Role of Sodium dependent SLC13 transporter and its inhibitors in various metabolic disorders

MOHAMMAD AKHTAR[1], Shah Khan[1], Bhupinder Kumar[2], Pooja Chawla[2], Rohit Bhatia[2], and Karanvir Singh[2]

[1]Oman Medical College Department of Pharmacy
[2]ISF College of Pharmacy

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Abstract

The sodium dependent SLC13 family transporters comprise of the five genes SLC13A1, SLC13A2 (NaDC1), SLC13A3 (NaDC3), SLC13A4 and SLC13A5 (NaCT). Among them the three NaDC1, NaDC3 and NaCT are sodium dependent transporters such as di-carboxylates (succinate, malate, α-ketoglutarate) and tricarboxylates (citrate). The mouse and the human NaCT structures have still not been crystallized, the information to the structures is taken from the related bacterial transporter of VcINDY. Citrate in the cytosol works as precursor for the fatty acid synthesis, cholesterol, and low-density lipoproteins. The excess citrate from the matrix is translocated to the cytosol for fatty acid synthesis through these receptors and thus controls the energy balance by downregulating the glycolysis, tricarboxylic acid (TCA), and fatty acid breakdown. These transporters play an important role in regulating various metabolic diseases including cancer, diabetes, obesity, fatty liver diseases and CNS disorders. These di and tricarboxylate transporters are emerging as new targets for metabolic disorders such as obesity and diabetes. The mutation in the function of the NaCT causes several neurological diseases including neonatal epilepsy and impaired brain development whereas mutation of the citrate present in the liver may provide positive effect. Therefore, continued efforts from the earlier work on citrate transporter are required for the development of citrate inhibitors. In this review the structure, function, and regulation of the NaCT receptors are discussed. The review also highlights citrate role in diagnosing diseases such as cancer, diabetes, fatty liver, and diabetes. The therapeutic perspective of synthetic inhibitors against NaCT receptors are succinctly summarized.

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Md Jawaid Akhtar[1]*, Shah Alam Khan[1], Bhupinder Kumar[2], Pooja Chawla[2], Rohit Bhatia[2], Karanvir Singh[2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy,National University of Science and Technology, PO 620, PC 130, Azaiba, Bousher, Muscat, Sultanate of Oman

[2]Department of Pharmaceutical Chemistry,

ISF College of Pharmacy, Ghal Kalan, Ferozpur G.T. Road Moga-142001, Punjab, India

***Corresponding Author:**

Dr. Md Jawaid Akhtar

Department of Pharmaceutical Chemistry, College of Pharmacy, National University of Science and Technology, PO 620, PC 130, Azaiba, Bousher, Muscat, Sultanate of Oman

E-mail: mjawaid@nu.edu.om, jawaid.pharmacist@gmail.com,

Abstract

The sodium dependent SLC13 family transporters comprise of the five genes SLC13A1, SLC13A2 (NaDC1), SLC13A3 (NaDC3), SLC13A4 and SLC13A5 (NaCT). Among them the three NaDC1, NaDC3 and NaCT are sodium dependent transporters such as di-carboxylates (succinate, malate, ∝-ketoglutarate) and tricarboxylates (citrate). The mouse and the human NaCT structures have still not been crystallized, the information to the structures is taken from the related bacterial transporter of VcINDY. Citrate in the cytosol works as precursor for the fatty acid synthesis, cholesterol, and low-density lipoproteins. The excess citrate from the matrix is translocated to the cytosol for fatty acid synthesis through these receptors and thus controls the energy balance by downregulating the glycolysis, tricarboxylic acid (TCA), and fatty acid breakdown. These transporters play an important role in regulating various metabolic diseases including cancer, diabetes, obesity, fatty liver diseases and CNS disorders. These di and tricarboxylate transporters are emerging as new targets for metabolic disorders such as obesity and diabetes. The mutation in the function of the NaCT causes several neurological diseases including neonatal epilepsy and impaired brain development whereas mutation of the citrate present in the liver may provide positive effect. Therefore, continued efforts from the earlier work on citrate transporter are required for the development of citrate inhibitors. In this review the structure, function, and regulation of the NaCT receptors are discussed. The review also highlights citrate role in diagnosing diseases such as cancer, diabetes, fatty liver, and diabetes. The therapeutic perspective of synthetic inhibitors against NaCT receptors are succinctly summarized.

Keywords: Citrate, dicarboxylates, NaCT, ATP citrate lyase, citrate transporter

Introduction

Citrate, malate, succinate, and oxaloacetate are formed in steps of Citric acid cycle. These intermediates are precursors of anabolic pathways for fatty acids, cholesterol, glucose, pyruvate, amino acids, and nucleotides. These also help in the regulation of important biological processes including fatty acid synthesis and glycolysis [1]. They act as a source for the cytosolic acetyl CoA for the synthesis of fatty acids, cholesterol and isoprenoids [2]. Citrate metabolite involved in tricarboxylic acid (TCA) cycle is generated in the matrix of the mitochondria [3]. The steps involve carboxylation of the glucose derived pyruvate and then acetylation with Acetyl CoA. The citrate metabolite is involved in the TCA cycle to yield ATP*via* generated NADH and $FADH_2$ entering the electron transport chain (ETC). The excess citrate in the matrix is transported out to the cytoplasm through mitochondrial citrate carrier. Citrate inhibits the glucose catabolism by allosterically inhibiting phosphofructokinase-1 (rate-limiting enzyme in glycolysis) and stimulate gluconeogenesis by activating fructose 1,6-biphosphatase. It also acts as a carbon source for the lipid biosynthesis (fatty acids and cholesterol) with the generation of acetyl CoA. The acetyl CoA is the allosteric activator of the acetyl CoA carboxylase (rate limiting enzyme for fatty acid synthesis) and generated malonyl CoA. The malonyl CoA is the intermediate involved in the fatty acid synthesis in the cytoplasm. Thus, citrate acts as a building bridge between the fatty acid and carbohydrate metabolism [4] **(Figure 1)** . It was confirmed in*Mucor circinelloides* where citrate produced from the TCA cycle was translocated (mitochondrial citrate transporter) across mitochondrial inner membrane and cleaved to oxaloacetate (OAA) and acetyl-CoA by ATP citrate lyase. The acetyl CoA along with NADPH are used to produce fatty acid biosynthesis in the cytosol. The two genes responsible for coding of citrate transporters i.e., citrate transporter and tricarboxylate transporter are overexpressed, cause increased lipid accumulation whereas decrease in extracellular citrate concentration [5]. The blockade of two plasma membrane malate transporter 2-oxoglutarate: malate antiporters (SoDIT-a and SoDIT-b) in the fungus results in increased malate available for the lipid biosynthesis [6]. The citrate transport is promoted by ΔpNa^+(chemical gradient of sodium ions) and ΔpH (pH gradient across the membrane) and not by [?]ψ (electrical potential across membrane) showing that citrate transport is electroneutral process. The sodium-ion-dependent citrate carrier also causes sodium counterflow in the absence of citrate [7]. The cytosolic concentration of the citrate also determines the rate of the fatty acid synthesis. The NaCT (sodium-dependent citrate transporter) in liver cell plays an important role in transporting citrate and is used as a target for anti-obesity drugs. The extracellular citrate enters the plasma membrane of specific cells through Na^+-coupled citrate transporter (NaCT)*i.e.,* SLC13A5 [8]. The

inner mitochondrial membrane channel is blocked by the externally bound high affinity $Mg^{2+}$and may be activated by the citrate or other Mg^{2+}chelators. The presence of exogenous $MgCl_2$ strongly inhibits the citrate-induced depolarization and is independent of the presence of sodium, potassium, or chlorine ions. The exogenous citrate in the Mg^{2+} free medium has been shown to suppress the reactive oxygen species (ROS) and uncoupling of the mitochondria. It also suppresses the H_2O_2 and the stimulation of respiration of mitochondria (suppress the depolarized mitochondria and amount of the citrate transporter)[9]. The sodium dependent SLC13 family transporters carry these intermediates from plasma membranes into the cells and is comprises of five genes. The three sodium dependent transporters of the human SLC13 family includes hNaDC1 (SLC13A2), hNaDC3 (SLC13A3), and hNaCT (SLC13A5). These receptors are emerging as drug targets for various metabolic disorders [1]. Some of the recent patents on sodium citrate transporters are listed in**Table 1** . The other two genes include SLC13 co-transporters SLC13A1 and SLC13A4 which are involved in transportation of renal sodium dependent anions such as selenite, sulphate, and thiosulphate [10].

Figure 1: Citrate-pyruvate shuttle and linkage of citrate as fatty acids and carbohydrate metabolism.

Table 1: Some of the patents on sodium citrate transporters are listed below:

S.No	Patent no	Title	Inventors	Application/patent grant date
1	US2005095240A1 and EP1816139A1	NaCT as a target for lifespan expansion and weight reduction	Ganapathy Vadivel; Inoue Katsuhisa; Fei You-Jun	2005-05-05 and 2007-08-08
2	US2020087258A1	Inhibitor of citrate transporter and their use in therapy	Zahn, Grit; Bromidge, Steve; Yarnold, Chris; Schaertl, Sabine; Khor, Someina	2020-03-19
3	US2007259956A1 CA2525367 EP2633856	Compositions and methods for treating cancer Use of citrate lyase inhibitors and tricarboxylate transporter inhibitors in the treatment of cancer ATP citrate lyase inhibitors for treating Cancer	Thompson Craig.; Bauer Daniel; Hatzivassiliou Georgia	2007-11-08 25-11-2004 04-09-2013
4	US10768167B2	Plasma membrane citrate transporter for use in the diagnosis and treatment of cancer	Geissler, Edward; Mycielska, Maria; Rummele, Petra	2016-03-30

S.No	Patent no	Title	Inventors	Application/patent grant date
5	CN111239353A	Screening method of human citric acid transporter inhibitor compound	Peng Zhigang; Xu Jingjing; Tao Min; Wang Xiaohui; Liu Li; Lan Weiming; Zhou Yu; Feng Xiaoyu; Jin Tiantian; Jing Weiqian; Tu Chengjun; Pan Xinbei; Wang Yangyang; Wang Zhen	2020-06-05
6	WO2021009950A1	Agent for enhancing effect of anticancer agent	Wada Hiromi; Ishida Tatsuhiro; Eshima Kiyoshi	2021-01-21
7	US8865153B2	Microorganisms for imaging and/or treatment of tumors	Aladar A. Szalay Nanhai Chen Yong A. Yu Qian Zhang	2014-10-21
8	KR101504769B1	Solid citrate and tartrate salts of dpp-iv inhibitors	Jen-Ping WU, David Alan Campbell, Julie M. Cherington	2015-03-20
9	WO2020102616	ATP citrate lyase (ACLY) inhibitors and uses thereof	Leit de Moradei, Silvana Marcel Kreutter, Kevin Harwood, H. James Therrien, Eric	22-05-2020
10	WO2014039477	Citrate-rich Calcium-Magnesium Supplement and Uses Thereof	PAK, Charles, Y.C. Sakhaee, Kashayar MOE, Orson, W.	13-03-2014
11	CN101664412	Medicinal health agent for preventing and treating canine uric acid calculi	Wang Jinyong, Wang Lianmin	02-09-2008
12	WO2018104220	Sulfonamides as inhibitors of the uptake of extracellular citrate	Kley, Joerg Kauschke, Stefan Pautsch, Alexander Wiedenmayer, Dieter	14-06-2018
13	WO2015120272	Organic acids as growth inhibitors of pathological calcification and uses thereof	Rimer, Jeffrey, D. Asplin, John	13-08-2015

S.No	Patent no	Title	Inventors	Application/patent grant date
14	EP0639187	Phenylderivate as inhibitors of ATP citrate lyase	Gribble Andrew Derrick, Groot Pieter Hendrik Evert, Shaw Antony Nicholas, Dolle Roland Ellwood	29-04-1993
15	US20210244694	BHB-citrate combination products for renal health and treating disease	Thomas Weimbs, Jacob Torres	12-08-2021
16	EP0809483	Process for inhibiting sorbate-induced brown discolorations in cosmetics and foodstuffs	Jager Martin	03-12-1997

Structural composition of NaCT

The structure of either human or the mouse NaCTs has not been crystallized. The current knowledge for the structural basis of the NaCTs is based upon *Vibrio cholerae* bacterial dicarboxylate transporter VcINDY. The structure VcINDY greatly differs from the human NaCT but still acts as the best option for the modeling studies [11]. It has a total of 11 transmembrane (TM) helices hairpin loops marked as HP_{in} (hairpin in) and HP_{out}(hairpin out). The TM 4, 5, 9 and 10 are each broken into two parts as a and b. The HP_{in} towards the cytosolic side is connected to helix H4c to one side and TM5 on the other side. Similarly, HP_{out} coming out from the periplasm is connected to the TM10 and H9c. The hairpin along with the broken helix (intramembrane loop) plays a chief role in membrane transport. The NaCT (VcINDY) has two repeated folds with 26% similarity in amino acid sequence from the N-and C-terminal side i.e., TM2-TM6 from the N terminal side is related with TM7-TM11 of C terminal side. The binding pocket for the citrate is formed from the residues HP_{in}, TM5, HP_{out} and TM10. The citrate molecule binds between Na1 (N terminal side hairpin tip-capping loop motif formed by HP_{in} tip, TM5a and TM5b) and Na2 sites (C terminal side hairpin tip-capping loop motif formed by HP_{out} tip, TM10a and TM10b)[12] **Figure 2** . The zebrafish Na^+ coupled citrate transporter was cloned showing 61% identical and 77% similarity to the human NaCT. The cloned human NaCT showed 77% identity with rat NaCT and the gene is located on human chromosome 17 at p12-13 [13]. Khamaysi *et al.,* studied the slc13 transporter H4c as a dynamic anchor domain which controls the metabolite transport (succinate and citrate). It was observed that intracellular determinants interact with the H4c domain and control the transport. The interaction to both the intracellular determinants and the membrane phospholipids is attained by a sole basic residue*i.e* ., Arg108. The conclusions were drawn by carrying out several experimental studies in *Xenopus oocytes* and fluorescent microscopy of mammalian cells. The Arg108 present in highly conserved H4c was found to be critical for the metabolite transport. Neutralization of this basic residues inhibited the transport functions [14].

Figure 2: A NaCT representation of a VcINDY in which transport domain is represented by the binding sites hairpin loops and unwounded helices 5 and 10. The N terminal end is related to the C terminal end through an inverted repeat symmetry (shown in red and blue color box) except the TM1[12,15].

The receptors of the VcINDY can be expressed in two forms - the outward facing state and the inward-facing state for the transport mechanism to occur. The substrate binds to the outward facing state formed by the

transport domain (shown in orange) which slides 15Å downward (translocate) with 43° rotation to form the inward facing states (as such elevator is sliding downwards). The elevator is formed by the transport domain and the hydrophobic barrier provided by transmembrane 4 and 9 (oligomerization domain) and scaffold domain. This leads to the release of the substrate to the cytoplasm. After releasing the substrate, the transporter again returns to it outward facing state to start the next cycle [15] (**Figure 3)** . It was also observed that crosslinking VcINDY to either inward or outward facing state inhibit the transport activity. The test was done by reconstituting the double cysteine mutants in proteoliposomes and access the transport abilities of the transporter. The crosslink was induced with excess $HgCl_2$ in both cases of outward and inward stabilizing mutants. The transport activity was restored when the crosslinking was reduced. The transport activity was strong in $HgCl_2$ treated cysteine less proteins [15].

Figure 3: Substrate transport by elevator type mechanism. The substrate binds to the outward facing of the transport domain, translocation inhibited by the hydrophobic barrier (scaffold and oligomerization domain). The transport domain translocated to 15Å downward rotating 43° to the inward facing state releasing the substrate to the cytoplasm [16].

Although the receptors functional properties are extensively discussed but the different analogues bindings within these receptors for the treatment of various metabolic disorders has not been covered in detail in previously published reviews. This article encompasses different key intermediates analogues responsible for the blocking of these SLC family receptors for promising results.

1. **Distribution and function**The sodium-coupled with anionic transporters includes three transporters of dicarboxylates and tricarboxylates (NaDC1, NaDC3 and NaCT). The metabolic intermediates of TCA cycle such as citrate, ∝-ketoglutarate and succinate are the substrate for these transporters which maintains the levels of these intermediates in plasma, urine, and tissue levels [17].
2. **SLC13A5 (NaCT):** These receptors are widely distributed. NaCT found in the liver, brain, and salivary glands primarily transport citrate into these tissues. It is major carrier of citrate in the liver [18]. The NaCT (SLC13A5) is mammalian homolog of the Drosophila INDY. The partial loss in the citrate transport causes increase in lifespan in Drosophila [11]. The rat NaCT (Na^+ coupled citrate transporter) has 77% similarity with the cloned human NaCT. It mediates the Na^+ coupled citrate transport and is the first transporter recognized in the mammalian cells that showed higher affinity for citrates [19]. It is mostly expressed in liver but also found in brain and testis. The transport is electrogenic whereas its activation showed sigmoidal curve (more involvement of the Na^+ ions in activation). The citrates are found in high concentration in the human blood and are involved in the metabolic energy and synthesis of fatty acids and cholesterol [20]. NaCT is more selective to citrate than other dicarboxylates or tricarboxylates [11]. The primary neurons culture of mouse cerebral cortex showed the NaCT transport system, but it was found to be absent in the astrocytes. It is Na^+-dependent, Li^+-sensitive inhibited by the unlabeled tricarboxylate citrate and other dicarboxylates e.g., malate, fumarate, succinate, and ∝-ketoglutarate [21]. The mutation in SLC13A5 gene codes for Na^+/citrate co-transporter results in loss of function and causes neurological disorders that includes neonatal epilepsy, delayed brain development and tooth dysplasia in children. Targeting the NaCT deletion in mice has beneficial effect against diet induced obesity with no brain dysfunction. Mutation different from the brain located in SLC13A5 may have beneficial effect. Thus, there is greater emphasis in designing inhibitors which specifically cause the NaCT loss in the liver rather than NaCT loss in the brain [11]. The SLC13A5 mutation in nine epilepsy patients were identified. The drug acetazolamide (carbonic anhydrase inhibitors) and the atypical anti-seizures drugs decreases the seizures in four patients. Also, it was noticed that the ketogenic diet and fasting worsens the symptoms. The mutations of NaCT transport function and protein expression were further studied by transient transfection against COS-7 cells. It was observed that there was no transport activity from the mutant transporter proteins affecting helix packing or substrate bindings. Other treatment such as chaperones and low temperature were not able to improve the transport functions of mutated NaCT. A decreased protein expression and activity in wild-type transporter have been observed when both the NaCT and mutant NaCT are co-expressed [22]. Selch *et al.,* studied the mutation associated with the SLC13A5 gene NaCT mediate transport

functions. Taking HEK293 as wild type and eight mutation NaCT proteins, it was observed that proteins were synthesized identical to the wild types but for the mutants pG219R, pG219E, pT227M, pL420P and pL488P the citrate transport was completely inhibited. These conserved amino acids are present in the transmembrane pore and are important for the NaCT-mediated transport [23]. The VcINDY homolog of SLC13A5 represent the receptors for information on substrate binding sites and mechanism. It has large open cavity towards the cytosolic sides for sodium driven succinate transport and is inhibited by the dicarboxylates such as malate and fumarate. In this it requires four sodium to couple with the substrate (4:1). The NaCT has many functions to perform such as energy production (mitochondria Kreb's cycle), glycolysis, gluconeogenesis, fatty acid, and cholesterol synthesis in the cytosol. The SLC13A5 also regulates fibroblast growth factor receptors (FGF23) function leading to the overproduction of the FGF23 and abnormal mineralization of the extracellular matrix leading to hypophosphatemic rickets disease. The NaCT activity is stimulated by the presence of Li^+ ions [10].

3. *Citrate transporter of Lactic acid bacteria*The human sodium-dependent citrate transporters (NaCT) and succinate/dicarboxylate from *Lactobacillus acidophilus* has great similarity in the structure as VcINDY. All these results support for the VcINDY protein structure representing the overall protein structure in ion transporter superfamily[8]. The citrate transporter and the malate transporter of lactic acid bacteria *Leuconostoc mesenteroides* and *Lactococcus lactis* were analyzed and both showed transport of substrate containing 2-hydroxycarboxylates. The structural changes either of the OH and COO^- drastically reduced the affinity to the transporters. The dicarboxylate with the formula OH-CR-$(COO^-)_2$, the$S$ -enantiomer of which binds efficiently and translocated whereas the R -enantiomer has no affinity. The binding order magnitude of citrate or S -enantiomer is one order higher than other uncharged R groups containing compounds including lactate; this shows the importance of second carboxylate in greater affinity with the proteins. The binding preference for the$R$ -enantiomer is more in *Leuconostoc mesenteroides*when R groups are hydrophobic whereas only$S$ -enantiomer is translocated in *Lactococcus lactis*[24]. The 46 residues at the C-terminal region containing C-terminal putative transmembrane XI was analyzed for the substrate recognition through chimeric transporters. The replacement of the C-terminal region in *Leuconostoc mesenteroides* with *Lactococcus lactis* and *vice versa* did not alter the transport characteristics of the di and tri carboxylate *i.e* ., malate and citrate whereas monocarboxylate substrate mandelate and 2-hydroxyvalerate interactions with the proteins were changed. The incorporation of C-terminal residues of one bacterium into another and *vice versa* led to higher affinity. The interchanging of the C-termini has more complex effect on R enantiomers. It was indicated that binding pocket is located in other transmembrane and transmembrane XI [25]. The substrate transported by citrate transporter of *Leuconostoc mesenteroides* and malate transporter of *Lactococcus lactis* are shown in**Figure 4** .**Figure 4:** The substrate transported with citrate transporter of *Leuconostoc mesenteroides* and malate transporter of *Lactococcus lactis*
4. *Citrate Transporter of Klebsiella pneumoniae*

The loops of the transmembrane segments VIII and IX (AH loop) represent the amphipathic surface helix of *Klebsiella pneumoniae* . The mutation of the cysteine residues especially G324C, F331C, and F332C has very low citrate transport activity whereas other mutants I321C and S333C showed decreased activity with membrane permeable thiol reagent$N$ -ethylmaleimide (NEM) but not with membrane impermeable 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid and [2-(trimethylammonium)ethyl]methanethiosulfonate. The impermeability of the reagent 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid supports for the localization of the AH loop in the cytoplasmic region [26].

Inhibitors of NaCT

Higuchi *et al.,* showed that deletion of the genes that encode for SLC13A5 in mice protects against obesity and other metabolic disorders such as diabetes. Suppressing the transporter also suppresses the hepatocellular carcinoma. The author reported one irreversible and non-competitive inhibitor of human NaCT, and which does not affect the mouse NaCT. The compound **1** inhibits the constitutively transporter in HepG2 cells and ectopically expressed in HEK-293 cells. The inhibition is in nanomolar range of 100 nM (**Figure 5**). The molecular modelling studies generated human NaCT and mouse NaCT taking humanized variant of

VcINDY as the template. The compound and the citrate showed different inhibitors to human NaCT *versus* mouse NaCT [27].

Figure 5: The compound inhibits HepG2 cell transporter and ectopically expressed HEK-293 cells.

It was further evidenced that HepG2 cells represent the excellent cell models to study the regulatory mechanisms of endogenous di and tricarboxylates. Also, it was found that there is higher rate of succinate transport as compared to citrate. The human retinal pigments epithelial cells transfected with hNaDC-1, hNaDC-3 and hNaCT were compared with citrate and succinate in HepG2 cells and the results showed that HepG2 cells transport were constant with hNaDC-3 and hNaCT expressed in human retinal pigments epithelial cells. The high affinity transport for succinate is consistent with hNaDC-3 [2]. The blockade of hepatic extracellular citrate uptake through the blockade of NaCT may be regarded as potential treatment of metabolic disorders. This led Huard *et al.,* in 2015 to identify and characterize some novel small dicarboxylate molecules especially the active (R)-enantiomer **2** to be highly selective for NaCT compared to NaDC1 and NaDC3. The (S)-enantiomer was found to be inactive. The mechanism for the compound **2** was found to be competitive to the citrate, specific, stereo sensitive and direct to the NaCT receptors. The compound acts a substrate for active cellular uptake and inhibits both *in vitro* and *in vivo* by directly binding the compound to NaCT receptors. The IC_{50} of the compound**2** against HEK_{NaCT} was found to be 0.41 μM as compared to the racemate having IC_{50} value of 0.80 μM[18] **Figure 6** .

Figure 6: Compound **2** with their IC_{50}value against HEK_{NaCT}

The inhibition of sodium citrate transporter NaCT (SLC13A5) is a major target for metabolic disease. As discussed above citrate acts as signaling molecules that regulates fatty acid and glucose metabolizing enzymes. Two compounds **3** and **4** inhibits the human NaCT with very high affinity. Compound **3** is very specific for hNaCT whereas, compound **4** inhibits all the three transporters (NaCT, NaDC1 and NaDC3) but NaCT with greater affinity. Compound **3**inhibited the citrate uptake (hNaCT) with no inhibition of citrate C14 transport activity in NaDC1 and NaDC3 whereas **4** inhibited all the three transporters at 100 μM. The citrate uptake blockade by compound **3** in the liver inhibits the conversion of extracellular citrate to tricarboxylic acid cycle (TCA) intermediates. This results in reduction of high plasma glucose concentration in mice fed with high fat (**Figure 7**). Pajor *et al* ., 2016 explored the molecular modelling inside the citrate receptors and analyzed the amino acids involved in the binding sites. The amino acid residue (G228, V231, V232, and G409) near the citrate binding sites affect both the transport as well as inhibition of citrate uptake. The amino acid V231 distinguish between the compound **3** and**4** as inhibitors. Some of the residue outside the binding sites such as Q77 and T86 are important in NaCT inhibition[28].

Figure 7: Sodium citrate transporter NaCT (SLC13A5) inhibitors

One of the studies carried out by Huard *et al.,* optimized the earlier series of synthesized compound to more potent compound**5** . The compound showed potent inhibition of the [^{14}C] citrate uptake in the liver and kidney. The compound showed more suitable *in vivo* pharmacokinetic profile for better *in vivo* pharmacodynamics. The compound also reduced the plasma glucose concentrations[18] (**Figure 8**).

Figure 8: Optimization of the compound **1** to more active isomer **5** against NaCT receptors

It was demonstrated that metformin inhibits the NaCT plasma membrane citrate transporter in HepG2 cells with decrease in the levels of the citrate and decreases mRNA levels. It inhibits gluconeogenesis and thus has anti-diabetic effects. An 5' adenosine monophosphate-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) has similar effect. Activating AMPK inhibits mTOR which further inhibits rapamycin and thus decreases NaCT expression. The decrease in the cellular levels of the citrate stimulate glycolysis and inhibits gluconeogenesis (citrate suppresses glycolysis by inhibiting phosphofructokinase-1 and activate gluconeogenesis by activating fructose 1,6-biphosphate) [29] (**Figure 9**).

Figure 9: Flow diagram showing new mechanism of action of the metformin acting on SLC13A5 citrate receptor

The cytosolic citrate activates fatty acid synthesis and downregulates the glycolysis and β-oxidation of fatty acids. The cytosolic concentration of the citrate controls rate of synthesis of fatty acids in the liver and adipose tissue which in turn is controlled by the transport through NaCT transporter. It was evidenced that mutation in homologous fly gene results in reduced fat storage. The study in the mice (NaCT knockout) further confirms reduced lipogenesis, higher lipid oxidation and energy expenditure. This led to protection of mice from obesity and insulin resistance [12]. The divalent anion sodium symporter (DASS) includes *Staphylococcus aureus*Na^+/dicarboxylate symporter SdcS, mammalian SLC13 Na^+/dicarboxylate cotransporters NaDC1 and NaCT. The anthranilic acid derivatives such as *N* -(p-amylcinnamoyl) anthranilic acid **6** is a slow inhibitor of the mammalian members. The SdcS was also inhibited by the *N* -(p-amylcinnamoyl) anthranilic acid and nonsteroidal anti-inflammatory drugs (NSAIDs) such as flufenamate and niflumate as allosteric inhibitors. The IC_{50} was calculated as 55 μM for*N* -(*p* -amylcinnamoyl) anthranilic acid (**Figure 10**). With succinate the graph was sigmoidal with $K_{0.5}$ of 9 μM and Hill coefficient 1.5. When given with*N* -(*p* -amylcinnamoyl) anthranilic acid $K_{0.5}$remains to be 1.5 but there was decrease in the *V* max and increased in Hill coefficient. Also, the mutants (N108C) reactivity was not affected by the *N* -(*p* -amylcinnamoyl) anthranilic acid inhibition [30].

Figure 10 : The *N* -(p-amylcinnamoyl) anthranilic acid inhibitors of both mammalian and *Staphylococcus aureus*Na^+/dicarboxylate symporter

The classical inhibitor benzene tricarboxylate for the mitochondrial citrate transporter protein led Aluvila *et al.,* to identify competitive inhibitors against the mitochondrial citrate transporter protein through *in silico* screening of the ZINC database. A total of 10 compounds were identified which showed more than 50% inhibitions at the concentration of the 1 mM. One of the compounds showed the highest percentage inhibition of 85% as compared to the benzene tricarboxylate (% inhibition = 79%). **Figure 11** shows the compound **7** identified by the *in-silico* database screening taking benzene tricarboxylate as the reference. One of the compound **8** acts as potent inhibitors against both the bacterial citrate transporter protein as well as plasma membrane citrate transporter [31].

Figure 11: Competitive mitochondrial citrate inhibitors by*in silico* screening of the ZINC database.

Na^+/Dicarboxylate co-transporter, SLC13A2 (NaDC1) : The hNaDC1 found in renal proximal tubule and small intestine and has low affinity for both the di and tri-carboxylate (oxaloacetate, fumarate, and malate). The main substrate is succinate (K_m value of 0.9 mM), α-ketoglutarate and citrate (main physiological substrate). In renal proximal tubule and small intestine, it reabsorbs the intermediates of the TCA from urine and diet, maintains the urinary levels of citrate and plays a crucial role in the development of kidney stone [10,17]. Citrate can chelate the calcium (Ca^{2+}) and avoid its precipitation. Thus, low urinary citrate promotes the development of the kidney stones [3]. It involves bindings of three sodium ions and then the dicarboxylate ions results in passage of one positive ions. The ion lithium competitively inhibits the transport after binding at one of the sodium bindings sites, but the sensitivity varies with species-to-species [10]. Human NaCT is activated by the presence of Li^+ whereas rodents NaCT showed inhibition[13]. The stimulation of NaDC1 by metabolic acidosis through physical exercise, clinical treatments led to decrease in the citrate concentration [10]. The transporter of SLC-13 family transport di or tricarboxylate (succinate or citrate) along with two to four Na^+ ions. Colas *et al.,* in the year 2017 modeled outward facing conformation of Na^+/dicarboxylate co-transporters of both human (h) and rabbit (rb) using outward facing model of bacterial homolog VcINDY template. It was observed that mutagenesis in rbNaDC1 of T474 with cysteine results in inactive protein. The cysteine substitution in M539C results in low affinity to both sodium and lithium ions, indicates for the hNaDC3 sites. The mutants of Y432C and T86C led to increased K_m value for succinate suggesting for these amino acids in outward-facing substrate binding sites[32].

- **Inhibitors of NaDC1**Pajor *et al.,* 2010., showed that the transmembrane helix (TM11) is important for sodium and lithium bindings and citrate affinity. The amino acid sequence of the TM11 is between 543 and 563, where glycine 543 resides inside of the membrane and valine 563 to the outside. The amino acid was mutated to see whether it was affecting the transport activity and was observed that in many of them it decreases the transport activity. Some of the mutants were inactive such as G550C,

W561C and L568C. The receptor was inhibited by low concentration of lithium in the presence of sodium. Thus, the work showed that Ile554 is somewhat located in the middle of the helix and is determinants of the bindings of the lithium[33].

- **SLC13A3 (NaDC3):** The hNaDC3 has higher affinity for both the di-carboxylate and tri-carboxylate and binds to succinates (K_m value of 20-102 μM), α-ketoglutarate (important physiologically substrate for NaDC3), and citrate and inhibited by other TCA intermediates such as fumarate, oxaloacetate, and malate. Others physiological metabolites substrate for NaDC3 of Kreb's cycle includes glutarate and *N* -acetyl-$_L$-aspartate [10]. It is primarily found in brain, placenta, kidney, liver, and pancreas [2]. Citrate transport by NaDC3 is also stimulated by acidic pH. Some of the oral chelators such as*meso* -2,3-dimercaptosuccinate (succimer) and dimercaptopropane-1-sulfonate (Dimaval) interact with NaDC3, others are antibacterial benzylpenicillin, NSAID flufenazine and immunosuppressive drug mycophenolic acid [10]. The sodium dicarboxylate cotransporters NaDC1 located in the brush bordered and NaDC3 in basolateral cell membranes transport three sodium ions and one dicarboxylates in case of succinates and glutarate generating inward currents whereas, oxalate and malonate cannot. The*trans* dicarboxylate maleate generated current like the succinate as compared to the *cis* substrate. Both glutarate and α-ketoglutarate showed larger current as compared to succinates whereas folate and glutamate failed to do so. At 60 mV, the kinetic studies showed the value of $K_{0.5}$ to be 45 ± 13μM for α-ketoglutarate and 25 ± 12μM for succinate. At concentration of up to 5 mM lithium concentration do not inhibit rat and flounder orthologs hNaDC3 instead it mediates succinate dependent current in the absence of sodium [34].
- **Inhibitors of NaDC3**

Colas *et al* ., 2015 uses homology model for human hNaDC1, mouse mNaDC1 and mouse mNaDC3 and human hNaCT and predicted small molecules inhibitors by the virtual screening. The compound **9** was selected for generating the most potent hit. The compound **9**inhibited [^{14}C] transport activity in all the transporters except for NaCT. All the five compounds (**10-14**) inhibited one or other SLC13 members, compound **11** being the most potent inhibitors in mNaDC1 (IC_{50} = 72 μM in one experiment and 82 μM in second experiment) and the compound **15**most selective against hNaDC3 inhibition (*cis* -inhibition assay). The IC_{50} value of **11** against mNaDC1 was 85 μM (one experiment) and 100 μM in second experiment whereas it poorly inhibited the hNaDC3 (2 mM). The compound **11** was found to show the bindings with amino acid sidechains Thr236 of mNaDC1 and was specific to this transporter. The hNaCT binds with citrate and four sodium ions inhibited by the dicarboxylate **13** . The newly compounds showed inhibition at IC_{50} value in μM range[1] (**Figure 11**).

Figure 11: Compounds representing the SLC13 members inhibitors;**15** being the most selective against hNaDC3

SLC25A1/Citrate/isocitrate Inhibitors

Cytosol citrates provide the source of acetyl CoA for fatty acid and sterol biosynthesis and act as regulators controlling gluconeogenesis, glycolysis, and lipogenesis. Conversely citrate/isocitrate carrier promotes citrate to the mitochondria from the cytoplasm thus causing TCA and oxidative phosphorylation with the generation of NADPH. This reverse effect led to the cancer cell expansion and growth of the tumor cells. Undoubtedly citrate/isocitrate carrier utilizes the resources to meet the energetic demand whereas the loss of it is pathogenic. Benzene tricarboxylate **16** (1st generation; analogs of citrate) interferes with the citrate bindings proteins at high concentration (5 mM) acts as citrate transporter protein inhibitors (CTPI). The second inhibitors CTPI-1; **17** was effective also at higher concentration of 1-2 mM and acts as competitive inhibitor. There is 20-fold improvement in the activity with CTPI-2; **18** as compared to CTPI-1; **17** and inhibits the CIC at a lower dose of 10-50 μM. Also, CTPI-2; **18** reverts steatosis, reduces obesity, glucose intolerance, triglycerides, and cholesterol levels [35].**Figure 12** showed the structure of first, second and third generation of citrate/isocitrate inhibitors. High levels of SLC25A1 showed enhanced levels of immune checkpoints inhibitors for antibody targeting and pro-inflammatory response for tumor killings. The inhibition of SLC25A1 reduced gluconeogenesis (involved in regulation of blood glucose levels) and thereby

normalizes the hyperglycemia and glucose tolerance [36]. The SLC25A1 specific CTPI-2 inhibitors reduces macrophage infiltration, preventing steatohepatitis, revert symptoms of inflammatory steatohepatitis and also modify obesity related with high fat. Inhibition of SLC25A1 inhibits peroxisome proliferator-activated receptor gamma (PPARγ) which inhibits the gluconeogenic genes and thus normalizes the hyperglycemia and glucose intolerance [37].

Figure 12: First, second, and third generation citrate and isocitrate inhibitors

Citrate role in Cancer

The citrate exists as an essential molecule in the metabolism and its excess availability by the citrate transporters causes high growth and proliferation of cancer cells. Inhibiting the mitochondrial and the cytoplasm citrate transporters may provide a viable strategy to develop chemotherapeutic agents [38]. The plasma membrane citrate transporter may act as tumor marker and can help in diagnosis of cancer. Glutamine is a major source of citrate in the cancer cells as the citrate produced either by the Krebs cycle in mitochondria or by reductive carboxylation of glutamine. The mitochondria activity is reduced in cancer, so the major source of citrate synthesis is through increase reverse activity of the Kreb's cycle. The plasma membrane citrate transporter may be considered as a novel target for the cancer identification and treatment [39]. The extracellular citrate promotes the cancer metabolism by reversing the Kreb's cycle from glutamine. It is very much possible to be observed in citrate rich organs such as liver, brain and bones where it may enhance the secondary tumor growth, metastasis and in colonizing the cancer cells survival [40]. The cancer cells have increased levels of the ATP citrate lyase than normal cells such as colorectal, breast, non-small lung cell and hepatocellular cancers, etc. Overexpression of the ATP citrate lyase may be inhibited by either chemical inhibitors or knockdown of ATP citrate lyase [41]. Studies have demonstrated that increase in the citrate or inhibition of ATP citrate lyase stop the tumor growth, inhibit the key anti-apoptotic Mcl-1, and increase in cisplatin sensitivity. Reduced formation of acetyl CoA from decreased citrate concentration causes deacetylation of the proteins and inhibition of apoptosis and epigenetic changes thereby resulting in cancer aggressiveness [42]. The decreased acetyl CoA (reduced intracellular lipid levels and histone acetylation) was also observed *via*caspase-10-mediated cleavage of ATP citrate lyase. The decreased histone acetylation downregulates metastatic and proliferative genes [43].*In vitro* studies showed that cancer cells deprived of citrate with specific inhibitor gluconate (plasma membrane citrate transporter) inhibit the metastases and reduces the growth of human pancreatic cancer cells and angiogenesis *in vivo* . The above findings provide evidence that citrate metabolite is an important support for the progression of cancer [44]. Results of some preclinical studies showed that high doses of citrate exhibit anticancer effects that includes inhibition of signaling pathway (IGF-1R/AKT), inhibition of growth of tumors, and promotion of apoptosis [45]. The high dose of citrate inhibited the A549 lung cancer growth. The increased citrate concentration makes tumor cell differentiation, higher infiltrating T-cells, increased cytokines and inhibited IGF-1R-AKT-PTEN-elF2a phosphorylation. Both the glycolysis and the TCA cycle were found to be suppressed *in vitro* [46]. The cancer cells have more citrate oxidizing potential as compared to the normal prostate cells. The level of the citrate gets decrease while there is an increase in the levels of the choline moving from normal cells to the cancerous prostate analyzed by magnetic resonance spectral imaging [3]. Cancer cells development causes extracellular acidity which in turn makes many anticancer drugs ineffective as acidity promotes protonation and decreases internalization. The phosphofructokinase-1 (PFK1) and PFK2 (regulator of the glycolysis) may be inhibited by high concentration of citrate as these kinases enhances the glycolysis, signaling cascade and cell cycle progression against *in vitro* studies in HCC cell lines. These inhibition decreases the ATP in the cells, acts against the HIF-$_1\alpha$ and PI3K/AKT signaling. This promotes apoptosis, increase in the action of cisplatin treatment, and inhibits the cancer cell growth [47].

1. **Citrate role in Inflammation**Citrate/isocitrate may increase inflammation and thus inhibiting the citrate/isocitrate reduces the citrate transport and hence decrease levels of pro-inflammatory prostaglandins E2 (PGE2) and nitric oxide (NO; mediators of the inflammation response) [35]. Citrate is considered to produce pro-inflammatory molecules. Excess citrate gets cleaved into acetyl-CoA and oxaloacetate by ATP citrate lyase in the cytosol after translocating from the matrix *via* mitochondrial

citrate carrier. Acetyl CoA produces NADPH for NO and ROS production by using PGE2 and oxaloacetate. Itaconate derived through citrate results in the synthesis of inflammatory mediators and thus inhibiting either citrate or the ATP citrate lyase can results in reduction of inflammatory mediators [48]. Infantino *et al* ., in a study showed that citrate carrier has a role in the inflammation as citrate carrier mRNA and protein increase the lipopolysaccharide (LPS)-activated immune cells thus citrate carrier gene silencing and its inhibition significantly reduce nitric oxide and ROS and prostaglandins [49]. The inhibitors of ATP citrate lyase; tricarballylic acid could reduce the citrate induced TNF-α as ATP citrate lyase increased the citrate metabolism. The tricarballylic acid analogue of citrate has lower affinity to chelate with calcium and thus able to block ATP citrate lyase more effectively thereby reducing the augmentation efficiency of the citrate to LPS induced TNF-α inflammatory response [50]. In LPS stimulated RAW 264.7 macrophages calcium citrate treated cells showed reduced intracellular ROS and increased in activities of antioxidant enzymes. It also inhibits the NO production and other pro-inflammatory markers such as iNOS, COX-2 and NF-$_k$B. The pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) were also reduced indicating that calcium citrate has anti-inflammatory properties [51]. There was higher expression of SLC25A1 (mitochondrial citrate carrier) and ATP citrate lyase in Bechet's syndrome patients (multisystemic inflammatory disorder). Mitochondrial citrate or its metabolites such as acetyl-CoA and OAA are important mediators of inflammation [52]. Citrate metabolism into acetyl-coenzyme A is utilized for fatty acid synthesis and protein acetylation which in turn are linked with macrophage and active dendritic cells activation. One of the inhibitors itaconate or methylene succinate possesses antibacterial and anti-inflammatory agents by inhibiting succinate dehydrogenase [53]. **Figure 13** showed the structure of itaconic acid or methylene succinic acid.**Figure 13** : Structure of itaconic acid or methylene succinic acid

2. **Citrate role in CNS physiology**The astrocytes in the CNS produce a large amount of citrate. It can be reflected by the high concentration of the citrates in cerebrospinal fluids (CSF). The human SLC13A5 loss causes severe epilepsy and encephalopathy. This is not obligatory as SLC13A5-knockout mice do not show any evidence of epilepsy or encephalopathy [54]. In another study however it was confirmed by Henke *et al* ., that SLC13A5 deleted mouse showed increased frequency of seizure (observed in the 50% of the mice and low frequency in other 50%), alteration in the citrate concentration in CSF (lower than in the plasma but mutant mice citrate is higher than wild types) and brain tissue and pro-epileptogenic excitability changes in the hippocampus. The generalized seizure is mainly associated with the knockout mice. The histological changes in hippocampus and para hippocampus do not show any neuronal changes in either wild or knockout mice [55]. The overexpression of the SLC13A5 neuron-specific receptors in mice showed autistic-like behaviors. It also showed the disrupted white matter integrity and different synaptic functions and structures. The hippocampus and the cortex area showed exceptional adaptation in the complete sets of proteome and acetyl proteome. The study showed that there is clear link between the aberrant citrate/acetyl CoA flux and autistic-like behaviors [56]. Citrate has a role to chelate the divalent cations including Ca, Mg, and Zn and thus regulate the extracellular concentration of these ions for excitation in the neurons and further acts as modulator of glutamate receptors and NMDA subtypes of receptors in CNS. An increase in citrate or decrease in the glutamine concentration was confirmed both *in vivo* and*in vitro* with use of fluorocitrate which led to the inhibition of the TCA cycle and loss of GABA synthesis. There is reported correlation of increased CSF glutamine and Mg^{2+}concentration in depressed patient [57]. It also showed hyperfunction of NMDA receptor signaling through some literature studies. This can be better understood by the zinc chelation hypothesis. The SLC13A5 mutation causes extracellular citrate to rise which then start chelating zinc. Negative allosteric regulation of the NMDA receptors is lost with large Ca^{2+} influx and that leads to enhance NMDA receptor mediated synaptic transmission and pro-convulsant effect [54] **Figure 14.Figure 14:** Zinc chelation hypothesis; the normal SLC13A5 receptor transport of extracellular citrate to cytoplasm takes place, the NR2A subunits of NMDA receptors occupied by the zinc ion regulate the Ca^{2+} influx. Mutated SLC13A5 led to increase in the extracellular citrate, chelates the zinc and allosteric inhibitors to NMDA receptors is freed led to increased Ca^{2+}influx and enhanced NMDA receptor-mediated synaptic transmission. The SLC13A5 deficiency dysregulate the

hepatic metabolism and initiate the pediatric epilepsy. Kumar *et al.,* used the^{13}C isotopes to study the pathophysiology of the disease against SLC13A5 deficient hepatocellular carcinoma cells and primary rat cortical neurons and found that hypoxic HCC cells use extracellular citrate for fatty acids and TCA intermediates. Under limited conditions of glutamine/other nutrients and oxygen conditions, the citrate supports the growth and lipid synthesis of HCC cell lines. Also, it was evident that citrate uptake in Huh7 cells is protective against Zn^{2+} cytotoxicity [58]. The congenital disorder 22q11.2 deletion syndrome was studied by Napoli *et al* . and found to have serious role in the mitochondrial citrate transporter SLC25A1. There were metabolic differences with dysregulation in energy homeostasis in 22q11.2 deletion syndrome children. The children showed elevated levels of cholesterol and 2-hydroxyglutaric acid (2HG) and less than half were also associated with hyperprolinemia. It was also observed that there was a shift from oxidative phosphorylation to the glycolysis in 22q11.2 deletion syndrome children and increase in reductive metabolism of α-ketoglutarate and glucose as carbon source for TCA cycle [59].

3. **Role of citrate in fatty liver disease**

The SLC13A5 receptors present in the sinusoidal membrane of the hepatocyte's uptakes the circulating citrate for the metabolic use [54]. It was evident that dysregulated expression of ATP citrate lyase was found in the patient with non-alcoholic fatty liver disease (NAFLD; associated with lipid accumulation). The lipid accumulation increases the endoplasmic reticulum (ER) stress and upregulated ATP citrate lyase expression through internal ribosome entry site mediated translation. The translation of mRNA is Cap independent and stimulate*de novo* lipogenesis [60]. Guo *et al.,* showed that acetylation of the ATP citrate lyase at Lys-540, Lys-546 and Lys-554 (3K) increases protein stability and promotes cell proliferation and lipid synthesis in the lung cancer cells. The increased acetylation of 3K and decreased SIRT2 levels were also found in both livers of the mice and the human with NAFLD. Overexpression of hepatic SIRT2 decreased the acetylation of 3K and protein levels and thereby lessen the hepatic steatosis in high fat/high sugar diet-fed mice[61]. Dysregulation of hepatic ATP citrate lyase led to hepatic steatosis, insulin resistance and hyperglycemia. It was evaluated in leptin-deficient *db/db*mice and was found that ATP citrate lyase is selectively elevated in the liver and not in white adipose tissues. Abolition of ATP citrate lyase reduced the hepatic content of acetyl CoA and malonyl CoA and inhibited the *de novo* lipogenesis in hepatic steatosis in *db/db*mice. It also inhibited nuclear hormone proliferator-activated receptor-gamma coactivator (PPAR) γ receptors. In hepatic abrogated ATP citrate lyase improves systemic glucose metabolism by down regulated expression of gluconeogenic genes in the liver and enhanced insulin sensitivity [62]. Increased citrate levels were observed in the NAFLD may be due to increased fatty acids. The excess citrate excites hydrogen peroxide (hydroxy radicals) induced oxidative stress in HepG2 cells [63]. The cytoplasmic citrate by plasma citrate transporter (SLC13A5), ATP dependent lyase (ACLY), and mitochondrial citrate regulate *de novo* lipogenesis. The chronic liver disease caused by NAFLD progress due to upregulation of the *de novo* lipogenesis. Curcumin was investigated against NAFLD induced by oleic acid and palmitic acid in primary mouse hepatocyte and with high-fat and high-fructose diet. Curcumin was found to reduce oleic acid/palmitic acid or high fat and high fructose induced NAFLD (hyperlipidemia and hepatic lipid deposition). Curcumin may block the transporter SLC13A5, SLC25A1 and enzyme activity of ACLY and improves the metabolic lipid disorder. The mechanism by which curcumin acts is by blocking the citrate transport (improves dysregulated SLC13A5) and inhibitory effect of ACLY (cleaves citrate into acetyl-CoA and oxaloacetic acid) in oleic acid and palmitic acid stimulated HepG2 cells *via* AMPK-mTOR signaling pathway. Curcumin has significant lipid lowering effect and inhibit the overexpression of SLC13A5 and ACLY. This further reduces the upregulation of *de novo* lipogenesis and regulates the lipid accumulation in NAFLD in mice [64]. The hydroxy citric acid extract from the *Garcinia cambogia* was evaluated for the inflammatory, atherogenic and metabolic biomarkers in 40 obese women with NAFLD. The results showed decrease in the macronutrient intake and energy in both the hydroxy citric acid group and the control groups. There was also reduction in the levels of the triglycerides, total cholesterol, fasting blood sugar, low density lipoproteins cholesterol (LDL-C) and increase in high density lipoprotein cholesterol (HDL-C) in the hydroxy citric acid. The ratio of TG/HDL-C ratio was decreased as compared to the control. The inflammation factors were not reduced as such in the hydroxy citric group but it improved metabolic factors [65].

Role of citrate in the treatment of obesity and type 2 diabetes mellitus (T2DM) The ATP citrate lyase, the one converting citrate to acetyl CoA, is highly induced in the obese patient with chronic kidney disease. The increase activity of ATP citrate lyase leads to the ectopic accumulation of lipid, glomerulosclerosis, and albuminuria. It also upregulates many rate-limiting lipogenic enzymes and promotes *de novo* lipogenesis for lipid accumulation. The raised acetyl CoA also causes hyperacetylation at the sites of H3K9/14 and H3K27. The citrate inhibitor completely blocked the hyperacetylation as well as induction of lipogenic enzymes. The ATP citrate lyase promotes renal lipid accumulation, fibrogenesis and renal injury in obesity [66]. A clinical study was conducted for 3 months in 100 obese patients to evaluate the effects of hydroxy citric acid and one of the major ingredients of *Garcinia cambogia* extract. Study results showed that there was significant reduction in the body weight, serum triglycerides, HDL, and LDL levels. The hydroxy citric acid treatment may reduce the body weight gain and fat deposits in obese patients [67]. The enzymatic activities and proteins levels of the citrate synthase were reduced in obese patients in another study of human omental adipose tissue [68]. The increased activity of the ATP citrate lyase in the livers of the hereditary obese mice is two to four times more than non-obese siblings and the activity was observed to be reduced in starvation and increased by refeeding. The increased in activity is also found to be increased after the onset of lactation [69]. The hypothesis that exogenously taken citrate in the form of processed foods and drinks contribute to the weight gain was evaluated in mice fed with citrate with or without sucrose. The results showed that neither it increased the weight gain nor change the lipid pattern in any of the tested groups (citrate or citrate + sucrose). The group with citrate + sucrose enhanced the pro-inflammatory cytokines (TNF-α, IL-6, IL-10 and IL-1β), fasting glycemia and glucose intolerance. Thus, citrate consumption may act as contributing agent for the complication associated with obesity such as altered glucose metabolism or adipose tissue inflammation [70]. Patients with obesity and diabetes mellitus are more likely to be associated with low urine citrate excretion among nephrolithiasis (15% more than 7% control). Uric stone and calcium oxalate monohydrate are two most common stone in citrate wasting groups (low urine citrate excretion). It was evident that citrate wasting groups showed higher uric acid, calcium, oxalate, and sodium [71]. In diabetes patients, the enzymatic activity was reduced rather than proteins levels. The key enzymes of the TCA cycle (citrate synthase) were affected. The compounds shown in the **Figure 15** with the general formula**19** claims for treating diseases related with the citrate transporter. The compound is used for treating diabetes especially T2DM, obesity, and other age-related disorders. Among them the compound **20** and **21** showed highest functional activity against citrate INDY in HEK293 and HepG2 cell lines [72]. The low citrate synthase activity as tested in A/J strain variant of H55N polymorphism accumulated more fats and was tolerant to glucose. The knockdown citrate synthase activity accelerated palmitate induced apoptosis in 50% of the tested in C2C12 mouse muscle cells [73]. Citrate administration could also decrease diabetic adverse cardiac changes. It decreased mean glucose levels whereas glucose levels are less affected in streptozotocin induced diabetic rats. Citrate application with the antioxidants decreased blood glucose levels protecting the β-cells of the pancreas from damage. The citrate groups reduced the harmful effect on the heart tissue of the diabetes. Also, caspase 3 immunoreaction optical density was decreased in citrate group and integrity of the intercalated discs was found to be maintained as compared to the diabetic groups [74]. The rate of citrate transport was reduced to 35% in diabetic mitochondria. There was also an increase in cholesterol contents and ratio of cholesterol/phospholipid ratio. The analysis confirmed the accumulation of hepatic citrate carrier mRNA, and decreased levels of the protein. The lower contents of both the mitochondrial citrate carrier and protein is because of decrease citrate carrier in diabetes [75].**Figure 15:** General formula and most potent compound for treating type 2 diabetes, obesity, and age-related disorders

Conclusion

The sodium dependent SLC13 transporter of di and tricarboxylates inhibitors are intended to treat various metabolic disorders including diabetes and obesity. The drugs mechanism and favorable risk-benefit ratio needs to be understood to further characterize their role against neurological disorders and cancer. In the recent past, significant efforts have been made in characterization of the proteins and discovery of novel inhibitors against these transporters. Reviewed literature revealed that in recent years there is more focus on basic residue and neutralization of it which may results in discovering the beneficial effects. The emphasis

on mutation unlike from the brain alleviates the symptoms associated with the metabolic diseases. The NaCT has many functional roles to play such as glycolysis, fatty acids and cholesterol and regulating it with exogenous di and tri carboxylates regarded as potential treatment of various disorders. More specific ligands may be designed that may act as a lead molecule for future drug developments. Some of the key findings of the review are firstly, the amino acid residue Ar108 is present in highly conserved H4c domain of the NaCT receptors and neutralization of this basic residue inhibited the transport activity. Secondly crosslinking either the inwards or outward facing state of the receptors by cysteine mutants or excess $HgCl_2$ reduced the transport activity. Thirdly mutation of amino acids presents in the transmembrane pore and citrate binding sites completely inhibited the citrate transport. Similarly, NaCT knockout mice also confirmed for reduced lipogenesis and higher energy expenditure. Fourth the 2-hydroxycarboxylate inhibitors modifications of either-OH or -COOH reduce the affinity of the transporter and *S*enantiomers binds efficiently while *R* enantiomer is devoid of any affinity. Citrate has several roles to play in the normal physiological process of the body and therefore its regulation may alleviate the symptoms associated with the cancer, inflammation, fatty liver diseases, obesity, diabetes, and CNS disorders. Consequently, both the receptor structure and the inhibitors design of new molecules needs to be taken in account to mitigate metabolic disorders.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the writing of this paper.

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