 value of S31 inhibiting HBV DNA replication was 53.1 μM [54]. Ester S32 (Fig. 8), which was an esterified derivative from andrographolide, also performed moderate anti-HBV effect, with the CC50 value of 211 μM in HepG2 2.2.15 cells, and the IC50 values of HBsAg and HBeAg were 753 and 518 μM, respectively. The IC50 value of S32 inhibiting HBV DNA replication was 53.1 μM. TMCA was considered as one of the group which was favourable to enhance anti-HBV activity according to SAR [55].

p-Hydroxyacetophenone, isolated from Artemisia capillaris, was explored as the precursor for the anti-HBV agent. A series of analogues were synthesized and identified through the Mitsu-nobu reaction on the primary hydroxyl group of pyranose. Among the synthetic compounds, ester S33 (Fig. 8) exhibited strongest anti-HBV DNA replication effect, with the CC50 value of 1821.75 μM in HepG2 2.2.15 cells, meanwhile, the IC50 value of S33 inhibiting HBV DNA replication was 5.8 μM. SI = 330 [56]. The SAR can be concluded that glycosides showed stronger inhibitory activity than aglycones in general, meanwhile, the cinnamic acid analogues positioned with methoxyl or fluoro group exhibited more potential activity than other substituted analogues.

According to the structure of effective ester tetrapeptide aldehyde against SARS, a series of derivatives were synthesized. Ester S34 (Fig. 8) showed weak anti-SARS CoV 3CL R188I mutant protease effect (IC50: 250 μM) [57]. Interestingly, according to another research result from the author [58], TMCA amide analogue was reported to be the most promising compound among the designed derivatives. The author considered that planar aromatic ring and its hydrophobic functionality on the structure of substituted group were essential for the inhibitory activity.

Inspired by the structure of penta-galloyl-β-D-glucose, several derivatives were synthesized and determined for the anti-influenza A activity [59]. Compared with 3,4,5-trimethoxy benzoic acid...
substituted derivatives, ester S35 showed stronger inhibition against influenza A. Ester S35 (Fig. 8), which was composed by D-Mannose and five units of TMCA, showed most potent anti-influenza A effect. As for the configuration, ester S35 was the mixture of α- and β-anomer, with the ratio of α/β = 63/37.

3.3. CNS agents of synthetic TMCA derivative

CNS disorders, which are made up of multiple diseases whose symptoms contain cognitive impairment and maniac or depressive behavior, affected millions of people around the world [60]. Because of the complexity of pathogenesis, development of CNS agents is high investment but low returns. Coherent with the bioactivity of precursor TMCA, TMCA ester and amide analogues have been reported to show CNS activity including antinarcotic, neuroprotective anti-Alzheimer and anticonvulsant effect. Several targets are involved to such as 5-HT (5-hydroxytryptamine), Ache (acetylcholine), BuChe (butyrocholinesterase), Aβ (1–42), EP2 and Nrf2 (nuclear factor 2).

3.3.1. Synthetic TMCA esters as CNS agents

In 2013, a series of TMCA analoges were synthesized and examined the antinarcotic activity [61]. Ester derivatives were synthesized by acyl chlorination of TMCA, among which ester S36 (Fig. 9) exhibited moderate antinarcotic in vivo and in vitro. At the dose of 20 kg/mg. S36 suppressed naloxone-stimulated jumping behavior in morphine-dependent mice. Moreover, S36 inhibited 5-HT1A with the IC50 value of 9.4 μM.

According to the structure of Sintenin, a lignanoid isolated from Piper sintenense, Jung et al. [62] synthesized multiple ester analogues. The neuroprotective effect of the synthetic esters was determined in in vitro models. Ester S37 (Fig. 9) showed moderate neuroprotective activity in DPPH (1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radicals scavenging model, with the inhibition for 18.60% at 50 μg/mL, which was higher than the positive control quercetin. Further study revealed that caffeic acid substituted ester showed the most potent neuroprotective effect through suppressing the H2O2-induced oxidative injury in PC12 cells.

Ester S38 (Fig. 9), which was a esterified derivative of active lead compound-tacrine, was confirmed to show potential against

**Table 5**

| Com. | CC50 (μM) HbsAg | IC50 (μM) HbsAg | DNA replication |
|------|----------------|----------------|-----------------|
| S30  | 506.99         | 107.19         | 4.73            |
| S31  | >1821.75       | >330.0         | 5.52            |
| S32  | 211            | 753            | 518             |
| S33  | >4203.5        | >8.5           | 114.9           |

Fig. 8. Structure of synthetic TMCA derivatives as antiviral agents (S30-S35).

Fig. 9. Structure of synthetic TMCA ester derivatives as CNS agents (S36-S39).
Alzheimer’s disease [63]. To detail, S38 showed inhibitor against AChE, BuChE and Aβ (1–42) aggregation with IC₅₀ values of 16.88, 298.9 and 45.88 μM, respectively. The most active ester S39, which also containing trimethoxybenzene moiety, showed AChE inhibition (IC₅₀: 5.63 μM, 13 times stronger than tacrine). The SAR investigation suggested that electron-withdrawing substituents on the benzene ring of cinamate were not conducive to improve the inhibitory activity of AChE, BuChE and Aβ (1–42) aggregation.

3.3.2. Synthetic TMCA amides as CNS agents

Jung et al. [5] synthesized several TMCA amides and measured the antinarcotic activity. The reaction details about preparation of TMCA were described. Among the homologous, amides S40 and S41(Fig. 10) were the most potential amides in vivo and in vitro. At the dose of 5 mg/kg (i.p.), S40 and S41 significantly decreased the naloxone-induced jumping behavior in morphine-dependent mice, with the inhibition ratio values of 88% and 80%, respectively. In vitro, S40 and S41 exhibited favourable binding affinity to serotonergic receptors such as 5-HT₁A, 5-HT₂A, 5-HT₂C, 5-HT₆, 5-HT₇, and the 5-HT transporter (Table 6). Amide S40 displayed the most significant binding affinity to the 5-HT₁A receptor. Additionally, S41 possessed different binding affinity to mentioned receptors. Ability of activating the pERK expression for the amides was also determined. The result indicated that amides S40 and S41 moderately increased the expression of pERK.

Amide S42 (Fig. 10) was reported to be effective in inhibiting seizure-induced mediation of neuronal injury by PGE₂ (prostaglandin E2) receptor subtype EP2 [64]. As the most potent molecular in the synthetic analogues, S42 possessed competitive antagonism of EP2 receptor (Kᵦ: 2.4 nM), meanwhile, the EP4 (prostaglandin E2 receptor 4) receptor Kᵦ value was 11.4 μM, and the SI was 4730. Furhermore, S42 as a brain-permeant agent inhibited the up-regulation of COX-2 (prostaglandin-endoperoxide synthase 2) mRNA in rat cultured microglia activated by EP2 and markedly decreased neuronal injury in hippocampus when administered in mice beginning 1 h after termination of pilocarpine-induced status epilepticus. The result revealed that S42 was effective in treating inflammation-related brain injury. Based on the structure of S42, amide S43 was synthesized and evaluated the selectivity against prostanoid receptor EP2 and DP1.

A series of pipartine analogues were synthesized and measured cytoprotection against hydrogen peroxide- and 6-hydroxydopamine-induced neuronal cell oxidative damage in PC12 cells [65]. Amides S44 and S45 (Fig. 10) showed low cytotoxicity and confer potent protection of PC12 cells from the oxidative injury via upregulation of a panel of cellular antioxidant molecules. Genetically silencing the transcription factor Nrf2, a master regulator of the cellular stress responses, suppresses the cytoprotection, indicating the critical involvement of Nrf2 for the cellular action of S44 and S45 in PC12 cells.

Cinepazide (S46) (Fig. 10) is a marketed drug using for the treatment of cardiovascular and cerebrovascular diseases, and peripheral vascular diseases. Cinepazide can also be regarded as a TMCA amide analogue. Amide S46 was suggested to protect PC12 neuronal cells by affecting mitochondrial functions [66]. To detail, S46 inhibited OGD (oxygen–glucose deprivation)-induced oxidative stress, as supported by its capability of reducing intracellular reactive oxygen species and malondialdehyde production and enhancing superoxide dismutase activity. Furthermore, S46 was found to sustain the function of mitochondrial function via stabilizing mitochondrial membrane potential, promoting OGD-induced suppression of mitochondrial respiratory complex activities and enhancing ATP (adenosine-triphosphate) production.

Jung and coworkers [67] presented a simple synthesis TMCA amides and evaluated the bioactivities. All the synthetic amides were determined the radical scavenging activity, neurotoxicity inhibition and antinarcotic activity. No significant radical scavenging activity was observed for the tested amides compared to the reference material in vitro. Amide S47 (Fig. 10) showed most potent neuroprotective activity in glutamate-induced primary cortical neuronal cells at the doses ranging from 5 to 20 μM. Meanwhile, all the analogues showed antinarcotic property in vivo. Amide S48 displayed strongest inhibition among the examined amides, which indicated that TMCA moiety was essential for the enhancement of antinarcotic activity.

![Fig. 10. Structure of synthetic TMCA amide derivatives as CNS agents (S40-S48).](image-url)
3.4. Antimicrobial activity of synthetic TMCA derivatives

TMCA ester ester and amide analogues are applied to synthesize antimicrobial agents as well. Active derivatives have been reported to suppressed the growth of strains including Ustilaginoidea oryzae, Pyricularia oryzae, P. falciparum, S. aureus, C. krusei and Trypanosoma cruzi.

3.4.1. Synthetic TMCA esters as antimicrobial agents

Trichodermin cinnamic acid ester derivatives were prepared and by Zheng et al. [68]. Among the obtained compounds, ester S49 (Fig. 11) exhibited moderate inhibition against Ustilaginoidea oryzae and Pyricularia oryzae in vitro, with the EC50 values of 11.04, and 11.07 μM, respectively. Ester S50, which was a derivative substituted with ortho-fluorine cinnamic acid, exhibited predominant inhibition against mentioned strains, with the EC50 values of 0.56, and 0.53 μM, respectively. The effect was even better than the marketed drug prochloraz, which could be related with the function of the fluorine moiety in inhibiting microbials [69].

Several studies have reported esters including TMCA moiety possessed antimalarial effect, which indicated that TMCA could be an important group for developing antimalarial agents. As an analogue of neolignane, ester S51 (Fig. 11) was identified and measured the antimalarial activity in vitro [70]. The result revealed that S51 exhibited moderate antimalarial activity against blood forms of chloroquine-resistant P. falciparum with both the IC50 values for 3H-hypoxantine and HRPII were 127.9 μM. Among the synthetic TMCA esters, ester S52 (Fig. 11) displayed the strongest inhibition of 0.56, and 0.53 μM, respectively. Ester S53, which was a derivative substituted on benzene ring exerted 7 and 20 times the efficiency of Kniphofiones A and B.

3.4.2. Synthetic TMCA amides as antimicrobial agents

Fregnan et al. [73] synthesized several analogues of pipartine and evaluated the antimicrobial activity of the analogues. Amides S55 and S56 (Fig. 12) were the most potent amides (Table 7). Amide S55 displayed three-fold more potent than pipartine in antibacterial evaluation against S. aureus and five-fold less toxic than pipartine. Amide S56 possessed fourfold more potent in antifungal evaluation against C. krusei and five-fold less toxic than pipartine. As for the SAR, it was possible to note that an aromatic ring lacking methoxyl moieties is important for the antibacterial activity of these compounds. On the other hand, trimethoxyphenyl group substituted on benzene ring was imperative for the antifungal activity.

Carvalho et al. [74] synthesized several cinnamic N-acylhydrazones and measured the antitypansosomal effect. Amide S57 (Fig. 12) exhibited modest antitypanosomal activity against trypanostigote forms of Trypanosoma cruzi with the IC50 value of 18.4 μM. The value of SI of S57 was the highest of 134. Moreover, possessed favourable cruzain inhibition with the IC50 value of 45.9 μM.

Derivatives of 4"-O-(trans-β-arylacrylamido)carbamoyl azithromycin were synthesized and assessed for their antibacterial effect against nine significant pathogens [75]. Amide S58 (Fig. 12) exhibited moderate antibacterial activity against susceptible and resistant strains (Table 8). The most potent amide S59 was structurally close to S58, which revealed that 3,4-dimethoxyl substituted moiety enhanced the antibacterial activity for the lead compound.

3.5. Anti-inflammatory activity of synthetic TMCA derivatives

Inflammation is body's natural response against external infection. [76]. TMCA ester and amide derivatives have been reported to show anti-inflammatory activity through the targets including TNF-α (tumor necrosis factor), NO (nitric oxide) and NF-κB.

3.5.1. Synthetic TMCA esters as anti-inflammatory agents

Ku et al. [61] combined carbazole with cinnamoyl group and measured the vascular barrier protective effects of derivatives.

![Fig. 11. Structure of synthetic TMCA amide derivatives as antimicrobial agents (S49-S54).](image-url)
Ester S60 (Fig. 13) exhibited marked inhibition on HMGB1 (high mobility group box-1 protein)-mediated hyperpermeability. At the dose of 10 μM, S60 inhibited hyperpermeability with the most remarkable inhibition of 70.2% and ELISA OD650 value of 0.158. On mice model, also suppressed HMGB1-mediated hyperpermeability with the inhibition of 58.9%. The result demonstrated that S60 could be a potent agent for inhibiting HMGB1-mediated inflammatory responses.

Kumar et al. [77] reported that ester S61 (Fig. 13) isolated from Piper longum inhibited ICAM-1 (intercellular cell adhesion molecule-1), VCAM-1 and E-selectin by the induction of TNF-α. As one of the thionocinnamate homologs, S62 exhibited better inhibition than S61. On the concentration of 20 μg/mL, S62 exerted 95% inhibition of ICAM-1 expression (IC50: 10 μg/mL). Consequently, S62 abolished adhesion of neutrophils to endothelial monolayer by the induction of TNF-α. SAR investigation indicated that the critical role of the chain-length of the alkyl moiety in the alcohol moiety, number of methoxy groups in the aromatic ring of the cinnamoyl moiety and the presence of the α, β-C-C double bond in the thionocinnamates and thionocinnamates.

3.5.2. Synthetic TMCA amides as anti-inflammatory agents

A series analogues of piprartine (S63) were synthesized and investigated the anti-inflammatory activity [76]. Among them, amide S63-66 (Fig. 14) exhibited better inhibition. At the dose of 10 μM, LPS (lipopolysaccharide)-induced NO production was inhibited by four mentioned amides with the inhibition of 91%, 46%, 65% and 41%, respectively. Additionally, the cytotoxicity of four amides in RAW264.7 macrophages was measured with the IC50 values of 3, 6, 14 and 17 μM, respectively.

Sun et al. [78] designed and synthesized several piprartine derivatives. Analogue S67 (Fig. 14), which was the ketone analogue with amide group replaced by carbonyl to increase its electrophilicity, was certified to show more potential than the lead amide piprartine in blocking LPS-induced secretion of NO and PGE2 as well as COX-2 and iNOS (inductive nitric oxide synthase) expressions in RAW264.7 macrophages.

3.6. Hematologic activity of synthetic TMCA derivatives

TMCA amides have been revealed to show hematologic activity, in which anti-aggregatory and haemostatic effect are the relative effects. Substituted cinnamoyl-tyramine analogues were synthesized and evaluated the platelet anti-aggregatory activity [79]. Among the synthetic derivatives, amides S68 and S69 suppressed PAF (platelet-activating factor) receptor binding to rabbit platelet with the inhibition of 12 and 19%, respectively (Table 9). On the concentration of 30 μg/mL, S68 and S69 inhibited PAF induced platelet aggregation with
Among the studied series, amide tion (IC$_{50}$: 18.09 activity to a certain extent. Amide PT (prothrombin time), suggesting that while promoting APTT (activated partial thromboplastin time) and moreover, showed lowest TT (thrombin time) value among the analogues, substructure were presented as potent haemostatic agents [81].

Collagen-induced platelet aggregation but reducing inhibition to different models (Table 10). SAR research revealed that adding a methyl group to the C-2 position of the piperidine ring exerted mixed effects, promoting inhibitory effect for thrombin and collagen-induced platelet aggregation but reducing inhibition to arachidonic acid-induced platelet aggregation.

Ten new cinnamamide derivatives containing a 2-aminothiazole substructure were presented as potent haemostatic agents [81]. Among the studied series, amide S70 (Fig. 15) displayed the most promising platelet aggregation inhibitory effect in different models (Table 10). SAR research revealed that adding a methyl group to the C-2 position of the piperidine ring exerted mixed effects, promoting inhibitory effect for thrombin and collagen-induced platelet aggregation but reducing inhibition to arachidonic acid-induced platelet aggregation.

3.7. Other activities of synthetic TMCA derivatives

Apart from the bioactivity described above, TMCA amide derivatives exhibited ACAT (O-acyltransferase) and ALR2 (aldose reductase) inhibitory as well. A series of Yakuchinone B derivatives were synthesized and assessed the lipid-lowering activity [5]. As the most promising amide, in vivo, amide S72 (Fig. 16) inhibited rat hepatic cholesterol ACAT more significant than positive control and it exerted remarkable hypcholesterolemic activity. Subsequent research implicated that S72 from male rats could be better metabolized than those from females [82]. Sex-related different CYP3A2 expression in the toxicology research relevant to decreased accumulation and metabolism of S72 in female rats.

Piplartine was proved to suppress recombinant human ALR2 (IC$_{50}$: 160 M) [6]. To improve the activity, multiple derivatives were prepared by modifying styril/aromatic and heterocyclic ring functionalities. S73 and S74(Fig. 16) synthesized by Michael addition exhibited aldose reductase inhibitor effect, with the IC$_{50}$ value for 4 M. Notably, according to SAR study, double bond and 3,4,5-trimethoxy substitutions at aromatic ring are important characteristics for ARI effect.

4. Conclusion

Up to now, esterification and amidation still play a significant role in discovering and developing new drugs. Amide bond formation dominated the most frequently used reaction to give the production even though the new synthetic reactions are spring up [83]. Compared with other modifications, esterification and amidation are easy to exert the metabolic characteristic of lead compounds, moreover, esterification and amidation can be easily controlled for industrial scale production for the targets compounds. Currently, the difficulty to develop new drugs is to discover novel lead compounds instead of synthesis.

As for the promising precursor TMCA, it is clearly evident that TMCA ester and amide analogues possess diversified biological activities and have immense potentiality in the field of medicinal chemistry from the above discussion. This review article is focused on the pharmacological activities of natural and synthetic TMCA ester and amide derivatives for various therapeutic targets reported recently. The present survey indicates that TMCA ester and amide derivatives have been targeted for their antitumor, antiviral, CNS agents, antimicrobial, anti-inflammatory and hematologic agents. There is much scope in this potent TMCA ester and amide moiety for other therapeutic targets, future investigations of the scaffolds could give some more encouraging results in the field of medicinal chemistry. It is not to be neglected that esters generally perform poor pharmacokinetics and limited druggability [84], so there are still gaps waiting for overcoming when TMCA derivatives are developed to the marketed drugs.

It is anticipated that the information compiled in this review article not only update researchers with the recent reported biological activities of TMCA ester and amide analogues derivatives, but also motivate them to design and synthesize promising TMCA.
ester and amide with improved medicinal properties.

Disclosure

None of the authors have any conflict of interest to disclose.

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Abbreviations

5-HT 5-hydroxytryptamine
ACh acetylcholine
ATP adenosine-triphosphate
APTT activated partial thromboplastin time
ACAT O-acyltransferase
ALR2 aldose reductase
APD50 action potential duration at 50% repolarization
APD90 action potential duration at 90% repolarization
BuChe butyrylcholinesterase
BDNF brain derived neurotrophic factor
CC50 half cytotoxicity concentration
COX-2 prostaglandin-endoperoxide synthase 2
C. kruzie Candida kruzie
CNS central nervous system
c-Met tyrosine-protein kinase Met
DCC Dicyclohexylcarbodiimide
DMAP 4-dimethylaminopyridine
DADs delayed afterdepolarizations
DPPH 1,1-Diphenyl-2-picrylhydrazyl radical

Table 10 Platelet anti-aggregatory activity of amide S70.

| Com.   | Conc. (μM) | Inhibition (%) | Collagen (2 μg/mL) | Arachidonic acid (100 μM) | PAF (10 nM) | Thrombin (100 μM) |
|--------|------------|----------------|--------------------|--------------------------|-------------|-------------------|
| S70    | 300        | 98.6           | 100                | 94.8                     | –           | –                 |
|        | 150        | 97.2           | 100                | 56.9                     | –           | –                 |
| Pipartine | 300        | 100            | 100                | 100                      | 23.5        | –                 |
|        | 150        | 100            | 76.4               | 100                      | –           | –                 |
| Acetylsalicylic acid | 300        | 5.8            | 100                | 0.3                      | –           | –                 |
|        | 150        | –              | 75                 | 0.3                      | –           | –                 |

Fig. 16. Structure of synthetic TMCA derivatives for other activities (S72-S74).

ERK extracellular signal-regulated kinase
EADs early afterdepolarizations
EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EC50 half effective concentration
EP4 prostaglandin E2 receptor 4
GSH glutathione
HPLC high performance liquid chromatography
HMGB1 high mobility group box 1 protein
APTT activated partial thromboplastin time
HDACi histone deacetylase inhibitor
HBV hepatitis B virus
HbAg hepatitis B surface antigen
HBeAg hepatitis Be Antigen
HUVECs human umbilical vein endothelial cells
ICAM-1 intercellular adhesion molecule-1
IC50 half maximal inhibitory concentration
iNOS inductive nitric oxide synthase
IL-6 interleukin-6
Ito transient outward potassium current
Km substrate concentration at which the reaction rate is half of Vmax
Kcat limiting rate of any enzyme-catalyzed reaction at saturation
LPS lipopolysaccharide
MAO monoamine oxidase
MAPK mitogen-activated protein kinase
MDR multi-drug resistant
MetAP2 human methionine aminopeptidase-2
NF-κB nuclear transcription factor-κB
NOS nitric oxide
NR2 nuclear factor 2
OGD oxygen–glucose deprivation
P. falciparum Plasmodium falciparum
P. tenuifoila Polygala tenuifoila Willd. (Polygalaceae)
ROS reactive oxygen species
SAR structure-activity relationship
SARS severe acute respiratory syndrome
SARS-CoV SARS coronavirus
P-gp P-glycoprotein 1

Table 10 Platelet anti-aggregatory activity of amide S70.

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|        | 150        | 97.2           | 100                | 56.9                     | –           | –                 |
| Pipartine | 300        | 100            | 100                | 100                      | 23.5        | –                 |
|        | 150        | 100            | 76.4               | 100                      | –           | –                 |
| Acetylsalicylic acid | 300        | 5.8            | 100                | 0.3                      | –           | –                 |
|        | 150        | –              | 75                 | 0.3                      | –           | –                 |
