Review

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Future Medicinal Chemistry

Store-operated CRAC channel inhibitors: opportunities and challenges

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Aberrant Ca^{2+} release-activated Ca^{2+} (CRAC) channel activity has been implicated in a number of human disorders, including immunodeficiency, autoimmunity, occlusive vascular diseases and cancer, thus placing CRAC channels among the important targets for the treatment of these disorders. We briefly summarize herein the molecular basis and activation mechanism of CRAC channel and focus on discussing several pharmacological inhibitors of CRAC channels with respect to their biological activity, mechanisms of action and selectivity over other types of Ca^{2+} channel in different types of cells.

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As a universal and remarkably versatile second messenger, cytoplasmic Ca^{2+} is important in mediating fundamental biological processes including gene expression, cell proliferation, differentiation and apoptosis [1,2]. There are two major sources contributing to the increase of cytoplasmic Ca^{2+} concentration, in other words, the release of stored Ca^{2+} within the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) and the influx of extracellular Ca^{2+} across the plasma membrane. Store-operated Ca^{2+} entry (SOCE) is a unique mechanism to generate cytoplasmic Ca^{2+} signals that combines these two processes, which is triggered by depletion of intracellular Ca^{2+} stores (mainly ER), and subsequently followed by Ca^{2+} influx across the plasma membrane by the opening of Ca^{2+} channels [3–5].

The prototypical store-operated Ca^{2+} channel is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel [6–9], which is widely distributed and involved in the regulation of a myriad of cellular activities in different cell types, including various subsets of T cells [10], B cells [11], mast cells [12], endothelial cells [13], platelets [14], vascular smooth muscle cells [15] and skeletal muscle cells [16]. It has an extremely low conductance (in the range of fS compared with pS of most Ca^{2+} channels) but is a highly Ca^{2+}-selective channel (P_{Ca}/P_{Na}: >1000) that opens in response to Ca^{2+} depletion in intracellular Ca^{2+} stores [17]. The opening of CRAC channel leads to the activation of diverse downstream signaling pathways that regulate cytokine production, gene expression, cell growth, proliferation, differentiation and even cell death.

In recent years, aberrant CRAC channel activity has been noted in several human diseases, including severe combined immunodeficiency (SCID) disorders [18], allergy [19], thrombosis [20], acute pancreatitis [21], inflammatory bowel disease [22] and cancer [23–25], which leads to an increasing interest in developing small molecule compounds that suppress aberrant CRAC channel function.

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Molecular basis & activation of CRAC channels

CRAC channel is composed of two key components, STIM (STIM1 and STIM2) [26,27] and ORAI (ORAI1, ORAI2 and ORAI3) [18,28–29], with the combination of STIM1/ORAI1 prevails in most cells and thus best characterized.

STIM1, originally identified as a tumor suppressor protein [30], is one of the two elementary components of SOCE, and functions as an ER Ca^{2+} sensor by detecting the fluctuation of Ca^{2+} concentration in the ER stores [26,27]. STIM1 is a single-pass transmembrane protein located in the ER membrane. The amino-terminal portion of STIM1 is located within the ER lumen [17], composed of an ER retention sequence, a canonical Ca^{2+}-binding EF-hand domain, a 'hidden' EF-hand domain [31] that does not bind Ca^{2+} and a sterile α-motif (SAM) domain that mediates STIM1 dimerization/oligomerization [32]. The cytosolic domain of STIM1 includes three putative coiled-coiled domains (CC1-3), the CRAC activation domain (SOAR/CAD) that is essential for the gating of ORAI1 [33,34], a serine/proline-rich domain, a TxIP motif associated with microtubule plus-end-tracking protein EB1, and a polybasic C-tail that facilitates efficient targeting of STIM1 toward the plasma membrane through physical association with phosphoinositides embedded in the inner leaflet of the plasma membrane [7,35]. As a homolog of STIM1, STIM2 has also been found to act as ER Ca^{2+} sensor but has a lower affinity to luminal Ca^{2+} and also associates with ORAI proteins to regulate Ca^{2+} influx [36]. However, its physiological functions are less well understood [37].

ORAI1 protein has been recognized as the ion pore-forming subunit of CRAC channels [7,29]. ORAI2 and ORAI3, human homologs of ORAI1, also form Ca^{2+}-

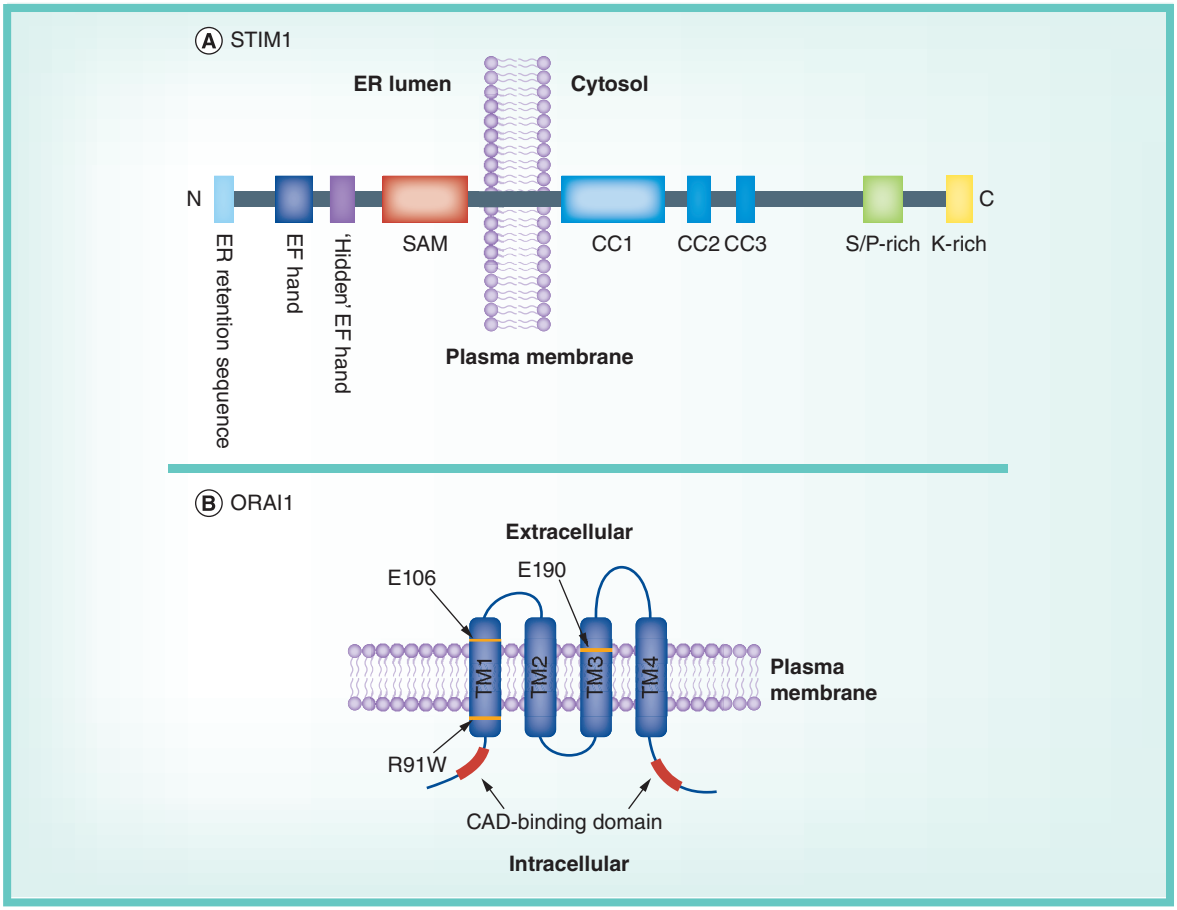

Figure 1. Domain architecture of STIM1 and ORAI1. (A) STIM1 is a single-pass transmembrane protein located in the ER membrane. The N terminus is located within the ER lumen and contains an ER-retention sequence, a canonical Ca^{2+}-binding EF-hand domain, a 'hidden' EF-hand domain and a sterile α-motif domain. The C terminus contains three putative coiled-coiled domains (CC1–3), a serine/proline-rich domain, and a polybasic C-tail. **(B)** ORAI1 bears four putative transmembrane-spanning domains (TM1-4), one intracellular and two extracellular loop regions. Residues at position E106 and E190 determine the channel selectivity and the dominant-negative mutant R91W has been found to be related to severe combined immunodeficiency.
ER: Endoplasmic reticulum; SAM: Sterile α-motif.

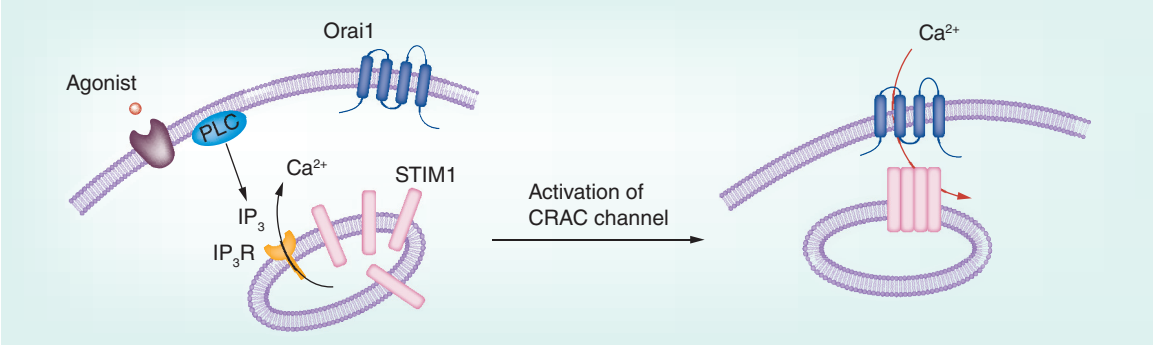

Figure 2. Activation of release-activated Ca^{2+} channel. The increase of IP_3 concentration induced by activation of PLC activates ER endoplasmic reticulum (ER)-resident IP_3 receptors (IP_3R) and causes the release of Ca^{2+} from ER, which leads to oligomerization and conformational switch of STIM1. The activated STIM1 oligomers then move toward the ER–plasma membrane junctions and trigger Ca^{2+} influx through direct interaction with an opening of ORAI1 Ca^{2+} channels in the plasma membrane.
CRAC: Release-activated Ca2+; PLC: Phospholipase C.

selective store-operated channels when co-expressed with STIM1 [38]. All three ORAI proteins have four putative transmembrane-spanning domains (TM1–4), one intracellular and two extracellular loop regions, and they are localized to the plasma membrane with their N- and C-termini facing the cytoplasm (Figure 1) [39].

Upon binding of their cognate ligands or antigens, the cell-surface receptors such as receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs) activate phospholipase C (PLC) to hydrolyze the membrane phospholipid phosphatidyl-4,5-bisphosphate to generate inositol-1,4,5-trisphosphate (IP_3) [40], followed by release of Ca^{2+} from the Ca^{2+} stores. So far, the IP_3-sensitive ER is the major store that is coupled to CRAC channel activation. The loss of Ca^{2+} from the ER results in Ca^{2+} dissociation from the luminal Ca^{2+}-binding EF hand of STIM1 [26,27], leading to the unfolding of EF-SAM domain, followed by STIM1 oligomerization through a mechanism that involves both the luminal and cytosolic domains [32,41–42], which is the essential step responsible for STIM1 conformational switch [43–45], subsequent accumulation at endoplasmic reticulum–plasma membrane (ER–PM) junctions and ultimate activation of ORAI channels [46]. The STIM1 oligomers then migrate from the bulk ER to specialized ER–PM junctions [47,48], during which STIM1 captures diffusing ORAI1 channels, and interaction between the amino and carboxyl termini of ORAI1 with SOAR/CAD on STIM1 leads to the opening of CRAC channel (Figure 2) [49–54].

Pharmacological inhibitors of CRAC channels

The identification of the molecular identities of CRAC channel kindled an intense interest in the search of small molecule modulators of CRAC channel. CRAC channel modulators may work by targeting either ORAI or STIM to regulate the overall level of CRAC channel activity. The compounds may either modulate channel activity by targeting STIM1 or acting directly at the pore of the ORAI channel by blocking the pore or interfering with the STIM–ORAI interaction.

Although both STIM1 and ORAI1 are widely expressed in a variety of tissues, the major clinical manifestations of patient with CRAC channelopathies are surprisingly limited to the immune system, skeletal muscle and ectodermally derived tissues [7], which agrees well with phenotypes observed in mice with targeted disruptions of the murine *Orai1, Stim1* and/or *Stim2* [55,56]. These findings indicated that therapies specifically targeting CRAC channels may serve as improved immunomodulators with high selectivity and low toxicity compared with currently US FDA approved immunosuppressive agents, such as cyclosporin A and FK506 that often cause undesired off-target toxicity in patients [57].

Although a number of agents that inhibit CRAC channels have been developed [58–61], most of them by far have not reached clinical trials, primarily owing to their poor selectivity and high toxicity. Nonetheless, a member of the CalciMedica series has reached Phase I clinical trials and it is highly anticipated to reach the milestone of FDA approval in drug development [62]. Apart from this, some CRAC modulators may provide promising lead structures for developing CRAC channel inhibitors with improved specificity and higher potency in the near future. Here we discuss a number of pharmacological agents that are most commonly used to inhibit CRAC channel activity, which are also helpful for understanding the physiological roles and dissecting the structure–function relation of the CRAC channel.

Lanthanides

Similar to other Ca^{2+} entry pathways, store-operated Ca^{2+} channels could also be inhibited by divalent and

trivalent cations. Particularly, CRAC channels show high sensitivity to complete blockade by the trivalent ion La^{3+} (lanthanum) and Gd^{3+} (gadolinium) at submicromolar concentration range [63]. This unique feature has been often used to distinguish CRAC channels from other types of less Ca^{2+} selective channels (e.g., TRP channels) [64–66]. The concentrations of Gd^{3+} used to effectively block the endogenous CRAC channel exert no significant inhibitory effect on TRP channels.

Mutation of several key acidic residues in the TM1–TM2 loop of ORAI1 (D110, D112 and D114) reduced the CRAC channel's selectivity for Ca^{2+} and decreased the inhibitory potency of the lanthanides, implying that the binding site of the trivalent ion La^{3+} and Gd^{3+} is located at or nearby that region of ORAI1 [67,68]. However, in the recent determined x-ray crystal structure of *Drosophila* Orai, Gd^{3+} situates at the same site (E106 in human ORAI1), rather than the acidic region in the first extracellular loop that is proposed to coordinate Ca^{2+} [69].

Lanthanides also showed inhibitory activity against other cationic ion channels, for example, voltage-gated calcium channels and TRP channels [70,71], which limited their potential use in developing CRAC channel inhibitors. Moreover, because the lanthanide salts of other multivalent anions and proteins are insoluble, their utility is also limited in many other applications.

Imidazole compounds

Imidazole antimycotic SKF-96365 (**1**) was one of the first identified CRAC channel inhibitors for experimental use [58,72], and the structurally related imidazole compounds econazole (**2**) and miconazole (**3**), which are primarily used as antimycotics [58], also suppress CRAC channel activity (Figure 3).

SKF-96365 inhibited thapsigargin-induced SOCE in Jurkat T cells with an IC_{50} value (measured by I_{CRAC}, the current generated by the opening of CRAC channels) of 12 μM in a dose-dependent manner (different groups reported varying IC_{50} values, which might be mainly due to the methods used to measure CRAC channel activity and the potencies are also cell-type dependent) [72]. Although this compound inhibits agonist-mediated Ca^{2+} influx in many cell types, it also suppresses the activity of other ion channels, such as voltage-gated calcium channels, nonselective cation channels and cyclic AMP-gated Cl^- channels with comparable potencies [72,73]. Econazole and miconazole also exhibit a lack of specificity to CRAC channel, thereby limiting its further clinical use as specific CRAC channel modulators.

Diphenylboronate compounds

2-Aminoethyldiphenyl borate (2-APB, **4**) has been widely used to characterize the activity of CRAC channel [74]. Its pharmacology is complex with an intriguing biphasic effect on CRAC channel activation. At low concentrations (1–10 μM), 2-APB potentiates CRAC channel activity; while at higher concentrations (20–100 μM), it often causes a transient activation of CRAC channel followed by strong inhibition [75,76]. In recent years, two 2-APB analogs DPB162-AE (**5**) and DPB163-AE (**6**), which were identified by Mikoshiba's group, have drawn attention for their higher potencies and greater specificity than 2-APB in terms of suppressing SOCE (Figure 4) [77].

2-APB was initially speculated to inhibit SOCE through its inhibitory effect for IP_3 receptors [78–81]. However, it has been clarified that the inhibition of SOCs has no direct relation with IP_3 [75,82–83].

In recent years, it has been found that formation of STIM1 puncta (clusters of STIM1 at ER–PM regions just below the PM) could be prevented by high concentrations (50 μM) of 2-APB, suggesting that 2-APB

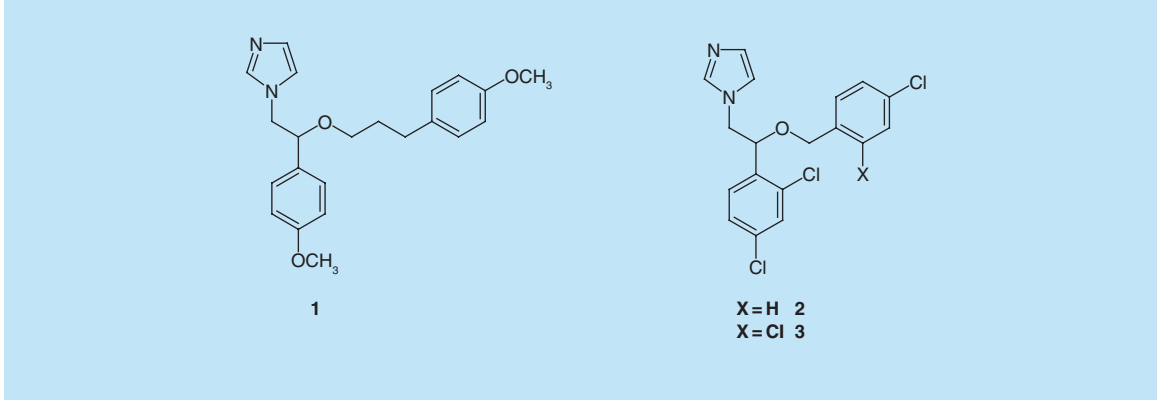

Figure 3. Chemical structures of typical imidazole release-activated Ca^{2+} channel inhibitors. SKF-96365 (**1**); econazole (**2**); miconazole (**3**).

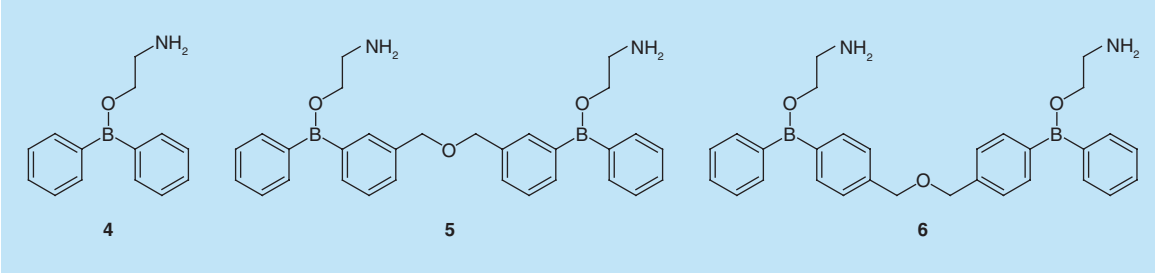

Figure 4. Chemical structures of 2-APB and its analogs. 2-APB (**4**); DPB162-AE (**5**); DPB163-AE (**6**).

could inhibit SOCE through affecting movement of STIM1 [84,85]. However, the inhibition of STIM1-puncta formation by 2-APB could be overcome by coexpression with ORAI1, even though the inhibition of SOCE is still effective [85,86]. These findings imply that the inhibitory effect of 2-APB on SOCE might be due to its effects on one or more steps in the following molecular events: STIM1 multimerization, STIM1 conformational switch, STIM1–ORAI1 interaction or the ORAI channel itself.

Interestingly, the effects of 2-APB on ORAI vary between different ORAI isoforms [85,87]. For example, at high concentrations (50 μM) of 2-APB, ORAI1-mediated Ca^{2+} entry is initially activated, which is quickly followed by a complete inhibition. 2-APB only partially inhibits ORAI2-mediated SOCE. Surprisingly, 2-APB at high concentrations activate, rather than inhibit, ORAI3 channel [88,89]. The different properties of these three ORAI proteins give hope to develop small molecules which could selectively target one specific ORAI protein.

Although 2-APB is widely used to modulate CRAC channel activity, it could also affect the activities of potassium channels [90], SERCA pumps [91] and mitochondrial Ca^{2+} efflux [75]. Notably, 2-APB has been reported to activate the heat-gated recombinant TRPV1, TRPV2 and TRPV3 channels [92].

2-APB derivatives DPB162-AE and DPB163-AE are isomers in chemical structure, which only differ in the linker chain between their two diphenyl groups. In STIM1–ORAI1 overexpressing cells, DPB163-AE had a biphasic effect on SOCE, which is similar to 2-APB but exhibits a higher potency with an IC_{50} of about 600 nM. DPB162-AE, on the other hand, solely inhibited SCOE with an IC_{50} of approximately 200 nM, which is two orders of magnitude more potent than 2-APB [76,93]. Similar to 2-APB, both DPB162-AE and DPB163-AE suppressed ORAI1 currents and partially inhibited ORAI2 currents. However, unlike 2-APB, they failed to activate ORAI3 channels at higher concentrations in the absence of STIM1, an observation that could be attributed to their larger size, relative to 2-APB, that likely prohibits access to the ORAI3 pore [77].

The interaction between the STIM1–ORAI1 activating region (SOAR) of STIM1 and a combination of the C- and N-termini of ORAI1 leads to the coupling of STIM1 and ORAI1. The single binding pocket formed by C- and N-termini of ORAI1 plays an important role in both SOAR-binding and gating of the channel [94]. Recent researches have shown that DPB162-AE, not acting as an ORAI1 channel pore blocker, does not prevent the STIM1–ORAI1 interaction but potently inhibits the activation of STIM1-mediated ORAI1 channel [93,94]. Using a unique point mutation in the SOAR of STIM1 (F394H), which prevents both physical binding between SOAR and ORAI1 as well as functional coupling to activate the ORAI1 channel [94], DPB162-AE was found to restore SOAR–ORAI1 binding rapidly but restore ORAI1-mediated Ca^{2+} entry slowly. These findings reveal that DPB162-AE seems to be a potent and relatively specific STIM1–ORAI1 functional uncoupler, and probably acts directly on the coupling interface between SOAR and ORAI1.

In contrast to 2-APB, DPB162-AE has little effect on TRPC channels, L-type Ca^{2+} channels or Ca^{2+} pumps at 2 μM, the maximal CRAC channel-mediated SOCE inhibitory level of DPB162-AE [93,95–97].

Pyrazole compounds: the BTPs

A series of bis(trifluoromethyl)pyrazoles compounds, known as BTP1 (**7**), BTP2 (**8**) and BTP3 (**9**), were initially identified as inhibitors of NFAT activation and T-cell cytokine production by Abbott Laboratories [98–100]. Interestingly, unlike other well-known NFAT inhibitors such as FK506 and cyclosporin A, the BTPs inhibited NFAT nuclear translocation without direct effect on the phosphatase activity of calcineurin, implying a probable effect on the upstream Ca^{2+} signal [98,99]. Indeed, the BTPs were later found to be capable of inhibiting SOCE in many cells at low micromolar to nanomolar concentrations with considerable selectivity over voltage-gated Ca^{2+} entry [61]. In particular, BTP2 (also known as YM-58483) inhibited thapsigargin-induced Ca^{2+} influx in Jurkat T cells with an IC_{50} value of 100 nM and did not inhibit phosphorylation of PLCγ1 and TCR-mediated Ca^{2+} release from the stores (Figure 5) [101].

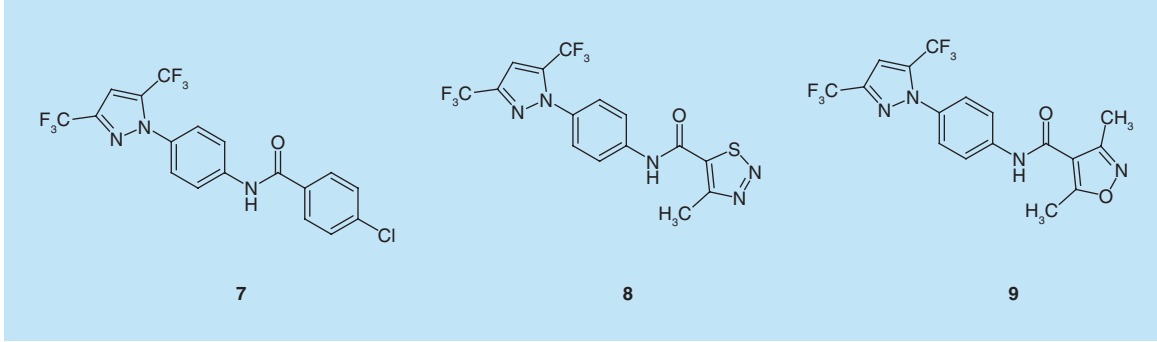

Figure 5. Chemical structures of the BTPs. BTP1 (**7**); BTP2 (**8**); BTP3 (**9**).

BTP2 has been reported to inhibit CRAC channels in human T cells with an IC_{50} of about 10 nM [102], which is one order of magnitude higher than the IC_{50} value described above. The most probable reason for the discrepancy in IC_{50} values is that the former experiment was performed via preincubation of cells with BTP2 for 18–24 h to reach full inhibition [102], while cytoplasmic Ca^{2+} concentrations in the later study were measured shortly (only a few minutes) after BTP2 treatment [101]. Moreover, it was also found that CRAC channel inhibition mediated by BTP2 was affected by the external Ca^{2+} concentration: higher external Ca^{2+} concentrations are correlated with reduced inhibitory effect on the CRAC channel [102].

A recent study has shown that drebrin, an actin reorganizing protein, is identified as a potential binding site for BTP2 [103]. Knockdown of drebrin by siRNA inhibited SOCE to the same extent as inhibition by BTP2. Thus, the authors of the report concluded that drebrin may play a role in regulating SOCs by affecting the actin cytoskeleton, and that BTP2 may act by inhibiting drebrin. However, previous studies have shown that the actin cytoskeleton is not considered to play a major role in SOCE [104].

Although BTP2 does not inhibit voltage-gated Ca^{2+} channels or K^+ channels [101,102], it activates TRPM4 channels and inhibits the activities of TRPC3 and TRPC5 channels [105,106].

Pyrazole compounds: the Pyrs

As discussed above, BTP2 (also known as Pyr2, **8**) could also inhibit TRPC3 channel activity while it acts on CRAC channels. Recently, the ability of three pyrazole derivative compounds Pyr3 (**10**), Pyr6 (**11**) and Pyr10 (**12**) in inhibiting Ca^{2+} entry have been examined in terms of TRPC/CRAC selectivity in HEK293 cells overexpressing TRPC3 channels and RBL-2H3 cells expressing CRAC channels (Figure 6) [107].

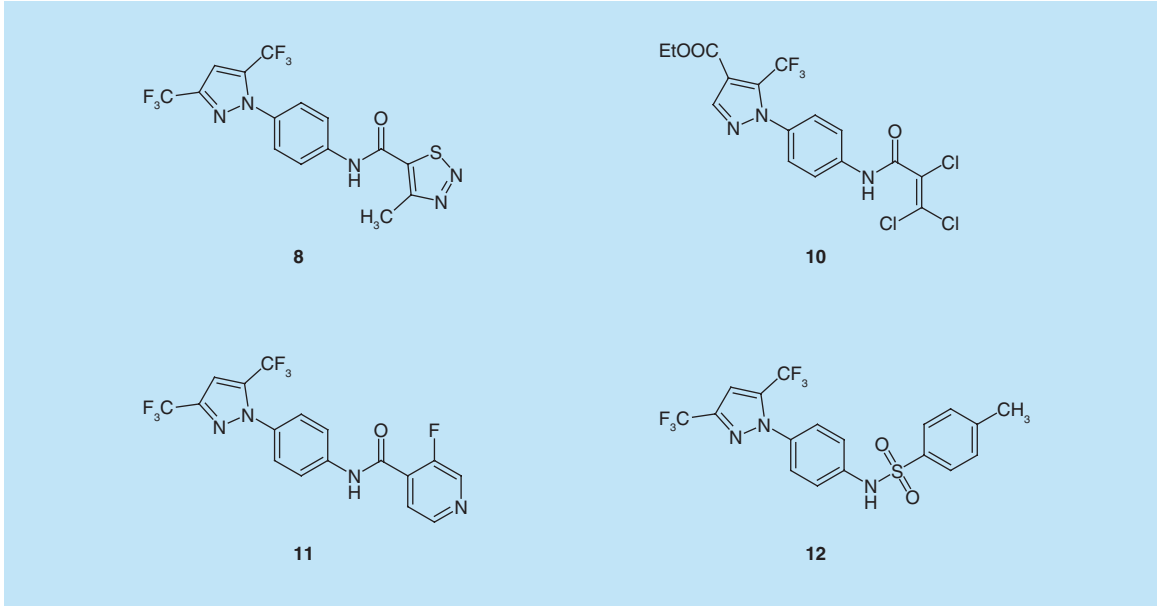

Figure 6. Chemical structures of the Pyrs. Pyr2 (**8**); Pyr3 (**10**); Pyr6 (**11**); Pyr10 (**12**).

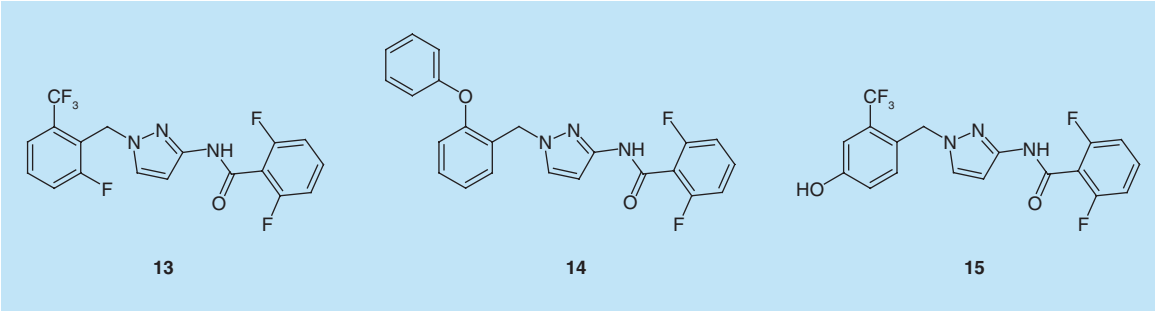

Figure 7. Chemical structures of the GSKs. GSK-5498A (**13**); GSK-5503A (**14**); GSK-7975A (**15**).

Structurally, Pyr6 and Pyr10 have similar structures to the BTPs bearing two trifluoromethyl groups in the C3 and C5 position of the pyrazole ring, which are important in CRAC channel inhibitory activity of BTP2 [108]. Interestingly, Pyr3 shares a carboxylate group in the C4 position of the pyrazole ring instead of the trifluoromethyl group in the C3 position, which appears to contribute to maintaining its inhibitory activity, and the trichloroacryl group of the side chain also seems to be necessary for its inhibitory activity toward both CRAC and TRPC3 channel [107].

Interestingly, Pyr6 displays higher potency to inhibit Ca^{2+} entry mediated by CRAC channel than TRPC3, while Pyr10 exhibits significant selectivity for TRPC3-mediated Ca^{2+} entry. By comparison, Pyr2 and Pyr3 do not show any appreciable selectivity for them [107].

Pyrazole compounds: the GSKs

Recently, several novel pyrazole compounds, GSK-5498A (**13**), GSK-5503A (**14**) and GSK-7975A (**15**), which were developed by GlaxoSmithKline, have been identified as selective CRAC channel inhibitors (Figure 7) [109–111].

Electrophysiological experiment showed that GSK-5498A inhibits I_{CRAC} with an IC_{50} value of about 1 μM in human embryonic kidney cells stably expressing STIM1 and ORAI1 [110]. GSK-5503A and GSK-7975A inhibited STIM1 mediated ORAI1 and ORAI3 currents with an IC_{50} value of about 4 μM in HEK293 cells [111].

FRET experiments implied that the GSK compounds did not affect STIM1 oligomerization or STIM1–ORAI1 interaction. Compared with wild-type ORAI1, the less Ca^{2+}-selective mutant E106D ORAI1 pore requires at least tenfold higher concentrations of GSKs for inhibition, thus pointing to the possibility that these compounds may act by altering the ORAI pore geometry [111].

A recent study has found that blockade of CRAC channels by GSK-7975A effectively precludes palmitoleic acid ethyl ester (POAEE), an important mediator of alcohol-related pancreatitis, from evoking sustained elevation of the Ca^{2+} concentration in the pancreatic acinar cells, activation of protease and necrosis of pancreatic acinar cell [21]. Given these findings, the authors indicated that pharmacological CRAC channel blockade could be applied as a potentially rational therapy against severe acute pancreatitis, which is life-threatening but lacks effective treatment thus far [21,112].

Interestingly, although these GSK compounds were found to have little or no effect on many other ion channels, they potently block TRPV6 channels [111,113].

Synta 66

Synta 66 (**16**), a selective CRAC channel inhibitor developed by Synta pharmaceuticals, has drawn extensive attentions in recent years [114]. The structure of Synta 66 is similar to Pyr6 (**11**), whose 3,5-bistrifluoromethyl pyrazole ring is replaced with 2,5-dimethoxy benzene ring (Figure 8).

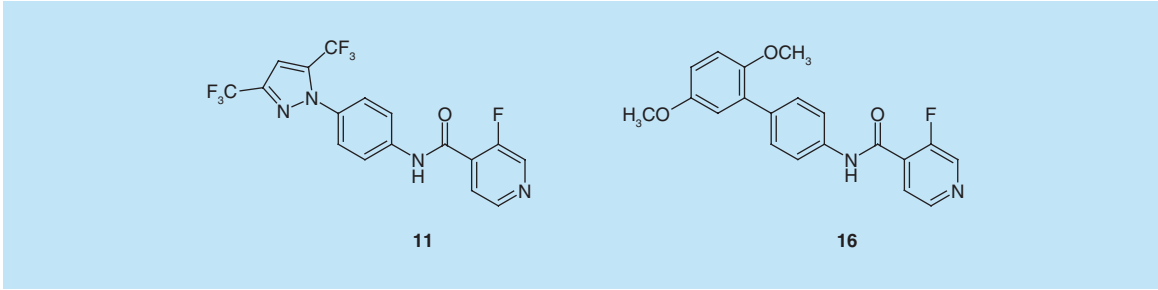

Figure 8. Comparison of the chemical structures of Synta 66 and Pyr6. Pyr6 (**11**); Synta 66 (**16**).

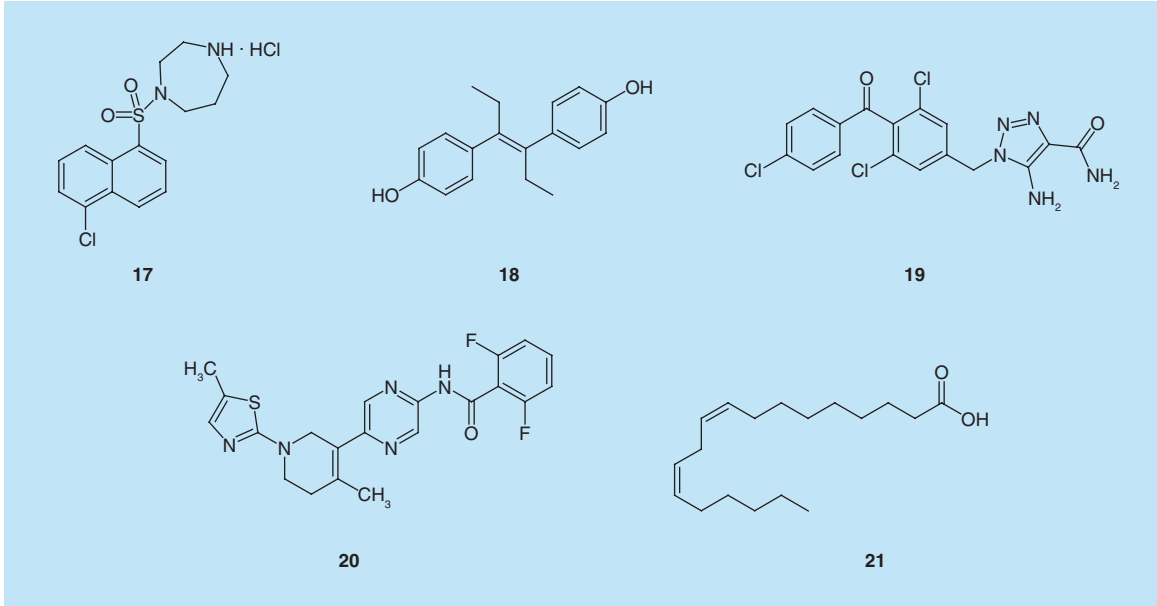

Figure 9. Chemical structures of several pharmacological inhibitors of release-activated Ca^{2+} channels. ML-9 (**17**); Diethylstilbestrol (**18**); Carboxyamidotriazole (**19**); RO2959 (**20**); linoleic acid (**21**).

This compound inhibits I_{CRAC} with an IC_{50} value of 1.4 μM in RBL cells and has no effect on plasma membrane Ca^{2+} ATPase pump and inwardly rectifying K^+ channels [114,115]. It has also been found that the compound inhibits expression of T-bet and production of IL-2, IL-17 and IFN-γ in biopsy specimens isolated from inflamed areas of IBD patients [115]. The results suggested that Synta 66 could be applied to further investigation of the CRAC channels functions in T-cell signaling and IBD.

A scrutiny of the selectivity of Synta 66 assessed by a panel of 50 specific radioligand-binding assays suggests that, at a concentration of 10 μM, it exerts no significant effect on a series of receptors, enzymes and ion channel targets [115]. Although there are more and more studies employing Synta 66 for probing the physiological role of CRAC channels, the mechanism of action for this compound has not yet been fully clarified.

ML-9

ML-9 (**17**), an inhibitor of myosin light chain kinase (MLCK), has been found to reversibly inhibit SOCE with an IC_{50} of approximately 10 μM [116,117]. In HEK293 cells, ML-9 was found to similarly inhibit SOCE and I_{CRAC} [117].

ML-9 inhibits SOCE at least partially by reversing the formation of STIM1 puncta and blocking its movement to ER–PM junctions. Interestingly, the inhibitory effect of ML-9 does not seem to be related to its well-known inhibition of MLCK [117]. Although STIM1 appears to be the molecular target of ML-9-mediated inhibition on SOCE, it is still unclear on the specific site in STIM1 that ML-9 may act on.

Diethylstilbestrol

Diethylstilbestrol (DES; **18**), a synthetic estrogen agonist, inhibits SOCE in a range of cell types including mast cells, vascular smooth muscle cells and rat microglia [118,119]. DES inhibits I_{CRAC} in RBL cells with an IC_{50} of approximately 0.6 μM and does not affect TRPM7 channels at a similar concentration range. Interestingly, if it is applied intracellularly, its inhibitory effect on I_{CRAC} disappears, thus raising the speculation that it might act on the extracellular regions on CRAC channel. Although it might be applied to investigate the function of CRAC channels *in vitro*, it could not be used in clinical setting due to its activation on estrogen receptors.

Carboxyamidotriazole

Carboxyamidotriazole (CAI; **19**) is a potential anticancer drug which has been tested in Phase I and Phase II clinical trials for its activity of inhibiting angiogenesis, tumor growth, invasion and metastasis [120,121]. CAI was initially identified as an inhibitor of SOCE in nonexcitable cells [122]. CAI inhibits I_{CRAC} with an IC_{50} value of approximately 0.5 μM in HEK293 cells [120,121]. Carboxyamidotriazole suppresses I_{CRAC} by reducing the production of IP_3 and depolarizing mitochondria, which induces Ca^{2+}-dependent inactivation of the CRAC channels [123–125]. Therefore, although CAI does inhibit I_{CRAC}, it seems to act in an indirect manner.

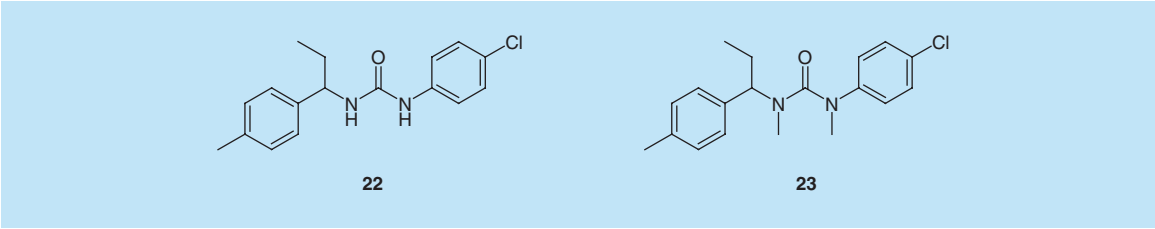

Figure 10. Chemical structures of two 1-phenyl-3-(1-phenylethyl)urea derivatives. Compound **22** and compound **23**.

RO2959

RO2959 (**20**), synthesized by Synta Pharmaceutical Corp., has been identified as a novel, potent and selective I_{CRAC} inhibitor by Roche [126]. RO2959 inhibits I_{CRAC} with an IC_{50} value of about 400 nM in RBL-2H3 cells, which has been preincubated with RO2959 [126]. Moreover, due to its ability of inhibiting I_{CRAC}, RO2959 could potently inhibit human TCR-mediated SOCE, T-cell proliferation, cytokine production and gene expression [126]. In T-REx-CHO cells which could stably express STIM1/ORAI1, STIM1/ORAI2 or STIM1/ORAI3, RO2959 inhibits ORAI1 to a greater extent than ORAI2 and ORAI3 [126], which implies that the compound could be applied as a selective ORAI1 inhibitor. RO2959 had no significant inhibitory effect on a variety of cellular receptors, transporters and ion channels, such as GABA receptors, dopamine transporter, 5-HT transporter, K_v channels, Cl^- channels, TRPC1, TRPM2, TRPM4 and Cav1.2 channels, which showed the high action selectivity of the compound [126]. Notably, TRPC1 channel has some similarities with CRAC channel, a number of drug candidates developed as CRAC channel inhibitors also act on TRPC1 channel. Although TRPC1 can contribute to SOCE along with ORAI1 and STIM1, it also participates in other signaling events that are independent on store depletion. If the CRAC channel inhibitors could potently act on TRPC1, one would therefore expect undesired off-target side effects. Thus, for CRAC channel inhibitors, it is necessary to examine the effect on TRPC1 channels.

Although RO2959 has been shown to be a potent and selective CRAC channel inhibitor, the *in vivo* efficacy and the exact mechanism of action warrants further investigation.

Linoleic acid

More recently, linoleic acid (**21**), an 18-C polyunsaturated fatty acid (PUFA), has been reported to effectively inhibit antigen- or thapsigargin-mediated SOCE in mast cells by acute addition at micromolar concentrations [127]. Interestingly, stearic acid, the 18-C saturated fatty acid, does not inhibit SOCE. The authors found that linoleic acid inhibited SOCE by affecting STIM1 oligomerization and subsequent STIM1/ORAI1 coupling. The authors further argue that linoleic acid inhibited STIM1/ORAI1 coupling by disrupting potential electrostatic interactions between STIM1 and ORAI1 [127]. Further studies are needed to delineate its mechanism of action and examine its selectivity over other types of ion channels (Figure 9).

1-Phenyl-3-(1-phenylethyl)urea derivatives

A series of 1-phenyl-3-(1-phenylethyl)urea derivatives has been recently identified as CRAC channel inhibitors. As the lead compound, compound **22** could inhibit Ca^{2+} influx with IC_{50} of 3.25 ± 0.17 μM in HEK293 cells stably co-expressing ORAI1 and STIM1 [128]. The Ca^{2+} influx assay and electrophysiological experiments showed that compound **22** could partially inhibit Ca^{2+} entry in constitutively opened CRAC channels which were formed by ORAI1-SS (monomer ORAI1 covalently linked with two S336–485 domains) and completely inhibit the Ca^{2+} entry and the current mediated by the opened STIM1-free V102A channel (a mutant of ORAI1), which is a constitutively opened CRAC channel, even in the absence of STIM1. Furthermore, this compound could specifically reduce ORAI1/STIM1-mediated Ca^{2+} entry, while exhibited no inhibitory effect on other ORAI channels. These results indicated that

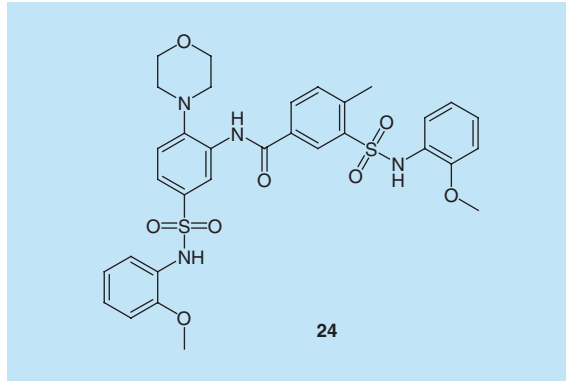

Figure 11. An example of release-activated Ca^{2+} channel modulators developed by CalciMedica. Compound **24**.

compound **22** inhibits CRAC channel by specifically targeting ORAI1 [128].

A total of 40 derivatives have been synthesized, and their primary structure–activity relationships (SARs) study showed that the alkyl substituent on the α-position of the N-phenylethyl group is vital for their inhibition on Ca^{2+} influx [128]. Notably, among these derivatives, compound **23** exhibited low cytotoxicity and relatively improved inhibition of IL-2 production in the Jurkat cell line [128].

It is encouraging that compound **22** could inhibit CRAC channel by specifically targeting ORAI1, however, its mechanism of action and selectivity over other types of ion channels still warrants further studies (Figure 10).

The CalciMedica series

CalciMedica has been actively developing novel, potent and specific CRAC channel inhibitors in the past decade. It is worth noting that CM2489, developed by this biotech company, is the only CRAC channel inhibitor tested in human and has completed Phase I clinical trials for treating moderate-to-severe plaque psoriasis [129]. Although there is no chemical structure information disclosed for this compound, through the patent published by CalciMedica, we

Table 1. The potencies of leads from each series and their proposed mechanism of action.

Species	CRAC channel inhibitory activity	Selectivity	Proposed mechanism of action
Lanthanides	Complete blockade at submicromolar concentration range [63]	Block other cationic ion channels, such as voltage-gated calcium channels and TRP channels [70,71]	Directly block ORAI1 [69]
SKF-96365	IC_{50}: 12 μM (I_{CRAC}) [72]	Suppresses voltage-gated calcium channels, nonselective cation channels and cyclic AMP-gated Cl^- channels [72,73]	Has not yet been fully clarified
2-APB	Activates at low micromolar and inhibits at high micromolar [75,76]	Affect the activities of potassium channels, SERCA pumps, heat-gated recombinant TRPV1, TRPV2 and TRPV3 channels [90–92]	Might act on STIM1 multimerization, STIM1–ORAI1 interaction or the ORAI channel itself [85,86]
DPB162-AE	IC_{50}: 200 nM [93]	Relatively selective [93,95–97]	Probably acts directly on the coupling interface between SOAR and ORAI1 [93,94]
BTP2	Inhibited thapsigargin-induced Ca^{2+} influx in Jurkat T cells with an IC_{50} of 100 nM [101]	Activates TRPM4 channels and inhibits the activities of TRPC3 and TRPC5 channels [105,106]	Has not yet been fully clarified
GSK-7975A	IC_{50}: 4 μM (I_{CRAC} in HEK293 cells) [111]	Potently blocks TRPV6 channels [111,113]	May act by altering the ORAI pore geometry [111]
Synta 66	IC_{50}: 1.4 μM (I_{CRAC} in RBL cells) [114,115]	Relatively selective [115]	Has not yet been fully clarified
ML-9	Reversibly inhibit SOCE with an IC_{50} of approximately 10 μM [116,117]	Inhibits MLCK [116]	Might target STIM1 [117]
DES	IC_{50}: 0.6 μM (I_{CRAC} in RBL cells) [118,119]	Activates estrogen receptors [118]	Might act on the extracellular regions on CRAC channel [118,119]
CAI	IC_{50}: ~0.5 μM (I_{CRAC} in HEK293 cells) [120,121]	Not very selective	Reduces the production of IP3 and depolarizes mitochondria [123–125]
RO2959	IC_{50}: 400 nM (I_{CRAC} in RBL-2H3 cells) [126]	Relatively selective [126]	Has not yet been fully clarified
Linoleic acid	Inhibit antigen- or thapsigargin-mediated SOCE in mast cells by acute addition at micromolar concentrations [127]	Has not yet been examined	Inhibits SOCE by affecting STIM1 oligomerization and subsequent STIM1/ORAI1 coupling [127]
1-phenyl-3-(1-phenylethyl) urea	Inhibits Ca^{2+} influx with IC_{50} of ~3 μM in HEK293 cells [128]	Has not yet been examined	Targets ORAI1 [128]

could find that most compounds bear phenyl or heterocyclic groups linked by carboxyamide, sulfoxamide or alkyl chain (take compound **24** for example) [62], which may help us to estimate the skeleton structure of CM2489 for designing novel CRAC channel inhibitors (Figure 11).

Conclusion

CRAC channels, fundamental to human immune cell function, have shown the physiological importance in many cell types. With the discoveries of STIM1 and ORAI1 proteins, the molecular components of CRAC channel have been identified, which facilitates the functional studies of CRAC channels in a wide range of cellular systems. In the last three decades, several classes of CRAC channel inhibitors have been developed. Although most of them have not reached clinical trials due to their poor selectivity and high toxicity, there are some selective CRAC channel inhibitors that might hold promise for further drug development. Most notably, CM2489 [129], developed by CalciMedica, has reached clinical trials, which is the first CRAC channel inhibitor that has completed the Phase I clinical trials. Unfortunately, there is no sufficient public information available for us to have a thorough study of this compound.

In addition to CM2489, RO2959, recently developed by Synta, has also been shown to act as a selective CRAC channel inhibitor by targeting ORAI1. Thus, developing drugs that target particular component(s) of CRAC channel might be the most efficient and effective way to improve the selectivity and specificity.

Future perspective

Given that CRAC channels have emerged as an attractive target for developing new therapies for autoimmune disorders, allergy, thrombosis and cancer, more and more pharmaceutical companies including Hoffmann-La Roche and GSK are joining the efforts to develop CRAC channel inhibitors. As summarized by Pevarello and colleagues [62], the publication of related patents has been kept growing. Although no CRAC channel inhibitors have reached the milestone of FDA approval and clinical use, the increasing attention paid by pharmaceutical companies, together with our deeper understanding of the activation and regulatory mechanisms of CRAC channel and the advent of novel optogenetic tools to manipulate CRAC channel activity [130–132], would certainly expedite the quest for new drugs that specifically target CRAC channels to treat human disorders associated with dysregulated Ca^{2+} influx (Table 1).

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Executive summary

- Store-operated Ca^{2+} entry (SOCE) constitutes one of the major Ca^{2+} entry routes in nonexcitable cells and is implicated in a variety of fundamental biological processes.
- Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which is widely distributed and involved in the regulation of many cellular functions in different cell types, is one of the most well-studied prototypical form of store-operated Ca^{2+} channels.
- Aberrant CRAC channel activity is associated with human disorders involving the immune system, as well as tumor growth and cancer metastasis.
- CRAC channel is composed of stromal interaction molecule (STIM) and ORAI, with the combination of STIM1/ORAI1 most well characterized.
- STIM1 functions as an ER Ca^{2+} sensor and ORAI1 is the ion pore-forming subunit of CRAC channel. The dynamic coupling between STIM1 and ORAI1 is the key step for the opening of CRAC channel.
- Major clinical manifestations of CRAC channelopathies in human are limited to the immune system, skeletal muscle and ectodermally derived tissues. CRAC channels can thus serve as an ideal target for developing novel immunomodulators with improved biosafety profiles.
- Although most agents developed as CRAC channel inhibitors have not reached clinical trials owing to their poor selectivity and high toxicity, the clarification of molecular basis of CRAC channel is anticipated to expedite the development of drug candidates that specially target STIM and/or ORAI. Such compounds might hold great promise to serve as selective CRAC channel inhibitors to treat human disorders arising from imbalanced Ca^{2+} homeostasis.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Clapham DE. Calcium signaling. *Cell* 131(6), 1047–1058 (2007).

2 Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 4(7), 517–529 (2003).

3 Putney JW. A model for receptor-regulated calcium entry. *Cell Calcium* 7(1), 1–12 (1986).

4 Putney JW. Capacitative calcium entry: from concept to molecules. *Immunol. Rev.* 231(1), 10–22 (2009).

5 Prakriya M, Lewis RS. Store-operated calcium channels. *Physiol. Rev.* 95(4), 1383–1436 (2015).

6 Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol. Rev.* 231(1), 59–87 (2009).

7 Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu. Rev. Immunol.* 28, 491–533 (2010).

8 Lewis RS. Store-operated calcium channels: new perspectives on mechanism and function. *Cold Spring Harb. Perspect. Biol.* 3(12), a003970 (2011).

9 Parekh AB. Store-operated CRAC channels: function in health and disease. *Nat. Rev. Drug Discov.* 9(5), 399–410 (2010).

•• **Summarized the progress in the study of the gating and function of Ca^{2+} release-activated Ca^{2+} (CRAC) channels with their relation with human disease prior to 2010.**

10 Shaw PJ, Feske S. Regulation of lymphocyte function by ORAI and STIM proteins in infection and autoimmunity. *J. Physiol.* 590(Pt 17), 4157–4167 (2012).

11 Maus M, Medgyesi D, Kiss E *et al.* B cell receptor-induced Ca^{2+} mobilization mediates F-actin rearrangements and is indispensable for adhesion and spreading of B lymphocytes. *J. Leukoc. Biol.* 93(4), 537–547 (2013).

12 Vig M, Dehaven WI, Bird GS *et al.* Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat. Immunol.* 9(1), 89–96 (2008).

13 Trebak M. STIM1/Orai1, I(CRAC) and endothelial SOC. *Circ. Res.* 104(9), e56–e57 (2009).

14 Tolhurst G, Carter RN, Amisten S, Holdich JP, Erlinge D, Mahaut-Smith MP. Expression profiling and electrophysiological studies suggest a major role for Orai1 in the store-operated $Ca(2^+)$ influx pathway of platelets and megakaryocytes. *Platelets* 19(4), 308–313 (2008).

15 Gandhirajan RK, Meng S, Chandramoorthy HC *et al.* Blockade of NOX2 and STIM1 signaling limits lipopolysaccharide-induced vascular inflammation. *J. Clin. Invest.* 123(2), 887–902 (2013).

16 Lyfenko AD, Dirksen RT. Differential dependence of store-operated and excitation-coupled $Ca(2^+)$ entry in skeletal muscle on STIM1 and Orai1. *J. Physiol.* 586(Pt 20), 4815–4824 (2008).

17 Cahalan MD. STIMulating store-operated $Ca(2^+)$ entry. *Nat. Cell Biol.* 11(6), 669–677 (2009).

18 Feske S, Gwack Y, Prakriya M *et al.* A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441(7090), 179–185 (2006).

19 Peters-Golden M, Gleason MM, Togias A. Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. *Clin. Exp. Allergy* 36(6), 689–703 (2006).

20 Braun A, Varga-Szabo D, Kleinschnitz C *et al.* Orai1 (CRACM1) is the platelet SOC channel and essential for pathological thrombus formation. *Blood* 113(9), 2056–2063 (2008).

21 Gerasimenko JV, Gryshchenko O, Ferdek PE *et al.* $Ca(2^+)$ release-activated $Ca(2^+)$ channel blockade as a potential tool in antipancreatitis therapy. *Proc. Natl Acad. Sci. USA* 110(32), 13186–13191 (2013).

22 Feske S. Calcium signalling in lymphocyte activation and disease. *Nat. Rev. Immunol.* 7(9), 690–702 (2007).

23 Yang S, Zhang JJ, Huang X-Y. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 15(2), 124–134 (2009).

24 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 100(1), 57–70 (2000).

25 Xie J, Pan H, Yao J, Zhou Y, Han W. SOCE and cancer: recent progress and new perspectives. *Int. J. Cancer* 138(9), 2067–2077 (2016).

26 Liou J, Kim ML, Heo WD *et al.* STIM is a $Ca(2^+)$ sensor essential for $Ca(2^+)$-store-depletion-triggered $Ca(2^+)$ influx. *Curr. Biol.* 15(13), 1235–1241 (2005).

27 Zhang SL, Yu Y, Roos J *et al.* STIM1 is a $Ca(2^+)$ sensor that activates CRAC channels and migrates from the $Ca(2^+)$ store to the plasma membrane. *Nature* 437(7060), 902–905 (2005).

28 Vig M, Peinelt C, Beck A *et al.* CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 312(5777), 1220–1223 (2006).

29 Zhang SL, Yeromin AV, Zhang XHF *et al.* Genome-wide RNAi screen of $Ca(2^+)$ influx identifies genes that regulate $Ca(2^+)$ release-activated $Ca(2^+)$ channel activity. *Proc. Natl Acad. Sci. USA* 103(24), 9357–9362 (2006).

30 Sabbioni S, Veronese A, Trubia M *et al.* Exon structure and promoter identification of STIM1 (alias GOK), a human gene causing growth arrest of the human tumor cell lines G401 and RD. *Cytogenet. Cell Genet.* 86(3–4), 214–218 (1999).

31 Lewis RS. The molecular choreography of a store-operated calcium channel. *Nature* 446(7133), 284–287 (2007).

32 Stathopulos PB, Zheng L, Li G-Y, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell* 135(1), 110–122 (2008).

33 Baba Y, Hayashi K, Fujii Y *et al.* Coupling of STIM1 to store-operated Ca(2+) entry through its constitutive and inducible movement in the endoplasmic reticulum. *Proc. Natl Acad. Sci. USA* 103(45), 16704–16709 (2006).

34 Yuan JP, Zeng W, Dorwart MR, Choi Y-J, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains

gate and regulate the Orai channels. *Nat. Cell Biol.* 11(3), 337–343 (2009).

35 Zhou Y, Srinivasan P, Razavi S *et al.* Initial activation of STIM1, the regulator of store-operated calcium entry. *Nat. Struct. Mol. Biol.* 20(8), 973–981 (2013).

36 Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca(2+) levels. *Cell* 131(7), 1327–1339 (2007).

37 Hoth M, Niemeyer BA. Chapter ten – the neglected CRAC proteins: Orai2, Orai3, and STIM2. *Curr. Top. Membr.* 71, 237–271 (2013).

38 Gwack Y, Srikanth S, Feske S *et al.* Biochemical and functional characterization of Orai proteins. *J. Biol. Chem.* 282(22), 16232–16243 (2007).

39 Prakriya M. The molecular physiology of CRAC channels. *Immunol. Rev.* 231(1), 88–98 (2009).

40 Berridge MJ. Inositol trisphosphate and calcium signalling. *Nature* 361(6410), 315–325 (1993).

41 Stathopulos PB, Li G-Y, Plevin MJ, Ames JB, Ikura M. Stored Ca^{2+} depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: an initiation mechanism for capacitive Ca^{2+} entry. *J. Biol. Chem.* 281(47), 35855–35862 (2006).

42 Covington ED, Wu MM, Lewis RS. Essential role for the CRAC activation domain in store-dependent oligomerization of STIM1. *Mol. Biol. Cell* 21(11), 1897–1907 (2010).

43 Soboloff J, Rothberg BS, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. *Nat. Rev. Mol. Cell Biol.* 13(9), 549–565 (2012).

• **A comprehensive review on STIM1 and regulatory mechanisms of SOCE.**

44 Ma G, Wei M, He L *et al.* Inside-out Ca(2+) signalling prompted by STIM1 conformational switch. *Nat. Commun.* 6, 7826 (2015).

45 Fahrner M, Muik M, Schindl R *et al.* A coiled-coil clamp controls both conformation and clustering of stromal interaction molecule 1 (STIM1). *J. Biol. Chem.* 289(48), 33231–33244 (2014).

46 Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* 454(7203), 538–542 (2008).

47 Liou J, Fivaz M, Inoue T, Meyer T. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca(2+) store depletion. *Proc. Natl Acad. Sci. USA* 104(22), 9301–9306 (2007).

48 Várnai P, Tóth B, Tóth DJ, Hunyady L, Balla T. Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1–Orai1 complex. *J. Biol. Chem.* 282(40), 29678–29690 (2007).

49 Gudlur A, Zhou Y, Hogan PG. Chapter two – STIM–ORAI interactions that control the CRAC channel. *Curr. Top. Membr.* 71, 33–58 (2013).

• **An authoritative summary on protein–protein interactions between STIM and ORAI proteins that control CRAC channel activation.**

50 Zhou Y, Meraner P, Kwon HT *et al.* STIM1 gates the store-operated calcium channel ORAI1 *in vitro*. *Nat. Struct. Mol. Biol.* 17(1), 112–116 (2010).

• **First demonstration of direct gating and opening of ORAI1 Ca^{2+} channel by recombinant STIM1.**

51 Yang X, Jin H, Cai X, Li S, Shen Y. Structural and mechanistic insights into the activation of stromal interaction molecule 1 (STIM1). *Proc. Natl Acad. Sci. USA* 109(15), 5657–5662 (2012).

52 Kawasaki T, Lange I, Feske S. A minimal regulatory domain in the C terminus of STIM1 binds to and activates ORAI1 CRAC channels. *Biochem. Biophys. Res. Commun.* 385(1), 49 (2009).

53 Muik M, Fahrner M, Derler I *et al.* A cytosolic homomerization and a modulatory domain within STIM1 C terminus determine coupling to ORAI1 channels. *J. Biol. Chem.* 284(13), 8421–8426 (2009).

54 Park CY, Hoover PJ, Mullins FM *et al.* STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136(5), 876–890 (2009).

55 Oh-Hora M, Yamashita M, Hogan PG *et al.* Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat. Immunol.* 9(4), 432–443 (2008).

56 Gwack Y, Srikanth S, Oh-Hora M *et al.* Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol. Cell. Biol.* 28(17), 5209–5222 (2008).

57 Kiani A, Rao A, Aramburu J. Manipulating immune responses with immunosuppressive agents that target *NFAT*. *Immunity* 12(4), 359–372 (2000).

58 Franzius D, Hoth M, Penner R. Non-specific effects of calcium entry antagonists in mast cells. *Pflügers Arch.* 428(5), 433–438 (1994).

59 Clementi E, Meldolesi J. Pharmacological and functional properties of voltagemi independent Ca^{2+} channels. *Cell Calcium* 19(4), 269–279 (1996).

60 Putney JW. Pharmacology of capacitative calcium entry. *Mol. Interv.* 1(2), 84–94 (2001).

61 Sweeney ZK, Minatti A, Button DC, Patrick S. Small-molecule inhibitors of store-operated calcium entry. *ChemMedChem* 4(5), 706–718 (2009).

• **An excellent review on SOCE inhibitors developed before 2010.**

62 Pevarello P, Cainarca S, Liberati C, Tarroni P, Piscitelli F, Severi E. Ca^{2+} release-activated Ca^{2+} channel inhibitors. *Pharm. Pat. Anal.* 3(2), 171–182 (2014).

•• **An excellent patent review on CRAC channel modulators developed by pharmaceutical companies within the recent 5 years.**

63 Hoth M, Penner R. Calcium release-activated calcium current in rat mast cells. *J. Physiol.* 465 359–386 (1993).

64 Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by

altered ion selectivity in a mutant of Orai. *Nature* 443(7108), 226–229 (2006).

65 Mcnally BA, Prakriya M. Permeation, selectivity and gating in store-operated CRAC channels. *J. Physiol.* 590(Pt 17), 4179–4191 (2012).

66 Mason MJ, Mahaut-Smith MP, Grinstein S. The role of intracellular Ca^{2+} in the regulation of the plasma membrane Ca^{2+} permeability of unstimulated rat lymphocytes. *J. Biol. Chem.* 266(17), 10872–10879 (1991).

67 Mcnally BA, Yamashita M, Engh A, Prakriya M. Structural determinants of ion permeation in CRAC channels. *Proc. Natl Acad. Sci. USA* 106(52), 22516–22521 (2009).

68 Vig M, Beck A, Billingsley JM *et al.* CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr. Biol.* 16(20), 2073–2079 (2006).

69 Hou X, Pedi L, Diver MM, Long SB. Crystal structure of the calcium release-activated calcium channel Orai. *Science (New York)* 338(6112), 1308–1313 (2012).

70 Reichling DB, Macdermott AB. Lanthanum actions on excitatory amino acid-gated currents and voltage-gated calcium currents in rat dorsal horn neurons. *J. Physiol.* 441, 199–218 (1991).

71 Clapham DE, Runnels LW, Strubing C. The trp ion channel family. *Nat. Rev. Neurosci.* 2(6), 387–396 (2001).

72 Chung SC, Mcdonald TV, Gardner P. Inhibition by SK&F 96365 of Ca^{2+} current, IL-2 production and activation in T lymphocytes. *Br. J. Pharmacol.* 113(3), 861–868 (1994).

73 Singh A, Hildebrand ME, Garcia E, Snutch TP. The transient receptor potential channel antagonist SKF96365 is a potent blocker of low-voltage-activated T-type calcium channels. *Br. J. Pharmacol.* 160(6), 1464–1475 (2010).

74 Ma H-T, Patterson RL, Van DB *et al.* Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. *Science* 287(5458), 1647–1651 (2000).

75 Prakriya M, Lewis RS. Potentiation and inhibition of $Ca(2^{+})$ release-activated $Ca(2^{+})$ channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP(3) receptors. *J. Physiol.* 536(Pt 1), 3–19 (2001).

76 Ma H-T, Venkatachalam K, Parys JB, Gill DL. Modification of store-operated channel coupling and inositol trisphosphate receptor function by 2-aminoethoxydiphenyl borate in DT40 lymphocytes. *J. Biol. Chem.* 277(9), 6915–6922 (2002).

77 Goto J-I, Suzuki AZ, Ozaki S *et al.* Two novel 2-aminoethyl diphenylborinate (2-APB) analogs differentially activate and inhibit store-operated $Ca(2^{+})$ entry via STIM proteins. *Cell Calcium* 47(1), 1–10 (2010).

78 Maruyama T, Kanaji T, Nakade S, Kanno T, Mikoshiba K. 2APB, 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of Ins(1,4,5)P3-induced Ca^{2+} release. *J. Biochem.* 122(3), 498–505 (1997).

79 Sugawara H, Kurosaki M, Takata M, Kurosaki T. Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. *EMBO J.* 16(11), 3078–3088 (1997).

80 Van Rossum DB, Patterson RL, Ma H-T, Gill DL. Ca^{2+} entry mediated by store depletion, S-nitrosylation, and TRP3 channels: comparison of coupling and function. *J. Biol. Chem.* 275(37), 28562–28568 (2000).

81 Bootman MD, Collins TJ, Mackenzie L, Roderick HL, Berridge MJ, Peppiatt CM. 2-aminoethoxydiphenyl borate (2-APB) is a reliable blocker of store-operated Ca^{2+} entry but an inconsistent inhibitor of InsP3-induced Ca^{2+} release. *FASEB J.* 16(10), 1145–1150 (2002).

82 Gregory RB, Rychkov G, Barritt GJ. Evidence that 2-aminoethyl diphenylborate is a novel inhibitor of store-operated Ca^{2+} channels in liver cells, and acts through a mechanism which does not involve inositol trisphosphate receptors. *Biochem. J.* 354(Pt 2), 285–290 (2001).

83 Iwasaki H, Mori Y, Hara Y, Uchida K, Zhou H, Mikoshiba K. 2-aminoethoxydiphenyl borate (2-APB) inhibits capacitative calcium entry independently of the function of inositol 1,4,5-trisphosphate receptors. *Receptors Channels* 7(6), 429–439 (2001).

84 Peinelt C, Lis A, Beck A, Fleig A, Penner R. 2-aminoethoxydiphenyl borate directly facilitates and indirectly inhibits STIM1-dependent gating of CRAC channels. *J. Physiol.* 586(Pt 13), 3061–3073 (2008).

85 Dehaven WI, Smyth JT, Boyles RR, Bird GS, Putney JW. Complex actions of 2-Aminoethyldiphenyl borate on store-operated calcium entry. *J. Biol. Chem.* 283(28), 19265–19273 (2008).

86 Navarro-Borelly L, Somasundaram A, Yamashita M, Ren D, Miller RJ, Prakriya M. STIM1–Orai1 interactions and Orai1 conformational changes revealed by live-cell FRET microscopy. *J. Physiol.* 586(Pt 22), 5383–5401 (2008).

87 Lis A, Peinelt C, Beck A *et al.* CRACM1, CRACM2, and CRACM3 are store-operated Ca^{+} channels with distinct functional properties. *Curr. Biol.* 17(9), 794–800 (2007).

88 Zhang SL, Kozak JA, Jiang W *et al.* Store-dependent and -independent modes regulating $Ca(^{2+})$ release-activated $Ca(^{2+})$ channel activity of human Orai1 and Orai3. *J. Biol. Chem.* 283(25), 17662–17671 (2008).

89 Schindl R, Bergsmann J, Frischauf I *et al.* 2-aminoethoxydiphenyl borate alters selectivity of Orai3 channels by increasing their pore size. *J. Biol. Chem.* 283(29), 20261–20267 (2008).

90 Wang Y, Deshpande M, Payne R. 2-aminoethoxydiphenyl borate inhibits phototransduction and blocks voltage-gated potassium channels in Limulus ventral photoreceptors. *Cell Calcium* 32(4), 209–216 (2002).

91 Missiaen L, Callewaert G, De Smedt H, Parys JB. 2-aminoethoxydiphenyl borate affects the inositol 1,4,5-trisphosphate receptor, the intracellular Ca2+pump and the non-specific Ca2+leak from the non-mitochondrial Ca2+stores in permeabilized A7r5 cells. *Cell Calcium* 29(2), 111–116 (2001).

92 Hu H-Z, Gu Q, Wang C *et al.* 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. *J. Biol. Chem.* 279(34), 35741–35748 (2004).

93 Hendron E, Wang X, Zhou Y *et al.* Potent functional uncoupling between STIM1 and Orai1 by dimeric 2-aminodiphenyl borinate analogs. *Cell Calcium* 56(6), 482–492 (2014).

94 Wang X, Wang Y, Zhou Y *et al.* Distinct Orai-coupling domains in STIM1 and STIM2 define the Orai-activating site. *Nat. Commun.* 5 3183–3183 (2014).

95 Nazıroğlu M, Özgül C, Çelik Ö, Çiğ B, Sözbir E. Aminoethoxydiphenyl borate and flufenamic acid inhibit Ca^{2+} influx through TRPM2 channels in Rat dorsal root ganglion neurons activated by ADP-ribose and rotenone. *J. Membr. Biol.* 241(2), 69–75 (2011).

96 Chokshi R, Fruasaha P, Kozak JA. 2-Aminoethyl diphenyl borinate (2-APB) inhibits TRPM7 channels through an intracellular acidification mechanism. *Channels* 6(5), 362–369 (2012).

97 Kovacs G, Montalbetti N, Simonin A *et al.* Inhibition of the human epithelial calcium channel TRPV6 by 2-aminoethoxydiphenyl borate (2-APB). *Cell Calcium* 52(6), 468–480 (2012).

98 Djuric SW, Bamaung NY, Basha A *et al.* 3,5-bis(trifluoromethyl)pyrazoles: a novel class of NFAT transcription factor regulator. *J. Med. Chem.* 43(16), 2975–2981 (2000).

99 Trevillyan JM, Chiou XG, Chen Y-W *et al.* Potent inhibition of NFAT activation and T cell cytokine production by novel low molecular weight pyrazole compounds. *J. Biol. Chem.* 276(51), 48118–48126 (2001).

100 Chen Y-W, Smith ML, Chiou GX *et al.* TH1 and TH2 cytokine inhibition by 3,5-bis(trifluoromethyl)pyrazoles, a novel class of immunomodulators. *Cell. Immunol.* 220(2), 134–142 (2002).

101 Ishikawa J, Ohga K, Yoshino T *et al.* A pyrazole derivative, YM-58483, potently inhibits store-operated sustained Ca^{2+} influx and IL-2 production in T lymphocytes. *J. Immunol.* 170(9), 4441–4449 (2003).

102 Zitt C, Strauss B, Schwarz EC *et al.* Potent inhibition of Ca^{2+} release-activated Ca^{2+} channels and T-lymphocyte activation by the pyrazole derivative BTP2. *J. Biol. Chem.* 279(13), 12427–12437 (2004).

103 Mercer JC, Qi Q, Mottram LF *et al.* Chemico-genetic identification of drebrin as a regulator of calcium responses. *Int. J. Biochem. Cell Biol.* 42(2), 337–345 (2010).

104 Ribeiro CMP, Reece J, Putney JW. Role of the cytoskeleton in calcium signaling in NIH 3T3 cells: an intact cytoskeleton is required for agonist-induced [Ca^{2+}] i signaling, but not for capacitative calcium entry. *J. Biol. Chem.* 272(42), 26555–26561 (1997).

105 Takezawa R, Cheng H, Beck A *et al.* A pyrazole derivative potently inhibits lymphocyte Ca^{2+} influx and cytokine production by facilitating transient receptor potential melastatin 4 channel activity. *Mol. Pharmacol.* 69(4), 1413–1420 (2006).

106 He L-P, Hewavitharana T, Soboloff J, Spassova MA, Gill DL. A functional link between store-operated and TRPC channels revealed by the 3,5-bis(trifluoromethyl)pyrazole derivative, BTP2. *J. Biol. Chem.* 280(12), 10997–11006 (2005).

107 Schleifer H, Doleschal B, Lichtenegger M *et al.* Novel pyrazole compounds for pharmacological discrimination between receptor-operated and store-operated $Ca(2^{+})$ entry pathways. *Br. J. Pharmacol.* 167(8), 1712–1722 (2012).

108 Law M, Morales JL, Mottram LF, Iyer A, Peterson BR, August A. Structural requirements for the inhibition of calcium mobilization and mast cell activation by the pyrazole derivative BTP2. *Int. J. Biochem. Cell Biol.* 43(8), 1228–1239 (2011).

109 Ashmole I, Duffy SM, Leyland ML, Morrison VS, Begg M, Bradding P. CRACM/Orai ion channel expression and function in human lung mast cells. *J. Allergy Clin. Immunol.* 129(6–3), 1628–1635.e1622 (2012).

110 Rice LV, Bax HJ, Russell LJ *et al.* Characterization of selective calcium-release activated calcium channel blockers in mast cells and T-cells from human, rat, mouse and guinea-pig preparations. *Eur. J. Pharmacol.* 704(1–3), 49–57 (2013).

111 Derler I, Schindl R, Fritsch R *et al.* The action of selective CRAC channel blockers is affected by the Orai pore geometry. *Cell Calcium* 53(2), 139–151 (2013).

112 Pandol SJ, Saluja AK, Imrie CW, Banks PA. Acute pancreatitis: bench to the bedside. *Gastroenterology* 132(3), 1127–1151 (2007).

113 Owsianik G, D'hoedt D, Voets T, Nilius B. Structure–function relationship of the TRP channel superfamily. *Rev. Physiol. Biochem. Pharmacol.* 156, 61–90 (2006).

114 Ng SW, Di Capite J, Singaravelu K, Parekh AB. Sustained activation of the tyrosine kinase Syk by antigen in mast cells requires local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels. *J. Biol. Chem.* 283(46), 31348–31355 (2008).

115 Di Sabatino A, Rovedatti L, Kaur R *et al.* Targeting gut T cell Ca^{2+} release-activated Ca^{2+} channels inhibits T cell cytokine production and T-Box transcription factor T-Bet in inflammatory bowel disease. *J. Immunol.* 183(5), 3454–3462 (2009).

116 Watanabe H, Takahashi R, Zhang X-X, Kakizawa H, Hayashi H, Ohno R. Inhibition of agonist-induced Ca^{2+} entry in endothelial cells by myosin light-chain kinase inhibitor. *Biochem. Biophys. Res. Commun.* 225(3), 777–784 (1996).

117 Smyth JT, Dehaven WI, Bird GS, Putney JW. Calcium store-dependent and independent reversal of stim1 localization and function. *J. Cell Sci.* 121(Pt 6), 762–772 (2008).

118 Zakharov SI, Smani T, Dobrydneva Y *et al.* Diethylstilbestrol is a potent inhibitor of store-operated channels and capacitative Ca^{2+} influx. *Mol. Pharmacol.* 66(3), 702–707 (2004).

119 Ohana L, Newell EW, Stanley EF, Schlichter LC. The Ca^{2+} release-activated Ca^{2+} current (ICRAC) mediates store-operated Ca^{2+} entry in rat microglia. *Channels* 3(2), 129–139 (2009).

120 Kohn EC, Reed E, Sarosy GA *et al.* A phase I trial of carboxyamido-triazole and paclitaxel for relapsed solid tumors: potential efficacy of the combination and demonstration of pharmacokinetic interaction. *Clin. Cancer Res.* 7(6), 1600–1609 (2001).

121 Hussain MM, Kotz H, Minasian L *et al.* Phase II trial of carboxyamidotriazole in patients with relapsed epithelial ovarian cancer. *J. Clin. Oncol.* 21(23), 4356–4363 (2003).

122 Rodland KD, Wersto RP, Hobson S, Kohn EC. Thapsigargin-induced gene expression in nonexcitable cells is dependent on calcium influx. *Mol. Endocrinol.* 11(3), 281–291 (1997).

123 Hoth M, Button DC, Lewis RS. Mitochondrial control of calcium-channel gating: a mechanism for sustained signaling and transcriptional activation in T lymphocytes. *Proc. Natl Acad. Sci. USA* 97(19), 10607–10612 (2000).

124 Glitsch MD, Bakowski D, Parekh AB. Store-operated $Ca(2^+)$ entry depends on mitochondrial $Ca(2^+)$ uptake. *EMBO J.* 21(24), 6744–6754 (2002).

125 Mignen O, Brink C, Enfissi A *et al.* Carboxyamidotriazole-induced inhibition of mitochondrial calcium import blocks capacitative calcium entry and cell proliferation in HEK-293 cells. *J. Cell Sci.* 118(23), 5615–5623 (2005).

126 Chen G, Panicker S, Lau K-Y *et al.* Characterization of a novel CRAC inhibitor that potently blocks human T cell activation and effector functions. *Mol. Immunol.* 54(3–4), 355–367 (2013).

127 Holowka D, Korzeniowski MK, Bryant KL, Baird B. Polyunsaturated fatty acids inhibit stimulated coupling between the ER $Ca(2^+)$ sensor STIM1 and the $Ca(2^+)$ channel protein Orai1 in a process that correlates with inhibition of stimulated STIM1 oligomerization. *Biochim. Biophys. Acta* 1841(8), 1210–1216 (2014).

128 Zhang H-Z, Xu X-L, Chen H-Y *et al.* Discovery and structural optimization of 1-phenyl-3-(1-phenylethyl)urea derivatives as novel inhibitors of CRAC channel. *Acta. Pharmacol. Sin.* 36(9), 1137–1144 (2015).

129 Jairaman A, Prakriya M. Molecular pharmacology of store-operated CRAC channels. *Channels* 7(5), 402–414 (2013).

•• **Has introduced the molecular basis of CRAC channels in detail and summarized some traditional CRAC channel inhibitors.**

130 He L, Zhang Y, Ma G *et al.* Near-infrared photoactivatable control of Ca^{2+} signaling and optogenetic immunomodulation. *eLife* 4 (2015). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4737651/

131 Kyung T, Lee S, Kim JE *et al.* Optogenetic control of endogenous Ca^{2+} channels *in vivo*. *Nat. Biotech.* 33(10), 1092–1096 (2015).

132 Ishii T, Sato K, Kakumoto T *et al.* Light generation of intracellular $Ca(2^+)$ signals by a genetically encoded protein BACCS. *Nat. Commun.* 6, 8021 (2015).