

Discovery of lipophilic two-pore channel agonists

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Keywords

endosome; lysosome; small-molecule activator; TPC1; TPC2; TRPML

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(Received 3 April 2020, revised 14 May 2020, accepted 27 May 2020)

doi:10.1111/febs.15432

Two-pore channels (TPCs) have been a hot topic in recent literature. Their involvement in various diseases such as viral infections and cancer is of great interest for drug research. Due to their localization in the endolysosomal system and the lack of cell-permeable activators, complex techniques were required for studying channel functions. Here, we review the first published lipophilic small-molecule activators of TPCs. In independent high-throughput screens, several new agonists were discovered, which now allow simple and fast investigation of TPCs in more detail in intact cells and *in vivo*. Zhang *et al.* identified tricyclic and phenothiazine antidepressants as TPC1 and TPC2 activators by screening a library of approved drugs. In contrast, Gerndt *et al.* screened an extensive compound library with mostly new chemotypes and drug structures. The latter resulted in two structurally distinct high-affinity agonists, which are able to selectively activate TPC2 in either an NAADP- or PI(3,5)P₂-like manner. Here, we discuss the advantages and drawbacks of the identified molecules and their structural features. The versatility by which TPCs can be activated indicates many opportunities for future studies.

Introduction

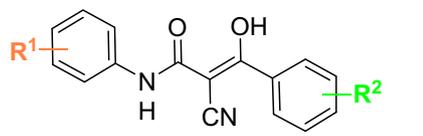
In 2009/2010, TPCs were first described as NAADP-activated nonselective, calcium-permeable cation channels in endolysosomes [1–5]. In 2012/2013, two groups challenged this view and claimed TPCs to be sodium-selective channels activated by the endolysosomal phosphoinositide PI(3,5)P₂ which also activates the related endolysosomal cation channels TRPML1, TRPML2, and TRPML3 (mucolipins 1, 2, and 3) [6,7]. Both views received independent support in the proceeding years [8–13] without really solving the debate.

Despite this, TPCs have emerged in recent years as highly exciting potential novel drug targets for a number of diseases associated with the endolysosomal system. Thus, TPCs have been demonstrated to play a role in various infectious diseases such as Ebola filovirus, Middle East respiratory syndrome coronavirus (MERS-CoV), COVID-19 coronavirus, or HIV-1 retrovirus infections [16–20]. In addition, several bacterial toxins such as diphtheria toxin, anthrax toxin, cholera toxin, or pasteurilla multocida toxin [21,22]

Abbreviations

5-HT, 5-hydroxytryptamine receptors; ALS, amyotrophic lateral sclerosis; CB1R, cannabinoid-1 receptor; CLN3, battenin; cy, cyclohexyl; CYP450, cytochrome P450; EGF, epidermal growth factor; GABA_A, receptors that respond to gamma-aminobutyric acid; HIV, human immunodeficiency virus; LOPAC, Library of Pharmacologically Active Compounds; LRRK2, leucine-rich repeat kinase 2; MERS-CoV, Middle East respiratory syndrome coronavirus; ML2-SA1, (3a*RS*,4*RS*,7*SR*,7a*RS*)-3-(2,6-dichlorophenyl)-3a,4,5,6,7,7a-hexahydro-4,7-methanobenzo[d]isoxazole; ML-SA1, mucolipin synthetic agonist 1; NAADP, nicotinic acid adenine dinucleotide phosphate; NMDA, *N*-methyl-D-aspartate receptor; PDGF, platelet-derived growth factor; PI(3,5)P₂, phosphatidylinositol 3,5-bisphosphate; SSRI, selective serotonin reuptake inhibitors; TPA, tricyclic antidepressants; TPC, two-pore channel; TRPML, transient receptor potential cation channel; TTX, tetrodotoxin; VSD, voltage-sensing domain.

Table 1. Structure variations and EC₅₀ values of TPC2-A1-N (**1**) and the most potent analogs. No significantly increased efficacies were observed by EC₅₀ values (Fluo-4-based Ca²⁺ imaging experiments) [15].



Compound	R ¹	R ²	EC ₅₀
TPC2-A1-N (1)	4-CF ₃	3,5-Cl ₂	7.8 μM
SGA-33 (11)	2,4-F ₂ , 3-Cl	3,5-Cl ₂	23 μM
SGA-85 (18)	3,5-(CF ₃) ₂	3,5-Cl ₂	3.0 μM
SGA-86 (19)	4-OCF ₃	3,5-Cl ₂	9.5 μM
SGA-90 (20)	2,4-F ₂ , 3-Cl	3,5-Br ₂	12 μM
SGA-108 (21)	4-CH ₃	3,5-(CF ₃) ₂	7.1 μM
SGA-111 (22)	4-CF ₃	3,5-(CF ₃) ₂	6.2 μM
SGA-132 (24)	4-CF ₃	3-Br, 5-I	5.3 μM

have been shown to require functional TPCs for trafficking and release of the toxins into the cytosol.

Besides their role in viral and bacterial infections, loss of TPCs has also been found to inhibit cancer cell migration and neoangiogenesis [23,24], to result in the endolysosomal accumulation of cholesterol [9] and to delay growth factor trafficking (EGF, PDGF) [9,21], to reduce glucagon secretion [25], and to increase melanin production and pigmentation [26–29]. Finally, Parkinson's disease caused by LRRK2 mutations [30], mature-onset obesity [31], and β-adrenoceptor signaling in the heart [32] has all been linked to TPC function.

Selective, potent, lipophilic small-molecule inhibitors and agonists are urgently needed to better understand the different physiological and pathophysiological roles of TPCs and to further establish TPCs as novel drug targets. In 2019/2020, two groups performed independent compound library screenings to identify novel lipophilic small-molecule activators of TPCs [14,15]. While Gerndt *et al.* identified TPC2-selective agonists, Zhang *et al.* found nonselective TPC1/2 activators. Here, we summarize these recent developments.

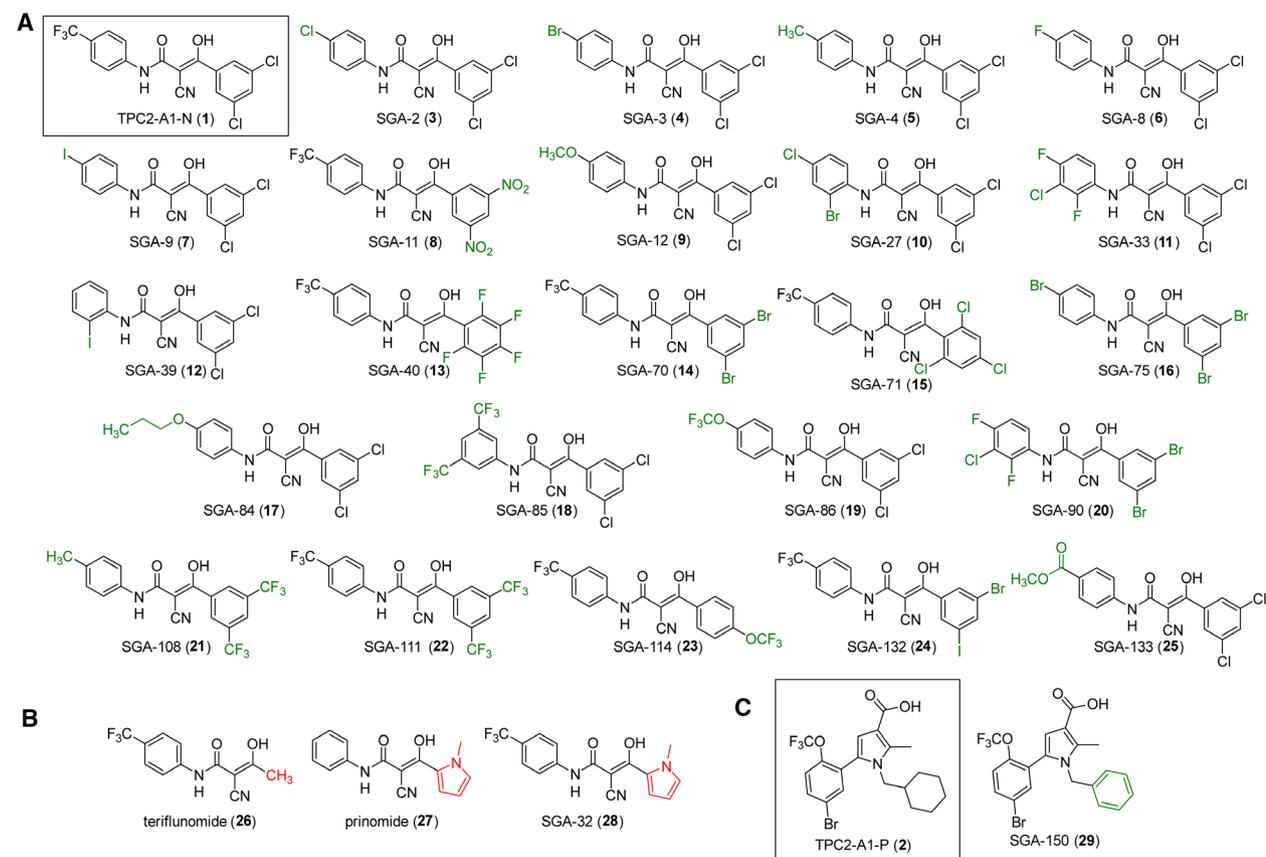


Fig. 1. Structures of TPC2 activators, identified by Gerndt *et al.* [15]. (A) Structure of TPC2-A1-N (**1**) and the 23 active analogs. Nonactive analogs are not shown. Differences in structure are marked in green. (B) Structures of the approved drugs teriflunomide (**26**) and prinomide (**27**) and analog SGA-32 (**28**), all of which were not able to activate TPC2 in Ca²⁺ imaging experiments.

Novel small-molecule activators of TPCs

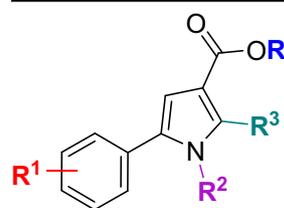
Selective TPC2 activators—modulation of Na⁺/Ca²⁺ permeability

Gerndt *et al.* [15] published two novel, yet very differently acting lipophilic small-molecule agonists of TPC2, one called TPC2-A1-N (**1**, 2-cyano-3-(3,5-dichlorophenyl)-3-oxo-*N*-(4-(trifluoromethyl)phenyl)propanamide), and the other TPC2-A1-P (**2**, 5-(5-bromo-2-(trifluoromethoxy)phenyl)-1-(cyclohexylmethyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid). Both compounds were identified in a high-throughput screening approach using the calcium indicator dye, Fluo-4. Two libraries from Roche comprising in total 80 000 natural and synthetic small molecules (X30 and X50; Roche, Basel, Switzerland) were screened with a HEK293 cell line stably expressing a plasma membrane variant of human TPC2 containing mutations in its N-terminal lysosomal targeting motif (TPC2^{L111A/L12A}). As a control, HEK293 cells stably expressing a plasma membrane variant of the unrelated lysosomal membrane protein CLN3 was used, likewise containing mutations in its lysosomal targeting motif (CLN3^{L253A/I254A}). TPC2-A1-N (**1**) and TPC2-A1-P (**2**) showed EC₅₀ values of 7.8 and 10.5 μM, respectively, in Fluo-4 calcium imaging experiments. Results were subsequently confirmed in Fura-2 calcium imaging and endolysosomal patch-clamp experiments. EC₅₀ values in endolysosomal patch-clamp experiments were both 0.6 μM. The authors discovered that TPC2-A1-N (**1**) rendered the channel more calcium-permeable, whereas TPC2-A1-P (**2**) increased sodium permeability. The authors further demonstrated that TPC2-A1-P (**2**) has a reversal potential and a Ca²⁺/Na⁺ permeability similar to PI (3,5)P₂, while the corresponding values obtained with TPC2-A1-N (**1**) resembled those obtained with NAADP. In conclusion, TPC2 emerges as a highly unusual cation channel with malleable ion selectivity depending on the activating ligand. Both agonists neither activated TPC1 nor activated TRPML1, TRPML2, and TRPML3. Thus, a single screen identified high-affinity, isoform-selective probes that mimicked very different physiological cues.

In an attempt to identify more potent/efficacious variants of TPC2-A1-N (**1**) and TPC2-A1-P (**2**), several structure modifications were performed and the modified compounds subsequently tested. Surprisingly, none of the 46 modified versions of TPC-A1-N (**1**) showed significantly increased efficacies or potencies (Table 1). Replacing the *p*-trifluoromethyl group on

the aniline side of the molecule (R¹; orange) with other electron-withdrawing groups in *para*-position did not cause significant changes, even the introduction of electron-releasing groups in *para*-position was tolerated to some extent (SGA-4 (**5**), SGA-84 (**17**)). The apparent enhancement of activity of SGA-85 (**18**) is explained by the fact that control cells showed increased levels of activation in these experiments as well [15]. For the substitution pattern of the acylated phenyl ring system (R², green), *meta*-disubstitution patterns are most beneficial. More drastic changes in this aromatic region (replacement by methyl or pyrrole residues), as demonstrated for the approved drugs teriflunomide (**26**) and prinomide (**27**) as well as the 4-trifluoromethyl variant of prinomide (SGA-32; **28**), led to a complete loss of activity (Fig. 1). Teriflunomide (**26**) was introduced for the treatment of multiple sclerosis and prinomide (**27**) for rheumatoid arthritis [33,34]. TPC-A1-N (**1**) itself and some of its analogs bearing residues in *para*-position at the acylated aromatic ring are known anthelmintic agents [35]. In

Table 2. Structure variations (selected examples) of TPC2-A1-P (**2**) and EC₅₀ values on TPC2. Even slight changes in structure of the original hit lead to decrease or loss of activity. Activity on the ion channel was determined by Fura-2-based single-cell Ca²⁺ imaging experiments, as previously described [15]. cy, cyclohexyl.



Compound	R	R ¹	R ²	R ³	EC ₅₀
TPC2-A1-P (2)	H	2-OCF ₃ 5-Br	CH ₂ cy	CH ₃	10.5 μM
SGA-150 (29)	H	2-OCF ₃ 5-Br	benzyl	CH ₃	34 μM
SGA-50 (30)	H	-	CH ₂ cy	CH ₃	n.a.
SGA-55 (31)	H	2-OCH ₃ 5-Br	CH ₂ cy	CH ₃	n.a.
SGA-140 (32)	CH ₂ CH ₃	2-OCF ₃ 5-Br	CH ₂ cy	CH ₃	n.a.
SGA-149 (33)	H	2-OCF ₃ 5-Br	Pentyl	CH ₃	n.a.
SGA-152 (34)	H	2-OCF ₃ 5-Br	CH ₂ cy	CH ₂ CH ₃	n.a.
SGA-153 (35)	H	2-OCF ₃ 5-Br	<i>i</i> Pr	CH ₃	n.a.
SGA-154 (36)	H	2-OCF ₃ 5-Br	CH ₂ cy	phenyl	n.a.
SGA-162 (37)	H	2-OCF ₃	CH ₂ cy	CH ₃	n.a.

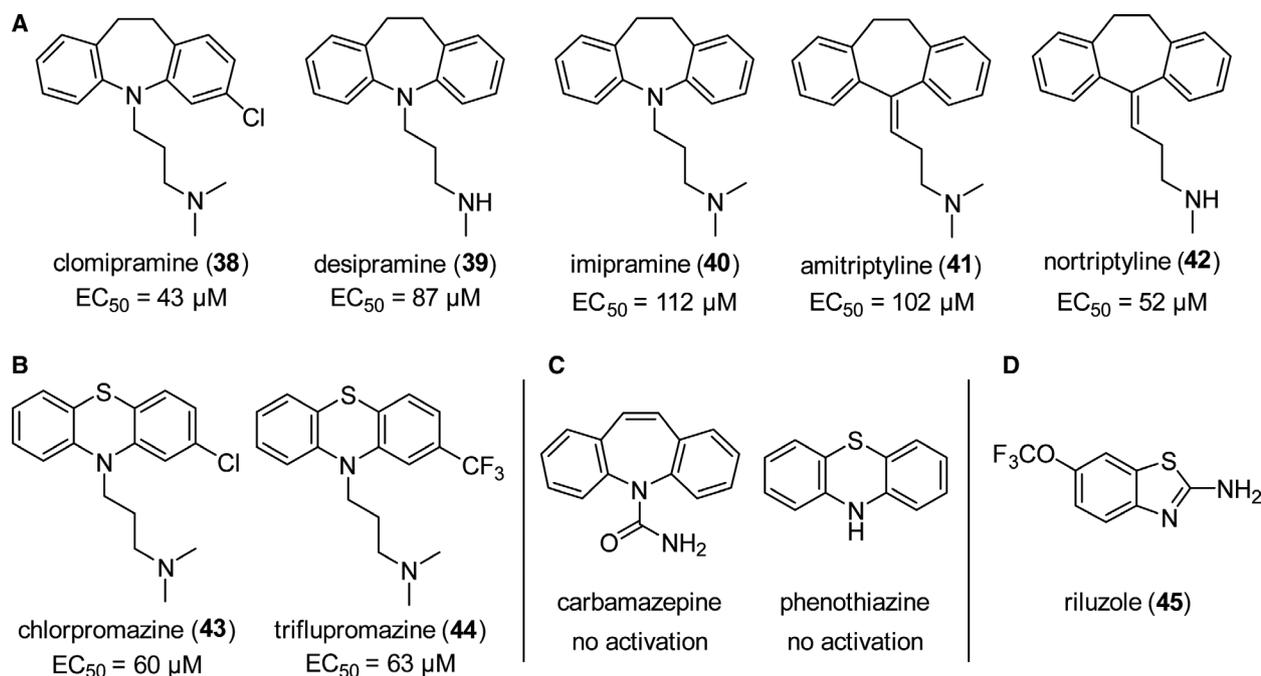


Fig. 2. Structures and EC₅₀ values of TACs (A), related phenothiazines (B), and riluzole (**45**, D) as TPC2 activators. EC₅₀ values were obtained from whole-cell recordings at −140 mV [14]. (C) Structure of inactive carbamazepine and phenothiazine.

conclusion, the TPC-A1-N (**1**) chemotype shows a very flat structure–activity relationship for TPC2.

Similarly, modified versions of TPC2-A1-P (**2**) showed no improvement of efficacy [15]. In a collection of 20 analogs, prepared by systematic variation of the substituents, every change in structure resulted in a decrease or total loss of function (Table 2). Analysis of structure–activity relationships revealed that the free carboxylic acid is essential for the activating effect, as the ester SGA-140 (**32**) is no longer active. Possibly, it might serve as a prodrug of TPC-A1-P in living systems, but this has not been investigated yet. Both the trifluoromethoxy and the bromine substituent at the phenyl ring are essential for activating TPC2, as

exemplified by the inactive methoxy (SGA-55, **31**) and des-bromo SGA-162 (**37**) analogs. Even moderate expansion of the size of the substituent at 2 position of the pyrrole (methyl in TPC2-A1-P (**2**) vs ethyl in SGA-152 (**34**); see also phenyl analog SGA-154 (**36**)) has the same effect. The only fairly tolerated structure modification was replacing the cyclohexylmethyl moiety in 1 position of the pyrrole ring by a benzyl residue (SGA-150, **29**), whereas linear or branched alkyl chains (SGA-149 (**33**), SGA-153 (**35**)) induced loss of activity.

There are only few reports in the literature about the biological activities of TPC2-A1-P-like compounds. TPC2-A1-P (**2**) itself is mentioned as a precursor in

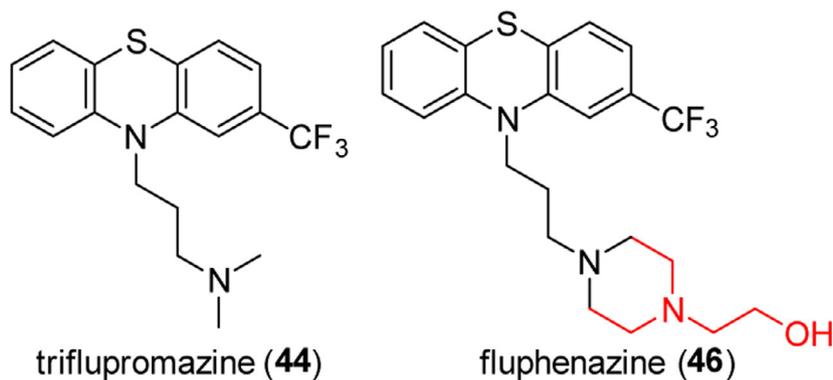


Fig. 3. Structures of the TPC2 activator triflupromazine (**44**) and the TPC2 inhibitor fluphenazine (**46**) [14,18]. Differences are marked in red.

the synthesis of cannabinoid-1 receptor (CB1R) inverse agonists, whereas the final active compounds contained a carboxamide group instead of the free carboxylic acid function [36]. Phenylpyrrolicarboxamides derived from SGA-50 (30) bind to 5-HT_{2A} and 5-HT_{2C} receptors and also the 5-HT transporter, and were thus evaluated as antidepressant compounds [37].

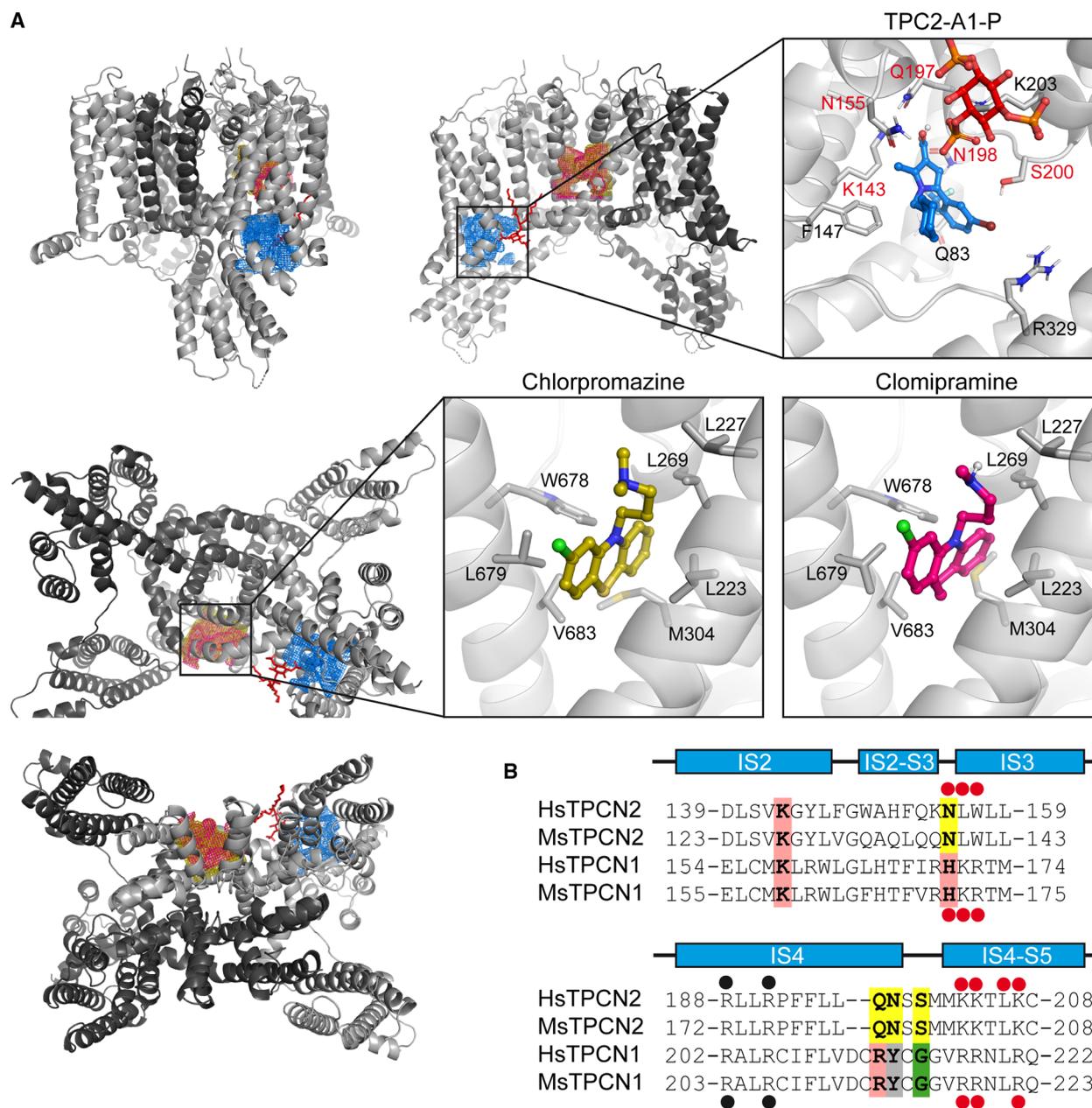
In summary, the two high-throughput screening hits TPC2-A1-N (1) and TPC2-A1-P (2) can be regarded as strong chemical tools with the need of fully analyzing their pharmacological properties.

Activators of TPC1 and TPC2—voltage-dependent gating

Zhang *et al.* [14] have likewise used a calcium imaging-based high-throughput screening approach to identify activators of TPCs. This was despite their claim in 2012 that TPCs were not calcium-permeable but rather sodium-permeable channels [6]. In contrast to the 80 000 compound-strong Roche Xplore libraries used by Gerndt *et al.*, Zhang *et al.* screened the Sigma LOPAC library which contains 1280 compounds. In this screening, 23 compounds induced calcium increases in TPC2-expressing cells but not in control cells expressing TRPML1 albeit in the plasma membrane. These hits from diverse chemical classes were further submitted to electrophysiological characterization using whole-cell recordings in TPC2^{LL/AA}-expressing HEK293 cells similar to those used by Gerndt *et al.* In this test, only five out of the 23 compounds showed significant currents, all of them belonging to the chemically closely related classes of dibenzazepine-type tricyclic antidepressants (TCAs) and

phenothiazine-based antidepressants. Subsequently, other TCAs were also tested. In summary, the TCAs clomipramine (38), desipramine (39), imipramine (40), amitriptyline (41), and nortriptyline (42) (named LyNa-VA1.1 to LyNa-VA1.5 by the authors), as well as the phenothiazines chlorpromazine (43) and triflupromazine (44) (named LyNa-VA2.1 and LyNa-VA2.2), were found to activate TPC2. The EC₅₀ values were between 43 and 112 μM and thus approximately two orders of magnitude less potent than TPC2-A1-N/P (Fig. 2A,B). None of the compounds were found to activate TRPML1 but TCA-induced currents exhibited strong inward rectification, which is characteristic of TRPML channels (TPCs normally show no rectification upon activation). In a separate screen, Zhang *et al.* identified another compound, riluzole (45, Fig. 2D) which also activates TPC2 but showing linear currents typical for TPC2 as reported before [6,7,10,29]. Currents elicited with TCAs were strongly voltage-dependent while riluzole (45) activation was voltage-independent, suggesting that the voltage dependence of TPC2 can be unmasked by extrinsic agonists rather than being a fixed intrinsic property of the channel. What the origin of the proposed agonist-mediated voltage dependence in the otherwise voltage-independent TPC2 is remains unclear. In contrast to Gerndt *et al.* [15] who identified two agonists (TPC2-A1-N (1) and TPC2-A1-P (2)) altering cation permeability in an agonist-dependent manner, the activators identified by Zhang *et al.* all showed similar cation selectivity, that is, low calcium, high sodium permeability. Likewise in contrast to Gerndt *et al.* who found that their compounds (1 and 2) only activate TPC2 but neither block nor activate TPC1, Zhang

Fig. 4. Modeling of TPC2 activators. The human TPC2 structure was recently resolved by cryo-EM in various states [44]. (A) Using the experimentally resolved apo-state hTPC2 structure (grey, accession 6nq1) [44], we docked TPC2-A1-P (2, blue), clomipramine (38, pink), and chlorpromazine (43, yellow) to the channel. PI(3,5)P₂ (red) was added to its cryo-EM-resolved site. Residues forming polar bonds with the ligand are highlighted in red letters. PyMOL v2.3.4 was used to assemble the structure. AutoDockTools (ADT) version 1.5.6 Sep_17_14 was utilized to prepare the protein and ligand. The channel pore was excluded from docking analyses by drawing two grid boxes, each demarcating one half of the protein, preserving peripheral pockets. AutoDock Vina 1.1.2 was used to carry out the docking simulation (exhaustiveness = 200). Binding sites were visualized in PyMOL v2.3.4. Following identification of agonist-binding sites, 'sticky' sites were excluded from further analysis: TPC2 agonist classes were previously discovered [15], showing TPC2 to be gated by distinct mechanisms (PI(3,5)P₂-like, NAADP-like gating). TCAs furthermore display another distinct, voltage-dependent gating mechanism. Since various modes of activation suggest distinct binding sites, sites of single-class binding were kept, and promiscuous binding sites excluded. Tricyclic antidepressants do not directly activate TPC2, rather rendering the channel voltage-gated. Subsequently, sites of individual agonist binding were removed, and binding sites where various activators bound maintained. Agonists were docked de novo within these binding pockets (30 × 30 × 30 Å search space), rendering residues within 6 Å of the docked agonist flexible. The following free energies were obtained by flexible docking (in kcal per mol): TPC2-A1-P (2, -7.4), clomipramine (38, -8.2), and chlorpromazine (43, -7.0). (B) Human and mouse TPCs were aligned using NCBI Protein BLAST to compare TPC2-A1-P-interacting residues. Residues found by docking to form polar bonds with TPC2-A1-P (bold) are fully conserved between human and mouse TPC2, but differ in TPC1. Red shade indicates positively charged residues, yellow polar residues, grey hydrophobic residues, and green glycine (no side chain). Dots indicate PI(3,5)P₂-interacting residues (red) and charge transfer center arginines (black), previously described for HsTPC2 (above, [44]) and MsTPC1 [45].



et al. found that some TCAs also activate TPC1 in a voltage-dependent manner, namely clomipramine (38) and desipramine (39), while the phenothiazine chlorpromazine (43) and riluzole (45) inhibit TPC1.

Unfortunately, the authors did not perform systematic structure variations of the hit compounds. Thus, within the seven identified structures it is barely possible to analyze structure–activity relationships. Nevertheless, the dibenzazepine carbamazepine and native phenothiazine (Fig. 2C) did not activate TPC2, highlighting the necessity of the aminoalkyl side chain at the central ring of the tricyclic core. At this stage,

more detailed structure–activity analyses would be desirable, as only slight changes in these structures are most likely to convert an activator into an inhibitor. The phenothiazines triflupromazine (44) and fluphenazine (46), recently published by Penny *et al.* [18], are a striking example for this phenomenon: Triflupromazine (44) activates TPC2, while fluphenazine (46) inhibits TPC2 currents evoked by PI(3,5)P₂ with an IC₅₀ of 8 μM (Fig. 3) [18].

Riluzole (45) blocks TTX-sensitive sodium channels, kainite receptors, and NMDA receptors [37–40]. At higher concentrations, it also strongly potentiates

postsynaptic GABA_A responses. Of interest, riluzole (**45**) has neuroprotective effects and it is currently approved for the treatment of amyotrophic lateral sclerosis (ALS) [41]. Riluzole (**45**) is the only drug to prolong survival for ALS, and it is associated with a 35% reduction in mortality [42]. TCAs were introduced into clinics in the 1950s and are used to treat, for example, depression, bipolar disorder, panic disorder, chronic pain, and insomnia. TCAs inhibit monoamine (serotonin, norepinephrine, dopamine) reuptake and block cholinergic, histaminic, and alpha-adrenergic transmission. Although TCAs have a wide range of unwanted effects, they served as first-line treatment for depression for 30 years, until the selective serotonin reuptake inhibitors (SSRI) were introduced. Of note, amitriptyline (**41**), imipramine (**40**), and clomipramine (**38**) are also potent CYP450 inhibitors, significantly inhibiting CYP450 2C19 and 1A2 [43].

Modeling of TPC activators

The recently published TPC2 agonists from both groups [14,15] were docked to the apo-state hTPC2 structure [44] to compare their binding sites (Fig. 4). We previously demonstrated that the TPC2^{K204A} mutation located within the PI(3,5)P₂ binding pocket blocks TPC2-A1-P (**2**) activity. Accordingly, our docking results show TPC2-A1-P (**2**) to dock in close proximity to experimentally resolved PI(3,5)P₂. Based on our docking results, K204 does not directly interact with TPC2-A1-P (**2**), but is likely still required to transduce TPC2-A1-P-evoked signals to the channel pore. The putative amino acids required for TPC2-A1-P binding are highlighted in Fig. 4. K143, N155, Q197, N198, and S200 highlighted in red appear to form hydrogen bonds with the docked agonist. In TPC1 (based on the alignment performed by She *et al.* [45]), these residues correspond to K143K (conserved), N155H, Q197V, N198D, and S200G. Thus, only one of the TPC2-A1-P-interacting amino acids is conserved in TPC1, while they are 100% conserved between human and mouse TPC2. This may potentially explain why TPC2-A1-P activates TPC2 but not TPC1. In contrast to TPC2-A1-P, TPC2-A1-N (**1**) did not dock within the PI(3,5)P₂ pocket. Supporting distinct binding sites, TPC2-A1-N (**1**) activity is unaffected by the TPC2^{K204A} mutation [15] and thus likely binds elsewhere on the channel. Similarly to TPC2-A1-N (**1**), riluzole (**45**) is also unaffected by the TPC2^{K204A} mutation [14]. Further investigations into the channel permeability evoked by riluzole (**45**) would be interesting, as its mechanism of action might mimic NAADP rather than PI(3,5)P₂. The voltage-dependent TPC2

activators clomipramine (**38**) and chlorpromazine (**43**) share one binding site, located between IS5, ISPH, and IIS6. This site is particularly interesting, as the homologous site forms the target of agonists activating the distantly related TRPML channels—ML-SA1 for TRPML3 [46] and ML2-SA1 for TRPML2 [47]. While several of these findings still require further validation, our analysis identifies numerous binding pockets on the TPC2 protein, and highlights the various means by which TPC2 can be activated, each with different outcomes.

Summary

Two independent groups identified small-molecule activators of TPCs by high-throughput screenings. Zhang *et al.* focused on drug repurposing and thereby identified tricyclic antidepressants (TCAs; Fig. 2A, **38**, **39**, **40**, **41**, **42**), phenothiazines (Fig. 2B, **43**, **44**), and the benzothiazole riluzole (Fig. 2D, **45**) that activate TPC2 with EC₅₀ values in the range of 43–112 μM. None of these compounds was found to activate TRPML1. Clomipramine (**38**) and desipramine (**39**) also activate TPC1 in a voltage-dependent manner, while chlorpromazine (**43**) inhibits TPC1 [14]. Hence, additional analysis of structure–activity relationships is needed, as slight changes in structures seemingly can reverse the activity on two-pore channel isoforms. Gerndt *et al.* performed a high-throughput screening using a library with 80 000 compounds and identified two TPC2 activators, TPC2-A1-N (**1**) and TPC2-A1-P (**2**). Both agonists did not activate or inhibit TPC1 or TRPML1, TRPML2, and TRPML3, which indicates a high selectivity for TPC2. Mimicking the physiological actions of NAADP and PI(3,5)P₂, respectively, TPC2-A1-N (**1**) rendered the channel more calcium-permeable, whereas TPC2-A1-P (**2**) increased sodium permeability. Numerous analogs of TPC2-A1-N (**1**) and TPC2-A1-P (**2**) were synthesized and tested yielding unusually flat and steep structure–activity relationships.

Comparing the results of both screenings, there is comprehensive knowledge on the pharmacological profiles (including undesired effects) of the repurposed TCA/phenothiazine-type TPC2 activators due to their long history in therapy, while the new activators identified by Gerndt *et al.* still need full pharmacokinetic and pharmacological characterization.

The two reviewed publications illustrate that researchers now have the opportunity to choose from an impressive and highly diverse collection of new lipophilic small-molecule activators for either TPC2 only or both TPC1 and TPC2 with the caveat that

some of the compounds also block TPC1. With cell-permeable small-molecule activators, an important milestone has been reached as physiology and pathophysiology of TPCs can now be studied in more detail. Most importantly, the novel tools allow studies in intact cells and they may also be applicable for in vivo studies and perhaps even for therapeutic purposes.

Acknowledgment

This work was supported, in part, by funding from the German Research Foundation, DFG (project number 239283807, SFB/TRR152 project P04 to C.G, DFG project GR-4315/4-1 to C.G., and DFG project BR-1034/7-1 to F.B.), and the Biotechnology and Biological Sciences Research Council, BBSRC (grants BB/N01524X/1 and BB/T015853/1 to S.P.).

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

CG designed the study and wrote the manuscript. SG and EK designed figures and tables, and wrote figure legends. SP and FB edited the manuscript. All of the authors discussed the results and commented on the manuscript.

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