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REVIEW

Modulating inflammatory prostaglandin E2 signaling to mitigate neurobehavioral comorbidities associated with seizure disorders

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Reactive gliosis;
Status epilepticus (SE)

Abstract Although epilepsy is first known as a disease of seizures and convulsions, most patients with epilepsy also suffer from seizure-associated behavioral abnormalities in motor functions, psychiatric status, and cognition. These neurobehavioral comorbidities may have greater impacts on the quality of life of people with epilepsy than the seizures themselves and can profoundly interfere with the treatment compliance. While repeated seizures often lead to behavioral comorbidities, certain types of comorbid conditions may potentially increase the risk for epileptic seizures, indicative of some common mechanisms that might underlie these two conditions. As such, emerging evidence supports that inflammation within the brain might represent a key component of such a shared mechanism, given that neuroinflammation can be induced by seizures and various behavioral stressors, and in turn may exacerbate both conditions. Among inflammatory pathways that arise after prolonged seizures, PGE_2 signaling *via* the EP2 receptor promotes cytokine induction, blood−brain barrier disruption, reactive gliosis, neuronal death, and eventually, contributes to behavioral dysfunctions. Pharmacological inhibition of EP2 by small-molecule drug-like antagonists affords broad therapeutic benefits including anti-inflammatory and neuroprotective effects in several rodent seizure models, leading to long-lasting alleviation of neurobehavioral comorbidities, particularly cognitive impairments. Targeting this key inflammatory prostaglandin receptor might provide an adjunctive strategy, along with the current anti-seizure medications, to mitigate cognitive dysfunctions associated with seizure disorders.

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1. Introduction

As one of the most common neurological diseases, epilepsy afflicts nearly 65 million people or about 1% of the population worldwide. The disease is primarily characterized by spontaneous repeated seizures that result from electrical disturbances within the brain due to synchronous hyperactivities of a group of brain neurons[1]. Although the causes of epilepsy in a majority of patients are not identifiable, the common known etiologies of epileptic seizures can be genetic mutations, developmental conditions, and other acute brain insults, such as *de novo* status epilepticus (SE), head trauma, brain tumor, stroke, brain infection, fever, and chemical exposure[2]. The pathogenic process converting a healthy brain into one that produces spontaneous seizures after these precipitating incidents is known as epileptogenesis[3]. Other than seizure burdens, behavioral abnormalities in locomotion, cognition, psychiatric status, and social-adaptive behaviors have long been known in epilepsy with a higher rate than that in many other neurological conditions, imposing substantial economic and social costs[4,5]. While repeated seizures can lead to neurobehavioral comorbidities, certain types of comorbid conditions in turn are known to increase the risk for seizures and epilepsy[6]. As such, it has been widely recognized that neurobehavioral comorbidities can have greater impacts on the quality of life of patients with epilepsy than the seizures themselves, which are largely controllable by current medications. Moreover, some unbearable comorbid conditions might profoundly interfere with the compliance of anti-seizure treatment[7,8].

The past few decades witnessed some exciting scientific advances in understanding the pathophysiological mechanisms underlying the seizure initiation, aggravation, and dissemination, leading to the introduction of a number of new third-generation anti-seizure drugs (ASDs) that engage some novel mechanisms of action[9-12]. However, there are still more than 30% of epilepsy patients who receive inadequate treatment and suffer from seizures that are refractory to the current frontline therapies[13]. Moreover, none of the drugs approved by FDA for seizure management can either mitigate the comorbid conditions, prevent epileptogenesis, or modify the disease progression. In fact, most of the commonly used ASDs are well known for their broad neurotoxic adverse effects that could result in medication nonadherence or exacerbation of behavioral comorbidities. In this regard, the new ASDs may not necessarily be safer than the older drugs[14]. In recent years, there is an increase in recognizing the effects and frequency of epilepsy-associated comorbidities, which have been acknowledged in the National Institute of Neurological Disorders and Stroke (NINDS) and American Epilepsy Society (AES) Benchmark Areas for research in epilepsy[15,16]. Among the most common behavioral conditions in epilepsy are anxiety, depression, and cognitive deficits[17,18], which are the main focus areas of this review article.

2. Neuroinflammation

The bidirectional relationship between epileptic seizures and behavioral comorbidities supports a common underlying pathophysiological basis for both conditions[6]. Emerging evidence from preclinical and clinal studies supports that inflammation might be a key element of such a shared mechanism given that inflammation can be induced by both seizure activities and common behavioral stressors[19,20]. As such, seizures can quickly trigger robust inflammatory processes within the brain, whereas concurrent inflammation increases seizure susceptibility and aggravates seizure severity in people with epilepsy[21]. Inflammatory processes, such as cytokine induction, blood−brain barrier (BBB) destruction, infiltration of immune cells and plasma proteins (*e.g.*, albumin and IgG), reactive gliosis, and neuronal death, are widely believed to play fundamental roles in acquired epileptogenesis after precipitating incidents such as traumatic brain injury, cerebrovascular accident, infection, fever, and new onset SE[22-25]. On the other hand, elevated pro-inflammatory mediators such as prototypical cytokines, such as IL-1β, IL-6, TNF-α, etc., can disrupt hippocampal neurogenesis, leading to cognitive impairments, learning deficits, mood disorders, decreased motor activities, and other behavioral disturbances[26]. Brain inflammation, particularly microglial activation, has also been found to highly correlate with the neuropsychiatric symptoms and cognitive impairment in human patients[27]. Therefore, targeting key neuroinflammatory pathways holds the potential to prevent the development to epileptic seizures after brain insults and relieve the neurobehavioral comorbidities in patients who have been diagnosed with epilepsy[28].

3. COX-2/mPGES-1/PGE_2/EP2 axis

In addition to pro-inflammatory cytokines that are well known for their pathogenic roles in brain excitability[29], the cyclooxygenase (COX) sits atop another large inflammatory system. As the inducible COX isoform, COX-2 is rapidly and robustly upregulated by acute brain insults such as SE, and in turn, contributes to seizure-induced neuroinflammation, neuronal death, behavioral abnormalities, and mortality (Table 1)[30,31]. However, administration of conventional nonsteroidal anti-inflammatory drugs (NSAIDs) or more selective COX-2 inhibitors (COXIBs) increased pentylenetetrazol-induced seizure threshold but diminished seizures triggered by kainic acid[32,33]. These contradictory outcomes from different animal models could be explained by the fact that COX-2 induction after SE can produce five different prostanoids that act on a total of nine G protein-coupled receptors (GPCRs), mediating complex physiological and pathological functions that might oppose each other[34]. The past two decades also witnessed a growing recognition of severe side effects of several prominent anti-inflammatory drugs targeting COXs due to their untoward inhibition on beneficial prostanoid pathways, leading to imbalance between thromboxane and prostacyclin[35]. As such, the COX-2 downstream signaling pathways might provide alternative molecular targets with higher specificity for epileptic seizures and other neurological conditions.

The induction of COX-2 is often accompanied by an elevation of membrane-associated microsomal prostaglandin E synthase-1 (mPGES-1)[36], which directly synthesizes prostaglandin E2 (PGE_2) from COX-2-derived PGH_2 (Fig. 1)[37,38]. PGE_2 activates four currently known GPCRs—EP1, EP2, EP3, and EP4, which are bound to the cell membrane[34]. The EP1 receptor is $G\alpha_q$-coupled to mediate the mobilization of cytosolic Ca^{2+} and the activation of protein kinase C (PKC); EP2 and EP4 receptors are linked to $G\alpha_s$ that activates adenylyl cyclase to generate intracellular cAMP for pathways mediated by protein kinase A (PKA) and exchange protein activated by cAMP (EPAC); EP3 subtype is mainly coupled to $G\alpha_i$ to down-regulate the cAMP signaling[39]. Interestingly, among these four GPCRs, EP2 receptor can be induced by seizures triggered by chemoconvulsants or traumatic brain injury (Fig. 1)[40-42], suggesting a pathophysiological role of

Table 1 Effects of conditional deletion of the COX-2/PGE_2/EP2 axis in animal models of SE.

Genotype	Animal model	Beneficial effect	Ref.
Deficiency of COX-2 in forebrain neurons (Syn1-Cre; COX-$2^{flox/flox}$)	SE was induced by pilocarpine (280 mg/kg, i.p.) in adult C57BL/6 mice	Decreased the induction of cytokines and chemokine in the brain; blunted gliosis; prevented the BBB disruption; protected hippocampal neurons.	30
Deficiency of COX-2 in forebrain neurons (Syn1-Cre; COX-$2^{flox/flox}$)	SE was induced by pilocarpine (275−310 mg/kg, i.p.) in adult C57BL/6 mice	Decreased overall mortality after SE; no significant effect on locomotor activity or anxiety-like behavior; enhanced the retrograde memory performance in Barnes maze test.	30
Deficiency of EP2 in microglia and monocytes (CD11b-Cre; EP$2^{flox/flox}$)	SE was induced by pilocarpine (328 mg/kg, i.p.) in adult male C57BL/6 mice	Accelerated recovery from weight loss; improved Irwin scores by 4 days after SE; reduced brain cytokines including IL-6; prevented the BBB disruption.	43

Abbreviations: BBB, blood−brain barrier; COX-2, cyclooxygenase-2; IL-6, interleukin 6; i.p., intraperitoneal injection; SE, status epilepticus.

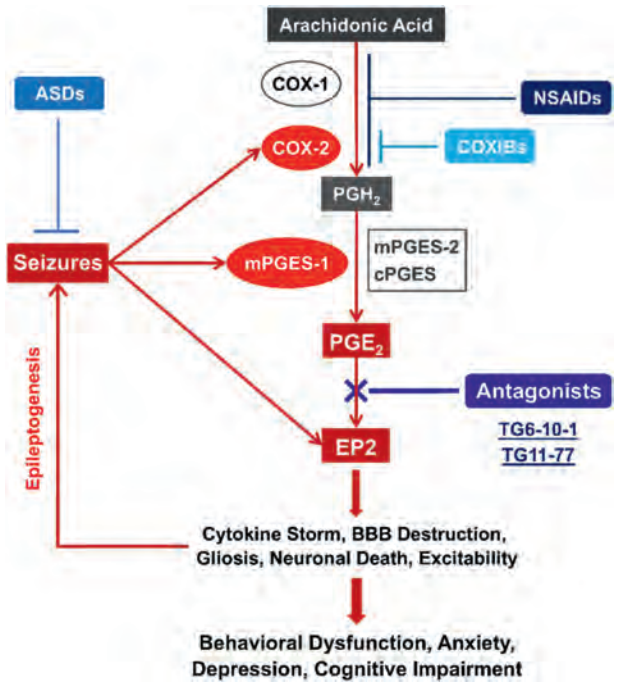

Figure 1 The COX-2/mPGES-1/PGE_2/EP2 signaling axis contributes to neuroinflammation and behavioral comorbidities after seizures. Upon seizure onset, membrane-bound arachidonic acid is converted to a short-lived intermediate named prostaglandin H2 (PGH_2) by COX, which consists of two isoforms: constitutive COX-1 and inducible COX-2. PGH_2 is then rapidly converted to five prostanoids by tissue-specific prostanoid synthases. Among these bioactive lipids, PGE_2 is directly synthesized by three prostaglandin E synthases (PGESs) including mPGES-1, mPGES-2, and cPGES. Elevated PGE_2 activates four receptors, namely, EP1, EP2, EP3, and EP4, and the inflammatory prostaglandin signaling triggered by seizures is mainly mediated by the EP2 receptor subtype. COX-2, mPGES-1, and EP2 are known to be upregulated by seizures, and in turn promote brain inflammation, seizure recurrence, and behavioral impairments. Targeting EP2 receptor by selective antagonists is emerging as a novel strategy to mitigate seizure-triggered neuroinflammation and behavioral comorbidities. Only the major signaling pathways are shown. Abbreviations: ASDs: anti-seizure drugs; BBB: blood−brain barrier; COX: cyclooxygenase; COXIBs: selective COX-2 inhibitors; cPGES: cytosolic prostaglandin E synthase, mPGES-1: microsomal prostaglandin E synthase-1; mPGES-2: microsomal prostaglandin E synthase-2; NSAIDs: nonsteroidal anti-inflammatory drugs; PG: prostaglandin.

EP2 in seizures and possibly acquired epilepsy. Indeed, conditional ablation of EP2 in $CD11b^+$ innate immune cells (microglia and monocytes) accelerated the recovery of mice from sickness behaviors, reduced brain cytokines such as IL-6, and prevented the BBB disruption following pilocarpine-induced SE (Table 1)[30,43]. These findings together suggest that the neuronal COX-2-mediated neuropathological alterations after prolonged seizures should largely be attributed to the elevated inflammatory PGE_2 signaling *via* EP2 receptors on immune myeloid cells (Table 1). Therefore, targeting EP2, instead of the upstream COX-2, might be able to prevent the PGE_2-promoted detrimental effects without affecting the likely benefits that are associated with other prostanoids and their receptors.

4. EP2 antagonists

Owing to its prominent pathogenic roles in a number of neurological disorders and many other chronic conditions, selective small-molecule modulators acting on EP2 with sufficient *in vivo* half-life and brain penetration have been developed (Fig. 2 and Table 2)[39,44-49]. Among these, compound TG4-155 was identified as a first-generation EP2-selective antagonist *via* the high-throughput screening of a small-molecule library containing 262,371 compounds[50]. Although TG4-155 is highly potent, it has relatively short plasma half-life and moderate brain penetration after systemic administration in mice. Introduction of fluorine atoms into its chemical scaffold created a lead compound TG6-10-1 that has improved metabolic stability but slightly reduced potency (Fig. 2 and Table 2). As a second-generation antagonist for the EP2 receptor, compound TG8-260 is more potent than TG6-10-1 and is at least 600-fold selective for the EP2 over other $G\alpha_s$-coupled prostaglandin receptors, *i.e.*, EP4 receptor for PGE_2, DP1 for prostaglandin D2 (PGD_2), and IP for prostaglandin I2 (PGI_2). In addition, it has excellent oral bioavailability (77.3%) and much-improved half-life in mice and rats. However, its brain penetration is quite limited (Table 2)[51], thereby impeding its use for neurological conditions. Continual efforts in medicinal chemistry also led to the development of compound TG11-77[52], which is another second-generation EP2 antagonist with overall better balanced pharmacodynamic and pharmacokinetic properties, particularly potency, selectivity, metabolic stability, *in vivo* half-life, and brain penetration (Table 2).

Molecular docking using the Schrödinger software reveals the dynamic interactions between these competitive antagonists and the human EP2 receptor-G_s protein complex (Fig. 2)[53], showing that the compound potency highly correlates with the docking

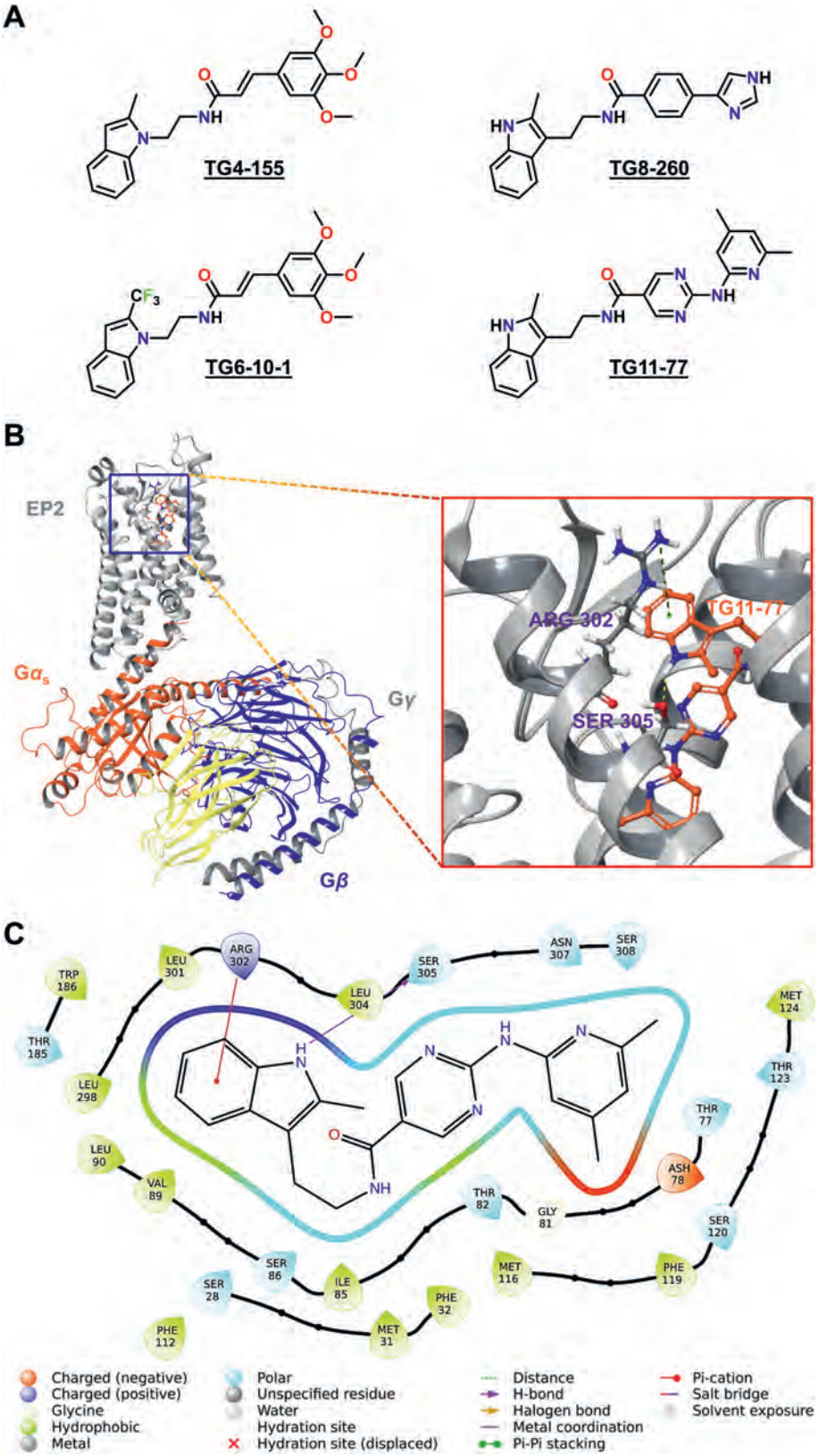

Figure 2 Selective EP2 antagonists that have been tested in animal models of SE for therapeutic effects. (A) As a first-generation EP2 antagonist, compound TG4-155 originated from a high-throughput screening (HTS). Introduction of fluorine atoms into its chemical scaffold created the lead compound TG6-10-1 with improved metabolic stability but reduced potency. As a second-generation antagonist for the EP2

Table 2 Key pharmacodynamic and pharmacokinetic properties of drug-like EP2 antagonists.

Compd.	Potency	Selectivity			Half-life	Brain penetration	Docking score	H-bond
	EP2 K_B (nmol/L)	DP1	EP4	IP	$t_{1/2}$ (h)	(Brain/Plasma)	(kcal/mol)	Score
TG4-155	2.1	14.4	>1000	>1000	0.6	0.3	−8.737	0
TG6-10-1	17.8	9.3	629	478	1.7	1.6	−7.477	−0.804
TG8-260	13.2	606	>1000	758	2.8	0.02	−8.164	−0.492
TG11-77	9.7	>1000	691	>1000	1.1	0.4	−8.677	−0.614

Compound potency on the EP2 receptor is indicated by Schild K_B value. Their selectivity for EP2 against other Gα_s-coupled prostanoid receptors, namely DP1 for PGD_2, EP4 for PGE_2, and IP for PGI_2, is also shown. The *in vivo* plasma half-life ($t_{1/2}$) and brain-to-plasma ratio (brain/plasma) are determined with intraperitoneal administration in mice. It is noted that the compound potency highly correlates with the docking score.

score (Table 2). Importantly, these small-molecule compounds are orally available and only show negligible effects on a panel of essential ion channels, enzymes, receptors, and neurotransmitter transporters, justifying their safe uses *in vivo*[50,54,55]. In line with the findings observed in conditional knockout mice, pharmacological inhibition of EP2 by these brain-penetrant compounds in rodents overall increased post-seizure survival and diminished SE-triggered neuroinflammatory processes, such as the BBB disruption, brain cytokine storm, and reactive gliosis, accompanied by reduced acute neuronal death[39,49,56]. Conversely, systemic administration of a selective EP2 agonist ONO-AE1-259-01 in pilocarpine-treated mice exacerbated cell death in the hippocampus, while the EP2 agonist alone also caused extensive neuronal death in naïve animals[57]. These results from EP2 deletion, inhibition, and activation together support the feasibility of targeting PGE_2/EP2 signaling as a new strategy to mitigate seizure-induced neuropathologies (Fig. 1). Also notably, these broad acute benefits by administration of EP2 antagonists after prolonged seizures are often followed by sequential behavioral improvements in both short term (days) and long term (weeks to months), highlighting a disease-modifying effect on epilepsy progression *via* modulating the EP2 receptor.

5. Comorbidities after SE

After experimental SE, animals enter post-ictal coma and remain relatively less active for a few days, accompanied by a considerable decrease in body weight (up to 20%) due to reduced intake of food and drink, then recover progressively[58]. This seizure-free period is essential to epileptogenesis and known as the latent period, which is prior to the appearance of unprovoked seizures that characterize the chronic phase of epilepsy[3]. A number of behavior tests have been commonly performed to monitor the recovery of animals from acute brain insults like prolonged seizures and to assess various short-term and long-term neurobehavioral alterations during and after the development of spontaneous seizures[5].

5.1. Functional recovery

Among several expedient behavioral tests for the immediate consequences of acute brain insults, nest construction from supplied nestlets has been commonly used to evaluate the functional recovery in numerous animal models owing to the quickness, simplicity, and effectiveness. Moreover, it can be performed daily without imposing any significant stress on the animals. For small rodents like mice, nests are essential to heat conservation and daily life, and impairment in nesting behavior is thought to correlate with lesions in brain areas, such as the medial preoptic area, septum, and hippocampus[59], which are involved in social behaviors, emotion generation, learning and memory, respectively. The Irwin test, an observational paradigm consisting of a battery of stress-limited examines represents another convenient way to assess the effects of test compounds on behavior and physiological functions[60]. This test can be fairly easily adapted to monitor the functional recovery after prolonged seizures in rodent models and can be performed on a daily basis *via* observing the animals for body posture, gait, walking, exploration, drinking, pooping, hypoactivity, hypothermia, ptosis, exophthalmia, and lacrimation[5].

Interestingly, in line with the weight change patterns, mice initially lost the capability of nest building for a couple of days after pilocarpine-induced SE, and then gradually recovered. However, treatment with a selective EP2 antagonist TG6-10-1, which is potent and has sufficient *in vivo* half-life and brain penetration (Fig. 2 and Table 2), twice daily helped the animals to regain their nesting activities faster than animals that were treated by vehicle only (Table 3)[40-43,54,55,61-65]. The improvement in nesting behavior by TG6-10-1 treatment was replicated in mice with kainic acid-induced SE[41] or lipopolysaccharide (LPS)-induced neuroinflammation[62], accompanied by therapeutic benefits on physiological functions evaluated using a modified Irwin test. To verify these pharmacological outcomes, the conditional ablation of EP2 receptor in myeloid cells (mainly microglia and monocytes) was generated and found to accelerate the recovery of mice from weight loss after SE, along with overall improved Irwin

receptor, TG8-260 has a much-improved half-life in rodents but its brain penetration is quite low. Compound TG11-77 is another second-generation EP2 antagonist with well-balanced pharmacodynamic and pharmacokinetic properties. (B) Simulated antagonist-EP2-G_s protein complex. Using the Schrödinger Software Maestro (Version 2024-1), EP2 antagonists, exemplified by compound TG11-77, were docked into the cryo-EM structure of the human EP2-G_s($\alpha/\beta/\gamma$) complex (PDB: 7CX3). The receptor and G proteins are displayed as ribbons (gray, orange, dark blue, yellow). Compound TG11-77 is depicted as the ball-and-stick model, where carbons are exhibited in orange, nitrogens in blue, and oxygens in red. Green dashed line represents $\pi-\pi$ stacking, and yellow dashed line indicates hydrogen bonding. The carbon atoms in key interacting residues are displayed in gray, with the corresponding residue names labeled in purple. The two residues are hypothetically important for the binding capabilities of ligands within the binding site of the EP2 receptor. (C) A two-dimensional representation of the ligand interactions within the binding site on the EP2 receptor is shown. The potency, selectivity, plasma half-life, brain penetration, docking score, and H-bond score of these compounds are shown in Table 2.

scores[43]. These findings from pharmacological and genetic studies validate each other and together reinforce the value of EP2 as a feasible target for mitigation of the neuropathogenesis triggered by SE.

However, systemic treatment with a second-generation EP2 antagonist with higher potency and selectivity, TG8-260 (Fig. 2 and Table 2), had no effects on either weight changes or Irwin scores in rats after pilocarpine-induced SE (Table 3). Albeit EP2 inhibition by TG8-260 was able to relieve neuroinflammation and reactive gliosis in the hippocampus, it did not prevent the associated neuronal damage or BBB breakdown in these SE rats[63]. Likewise, another second-generation EP2 antagonist, TG11-77 (also known as BPN300343, Fig. 2), which is also more potent and selective than TG6-10-1 (Table 2), did not show significant effects on nesting behavior or Irwin scores after SE in mice. However, it did improve the post-SE survival rates[55]. The lack of immediate effects on nesting or other physiological functions by treatment with TG8-260 or TG11-77 after SE might be explained by their relatively lower brain penetration than compound TG6-10-1 (Table 2), leading to less neuroprotective effects[55,63]. This could be especially the case when the scores in these observation tests are designated to indicate the levels of brain lesion and neurotoxicity[59,60].

5.2. *Anxiety and depression*

Anxiety- and depression-like behaviors are more common in patients with epilepsy than healthy individuals[66], and are largely recapitulated in experimental rodents after prolonged seizures. A battery of behavioral tests, such as open field, light−dark box, sucrose consumption, force swimming, and tail suspension tests, can be used to detect such behaviors in these animals[5]. In a rat model of SE triggered by exposure to diisopropyl fluorophosphate (DFP), a prototypical organophosphate targeting acetylcholinesterase to quickly elevate acetylcholine levels in the brain for seizure induction, animals showed anxiety-like behavior in a light−dark box test, evidenced by the significantly increased time spent in the light compartment. Treatment with EP2 antagonist TG6-10-1 did not decrease the overall time of rats in the light compartment after DFP-induced SE, nor did it reduce the number of their entries into the light compartment (Table 3). Similarly, TG6-10-1-treated DFP rats did not display any manifest

Table 3 Effects of EP2 inhibition on behavioral impairments in rodent models.

Animal model	Treatment	Behavioral test	Outcome	Ref.
Pilocarpine (280 mg/kg, i.p.)-induced SE in adult male C57BL/6 mice	TG6-10-1 (5 mg/kg, i.p.) at 2, 6, 21, and 30 h after SE onset	Nesting test	Decreased mortality after SE; accelerated recovery from weight loss and improved nesting behavior by 7 days after SE.	40
Kainic acid (30 mg/kg, i.p.)-induced SE in adult male C57BL/6 mice	TG6-10-1 (5 mg/kg, i.p.) at 2, 6, 21, and 30 h after SE onset	Irwin test	Accelerated recovery from weight loss; improved nesting behavior; improved Irwin scores by 4 days after SE.	41
Kainic acid (30 mg/kg, i.p.)-induced SE in adult male C57BL/6 mice	TG6-10-1 (5 mg/kg, i.p.) at 4, 21, and 30 h after SE onset		Accelerated recovery from weight loss.	43
Pilocarpine (280 mg/kg, i.p.)-induced SE in adult male C57BL/6 mice	TG6-10-1 (5 mg/kg, i.p.) at 4, 21, and 30 h after SE onset	Nesting test	Decreased mortality after SE; accelerated recovery from weight loss and improved nesting behavior by 4 days after SE.	54
Pilocarpine (280 mg/kg, i.p.)-induced SE in adult male C57BL/6 mice	TG11-77 (8.8 mg/kg, i.p.) at 4, 8, and 19 h after SE onset	Nesting test Irwin test Y-maze test	Decreased mortality after SE; no effect on weight loss; no effect on nesting behavior; no effect on Irwin scores; improved spatial working and reference memory in a Y-maze test; no effect on motor abilities.	55
LPS (3 mg/kg, i.p.)-induced neuroinflammation in adult C57/BL6 mice	TG6-10-1 (10 mg/kg, i.p) at 0.5 and 3.5 h after LPS injection	Nesting test	Accelerated recovery from weight loss; improved nesting behavior by 3 days after LPS injection.	62
LPS (5 mg/kg, i.p.)-induced neuroinflammation in adult C57/BL6 mice	TG6-10-1 (10 mg/kg, s.c.) at 0.5 h after LPS injection	SP test NOR test	Ameliorated depressive behavior in an SP test by 3 weeks after LPS injection; improved cognition in an NOR test by 4 weeks after LPS injection.	62
Pilocarpine (380 mg/kg, s.c.)-induced SE in adult male Sprague−Dawley rats	TG8-260 (10 mg/kg, i.p.) at 2, 8, and 20 h after SE onset	Irwin test	No effect on weight change; no effect on Irwin scores at either 24 h or 4 days after SE.	63
DFP (9.5 mg/kg, i.p.)-induced SE in adult male Sprague−Dawley rats	TG6-10-1 (5 mg/kg, i.p.) at 1.5, 6, 21, 30, 45−47, and 52−55 h after SE onset	Open field test Ligh-dark box test NOR test Irwin test	No effect on anxiety-like behavior in the open field and light−dark box tests by 4 weeks after SE; improved cognition in a NOR test by 6−12 weeks after SE; no effect on motor functions.	64
DFP (9.5 mg/kg, i.p.)-induced SE in adult male Sprague−Dawley rats	TG6-10-1 (5 mg/kg, i.p.) at 1.5−2.5, 5−6, 9−21, 24, 31−42, and 48 h after SE onset	Irwin test	Accelerated recovery from weight loss; no effect on Irwin scores by 24 h after SE.	65

Abbreviations: DFP, diisopropyl fluorophosphate; i.p., intraperitoneal injection; LPS, lipopolysaccharide; NOR, novel object recognition; s.c., subcutaneous injection; SE, status epilepticus; SP, sucrose preference.

behavioral changes in an open field test when compared to the vehicle-treated cohorts[64]. These results suggest that the anxiety-like behavior in rats after exposure to DFP was not alleviated by treatment of TG6-10-1, and in fact, are in alignment with its lack of significant effects on the Irwin scores[65]. These findings are also consistent with an early study showing that conditional ablation of COX-2 in the forebrain neurons had no effect on anxiety levels in mice after pilocarpine-induced SE (Table 1)[31], although EP2 inhibition by TG6-10-1 or genetic deficiency in COX-2 increased the overall animal survival rates after SE induced by DFP or pilocarpine, respectively[31,65].

Systemic treatment with LPS in mice led to extensive neuroinflammatory reactions and reduced sucrose consumption in a sucrose preference test performed three weeks later. However, a single subcutaneous administration of EP2 antagonist TG6-10-1 at 30 min after LPS-triggered inflammatory insults restored the consumption of sucrose in these animals (Table 3)[62]. Given that anhedonia is widely considered as a core symptom of depression in both animal models and human patients[5], these findings support a long-lasting antidepressant effect by compound TG6-10-1 under neuroinflammatory conditions. However, whether EP2 inhibition can relieve the depressive symptoms associated with seizures and epilepsy in animal models requires further investigation utilizing more sophisticated behavioral assessments, such as force swimming and tail suspension tests.

5.3. *Aggression*

It is widely believed that aggressive behavior might be associated with seizures, but its prevalence in patients with epilepsy is unlikely to be higher than that in people having other neurological conditions. Therefore, it is unfair to subjectively classify epilepsy as an aggressive disease[67]. Although seizures themselves usually do not directly cause aggressive behavior or even violent assault, it is evident that the post-seizure confusion can lead to anger and aggression. Just like depressive symptoms, aggressive behaviors are quite common in chemoconvulsant models of epilepsy and may correlate with post-SE hyperexcitability as well as the development of spontaneous recurrent seizures[68], suggestive of a common pathophysiological mechanism underlying both symptoms in epilepsy. Intriguingly, aggressive behaviors in animal models appear more prevalent in those with severe acute neuronal damage caused by the initial SE[69]. Post-SE administration of EP2 antagonists is known to prevent SE-triggered neuronal death in the hippocampus and many other brain regions[56]. However, whether the broad neuroprotection by these compounds can lead to any reduction in aggressive behaviors after SE requires further investigation.

5.4. *Cognitive impairment*

Among various behavioral comorbidities associated with seizures and epilepsy, cognitive dysfunctions, such as difficulties in learning, memory, attention, and processing, are the most common and problematic[70]. There is a corresponding high rate of cognitive complication in people with epilepsy, which often impedes their educational progress and career achievement. Further, patients with pharmacoresistant seizures are more likely to have cognitive difficulties than those with seizures that can be controlled by current anti-seizure medications. The key aspects of cognitive deficits in human epilepsy patients can largely be recapitulated and characterized in most commonly used animal models of epilepsy with various behavioral tests, such as novel object recognition, Barnes maze, Y-maze, and Morris water maze tasks[5]. Early studies on the mouse pilocarpine model of SE showed that conditional deletion of COX-2 from a restricted population of forebrain neurons reduced neuroinflammation and enhanced the retrograde memory performance in a Barnes maze test three weeks after SE (Table 1)[30,31], indicating an essential role of PGE_2 signaling in cognitive dysfunctions associated with seizure disorders.

Indeed, adult rats treated by EP2 antagonist TG6-10-1 were able to discriminate between a novel object and a familiar object even several weeks after DFP-induced SE when compared to vehicle-treated animals (Table 3)[64,71], suggesting the involvement of PGE_2/EP2 signaling in SE-triggered memory impairment. Likewise, treatment with TG6-10-1 in mice after LPS-mediated neuroinflammatory insult helped the animals to regain the preference for the novel object over the familial object[62]. Moreover, mice administered with TG11-77, showed improved spatial working and reference memory in a Y-maze test performed several weeks after pilocarpine-induced SE[55]. It should be noted that EP2 inhibition by these two compounds did not affect the total travel distances of tested animals, suggesting that the long-term restoration of cognitive functions was not due to any difference in motor abilities. Taken together, systemic EP2 inhibition appears to completely recapitulate the multiple effects of neuronal ablation of COX-2, even though COX-2 produces five different prostanoid products that act on nine different GPCRs[34]. Hence, it is likely that the COX-2-mediated long-term behavioral deficits after seizures are largely attributed to the downstream PGE_2/EP2 signaling axis.

6. Conclusions and perspectives

Several early studies show that, under normal physiological conditions, global deletion of the EP2 receptor in mice causes heightened anxiety, impaired social recognition memory, and abnormal hippocampal synaptic plasticity, but the animals show normal motor activity, exploratory behavior, and spatial reference memory[72,73]. These findings seemingly support an essential role of EP2 signaling in synaptic plasticity, cognition, and emotion. However, genetic deficiency in EP2 also causes complications such as reduced litter size and hypertension[74-77], and these developmental adjustments suggest that the congenital global EP2 knockout mice may not be ideal subjects for behavioral studies in seizure models. On the contrary, mice with postnatal or cell type-specific deletion of EP2, under healthy conditions, showed normal behaviors in a battery of tests including prepulse inhibition, open field test, elevated plus maze, and Morris water maze[78], demonstrating the safety of targeting EP2 receptor by selective pharmacological agents.

6.1. *Insights from other CNS conditions*

Interestingly, conditional deletion of EP2 in myeloid cells including brain-resident microglia decreased the spatial memory deficits in the APP-PS1 transgenic mice, a model for Alzheimer's disease (AD), revealed by novel object recognition and radial arm maze tests[79]. The result suggests that inhibition of the PGE_2/EP2 pathway might restore healthy microglial functions and prevent AD progression. In line with this finding, conditional deficiency in myeloid EP2 was able to revive cellular bioenergetics, inflammatory states, hippocampal synaptic plasticity, and spatial memory in aged mice. Furthermore, treatment with EP2-selective antagonists PF-04418948 (developed by Pfizer; Fig. 3A) and benzoxazepine-52 (developed by Amgen; Fig. 3A) rejuvenated

phagocytic functions of microglia in aged mice and improved their hippocampal synaptic plasticity and spatial memory, evaluated by novel object recognition, radial arm maze, and Barnes maze tests[80]. Likewise, compound benzoxazepine-52 has been shown to decrease brain infarction in mice after transient ischemic stroke, leading to improved neurological functions[78]. Consistently, post-stroke inhibition of EP2 by compound TG6-10-1 after transient cerebral ischemia was also able to reduce brain infarction and functional deficits[81]. In a more recent study, the second-generation EP2 antagonist TG11-77, when administered after thrombotic stroke, reduced cortical infarction. Interestingly, treatment with TG11-77 also improved the post-stroke neurological impairments in locomotor and cognitive functions, uncovered by a panel of behavioral tests including open field, novel object recognition, and corner tests[82]. All these findings from animal models of AD, aging, and strokes together support the EP2 receptor as a feasible

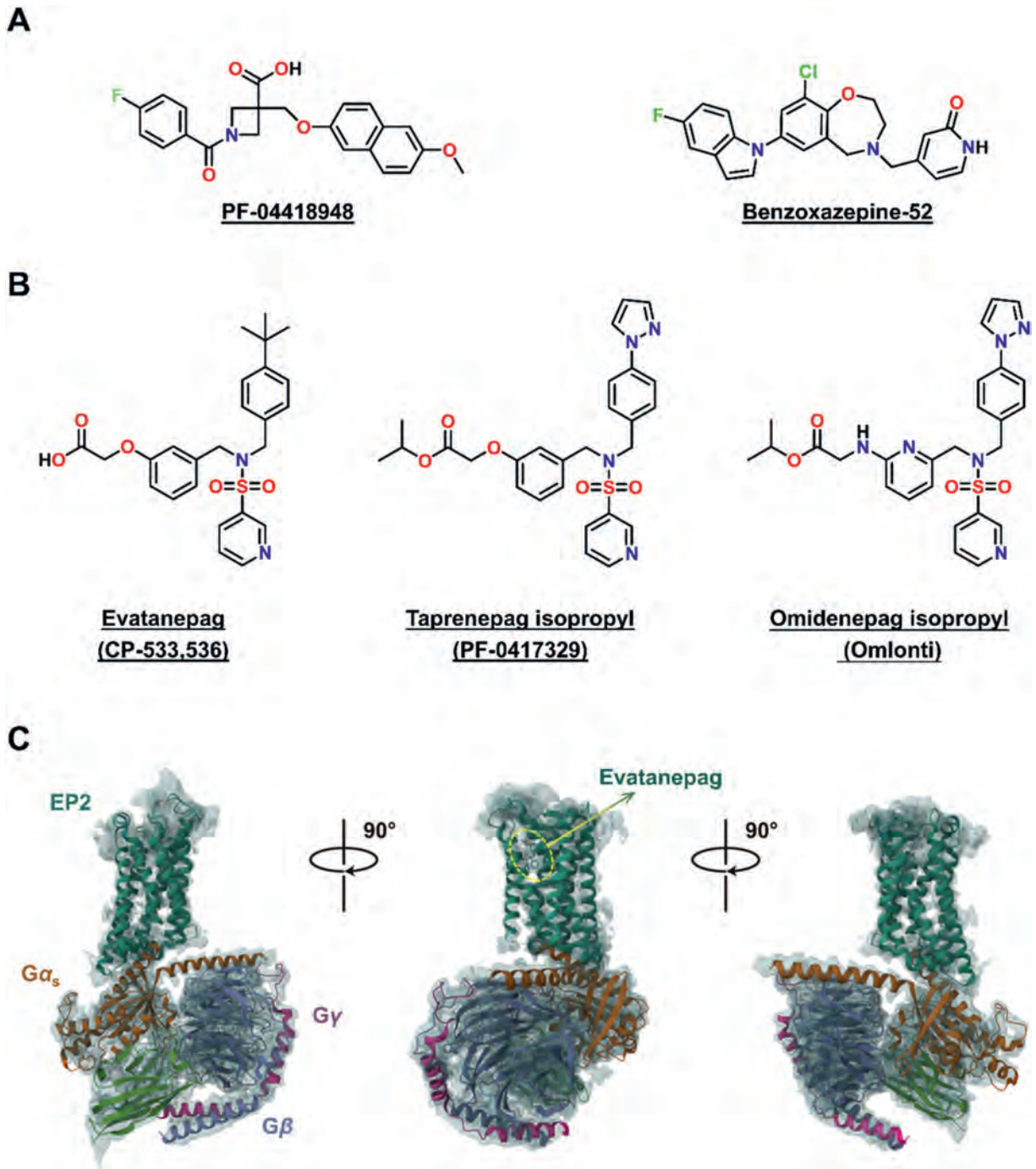

Figure 3 EP2 modulators that have been evaluated in human clinical trials. (A) Chemical structures of EP2-selective small-molecule antagonists PF-04418948 and benzoxazepine-52. (B) Chemical structures of selective EP2 agonists evatanepag (CP-533,536), taprenepag isopropyl (PF-0417329), and omidenepag isopropyl (Omlonti). (C) Cryo-EM density of EP2-G_s in complex with EP2-selective agonist evatanepag (PDB: 7CX4) to highlight the ligand−receptor interaction. Reproduced with permission[53].

target to alleviate neurobehavioral comorbidities, particularly the cognitive impairment, which is commonly observed in various neuroinflammatory conditions.

6.2. EP2 compounds in clinical studies

Evatanepag (also known as CP-533,536; Fig. 3B and C), a potent and selective EP2 agonist developed by Pfizer underwent a Phase 2 clinical trial for efficacy, safety and tolerability in patients with closed fracture of the tibial shaft (https://clinicaltrials.gov/study/NCT00533377). Although the results from this human study are not available, EP2 receptor activation by this small-molecule agonist was reported to promote local bone formation and enhance fracture healing in rat models of fracture healing[83]. Taprenepag isopropyl (also known as PF-0417329, Fig. 3B), another EP2-selective agonist developed by Pfizer, was studied in patients of primary open angle glaucoma and ocular hypertension (https://clinicaltrials.gov/study/NCT00572455). In another Phase 2 study, this EP2 agonist was further evaluated for effects on circadian intraocular pressure and blood pressure in glaucoma patients (https://clinicaltrials.gov/study/NCT00934089). It was found that PF-0417329 largely decreased intraocular pression and ocular hypertension[84,85]. Despite a number of clinical trials in the past couple of decades, to date, there is only one approved drug targeting the EP2 receptor—omidenepag isopropyl (Omlonti®; Fig. 3B), which is a selective agonist for EP2 co-developed by Santen Pharmaceutical and Ube Corporation. It was approved for clinical use to treat glaucoma and ocular hypertension on September 22, 2022 (https://www.fda.gov/drugs/novel-drug-approvals-fda/new-drug-therapy-approvals-2022). In contrast to the clinical success of these EP2 agonists, currently, there is no FDA-approved EP2-selective antagonist for any clinical use. PF-04418948 (Fig. 3A), a highly potent and selective EP2 antagonist developed by Pfizer, went through a Phase 1 human clinical trial for safety evaluation (https://clinicaltrials.gov/study/NCT01002963). The results suggest that the compound was well tolerated without causing any cardiovascular events or renal toxicity. Unexpectedly, it led to mild hyperbilirubinemia, which was likely associated with its off-target activity on organic anion transporting polypeptide 1B1 (OATP1B1)[49]. Consequently, its further clinical development was halted.

6.3. Caveats and considerations

In addition to its well-studied neuropathogenic roles in inflammation-associated conditions, PGE_2 signaling *via* the EP2 receptor has also long been known for some beneficial actions in a variety of tissues and organs. For instance, neuroprotection *via* allosterically activating the PGE_2/EP2 signaling has been reported in cell culture models of excitotoxicity[44,86], and is thought to be mediated by its downstream $G\alpha_s$-mediated cAMP/PKA/CREB pathway in neurons[77]. In the periphery, PGE_2 promotes repair and regeneration of various damaged tissues and organs *via* acting on its all four receptors EP1-EP4[87,88]. Particularly, the PGE_2/EP2 signaling is involved in the process of bone healing[89], mitigates renal inflammation and fibrosis[90,91], and regulates muscle progenitors in proliferation and differentiation[92]. Moreover, the EP2 receptor has been shown to mediate the PGE_2-promoted cardiomyocyte regeneration following myocardial ischemia[93]. This beneficial role of PGE_2/EP2 pathway is likely *via* regulating the macrophage activation, characterized by infiltration of macrophages toward the injured myocardium where they are transformed from phenotype M1 to M2 that promotes healing[94]. Overall, the PGE_2 signaling-mediated repair and regeneration of various organ systems following tissue injuries might be associated with the positive aspects of inflammation, such as activation of endogenous stem cells, immune regulation, and angiogenesis[88]. Nonetheless, these protective and favorable effects mediated by various PGE_2 pathways should be carefully considered when developing small-molecule antagonists inhibiting PGE_2 signaling for clinical uses.

6.4. Targeting PGE_2 synthase or signaling?

COX is responsible for the initial step of PGE_2 biosynthesis following prolonged seizures, whereas PGE_2 synthase, particularly the inducible form mPGES-1, catalyzes the terminal step. As such, targeting mPGES-1 is unlikely to cause any substantial changes on other types of prostanoids, thereby providing a more specific strategy to diminish PGE_2 than blocking the entire COX cascade by NSAIDs or COXIBs[95]. Indeed, systemic treatment with mPGES-1 inhibitor PBCH (also known as **7d** and MPO-0063) in mice after transient ischemic stroke reduced cerebral infarction, brain cytokines, locomotor dysfunction, anxiety-like behavior, and the long-term cognitive impairments[96]. In a mouse model of SE, PBCH treatment also decreased the SE-induced PGE_2, inflammatory cytokines, and reactive gliosis within the brain, accompanied by broad neuroprotection in various brain areas[97]. However, whether these anti-inflammatory and neuroprotective effects by mPGES-1 inhibition could lead to any benefits in post-SE behaviors remains to be determined.

PGE_2 plays various pathophysiological roles *via* acting on EP1-EP4 receptors, which diversely engage in downstream G protein-dependent and independent signaling pathways. Among these, EP1 receptor activation contributes to the pharmacoresistance in epilepsy *via* upregulating the P-glycoproteins[98]; PGE_2 signaling *via* EP2 promotes brain inflammation and injury and causes subsequent neurobehavioral deficits as described above. In contrast to the widely-investigated EP2 in animal models, EP3 and EP4 receptors are relatively unclear for their roles in neuropathogenesis after prolonged seizures[3,34]. Thus, targeting EP2 receptor seemingly provides higher therapeutic specificity than blocking PGE_2 synthesis by mPGES-1 inhibition, which could also affect EP3 and EP4-mediated effects that may not necessarily be pathogenic or deleterious.

6.5. Future perspectives

In the pilocarpine model of SE, conditional ablation of EP2 in the brain microglia and monocytes reduced behavioral deficits in mice, particularly ptosis and abnormal posture, determined by a modified Irwin test[43]. These disease-modifying effects derived from cell type-specific ablation of EP2 support the feasibility of targeting EP2 for seizure-promoted behavioral comorbidities and have been validated and extended by a number of pharmacological studies utilizing the selective EP2 antagonists including TG6-10-1 and TG11-77 (Table 3). These EP2 compounds are generally considered safe for clinical use because their acute or long-term administration did not alter the overall well-being, motor behavior, blood cell counts, bone morphology, cardiovascular or respiratory functions in normal mice or rats[99]. Nor did they cause any overt adverse clinical signs in a comprehensive test for dose range-finding toxicology in rats or

considerable inhibition on human cytochrome P450 enzymes[55]. Also important, they do not have any significant off-target activities, are metabolically stable in hepatocytes, and can be delivered orally with favorable bioavailability, plasma half-life, and brain penetration[39,55]. In sum, pharmacological modulation of EP2 receptor by these drug-like compounds as an adjunctive strategy along with the current ASDs after seizures might provide the first preventive treatment for neurobehavioral comorbidities of epilepsy, particularly the most troublesome cognitive deficits.

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Conflicts of interest

The authors declare no conflicts of interest.

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