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Design, Synthesis, and Evaluation of Bioactive Small Molecules

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ABSTRACT: Collaborative research projects between chemists, biologists, and medical scientists have inevitably produced many useful drugs, biosensors, and medical instrumentation. Organic chemistry lies at the heart of drug discovery and development. The current range of organic synthetic methodologies allows for the construction of unlimited libraries of small organic molecules for drug screening. In translational research projects, we have focused on the discovery of lead compounds for three major diseases: Alzheimer's disease (AD), breast cancer, and viral infections. In the AD project, we have taken a rational-design approach and synthesized a new class of tricyclic pyrone (TP) compounds that preserve memory and motor functions in amyloid precursor protein (APP)/presenilin-1 (PS1) mice. TPs could protect neuronal death through several possible mechanisms, including their ability to inhibit the formation of both intraneuronal and extracellular amyloid β (Aβ) aggregates, to increase cholesterol efflux, to restore axonal trafficking, and to enhance long-term potentiation (LTP) and restored LTP following treatment with Aβ oligomers. We have also synthesized a new class of gap-junction enhancers, based on substituted quinolines, that possess potent inhibitory activities against breast-cancer cells in vitro and in vivo. Although various antiviral drugs are available, the emergence of viral resistance to existing antiviral drugs and various understudied viral infections, such as norovirus and rotavirus, emphasizes the demand for the development of new antiviral agents against such infections and others. Our laboratories have undertaken these projects for the discovery of new antiviral inhibitors. The discussion of these aforementioned projects may shed light on the future development of drug candidates in the fields of AD, cancer, and viral infections. **DOI 10.1002/tcr.201200016**

Keywords: Alzheimer's disease, drug design, drug discovery, inhibitors, viruses

1. Introduction

This Personal Account describes three translational research projects that were carried out in our laboratories that involved the discovery of bioactive compounds or lead compounds for the treatment of AD, breast cancer, and viral infections. About 35 million people worldwide suffer from AD and the currently available treatments for AD, such as donepezil, rivastigmine, galantamine, and memantine, temporarily ameliorate some of the symptoms but do not modify the underlying disease. As such, there is an urgent need and challenge for the discovery of new drugs that treat this disease. We have discovered a new

class of tricyclic pyrones that possess high oral bioavailability, excellent permeability through the blood/brain barrier, and low toxicity; these pyrones substantially decrease the number of soluble and insoluble Aβ species in the brain and preserve memory and motor functions in AD transgenic mice. Tumor cells have impeded or altered cell-to-cell communication; one type of cell-cell communication is through a gap junction. We synthesized a library of substituted quinolines and found that several compounds possessed potent inhibitory activity against T47D breast-cancer cells through the enhancement of gap-junctional intercellular communication (GJIC); these compounds reduced xenografted breast tumors in mice and completely eradicated tumor formation in spontaneous transgenic mammary mice. Viral infections, including influenza, are responsible for over 3 million cases of illness and up to half a million deaths per year. Although antiviral drugs against certain viral infections are available, the emergence of viral resistance to existing antiviral drugs emphasizes the demand for the development of new antiviral drugs. We have synthesized and bioevaluated several classes of antiviral compounds, including triacsin C analogues, ACAT inhibitors, and antioxidant quercetin analogues.

Duy H. Hua received his BE degree under Professor Hitosi Nozaki from the Department of Industrial Chemistry, Kyoto University, Japan, in 1976 and his PhD under Professor Cal Y. Meyers from the Department of Chemistry, Southern Illinois University at Carbondale, in 1979. After postdoctoral training with Professor E. J. Corey at the Department of Chemistry, Harvard University, Hua started his academic career at Kansas State University in 1982 as an Assistant Professor. He was appointed a University Distinguished Professor at Kansas State University in 2007. In the first ten years of his research career, his interests were centered on the development of asymmetric induction reactions by using chiral allylic sulfoxides and phosphonamides, chiral sulfinyl ketimines, and chiral sulfinylimines, as well as the utilization of these reactions in the synthesis of natural products. In the last twenty years, his group has investigated the design, synthesis, and evaluation of bioactive small molecules and new compounds that are based on natural-product scaffolds, along with the synthesis of challenging molecules. Such bioactive compounds range from anticancer molecules that contain anthracenedione, triptycene bisquinone, and substituted-quinoline moieties to tricyclic pyrone molecules for the amelioration of AD, antimalarial agents, and anti-viral agents.

2. Translational Research

2.1. Design, Synthesis, and Bioevaluation of Anti-AD Compounds

AD, which is a progressive neurodegenerative disorder, is the most common form of dementia in the elderly. More than 5 million Americans and 35 million people worldwide suffer from AD. At present, available treatments for AD temporarily alleviate some of the symptoms but do not treat or cure the underlying disease.[1] The causes of AD remain elusive. The initial cholinergic hypothesis (decrease of the synthesis of neurotransmitter acetylcholine) cannot completely explain the pathogenesis. Amyloid and tau hypotheses have received the most attention. Brains of patients with AD present two classical lesions: Extracellular amyloid plaques and intracellular neurofibrillary tangles. Aggregates of amyloid-β (Aβ) peptide, a 39–43 residue protein, are widely considered to initiate a cascade of molecular changes that lead to AD.[2] The production of Aβ results from the sequential proteolytic processing of amyloid precursor protein (APP) by β- and γ-secretases. Presenilins are a major component of γ-secretase and comprise two isoforms, presenilin (PS)-1 and -2. Most autosomal-dominant familial forms of AD are attributed to mutations in APP, PS-1, and PS-2.[3] Emerging evidence supports the conclusion that soluble oligomers of Aβ–but not monomers or insoluble amyloid fibrils–may be the primary toxic amyloids that cause synaptic dysfunctions in brains and animal models with AD.[2] Low-*n*-oligomers, such as Aβ dimers, or high-*n*-oligomers, such as the 12 mer Aβ*56, were implicated in directly causing the impairment of synaptic plasticity and memory.[4] Most cases of AD, however, are sporadic (late onset). The major genetic risk factors include the inheritance of ε4 allele[5] of the apolipoprotein E (APOE) gene-encoding APOE, which increases the risk of AD 3-fold in heterozygotes and 15-fold in homozygotes,[6] thus suggesting an important role of cholesterol homeostasis in AD. Major strategies for experimental disease-modifying therapies include targeting multiple sites in the metabolism of Aβ (synthesis, aggregation, deposition, and clearance), targeting tau (inhibition of tau phosphorylation, prevention of tau aggregation and/or disaggregation of tau aggregates), regulating cholesterol levels, anti-excitotoxicity, anti-inflammation, and modulating calcium homeostasis, among others.[7] Because there are no FDA-approved drugs that can delay or halt the progression of the disease, a major focus of AD research is on the development of drug-targeting amyloid or tau. Our research goals have, therefore, been the discovery and development of new molecules that can slow or reverse AD progression by reducing amyloid pathology and maintaining cholesterol homeostasis in the brain. In our studies of the synthesis of new acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors based on natural product pyripyropene A,[8] we synthesized a new class of tricyclic pyrone

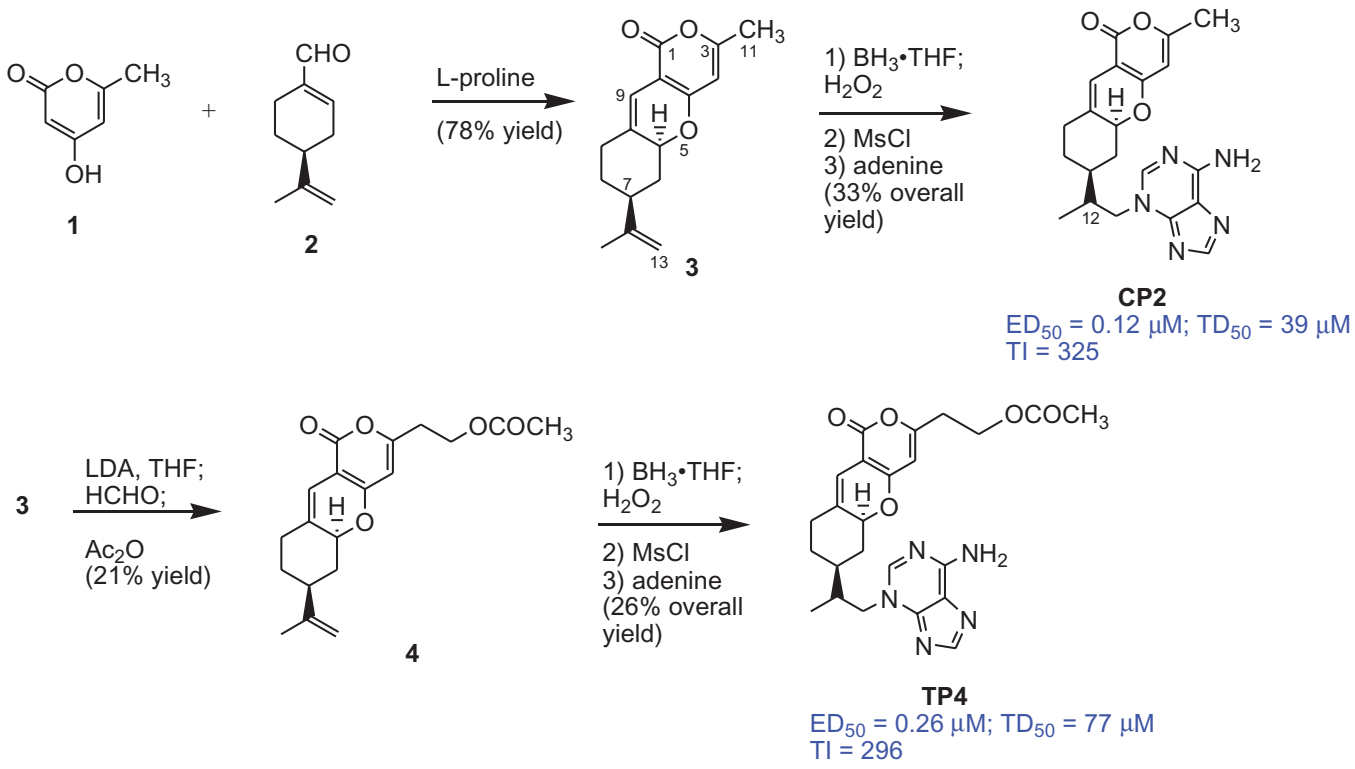

Fig. 1. Synthesis and ED_{50}, TD_{50}, and TI values of CP2 and TP4. Ms=mesyl, LDA=lithium diisopropylamide.

molecules that contained three of the four rings of the pyripyropene nucleus.[9–17] Based on our initial screening results of various tricyclic pyrone molecules by using MC65 cells and computational docking experiments with Aβ fibrils, CP2 (code name) and its analogues were designed. ACAT converts membrane cholesterol into cytoplasmic cholesteryl-ester droplets for storage and ACAT inhibitors have been implicated in antiatherosclerosis and in the reduction of amyloid pathology by regulating cholesterol homeostasis.[18–20] Tricyclic pyrone molecules, such as CP2, were synthesized in four steps:[9,12] 1) The condensation of commercially available 4-hydroxy-6-methyl-2-pyrone (**1**) with (*S*)-perillaldehyde (**2**); 2) selective hydroboration of the resulting tricyclic pyrone (**3**) with borane, followed by treatment with hydrogen peroxide; 3) mesylation with methanesulfonyl chloride; and 4) a displacement reaction with adenine (Figure 1). A single enantiomer of compound **3** was formed from the condensation reaction. However, a 1 : 1 mixture of two diastereomers, which possessed *R* and *S* configurations at the C12 position, was produced during the hydroboration process in the synthesis of CP2. We have synthesized a library of over 100 tricyclic pyrone (TP) molecules, screened them for neuronal-protective activities by using MC65 cells that were induced with Aβ oligomers,[13–15] and identified the seven most-active compounds, including CP2 and TP4, with the minimum effective dosage for 50% cell survival, ED_{50} values in the range 70–210 nM, and TD_{50} (toxic effect for 50% cell death in the presence of tetracycline) values in the range 14–77 μM. The therapeutic index (TI or therapeutic ratio = TD_{50}/ED_{50}) values for CP2 and TP4 are 325 and 296, respectively. MC65 is a line of human neuroblastoma that conditionally expresses C99, a 99-residue carboxyl terminal fragment that is derived from the β-secretase cleavage of APP. C99 is subsequently cleaved by γ-secretase to generate Aβ. Significant loss of cell viability occurs after 48 h following transgene induction (in the absence of tetracycline). Cell death is not caused by factors in the media, including secreted Aβ.[13,14] We demonstrated that cell death is dependent on the cellular production and the accumulation of Aβ, possibly in aggregated forms that are present as SDS-stable Aβ oligomeric complexes. MC65 cells are easily propagated and the cell toxicity is measured quantitatively by a simple 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

To understand the mechanism of action of this class of compounds, we used surface plasmon resonance spectroscopy to show that TP compounds bind to Aβ42 oligomers, atomic force microscopy to reveal that TP compounds inhibit the aggregation of Aβ42 peptide and disaggregate Aβ42

oligomers and protofibrils in real time, circular dichroism to monitor Aβ42 protein conformations (in the presence of TP, Aβ42 peptide remains as random coil structure), and protein quantification to verify that TP compounds inhibit the formation of Aβ42 fibrils.[14,15] Moreover, from cell studies, we found that TPs reduce the accumulation of Aβ peptides and Aβ oligomers,[14] normalize cholesterol level in cells,[17] and inhibit ACAT activity and increase cholesterol-transporter gene ABCA1, thereby resulting in the modulation of cholesterol efflux in MC65 neurons.[21] These in-solution and cell-based studies suggest that TP compounds possess anti-amyloid properties and modulate cholesterol homeostasis in neurons. For instance, CP2 was found to inhibit ACAT with an IC_{50} value of 1.2 μM and increase the ABCA1 cholesterol-transporter gene with an EC_{50} value of 0.9 μM. Other active TP compounds have similar or greater IC_{50} values for ACAT inhibition and EC_{50} values in enhancing the ABCA1 gene. We also investigated the effects of TP compounds on basal neurotransmission and synaptic plasticity in rat-hippocampal slices from the measurement of LTP. We found that the addition of amyloid β oligomers (AβO) blocked LTP induction on the nanomolar range. Perfusion of a TP compound (1 μM) prior to the co-application of AβO restored LTP to the non-AβO-treated level. Moreover, TP alone also significantly enhanced the magnitude of LTP, without affecting basal synaptic activity, thus implying that TP can block AβO-induced toxicity and preserve hippocampal synaptic plasticity. TP compounds appear to possess multiple beneficial effects.

To evaluate the bioavailability of these TP compounds, we employed a number of techniques. From octanol/water partition experiments, log P values of 2.20 and 1.90 were obtained for CP2 and TP4, respectively, which suggest that CP2 and TP4 readily enter the brain. Furthermore, by performing liquid scintillation analysis of the radioactivity of CP2 in the brain after i.p. injection of ^{14}C-labeled CP2 into C57BL/6 mice, we found that CP2 readily entered the brain after only a short amount of time (3–30 min).[14] We also used the ALOGPS 2.1 program to calculate log P values for CP2 and TP4, as well as other TP compounds, and their average log P values were in the range 1.91 (± 1.10) to 1.83 (± 1.13). These calculated log P values indicate that other TP compounds have similar brain-capillary permeabilities as CP2 and TP4 and should, therefore, also readily penetrate the blood/brain barrier (BBB). Because oral administration would be ideal for future therapeutic applications, we determined the amount of CP2, along with several other active TP compounds, in the plasma and brain after oral gavage administration in C57Bl/6 mice. A known amount of 1,2,4,5-benzenetetracarboxylic acid (BTA, as an internal standard) was added to the plasma or tissue. TP and BTA were extracted from the plasma or brain tissue (from mice) with EtOAc/1-propanol (9 : 1). The extract (which contained BTA and TP) was subjected to HPLC analysis and the amount of TP was deduced from the ratio of the integrated peaks of BTA and TP from the HPLC trace. Our results showed that the concentrations of CP2 reached 200 and 300 μM in the brain after 0.5 and 2 h, respectively, following administration at 25 mg kg^{-1}, thus indicating good BBB penetration. Other TP compounds have similar concentrations in the brain to that of CP2.

In vivo efficacy was evaluated by using AD transgenic mice in collaboration with Lee-Way Jin. Previously, a two-week intraventricular infusion of 100 μM CP2 (about 370 ng/day) into the brain of 5×FAD mice substantially decreased the levels of soluble and insoluble Aβ, by 40% and 50%, respectively.[15] Because CP2 and other TP compounds could reach brain concentrations above 200 μM following oral administration at 25 mg kg^{-1}, the effect of 8 weeks of twice-daily oral treatment on cerebral Aβ deposition was studied. This treatment eliminated over 83% of the amyloid load (Figure 2A-C). Because the Aβ dimer has been shown to cause memory deficit in rodents[4] and is the main species of soluble Aβ oligomers in 5×FAD mice, we evaluated the levels of Aβ dimer in TBS-soluble brain extracts by Western blot and found that the CP2-treated mice showed substantially lower levels of the soluble Aβ dimer (Figure 2D).

We further evaluated the improvement in memory and motor function in APP/PS1 transgenic mice on treatment with CP2 in collaboration with Eugenia Trishina. These mice were characterized by amyloid deposits and AD-like behavioral and memory deficits after as early as 13–16 weeks. CP2 was administered through drinking water (25 mg kg^{-1} daily) to APP/PS1 and non-transgenic (NTG) littermates ($n = 5$ per group of 3.5-month-old mice) for 2 months (for a new object-recognition test) and 12 months (for motor-abnormality studies by using "hanging bar" and "rotarod" tests). The CP2•trifluoroacetic acid salt that was used in the in vivo studies was completely water soluble and tasteless and did not interfere with animal's normal water consumption. CP2 treatment enhanced the memory performance of APP/PS1 mice to a level that was not different from that of the NTG control mice.[22] CP2 treatment improved motor abnormalities in the APP/PS1 mice as well. APP/PS1 mice that were treated with CP2 showed improved performance that was not different from that of the NTG mice. In the rotarod test, CP2-treated PS1 and APP mice outperformed vehicle-treated PS1 and APP mice and CP2-treated PS1 mice performed better than age-matched NTG mice. Notably, no apparent drug toxicity was observed during the course of the treatment with CP2 or other TPs. No abnormalities in histopathology, hematological counts, or blood chemistry were found on the necropsy of the mice. In summary, these encouraging pathological and behavioral data provide a strong rationale for future therapeutic studies.

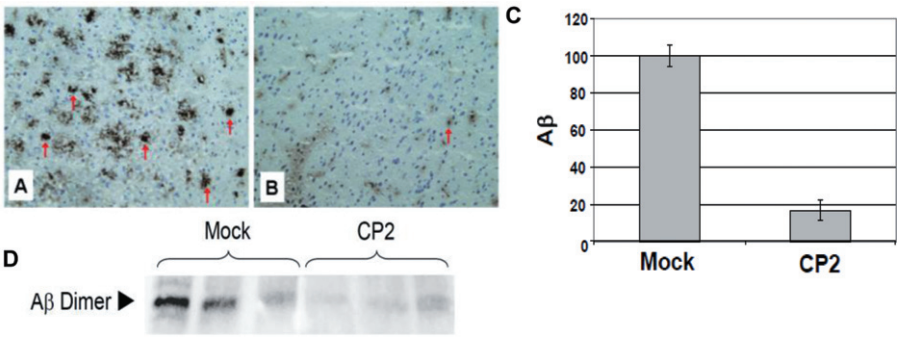

Fig. 2. Two-month-old 5×FAD mice were treated with CP2 or vehicle (mock) for 8 weeks as described. Cortical sections were immunostained for Aβ by using the monoclonal antibody 4G8. Numerous amyloid plaques (indicated by red arrows) were seen in the vehicle-treated mice (A), but only a scarce amount of small plaques were seen in CP2-treated mice (B). C) Quantification of the Aβ-immunoreactive areas showed a significant difference between the two samples; $n = 3$, $p < 0.001$. D) The soluble fractions of 5×FAD brains were analyzed for the Aβ dimer by Western blot.

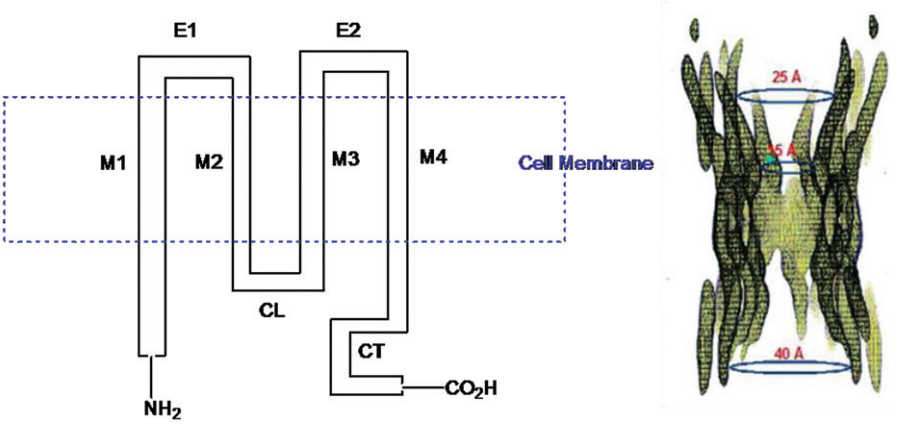

Fig. 3. The Left) membrane topology of connexin contains transmembrane domains (M1–M4, helical bundles), extracellular loops (E1 and E2), a cytoplasmic loop (CL), and a carboxy terminal (CT). Right) Representative gap-junction channel, which is composed of two hemichannels (from computational data) that are formed from six Cx43 proteins.

2.2. Enhancement of Gap-Junctional Intercellular Communication (GJIC)

Communication between cells is mediated by the gap junction (GJ), which regulates cell proliferation, apoptosis, and differentiation. GJ is an intercellular membrane channel and GJ channels contain one or more different connexin (Cx) proteins to form gap-junction plaques, which are large clusters of gap junctions with up to 10,000 channels.[23,24] Figure 3 shows the topology of Cx, along with a GJ channel.[25] Six Cxs aggregate to form a hemichannel, or connexon, and each hemichannel docks to another on an adjacent cell to form a dodecameric GJ channel. Channel activity can be measured by the passage of a small dye molecule (molecular weight <1000 Da) from one cell to another. It has been shown that defects in GJIC may be involved in the carcinogenesis process[26] and GJs are frequently decreased or absent in cancer cells. The loss of GJIC during carcinogenesis may not result from the direct disruption of a specific connexin gene or mRNA levels. Cx is synthesized at the endoplasmic reticulum and is transported through the Golgi apparatus and trans-Golgi network into the plasma membrane. When Cx reaches the plasma membrane, it oligomerizes into connexons. Newly synthesized connexons are added onto the peripheries of existing gap junctions and dock with connexons in the adjacent cell. Connexons are degraded by phosphorylation with protein kinase C (PKC)[27] or mitogen-activated protein kinases (MAPK) at serine residue 368 of Cx43 (molecular weight: 43 KDa), followed by ubiquitination. The phosphorylation step results in an altered conformation, Cx43, and Nedd4 binds to the altered connexin

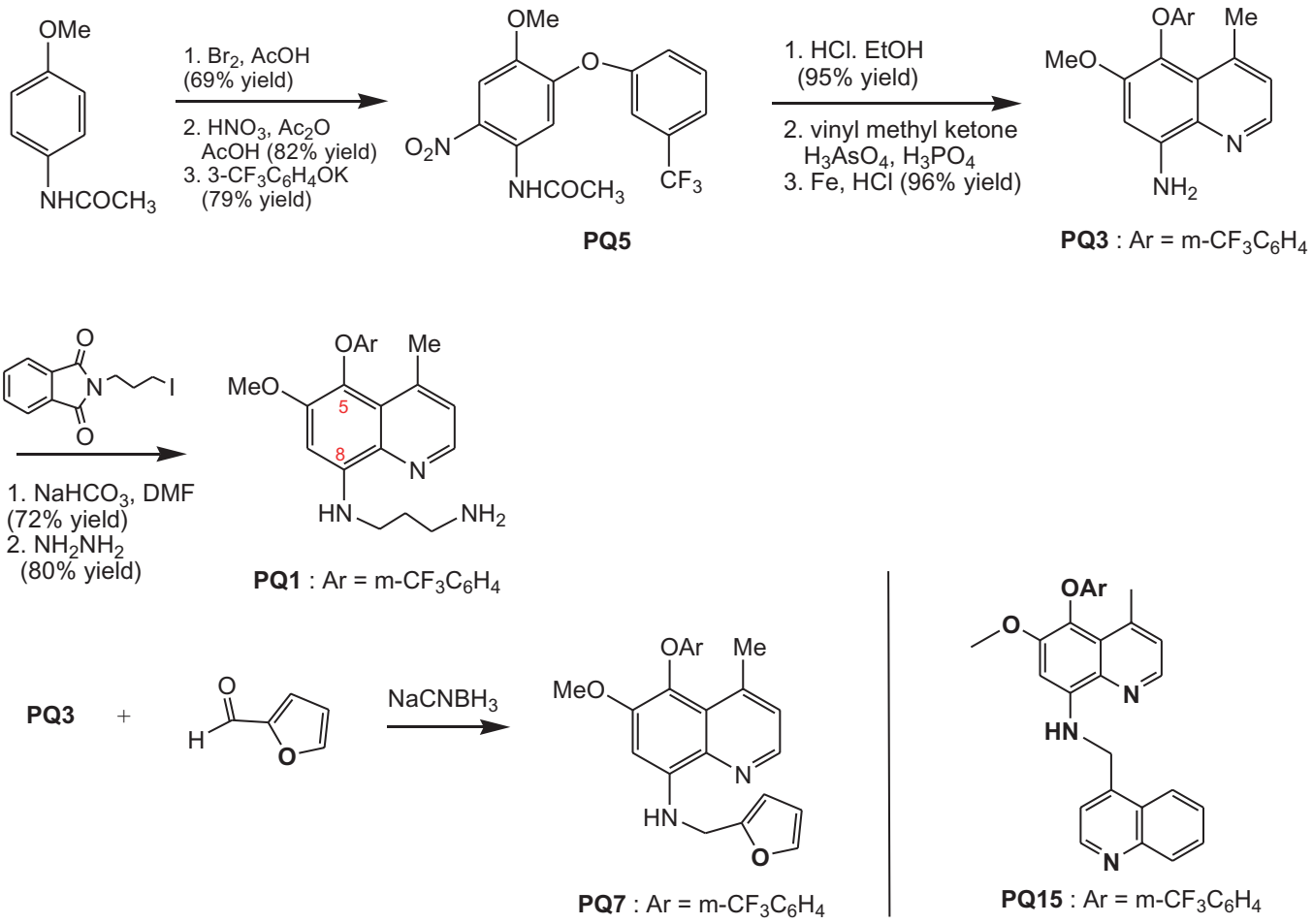

Fig. 4. Synthesis of PQ compounds.

and recruits ubiquitin, thereby leading to the ubiquitination of Cx43 and disassembling of connexon.[28] Furthermore, PKCα over-expression has been shown to cause tumor growth and tumor resistance to cytotoxic chemotherapy. Multiple in vitro studies confirm the loss of connexin and/or GJIC in breast-cancer cells and significantly, increase cell-cell communication in Cx43-overexpressing tumor cells, which enhances drug sensitivity.[29]

In search of GJIC enhancers, we carried out computational docking studies of connexon (from Cx43), PKCα, and Nedd4 with various small molecules and discovered a class of substituted quinolines that binds to connexon[30] and PKCα. A library of substituted-quinoline compounds, termed PQs, were synthesized and evaluated for their anticancer activities in vitro by using T47D breast-cancer cells.[31] We found that several PQ compounds, such as PQ1 and PQ7, possessed anticancer activities in the nanomolar range.[31–34] The synthesis and structures of PQ compounds are illustrated in Figure 4.[31] The in vivo anticancer efficacy of PQ compounds were evaluated by using nude mice. Nu/nu mice that were treated with PQ1 showed a significant reduction in xenograft tumor growth of T47D cells compared with control or tamoxifen-treated mice.[32] Moreover, PQ7-treated nu/nu mice showed a 100% regression of xenograft tumor growth.[33] Combinational treatment of tamoxifen and PQ1 enhances the anticancer effect of tamoxifen,[35] thus implying a deeper penetration of other anticancer drugs into the cancer mass from PQ-enhanced GJIC or synergistic anticancer activity. Our collaborator, Thu A. Nguyen, recently developed a transgenic mouse model with a mammary tumor that had a lower level of connexin expression compared to control non-transgenic mice. Preliminary results showed that the treatment of PQ1 for two weeks could completely prevent the tumor formation in 8-week-old mice compared to controls.

In the pursuit of the mechanism of action of PQs, we used dye (Lucifer yellow) experiments to demonstrate the enhancement of GJIC in PQ-treated T47D cells.[32] We found an increase of Cx43 expression in spontaneous mammary tumors upon treatment with PQ1 and a decrease in the phosphoryla-

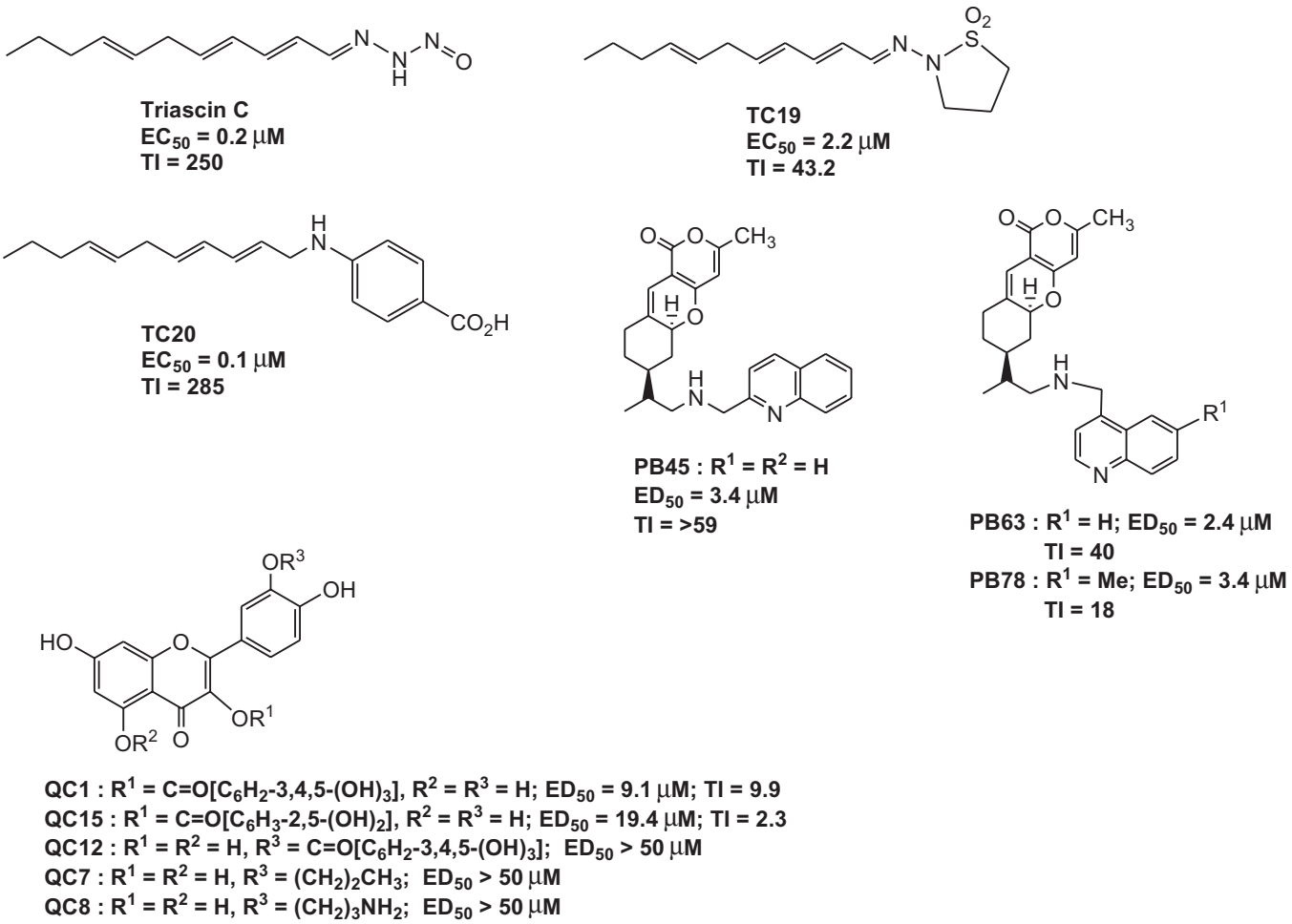

Fig. 5. Representative ACSL (TC compounds) and ACAT inhibitors (PB compounds) and quercetin analogues (QC compounds).

tion of Cx43 at the serine 368 residue in PQ1-treated T47D cells. Consequently, studies of the inhibition of the phosphorylation of PKC in an enzyme assay were conducted. A commercially available PepTag® non-radioactive PKC assay kit (Promega) was used to study the inhibition of PKC by PQ compounds and the IC_{50} values of PQ1, PQ7, PQ15 and staurosporine (a known PKC inhibitor) were found to be in the nanomolar range. An inactive PQ (PQ10) was used as a negative control and no inhibition of phosphorylation was found up to 4 μM. Hence, the PKC-inhibitory and T47D-cell-growth-inhibitory activities of the PQs appear to be correlated.

Overall, xenograft and spontaneous tumor studies show exciting results; they demonstrate that PQ compounds can attenuate tumor growth. The outcome of our findings provides a new class of anticancer molecules, as well as a tool for regulating GJIC.

2.3. Synthesis and Bioevaluation of Antiviral Compounds

Viral infections are caused by viruses that multiply on host cells. Viruses are made up of genetic DNA or RNA material, protective proteins, and outer envelope lipids; hence, these three components are the main targets for antiviral agents. Herein, we summarize our studies on inhibitors of long-chain fatty-acid acyl-CoA synthetase (ACSL)[36] and ACAT,[37] as well as analogues of the antioxidant quercetin[38] (Figure 5). For a number of years, our laboratories have been interested in lipogenesis (e.g., see Section 2.1.), which involves the synthesis of fatty acids from acetyl-CoA and subsequent esterification with glycerol to form triglycerides. Lipogenetic enzymes include fatty-acid synthase (FASN), ACSL 1–6, diacylglycerol acyltransferase (DGAT) 1 and 2, and Acyl-CoA:cholesterol

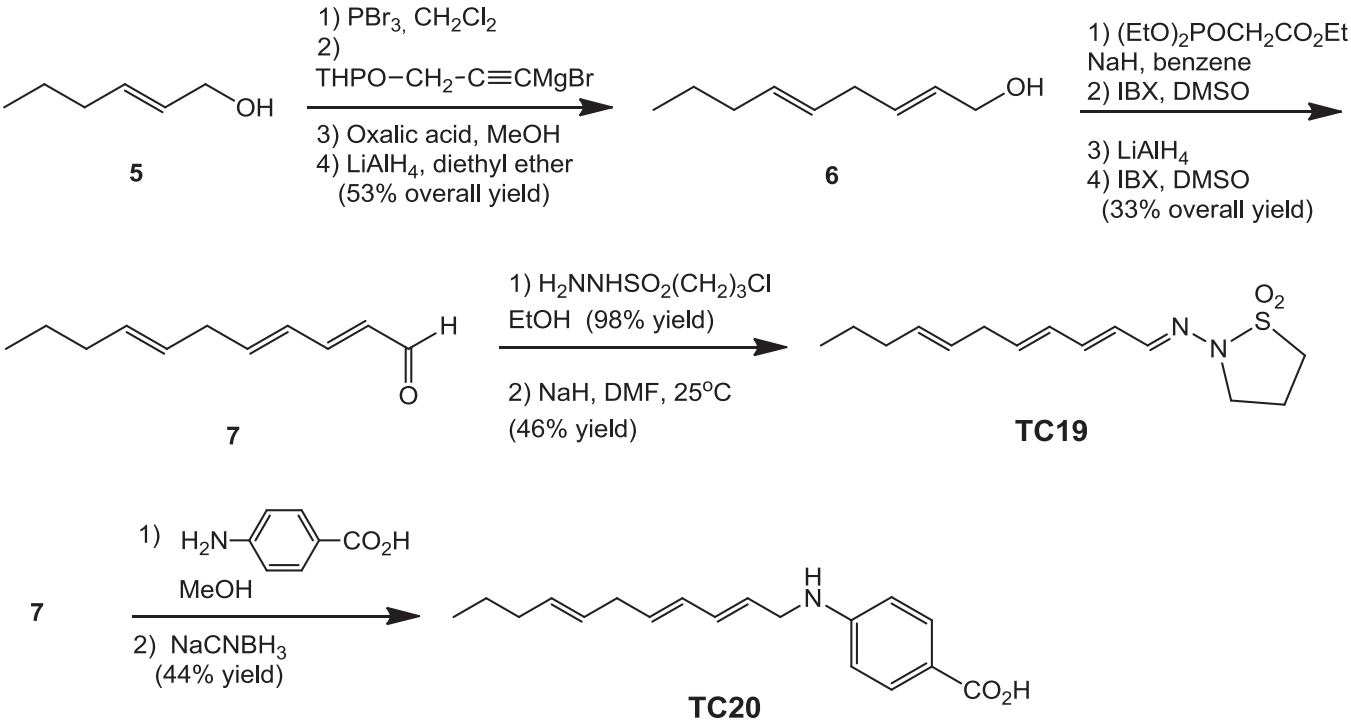

Fig. 6. Synthesis of TC compounds.

acyltransferase (ACAT) 1 and 2.[36] Because commercially available inhibitors of FASN, ACSL, DGAT, and ACAT were found to significantly reduce the replication of rotaviruses in vitro by our collaborator, Kyeong-Ok Chang, we investigated analogues of triacsin C (TCs), which is a fungal metabolite from *Streptomyces aureofaciens*,[39] that showed the most-potent inhibition of rotavirus replication. Overexpression and/or activation of lipogenic enzymes are found in most cancers;[40] hence, ACSL inhibitors may be useful in the reduction of cancer growth. The synthetic sequence of TC compounds is straightforward and efficient (Figure 6),[36] which provides flexibility for further optimization. Starting from known aldehyde **7**,[39] TC19 and TC20 were synthesized and evaluated along with their analogues. Notably, triacsin C is a difficult compound to prepare and sufficient amounts are not available for in vivo study. Among triacsin C and its analogues, TC20 is the most potent against rotavirus replication with an ED_{50} value (the effective dose that reduces 50% of virus replication) of 0.1 μM. Therefore, TC analogues may be useful as lead compounds for rotavirus infection and possibly cancer therapy and lipogenic enzymes, such as ACSL may represent potential therapeutic targets.

Norovirus belongs to the *Caliciviridae* family, which are small, non-enveloped RNA viruses of 27–35 nm in diameter. At present, there are no specific drugs for the treatment of norovirus infection. Because noroviruses do not grow in a cell culture, show high diversity, and demonstrate immunity to heterologous strains, the development of a vaccine has faced stiff challenges and is currently unavailable. By performing DNA microarray analysis of norovirus replicon-harboring cells (HG23) by an Affymetrix Gene Chip in collaboration with Kyeong-Ok Chang, increases in ACAT-1 and other cholesterol modulating genes (> ±1.5 fold) were found. Hence, a library of ACAT inhibitors, pyranobenzopyrones compounds (PBs), was synthesized and evaluated for their inhibitory activities of HG23 cells.[37] Compounds PB45, PB63, and PB78 possess the strongest activities among the tested library of over 120 compounds. Their ED_{50} and TI values are listed in Figure 5. Because these ED_{50} values are in the low micromolar range, further investigation of this class of compounds is needed to improve their potency.

Because viruses live inside the host cell, targeting lipogenetic enzymes will inevitably show toxicity or side effects. Antioxidant polyphenols, such as quercetin and its derivatives, quercetin-3-β-galactoside, quercetin 3-rhamnoside, and quercetin 3-β-D-glucoside, were found to be effective against influenza infections and quercetin-3-gallate (QC1) was effective for the treatment of inflammatory bowel disease by inhibiting Na^+-K^+-ATPase and/or Na^+/H^+ exchange activities.[41] Based on these results, we studied a hybrid of quercetin and gallate and other substituents for anti-H1N1 activity (Figure 5).[38] From a small library of over 20 derivatives, a QC1-containing gallate

moiety at C3 of quercetin showed comparable antiviral activity against influenza virus porcine H1N1 as that of EGCG with an improved in vitro therapeutic index. Other derivatives that possess various polyhydroxylbenzoate and aminohydroxybenzoate functions at the C3 and C3′ positions are less effective and compounds that contain aminoalkoxy and alkoxy substituent at the C3′ and C5 positions have lower antiviral activity. QC compounds were readily synthesized and further modification at the C3 position may improve their efficacy. Combinational treatment of inhibitor of lipogenesis and antioxidant may produce synergistic results.

3. Summary and Outlook

Synthetic organic chemistry has provided abundant methodologies for the construction of bioactive compounds and protein crystallography and computational docking software have been used to infuse predictive factors into their design. By utilizing these tools, various bioactive compounds, including anti-AD, anti-breast-cancer, and anti-viral agents, have been produced. Anti-AD compounds, such as CP2, have good bioavailability and have shown efficacy in AD transgenic mouse models by reducing amyloid plaques and improving cognitive and motor functions. GJIC enhancers, such as PQ1, kill breast-cancer cells both in vitro and in vivo. Further investigation of these two classes of compounds in larger-scale pharmacokinetic/pharmacodynamic and toxicity studies is warranted. In a continuation of our search for antiviral agents, viral protease inhibitors are being pursued. Despite the tremendous endeavors that have been made on chymotrypsin-like serine proteases of the hepatitis C virus and pepsin-like aspartic proteases of the human immunodeficiency virus (HIV), chymotrypsin-like and papain-like cysteine proteases of picornavirus, calicivirus, coronavirus, and hepatitis A virus, as well as coxsackievirus and enterovirus proteases of hand, foot, and mouth diseases and aseptic meningitis, to name a few, have been less studied.

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