Review

Thiazolidine-2,4-dione derivatives: Programmed chemical weapons for key protein targets of various pathological conditions

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Abstract

Thiazolidine-2,4-dione is an extensively explored heterocyclic nucleus for designing of novel agents implicated for a wide variety of pathophysiological conditions, that is, diabetes, diabetic complications, cancer, arthritis, inflammation, microbial infection, and melanoma, etc. The current paradigm of drug development has shifted to the structure-based drug design, since high-throughput screenings have continued to generate disappointing results. The gap between hit generation and drug establishment can be narrowed down by investigation of ligand interactions with its receptor protein. Therefore, it would always be highly beneficial to gain knowledge of molecular level interactions between specific protein target and developed ligands; since this information can be maneuvered to design new molecules with improved protein fitting. Thus, considering this aspect, we have corroborated the information about molecular (target) level implementations of thiazolidine-2,4-diones (TZD) derivatives having therapeutic implementations such as, but not limited to, anti-diabetic (glitazones), anti-cancer, anti-arthritic, anti-inflammatory, anti-oxidant and anti-microbial, etc. The structure based SAR of TZD derivatives for various protein targets would serve as a benchmark for the alteration of existing ligands to design new ones with better binding interactions.

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1. Introduction

Thiazolidine-2,4-dione (TZD) is an important heterocyclic ring system that exhibit a range of pharmacological activities, but not limited to, including anti-hyperglycemic, anti-cancer, anti-arthritic, anti-inflammatory and anti-microbial, etc. Among them, anti-hyperglycemic is the widely studied effect of TZD derivatives that has also been extended to the development of clinically used ‘glitazone’ drugs such as rosiglitazone, pioglitazone, lobeglitzazone and troglitazone, etc.

The first ever pharmacological evaluation, that is, anti-TB activity, of any TZD derivative was reported by an Italian scientist ‘Vistentini’ in 1954 and following this, in the same year, Marshall and Vallance reported the anti-convulsing activity of other TZD derivatives. Later on, in 1960s and 1970s, various other pharmacological and toxicological effects of TZD derivatives were explored by different research groups. In 1982, the research on TZD gained high standards when Sohda and co-workers selected ‘ciglitazone’ for clinical evaluation in hyperglycemia. Although ‘ciglitazone’ failed to achieve desired clinical standards, the continuous research on TZD resulted in ‘troglitazone’ that was approved by FDA in 1997 for hyperglycemic conditions. Thereafter, in 1999, two more TZD derivatives, that is, ‘rosiglitazone’ and ‘pioglitazone’ gained FDA approval, and most recently in 2013, ‘lobeglitzazone’ has been approved for use in Korea.

Chemically, TZD nucleus has two carbonyl moieties at 2nd and 4th position sparing behind –NH and methylene (–CH2) of thiazole ring for structural modifications to develop various analogues (molecule 1). In the literature, several TZD derivatives have been reported by structural variations at these two positions that in turn led to the development of biologically active molecules against a broad spectrum of protein targets, but not limited to, such as Peroxisome Proliferator activated receptor (PPARγ), Aldose reductase (ALR2), Phosphoinositide 3-kinase (PI3Kγ), Phosphoinositide 3-kinase (PI3Kα)/Mitogen activated protein kinase kinase (MEK), Pim kinase, Protein tyrosine phosphatase 1B (PTP1B), Cyclooxygenase (COX-2), UDP-N-acetylmuramoylalanine-β-glutamate Ligase (MurD ligase), Histone deacetylase (HDAC) and tyrosinases, etc. (Fig. 1).

Recently, review articles described the role of TZD nucleus in various disease conditions; however, our draft is strictly focused on the target level (i.e., specific proteins) therapeutic benefits of TZD derivatives. In this review article, we mainly tried to compile the structure activity relationship (SAR) of TZD derivatives with different protein targets involved in a wide variety of pathological conditions.

![Figure 1. Multi-targeted activity of thiazolidine-2,4-dione.](image)

![Scheme 1. Synthesis of thiazolidine-2,4-dione using thiocarbamate and chloroacetic acid.](image)
2. Chemistry

2.1. Possible ways to synthesize TZD nucleus

The synthesis of TZD nucleus has been carried out using different starting materials including thiocarbamates, thioureas, thiosemicarbazones and alkali thiocyanates, etc. The in situ preparation of alkyl thioncarbamate is carried out by reacting carbonyl sulfide with primary amine in the presence of potassium hydroxide. These alkyl thioncarbamates are then reacted with \( \alpha \)-haloalkanoic acids to produce thiolcarbamates, which cyclize to yield TZD nucleus (Scheme 1).

The most commonly used synthetic protocol is refluxing of \( \alpha \)-chloroacetic acid with thiourea for 12 h that yields TZD nucleus via 2-imino-4-thiazolidinone intermediate as described in Scheme 2. This reaction can be further accelerated using microwave (MW) assisted technique, in which initially \( \alpha \)-chloroacetic acid is reacted with thiourea in cold conditions to yield 2-imino-4-thiazolidinone that is further irradiated with microwave at 250 W for 5 min to obtain white crystals of TZD (Scheme 3).

The third synthetic protocol is the reaction of thiosemicarbazone of acetone with chloroacetic acid ester, which, in the presence of sodium ethoxide yields 2-hydrazino-4-thiazolidinone that further, in the presence of dilute hydrochloric acid, yields TZD nucleus (Scheme 4).

Due to the presence of two carbonyl groups and one \( \alpha \)-hydrogen, TZD nucleus undergoes different tautomerism such as amide-imidol and/or keto-enol (Fig. 2). The \( \text{pK}_a \) value of the TZD nucleus has been reported to be 6.82.

2.2. Physico-spectral properties of TZD moiety

The unsubstituted TZD nucleus exists as a white crystalline solid with melting point range of 120–122 °C. The infrared (IR) spectrum of the same depicts a NH stretch at 3145 cm\(^{-1}\), CH\(_2\) stretch at 2923 cm\(^{-1}\), carbonyl stretch at 1738 cm\(^{-1}\) and 1659 cm\(^{-1}\) (for C2 and C4 carbonyls, respectively), C-N stretch at 1318 cm\(^{-1}\) and 1165 cm\(^{-1}\), and C-S stretch at 808 cm\(^{-1}\) and 727 cm\(^{-1}\). The \( ^1 \text{H} \) NMR depicts a singlet for CH\(_2\) protons at \( \delta = 3.98 \) (ppm) and a broad singlet for NH protons at \( \delta = 12.51 \) (ppm), which is due to the deshielding effect of two carbonyl groups present on both sides. The mass spectrum of TZD depicts a base peak at m/z 116 (100%).

2.3. Synthesis of various TZD derivatives

The free –NH and –CH\(_2\) moieties of TZD core are referred to as substitution positions, which have been explored to develop a wide variety of TZD derivatives.
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Reactants</th>
<th>Products</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TZD + triethylamine (TEA) + Br</td>
<td><img src="image1" alt="Product 1" /></td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image2" alt="Product 2" /></td>
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<td></td>
<td><img src="image3" alt="Product 3" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image4" alt="Product 4" /></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TZD + BrCH₂COOCH₃ + K₂CO₃/acetone</td>
<td><img src="image5" alt="Product 5" /></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="image6" alt="Product 6" /></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TZD + BocNHCH₂CH₂Br + TBAI/acetone</td>
<td><img src="image7" alt="Product 7" /></td>
<td>34, 43</td>
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<tr>
<td></td>
<td></td>
<td><img src="image8" alt="Product 8" /></td>
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</tr>
<tr>
<td>4</td>
<td>TZD + K₂CO₃, TBAI/acetone + Br⁺Br⁻</td>
<td><img src="image9" alt="Product 9" /></td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>TZD + KOH/EtOH + Cl⁻Br⁺</td>
<td><img src="image10" alt="Product 10" /></td>
<td>45</td>
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<tr>
<td></td>
<td>R = 4-Me, 4-OMe</td>
<td><img src="image11" alt="Product 11" /></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>TZD + KOH/acetone +</td>
<td><img src="image12" alt="Product 12" /></td>
<td>46</td>
</tr>
<tr>
<td>7</td>
<td>TZD + K₂CO₃, TBAI/DMF +</td>
<td><img src="image13" alt="Product 13" /></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>R = H, 4-CH₃, 4-OCH₃, 4-Cl, 4-Br</td>
<td><img src="image14" alt="Product 14" /></td>
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<tr>
<td>8</td>
<td>TZD + K₂CO₃/acetone + R-Cl</td>
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<td>48</td>
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<tr>
<td></td>
<td>Where R = methyl, allyl, isopropyl, butyl</td>
<td><img src="image16" alt="Product 16" /></td>
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</tr>
<tr>
<td>9</td>
<td>TZD + K₂CO₃ +</td>
<td><img src="image17" alt="Product 17" /></td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>R = 4-Cl, 4-CH₃</td>
<td><img src="image18" alt="Product 18" /></td>
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TEA, triethylamine; TBAI, tetrabutylammonium iodide; K₂CO₃, potassium carbonate; KOH, potassium hydroxide; HCHO, formaldehyde; DMF, dimethylformamide.
2.3.1. Substitutions at –NH moiety of TZD core

The free –NH moiety of TZD has primarily been alkylated using alkyl or aryl halides in the presence of alkali including potassium carbonate\(^{33}\) (Scheme 6), tetrabutylammonium iodide,\(^{34}\) or sodium hydride\(^ {35}\) using acetone or DMF as solvent. The different derivatives that can be obtained with substituting free –NH of TZD core have been mentioned in Table 1.

2.3.2. Substitution at free –CH\(_2\) moiety of TZD core

The methylene moiety has critically been substituted with aldehydes or ketones leading to formation of arylidene derivatives, via ‘Knoevenagel’ condensation. The condensation of aldehyde and TZD has been carried out under different reaction conditions including, few drops of piperidine using ethanol or methanol as solvents for 7–42 h\(^ {36}\) (Scheme 7), or anhydrous sodium acetate in glacial acetic acid\(^ {37}\) while condensation of TZD with ketones has been carried out in the presence of ammonium acetate or piperidinium acetate in toluene or ethyl acetate.\(^ {38}\) Attempts have also been made to develop an eco-friendly reaction condition for Knoevenagel condensation using l-tyrosine in water\(^ {39}\) or β-alanine in acetic acid,\(^ {40}\) or baker’s yeast in ethanol.\(^ {41}\) These reactions have enabled coupling of TZD with various benzylidene derivatives as well as other heterocyclic ring moieties such as chalcones, flavones, acridines, furfurals, dibenzocycloheptanone, etc. Various aldehydes and ketones that are condensed with the –CH\(_2\) moiety of TZD are discussed in Table 2.

3. TZD derivatives: target-based SAR

In this section, we specifically described SAR of TZD derivatives against various protein targets including PPAR\(_γ\), PTP1B, ALR2, Pim

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**Table 2** Examples of some of the substitutions of aldehydes and ketones reported in literature

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Reactants</th>
<th>Products</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
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<td>TZD + R CHO</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TZD + N CHO</td>
<td><img src="image2" alt="Product Image" /></td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>TZD + H O</td>
<td><img src="image3" alt="Product Image" /></td>
<td>43,44</td>
</tr>
<tr>
<td>4</td>
<td>TZD + R CHO</td>
<td><img src="image4" alt="Product Image" /></td>
<td>51</td>
</tr>
<tr>
<td>R = H, 6-Br, 8-OMe; R(_2) = Me, H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TZD + R(_1) R(_2) R(_1) = H, 6-Br, 8-OMe; R(_2) = Me, H</td>
<td><img src="image5" alt="Product Image" /></td>
<td>52</td>
</tr>
<tr>
<td>6</td>
<td>TZD + H(_2)N CHO</td>
<td><img src="image6" alt="Product Image" /></td>
<td>45</td>
</tr>
<tr>
<td>(F) + α-bromoacrylic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>TZD + R CHO</td>
<td><img src="image7" alt="Product Image" /></td>
<td>47</td>
</tr>
<tr>
<td>R = H, CH(_2)</td>
<td></td>
<td></td>
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</tr>
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</table>

(continued on next page)
### Table 2 (continued)

<table>
<thead>
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<th>Example No.</th>
<th>Reactants</th>
<th>Products</th>
<th>References</th>
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<tbody>
<tr>
<td>8</td>
<td>TZD +</td>
<td><img src="image1" alt="Structure" /></td>
<td>R = F, Cl, Br, Me, t-Bu, Phenyl, methoxy, nitro, piperidene, morpholine</td>
</tr>
<tr>
<td>9</td>
<td>TZD + HOOC</td>
<td><img src="image2" alt="Structure" /></td>
<td>X = NH, O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(G)</td>
<td>(G) * γ-glutamic acid dimethyl ester hydrochloride + TBTU/TEA/DCM</td>
</tr>
<tr>
<td>10</td>
<td>TZD + <img src="image3" alt="Structure" /></td>
<td><img src="image4" alt="Structure" /></td>
<td>R = 2-OH, 3-OH, 4-OH, 2-OMe, 2-Cl, 2-Br, 2-0H, 2-F, 3-F, 4-F</td>
</tr>
<tr>
<td>11</td>
<td>TZD + <img src="image5" alt="Structure" /></td>
<td><img src="image6" alt="Structure" /></td>
<td>R = H, Cl, Br, OMe, OEt</td>
</tr>
<tr>
<td>12</td>
<td>TZD + <img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td>(H) * PPh3/DAED/aq acetic acid + <img src="image9" alt="Structure" /> or <img src="image10" alt="Structure" /></td>
</tr>
<tr>
<td>13</td>
<td>TZD + <img src="image11" alt="Structure" /></td>
<td><img src="image12" alt="Structure" /></td>
<td>R = Cl, F, Br, OMe</td>
</tr>
<tr>
<td></td>
<td>TZD + <img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
<td>R1 = 2-OCH2COOH, 4-OCH2COOH; R2 = H, 3-OMe, 3-OEt, 5-Br</td>
</tr>
<tr>
<td>14</td>
<td><img src="image15" alt="Structure" /></td>
<td><img src="image16" alt="Structure" /></td>
<td>(I) * or</td>
</tr>
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(continued on next page)
kinase, PI3Kα, PI3Kα/MEK, Tyrosinase, MurD, COX-2 and Histone deacetylase involved in different pathological conditions.

3.1. Peroxisome proliferator activated receptor (PPARγ)

PPARs are nuclear receptor proteins that function as transcription factor for the regulation of target genes in diabetes. Among the different isoforms of PPAR, PPARγ, encoded by PPARγ gene, is mainly expressed in adipose tissue, large intestine and spleen. The high expression of PPARγ in adipocytes plays an important role in the regulation of adipogenesis, energy balance and lipid biosynthesis. On the cellular level, PPARγ forms a heterodimer with retinoid X-receptor, and the binding of TZD derivatives induce conformational changes in this heterodimer to promote peroxisome proliferator response elements (PPREs), present on promoters of PPARγ target genes, to stimulate the transcription of PPARγ. The stimulation of PPARγ down-regulates the genes of adipocytes derived insulin-resistant factors, that in turn, intensify the signal transduction associated with insulin response. The domain structure of PPARγ starts with N-terminal that consists of A/B domain, which is involved in ligand independent activation via α-helix fragment AF-1, possessing ligand-independent activation ability (Fig. 3). The N-terminal domain is followed by a C-domain which is a characteristic for DNA binding. The C-domain contains two zinc finger-like motifs, responsible for binding of the receptor to DNA promoter of target genes. The N- and C-terminal domains are connected through a D-domain or ‘hinge’ region, which is regarded as flexible region. The domain structure continues to E/F-domain or ligand binding domain (LBD) composed of 13 α-helices and four stranded β-sheets. The ligand binding domain is primarily responsible for the dimerization and ligand dependent activation of PPARγ receptor. The shape of PPARγ binding pocket is Y-like, which consisted of an entrance pocket and two ‘Arm’ pockets (1 and II) along with a charge-clamp. This binding pocket exhibit recognition elements including a cationic or hydrophilic site, flat aromatic region and oxygen atom anchor site. Next to LBD is an F-domain, for which the function is still not clear and needed to be elucidated.

Earlier in 1982, TZD derivatives (AL-321, 2) have been reported to potentially reduce the levels of cholesterol and triglycerides in genetically obese and diabetic mice; however, the cellular basis of this effect was described by Lehmann and co-workers. They reported TZD derivatives as the potent and selective activators of PPARγ—later on termed as ‘glitazone’ class of PPARγ agonists. Further, the modifications of lipophilic tail of AL-321 resulted in the identification of ciglitazone (3), pioglitazone (4), rosiglitazone (5), troglitazone (6) and englitazone (7) that are potent agonists of PPARγ and acts as ‘insulin sensitizers’. Lobeglitazone (8), a pyrimidine aryl ether derivative of rosiglitazone, was discovered by Chon Kun Dang Pharmaceuticals™, discovered by Daiichi Sankyo Co.™, discovered by Daiichi Sankyo Co.™, showed 178-fold more efficacy as compared to pioglitazone, and also described beneficial effects in Phase II clinical trials. Recently, Balaglitazone (10), a product of Dr. Reddy’s Laboratories™, has been engaged in Phase III clinical evaluation that is five-fold more potent than rosiglitazone.

Although, glitazones have achieved clinical success for the treatment of diabetes, troglitazone (Rezulin®) and rosiglitazone (Avandia®) were withdrawn from the clinical practice in 2000 and 2010 due to severe hepatic and cardiac toxicity respectively. Recently in 2013, in light of the new re-evaluation of the Rosiglitazone evaluated for Cardiovascular Outcomes and Regulation of Glycemia in Diabetes (RECORD) trial, the prescribing
and dispensing restrictions of rosiglitazone have been withdrawn by FDA. Among other discovered glitazones, pioglitazone (Actos®), rosiglitazone (Avandia®) and lobeglitazone (Duvie®) are the only marketed drugs at present, since ciglitazone and englita-
zone failed clinical evaluation and troglitazone has been with-
drawn from market.
In addition to glitazones, some other TZD derivatives have also described the PPARγ agonism with less side effects and/or improved potency. For instance, oxime derivatives of TZD describe good activity for PPARγ with compound 11 showing the highest potency (IC\textsubscript{50} = 0.19 μM).\textsuperscript{59} For such derivatives, the aromatic group at the meta or para position of acetophenone oxime or acetylpyridine oxime, and the ethylene bridge between oxygen atoms of oxime moiety is required for PPARγ activation. The hybrids of TZD with quercetin through diether linkage (12)\textsuperscript{60} and with substituted chromones through methylene linkage (13) showed potent PPARγ agonism with reduced hepatotoxicity. The conjugation of TZD core with the oxadiazole ring resulted in compound 14 that exhibit no hepatic and cardiac toxicity.\textsuperscript{61} The other conjugates of TZD include with benzoxazine (15)\textsuperscript{62} benzothiathiazole (16)\textsuperscript{63} and chalcones (17)\textsuperscript{64} that possess potent PPARγ agonism, and anti-hyperglycemic potential.

To extract out the SAR of TZD derivatives for PPARγ agonism, the reported crystal structure of PPARγ co-crystallized with TZD derivative (PDB ID: 2PRG, 2.30 Å)\textsuperscript{65} and the docking interactions of TZD PPARγ agonists\textsuperscript{66} was analyzed (Fig. 4A). The TZD nucleus mainly accommodate in the cationic hydrophilic pocket primarily comprised of polar amino acid residues such as Gln286, Ser289, His323 and His449. The 2-carbonyl group of TZD depict interactions with Gln286 and His449, the 4-carbonyl group with Ser289 and His323 while the –NH group with hydroxyl moiety of Tyr473 amino acid residue. The central aromatic ring (benzene) of molecules settle into the hydrophobic environment composed of Cys285, Ile326, Leu330, Leu340 and Met364 amino acid residues. The aromatic benzylidene group show hydrophobic contacts specifically with Cys285 amino acid residue of the PPARγ binding site. An ether linkage, connecting the central aromatic ring and the terminal benzylidene moiety, provides conformational and dihedral flexibility that is required for the molecule to achieve appropriate orientation within the binding pocket. The binding site region close to the solvent accessible area consisted of hydrophobic amino acid residues, and the lipophilic interactions of molecule with these amino acid residues impart significant contribution for biological activity. Thus, a wide range of structural substitutions is possible at this position of molecules to consider such steric clashes for the improvement of PPARγ agonism. The general structural requirements of the TZD derivatives for PPARγ agonism are depicted in Figure 4B.

### 3.2. Protein tyrosine phosphatase 1B (PTP1B)

PTP1B, a non-receptor protein tyrosine phosphatase encoded by gene \textit{PTPN1},\textsuperscript{97} is involved in the negative regulation of insulin signaling pathway making it a promising target for the treatment of Type II diabetes mellitus and obesity.\textsuperscript{88} PTP1B is expressed ubiquitously especially on the cytosolic side of endoplasmic reticulum and functions to dephosphorylate the activated insulin receptor or insulin receptor substrates (IRS).\textsuperscript{89–91} Thus, the inhibition of PTP1B can be beneficial in improving the uptake and metabolism of glucose.

The crystal structure of PTP1B revealed the presence of characteristic active site signature motif (H/V)/C(X)\textsubscript{R/S/T} in its catalytic domain,\textsuperscript{89} and three loops within the active site present in catalytic domain. The three loops include a WPD loop with Asp181 residue, a pTyr loop with Tyr46 residue and a Q-loop with Glu262 residue. The active site of PTP1B exists inside the deep cleft of the protein, and the pTyr loop present on the outer part of active site functions to maintain the selectivity of small phospho-residues that cannot expose the base of the cleft.\textsuperscript{82,83} PTP1B mediates the catalysis of substrate through two steps: the first step include nucleophilic attack by sulfur atom of Cys215 on the phosphate of substrate followed by the protonation of tyrosyl-leaving group of substrate by the side chain of Asp181 that acts as a general acid, and lead to the formation of cysteinyl phosphate intermediate. The second step includes the hydrolysis of catalytic intermediate to release phosphate moiety that is mediated by Gln262, water molecule and Asp181—functions as a base for the hydrolysis.\textsuperscript{94}

The PTP1B inhibitory activity of TZD derivatives was first reported by Maccari and co-workers.\textsuperscript{95} The reported molecules bear p-methylbenzoic acid moiety at the –NH of TZD ring whereas different substitutions at the \textit{para} position of central arylidine ring afforded micromolar (μM) activity of the resulting molecules (compound 18, IC\textsubscript{50} = 1.1 μM). Later on, Bhattarai and co-workers reported the structural modifications at 0-benzyl group attached to benzylidine ring, and the highest potency was observed with 4-trifluoromethane (19, IC\textsubscript{50} = 5.0 μM).\textsuperscript{96} The further optimization of substitutions at the central benzylidene ring resulted in the improved potency, for example, molecule 20 with methyl-4-(trifluoromethyl)benzene sulfonate and 1-(methoxymethyl)-4-(trifluoromethyl)benzene group at the \textit{para} and ortho position, respectively displayed IC\textsubscript{50} value of 1.3 μM, and demonstrate the benefit of dual substitution for activity improvement.\textsuperscript{97} Moreover, the extension of hydrophobic tail at the \textit{para} position of benzylidene ring with benzoxoxyphenyl group drastically improved the potency, and yielded the most potent compound of TZD class of PTP1B inhibitors (compound 21, IC\textsubscript{50} = 0.69 μM).\textsuperscript{98}
The docking analysis of TZD derivative 20 within the active site of PTP1B (PDB ID: 2F71) showed the hydrogen bonding interactions of 2- and 4-carbonyl of TZD core with the side chains of Arg221 and Gln266, and the backbone of Phe182, Ser216, Ala217 and Gly218 amino acid residues (Fig. 5A). The central benzylidene ring is settled into the hydrophobic groove composed of Tyr46, Val49, Phe182 and Ala219 amino acid residues. The top of PTP1B active site consist of an YRD motif and a charged region, thus, the p–p interaction of molecule with Tyr46 or a salt bridge between basic nitrogen of ligand and Asp48 would be beneficial to infer selectivity of molecules for PTP1B. The general structural requirements of TZD derivatives to achieve PPTP1B inhibitory activity is displayed in Figure 5B.

3.3. Aldose reductase (ALR2)

Aldose reductase (ALR2)—expressed by AKR1B1 gene—is a ubiquitously expressed cytosolic enzyme of Aldo–keto reductase superfamily, and serves as an upstream (first) catalyst of the polyol metabolic pathway of diabetic complications.\textsuperscript{99–101} Basically, ALR2 is an oxido-reductase that catalyzes the reduction of aldehydes and carbonyls. At the physiological level, ALR2 is also responsible for the detoxification of a wide range of substrates including hydroxy-nonenols, catecholamines, lipid aldehydes generated during lipid peroxidation and their glutathione (GSH) conjugates, steroid metabolites (e.g., isocorticosteroids), phospholipids and atherogenic aldehydes.\textsuperscript{102} Under prolonged diabetic conditions, the marked increase in glucose level lead to the activation of ALR2 that catalyzes the NADPH-dependent reduction of glucose to sorbitol as the first step in polyol pathway.\textsuperscript{103} The excessive accumulation of sorbitol inside the cell membrane results in the increased osmotic and oxidative stress leading to the etiology of chronic diabetic complications.

The reported crystal structure of ALR2 (PDB ID: 1US0) exhibited that the single domain of this protein is folded in an eight-stranded parallel α/β barrel, which is composed of eight β-strands connected by peripheral eight α-helices.\textsuperscript{104–106} NADPH, the co-factor of ALR2, binds in an extended conformation with its nicotinamide ring lying at the bottom of the active site that is located at the center of eight-stranded parallel α/β barrel. The active site of ALR2 is primarily composed of hydrophobic aromatic amino acid residues (Trp20, Trp79, Tyr48, Trp111, Phe122, Trp219), apolar residues (Val47, Pro218, Leu300, Leu301) and some polar residues (Gln49, Cys298 and His110). Among the above described amino acid residues, Tyr48, His110 and Cys298 are reported to be possible proton donors.

Bruno and co-workers exhibited the ALR2 inhibitory potential of TZD derivatives, and reported a series of molecules by varying substituents at the para position of benzylidene ring. The substitution of phenoxy group at the benzylidene ring resulted in
A rhodanine derivative co-crystallized with protein ALR2 (PDB ID: 4JIR, 2.0 Å) depicted that the ligand forms particularly the lipophilic interactions with hydrophobic amino acid residues of the active site (Fig. 6A). The terminal aromatic ring of the crystal ligand exhibited \( \pi-\pi \) stacking interactions with the aromatic ring of Trp111 while the hydrogen bonding (HB) acceptor group present as acidic group attached to the NH of TZD ring contributes to the interaction with HB donor group of Tyr48, His110 and/or Trp111. The docking analysis of TZD derivatives was carried out in the same protein (PDB ID: 4JIR) to study the interactions between the ligand and the protein. The docked structure of inhibitor 22 showed similar interaction pattern to that of the crystal ligand, with acidic group attached to NH forming hydrogen bonding interactions with Tyr48, His110 and Trp111. The benzylidene ring is observed to fit into the hydrophobic pocket formed by Trp111, Phe122, Trp219 and Leu300 (Fig. 6B). The compd 23 without acidic carboxylate group was observed to form interactions with Tyr48 and His110 via carbonyl group of TZD. Experimentally, the molecules bearing acidic group at the –NH of TZD core were observed to exhibit high ALR2 inhibitory activity. On basis of the experimental and docking analysis, the structural requirements of TZD derivatives for ALR2 inhibition are mentioned in Figure 6C.

### 3.4. Pim kinase 1

Pim kinase 1, encoded by PIM1 gene, is a member of serine/threonine kinase family, and expressed primarily in spleen, thymus, bone marrow, prostate and epithelium. This kinase plays an important role in the development of various kinds of cancers—especially in leukemia, prostate and hematopoietic cancer. Pim kinase 1, in response to various cytokines, is activated through different pathways including JAK-STAT, NF-κB and PI3K/AKT, and exert oncogenic activity via regulation of MYC transcriptional activity, cell progression, cap-dependent protein translation and phosphorylation of pro-apoptotic protein BAD, which further induces the release of anti-apoptotic protein BCI-3/CI. Moreover, Pim1 regulates the progression of cell cycle by phosphorylation of Cdc25A and Cdc25C that in turn enhance G1/S and G2/M transition, respectively.

The crystal structure of Pim kinase 1 exhibited a typical kinase topology that starts with an N-terminal domain—composed of five antiparallel \( \beta \)-sheets—and ends up with a C-terminal domain—primarily built up of \( \alpha \)-helices. Like other kinases, the N- and C-terminal domain of Pim1 is connected through a hinge region that constructs a part of an ATP-binding pocket. Pim1 differs from other kinases due to some of its characteristic features including a \( \beta \)-hairpin insert—located at N-terminal to helix \( \alpha \)-C, and Pro123 in the hinge region. The presence of proline in the hinge region facilitates the formation of a single hydrogen bond with ATP molecules, unlike two interactions in other kinases that is a probable reason for its relatively high \( K_m \) value for ATP. The active site of Pim1 contains a hydrophobic sub-pocket—composed of Leu44, Gly45, Phe49 and Val52 that can efficiently accommodate a range of hydrophobic substitutions of binding ligands. There is presence of a number of acidic residues in the pocket consisting of Asp128, Asp131, Asp170, Glu171, Asp234 and Asp239 that are supposed to make interactions with positively charged inhibitor molecules.

The crystal structure of Pim1 co-crystallized with TZD derivative (PDB ID: 4DTK, 1.86 Å) revealed that the two carbonyl groups of TZD core form two hydrogen bonding interactions with Asp186 and Lys67 amino acid residues of Pim1 active site (Fig. 7A). The benzylidene ring of the crystal ligand settles into the hydrophobic pocket of Leu174, Ile185, Ala65 and Leu44 amino acid residues. The 3-amino functional group of the crystal ligand participates into two hydrogen bonding interactions with negatively charged amino acid residues, that is, Glu171 and Asp128. The substitution pattern for TZD derivatives to exhibit Pim inhibitory activity is proposed in Figure 7B.

### 3.5. Phosphoinositide-3-kinase \( \alpha \) (PI3K\( \alpha \)) and mitogen activated protein kinase kinase (MEK)

PI3K\( \alpha \) is a lipid kinase encoded by PIK3CA gene and expressed ubiquitously in the body. It is a prime contributor of PI3K/Akt pathway, which is highly activated in cancer, often due to deletions, activating mutations, and/or amplification of its players. PI3K\( \alpha \) phosphorylate and activates Akt, a serine/threonine protein kinase that is responsible for the activation of other downstream
effectors involved in many crucial cellular processes such as metabolism, growth, proliferation, survival and angiogenesis.

In addition to PI3K/Akt pathway, mitogen-activated protein kinase (MAPK) is another important pathway of kinase signaling cascade that is activated in cancer. This pathway involves a hierarchy of kinases at multiple levels including MAP kinase kinases (or MEKs, for MAPK/ERK kinases) and MAP kinase kinase kinases (or ERKs, for extracellular signal-regulated kinases).

Mitogen activated protein kinase kinase (MEK), encoded by MAP2K1 gene, is mainly activated through phosphorylation by upstream kinases such as Raf kinase. The activated MAPK proteins translocate into the nucleus, and activate downstream targets that result in the activation of cell growth and proliferation. Therefore, the attenuation of MAPK pathway provides an opportunity to control abnormal cell growth, and render beneficial therapeutic effect in cancer.

The significance of PI3K/Akt and Raf/MEK/ERK pathways in the etiology of human cancers has led to the consideration of different members of these signaling pathways for drug discovery research. Engelman and co-workers demonstrated that the simultaneous attenuation of PI3K and MEK is highly effective particularly in K-Ras G12D and H1047R murine lung cancers.

Thereafter, Liu and co-workers also reported the PI3K and MEK inhibitory potential of TZD derivatives. Modifications of molecule 27 including replacement of amine moiety of ethylamine with carboxyl and tertiary amino groups diminished biological activity suggesting the importance of primary amine; however, the presence of hydroxyl group at the same position resulted in equipotent anti-cancer activity to 27 in U937 cancer cell line. These results suggested the presence of hydrogen bonding interactions with active site amino acid residues instead of ionic interactions. Overall, the molecule 31 exhibited the highest efficacy in U937 cancer cell lines and in in vitro assay of PI3K and MEK1 enzyme inhibition.
In the absence of MEK1 and PI3Kα crystal structure co-crystallized with TZD, the docking analysis of molecule 31 was performed with crystal structures of MEK1 (PDB ID: 1S9J; 2.40 Å; Fig. 8A) and PI3Kα (PDB ID: 3HHM; 2.80 Å; Fig. 8B). The results of docking experiment showed that the ethylamine moiety of molecule 31 depicts hydrogen bonding interactions with Asp190, Asn195 and Asp208 while the two carbonyls of TZD core displayed H-bonding interactions with Lys97 and Ser194 amino acid residues of MEK1 (Fig. 8A). The terminal cyclohexane ring of the molecule 31 is well fitted into the hydrophobic pocket composed of Leu74, Val82, Ala95 and Leu197 amino acid residues.

The reported docking study of molecule 31 with PI3Kα showed two hydrogen bonding interactions of ethylamine with Glu768 and Glu798, and fitting of terminal cyclohexane ring into the hydrophobic pocket composed of Ile800, Ile848 and Ile932. However, when we docked the available TZD PI3Kα inhibitors to develop SAR, the molecules displayed different binding behavior as compared to the reported ones. The reported amino acids were present significantly away from the active site of PI3Kα (PDB ID: 3HHM); thus, may not participate in the ligand binding. Our docking experiment of molecule 31 with PI3Kα exhibited two hydrogen bonding interactions of ethylamine with Asp933 and Asp810, and of two TZD carbonyls with Asp933 and Lys802. Besides these interactions, the terminal cyclohexane group participated in the hydrophobic interactions with Ile800, Tyr867, Met953, Ile963, Ile831 and Ile876 amino acid residues. The results of docking analysis have been maneuvered to extract out the structural requirements of TZD for the PI3Kα and MEK1 dual inhibition (Fig. 8C).

3.6. Phosphoinositide3-kinase γ

PI3Kγ—encoded by PIK3CG gene—is primarily expressed in leucocytes, and its criticality in leucocyte chemotaxis and mast cell degranulation involves it in allergic and inflammatory conditions. Primarily, PI3Kγ is activated by G-protein coupled receptors via their regulatory subunit and G-protein βγ. The activated kinase participates in multiple signaling pathways including cytokine, JAK/STAT, B-cell receptor and Toll-like receptor signaling pathway. Like other protein kinases, the N- and C-terminal of PI3Kγ is connected through a hinge region that constructs a part of the archetypal ATP binding pocket. The active site of PI3Kγ consists of a polar pocket made up with Lys883, Asp836 and Asp964, and a hydrophobic pocket composed of Tyr867, Met953, Ile893, Ile831 and Ile876 amino acid residues.

Initially, Pomel and co-workers reported the discovery of TZD inhibitors of PI3Kγ with the help of a SAR study. The furan ring present at TZD was substituted with phenyl group, and the variation of hydroxyl group exhibited maximal PI3Kγ inhibitory activity at the ortho position of phenyl ring (compd 32, O > m > p, IC₅₀ 30 > 290 > 380 nM). Thereafter, the optimization process of molecule 32 exhibit that 1,4-di-substitution of hydroxyl groups at phenyl ring are much favorable (compd 34, IC₅₀ = 20 nM) as compared to o-hydroxy-p-fluoro (compd 33, IC₅₀ = 33 nM) and o-hydroxy-p-methylester (compd 35, IC₅₀ = 29 nM) substitutions. Furthermore, some TZD inhibitors of PI3Kγ including AS-605240 (IC₅₀ = 9 nM) and AS-604850 (IC₅₀ = 250 nM) are the patent molecules of Merk Sereno. The co-crystal structures of PI3Kγ were available with AS-604850 (PDB ID: 2A4Z, 2.90 Å, Fig. 9A) and AS-605240 (PDB ID: 2A5U, 2.70 Å, Fig. 9B). The analysis of these two structures showed that the carbonyl group of TZD ring forms hydrogen bond interaction with side chain of Ser806 whereas the backbone nitrogen of Val882 forms hydrogen bonding interaction with the oxygen of 1,3-benzodioxole ring of AS-604850 while TZD ring of AS-605240 is shown to interact with Lys883, Asp836 and Phe965. In addition, the aromatic ring system attached to TZD ring in both molecules fit well into the hydrophobic pocket of the enzyme. Docking analysis of molecule 33 revealed the molecular level interactions of TZD derivatives within the active site of PI3Kγ (Fig. 9C). The deprotonated nitrogen of TZD core forms a salt bridge with positively charged side chain of Lys833. The 2-hydroxyl functional group present at the phenol ring interacts with backbone NH of Val882 by acting as hydrogen bond acceptor. The phenyl and furan rings participate in forming hydrophobic interactions with several hydrophobic residues in the active site. Based on the above information, a probable pharmacophore for TZD based PI3Kγ inhibitors have been proposed in Figure 9D.
3.7. Tyrosinase

Tyrosinase—encoded by TYR gene—is a copper containing oxidase expressed primarily in melanocytes, and catalyzes the first two steps of melanin production. These two steps involve the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), which is further oxidized to yield L-DOPAquinone.\(^{141-143}\) The process of oxidation or oxygen transfer can progress via either of two mechanisms, that is, \(\mu^{-}\eta^{2}:\eta^{1}\)-peroxodicopper(II) or bis-\(\mu\)-oxodicopper(III) intermediate.\(^{144,145}\) Tyrosinase can be considered to be an attractive target for melanoma as it catalyzes the rate limiting steps of melanogenesis.

The crystal structure (PDB ID: 4P6T) revealed that the overall topology of tyrosinase is primarily maintained by electrostatic and \(\pi\)--cation interactions between its helical segments. The active site of tyrosinase that is hydrophobic in nature and is located inside a helical bundle comprised of four densely packed helices. The active site comprised of six highly conserved histidine residues that functions as copper binding regions. These histidine residues form two histidine rich regions, CuA and CuB that are responsible for the binding of two copper ions. Moreover, tyrosinase comprised of an N-terminal domain—responsible for intracellular trafficking and processing, cysteine-rich domains, a hydrophobic C-terminal transmembrane segment—responsible for targeting the enzyme to melanosome,\(^{146}\) and a short cytoplasmic tail.\(^{147,148}\)

Initially, Ha and co-workers developed TZD inhibitors of tyrosinase, and among them, molecule 36 exhibited prominent inhibition of tyrosinase (IC\(_{50}\) = 9.87 \(\mu\)M).\(^{36}\) The SAR analysis of these molecules described the importance of meta and para substitutions at the benzylidene ring. For instance, the removal of meta hydroxyl group from compound 36 compromised the biological activity against tyrosinase (compd 37 {24.08% inhibition} vs compd 36 {82.27% inhibition}), the replacement of para methoxy group with hydroxyl maintains the biological potential (compd 38 {62.68% inhibition} vs compd 36 {82.27% inhibition}) while the substitution of hydroxyl group at both meta and para positions potentiate the activity of resulting compound 39 (MHY498, IC\(_{50}\) = 3.55 \(\mu\)M) as compared to compound 36 (IC\(_{50}\) = 9.87 \(\mu\)M).\(^{51}\)

The docking analysis of compound MHY498 within the active site of tyrosinase (PDB ID: 4P6T, Fig. 10A) depicted one hydrogen bonding interaction of para hydroxyl group with Val218 and copper ion each while the meta hydroxyl group formed a hydrogen bond with His231. The phenyl ring of MHY498 molecule exhibited \(\pi\)--\(\pi\) stacking interactions with three histidine residues of the active site.
including His60, 204 and 208. Based on these observations, it can be interpreted that a polar moiety is necessary for the copper ligation while the hydrophobic (aromatic) segment of the inhibitor is required for optimal interactions within the histidine rich pocket. The general structural requirements of TZD derivatives to achieve tyrosinase inhibition are mentioned in Figure 10B.

3.8. Mutant thyroid hormone receptor-β (TR-β)

TR-β, encoded by THRB gene, is a nuclear receptor stimulated by thyroid hormones (THs)—triiodothyronine (T₃) and thyroxine (T₄). At the cellular level, TR-β provides a negative feedback mechanism to suppress TSH (thyroid stimulating hormone) that in turn regulates the secretion of THs. The mutational changes—specifically R320H—in TR-β would make it unresponsive for THs, and develop ‘generalized thyroid hormone resistance’ (GTHR) that is characterized by goiter, deafness, abnormal growth and bone maturation arising due to elevated levels of THs and TSH. Thus, the molecules or hormone analogs that can specifically activate this mutant form of TR-β are required for effective management of GTHR.

Till date, no X-ray crystal structure of mutant TR-β has been reported; however, a wild type TR-β is co-crystallized with agonist molecule GC-1 (PDB ID: 3IMY, 2.55 Å). The structure of wild type TR-β revealed that the binding site is structured of three regions referred to as region 1, 2 and 3. Region 1 is mostly hydrophobic with one polar residue (His435) that depicts a hydrogen bond with hydroxyl group (phenol) of most of thyromimetics or TH while the TH analogues devoid of hydroxyl group (phenol) are inert. Region 2 is mainly composed of apolar residues that interact with thyrionine ring of TH derivatives or phenolic ring of thyromimetics such as GC-1. Region 3 is primarily assembled of three electropositive arginine residues including Arg282, Arg316 and Arg320—also termed as arginine rich region—that interact with oxyacetic acid moiety of GC-1, and determine the selectivity of the thyromimetics. The mutation of Arg320 to His320 decrease the electropositive potential of binding site since pKa of histidine is 6.5 as compared to 12 of Arginine. Moreover, R320H mutation increases the volume of binding site, and also diminishes the interactions of −COOH moiety with binding site region 3.

Hashimoto and co-workers developed TZD derivatives as mutant TR-β inhibitors by replacing the oxyacetic acid moiety of GC-1 with TZD core, and molecule 40 (EC₅₀ = 0.46 nM) depicted the highest potency. The SAR of these molecules depicted that the nature of methylene linker at the 5th position of TZD core is crucial for mutated TR-β inhibitory activity, for example, Molecule 41 (−CH₂−, EC₅₀ = 80.1 nM) and 42 (no linker, EC₅₀ = 15.7 nM) showed lower activity as compared to 40 (−CH₂−, EC₅₀ = 0.46 nM). Additionally, the substitution of free −NH moiety of TZD core compromised the TR-β inhibitory activity of resulting molecules (43, EC₅₀ = 186.6 nM).
The docking analysis of TZD derivatives, using PDB ID: 3IMY, showed similar interactions with TR-β as of GC-1 (Fig. 11A). The phenolic –OH group depicted hydrogen bonding interactions with His435 while the electronegative TZD core settles into arginine rich region 3 forming interaction with Arg282. The linker of TZD derivatives is critical to extend the molecules into an extra space created by R320H mutation. Based on the crystal structure and docking analysis, the general SAR for TZD modulators of mutant TR-β is proposed in Figure 11B.

3.9. UDP-N-acetylmuramoylalanine—d-glutamate ligase (MurD ligase)

MurD ligase—encoded by murD gene—catalyzes the addition of glutamate to UDP-N-acetylmuramoyl-L-alanine (UMA) in the presence of ATP resulting in ADP, a phosphate and UDP-N-acetylmuramoyl-L-alanyl-d-glutamate—a peptidoglycan—involved in the formation of cell wall of bacteria. Thus, MurD inhibitors would tend to attenuate the cell wall synthesis of microorganisms, and exhibit anti-microbial potential.

The crystal structure of MurD ligase (PDB ID: 1UAG, 1.95 Å) revealed the presence of three globular domains—D1, D2, and D3. D1—primarily responsible for the fixation of UDP moiety of UMA—is composed of a five stranded parallel β-sheet, surrounded by four α-helices. D2 is formed of a central six stranded β-sheet, surrounded by seven α-helices, and a small flanking three stranded antiparallel β-sheet. D3 is composed of a six stranded β-sheet with parallel and antiparallel strands, and five neighboring α-helices. On the cellular level, the substrate molecule (UMA) binds to MurD ligase in the cleft architecture between D1 and D2. Upon binding, UMA (44) participates in many polar interactions including uracil ring contribution of two hydrogen bonds with Thr36. Furthermore, uracil ring also participates in interplane stacking with a salt bridge between Asp35 and Arg37. The N-acetylmuramic acid ring bridges the gap between D1 and D2, and the one carboxylate groups of UMA forms hydrogen bonds with side chain of Asp138 while the other one with His183.

Tomasic and co-workers reported TZD inhibitors of MurD as anti-microbial agents. However, the results showed that these compounds are inactive as compared to its rhodanine derivative 45 (S-isomer IC50 = 206 µM, R-isomer IC50 = 174 µM). Their investigations showed that methyl esters of carboxylic acid had reduced activities. Moreover, the substitution at 4th position reduced inhibitory activity (compd 46% inhibition = 74 at 250 µM). Further, derivatives were synthesized and TZD derivative 47 (R-isomer, IC50 = 35 µM) was observed to be potent than its rhodanine derivative (R-isomer, IC50 = 49 µM). Substitution of the benzyl amine linker with alkyl linkers (compd 48) gave compounds devoid of inhibitory activity. Modification of compound 47 was carried out by formulating its methyl ether derivatives. However, evaluation of these molecules showed that rhodanine derivatives were more potent than its TZD derivatives.
The X-ray crystallographic structure of MurD complexed with compd 47 has been reported (PDB ID: 2X5O, 1.5 Å) (Fig. 12A). The TZD core of the molecule was observed to fit into the uracil binding pocket forming two hydrogen bonding interactions with the Thr36 amino acid residue. The benzylidene moiety forms hydrophobic interactions with Gly73 while the phenyl group depicted hydrophobic interactions with Phe416 and π-π interactions with Phe161. The α-carboxyl group of the α-glutamate moiety of the inhibitor formed a salt bridge with side chain nitrogen of Lys348, and a hydrogen bond with His183 while γ-carboxyl group formed hydrogen bonds with Ser415 and Phe422. Similar results were also observed in the crystal structure solved with other TZD derivative containing methyloxy linker (compd 49; PDB ID: 2Y66, 1.49 Å). The information extracted out from the crystallographic and biological activity analysis was corroborated to propose a general SAR model for TZD inhibitors of MurD ligase (Fig. 12B).

3.10. Cyclooxygenase-2 (COX-2)

COX-2, encoded by PTGS2 gene, catalyze two reactions including cyclo-oxygenation of arachidonic acid to hydroperoxy-endoperoxide prostaglandin G2 proceeded by its (PGG2) reduction to prostaglandin H2. Further, the metabolism of PGH2 with different prostaglandin synthases lead to the biosynthesis of PGD2, PGE2, PGF2α, PGI2 (prostacyclin) and TXA2. Among them, PGE2 is the prime inflammatory mediator responsible for various inflammation-related pathological conditions including rheumatoid arthritis, asthma, inflammatory bowel disease, psoriasis and cancer, etc. Non-steroidal anti-inflammatory drugs (NSAIDs) are clinically successful candidates for the management of inflammatory conditions that acts through the attenuation of COX-2 functioning.

The analysis of crystal structure (PDB ID: 3KK6; 2.75 Å)—solved with selective ligand celecoxib—showed that COX-2 is composed of three structural domains termed as N-terminal epidermal growth factor (EGF)-like domain, a membrane binding domain (MBD) and a large C-terminal globular catalytic domain that contains an active site. In the active site of COX-2, the bulky groups present in COX-1, that is, Ile523, His513 and Ile434 are replaced with Val523, Arg513 and Val434 that tend to increase the volume of COX-2 active site as compared to COX-1. Furthermore, Arg513 of COX-2 active site is a critical residue for ligand interactions and biological activity. Another striking feature is the rotamer position of Leu384, which is oriented inside the active site of COX-1 while outward in COX-2; thus, provide more space for ligand binding in COX-2 as compared to COX-1. This explains the selectivity of celecoxib due to the presence of bulky group sulfonamide that prevent its entry into the active site of COX-1.

Ali and co-workers reported TZD derivatives (compd 50 and 51) as potent anti-inflammatory and analgesic compounds, and thereafter suggest that the biological activity of these molecules is conferred through COX-2 inhibition (Fig. 13A).
The docking simulations of compound 50 with COX-2 (PDB ID: 1CXX) revealed that hydrogen bonding interaction of TZD core (carbonyl group) with Arg513 (Fig. 13A), which is also depicted by the crystal ligand of 1CXX. The two bulky groups, that is, phenyl, attached at the –NH moiety of TZD core display hydrophobic interactions with Leu359, Leu384, Tyr385, Val523 and Ala527 amino acid residues of the COX-2 active site. The corroborated information of crystal structure and docking analysis is implemented to propose the SAR for TZD inhibitors of COX-2 (Fig. 13B).

3.11. Histone deacetylase 1

Histone deacetylase 1 (HDAC1), encoded by HDAC1 gene, catalyzes the removal of acetyl group from ε-N-acetyl lysine amino acid of histone molecule, and consequently allow it to tightly wrap the DNA. Histone is critical since DNA is wrapped around it and DNA expression is primarily dependent upon acetylation and deacetylation of histone. The therapeutic value of HDAC inhibition has been widely observed due to its critical role in cell differentiation in patients with hepatocellular carcinoma.\(^{162-164}\)

The reported crystal structure of HDAC1 (PDB ID: 1C3S; 2.50 Å) revealed a single-domain structure that belongs to an open α/β class of folds. The catalytic domain of HDAC1 has a tube like topology composed of seven loops, and the inner environment of active site is mainly constructed with hydrophobic and aromatic residues including Pro22, Gly140, Phe141, Phe198, Leu265 and Tyr297. HDAC1 uses zinc (\(\text{Zn}^{2+}\)) as cofactor, which is positioned near the bottom of active site, and coordinated by Asp168, His170 and Asp258. Furthermore, the active site also contains two histidines (His131 and His132), two aspartic acids (Asp166 and Asp173) and a tyrosine amino acid residue (Tyr297).\(^{165}\)

Mohan and co-researchers developed TZD inhibitors of HDAC1 (52 and 53), and subsequently evaluated their cancer cell line activity.\(^{32}\) These molecules exhibited 43.31% and 57.27% inhibition in HepG2 cell lines at a dose of 100 \(\mu\text{M}\), and 42.11% and 56.85% inhibition of HDAC1. The docking interactions of these molecules exhibited that TZD core serves as zinc chelating, hexyl chain as a linker, amide and sulphonamide as hydrogen bond conferring moieties, and the terminal phenyl group as a cap.

The docking interactions of molecule 52 within the active site of HDAC1 protein (PDB ID: 1C3S) are displayed in Figure 14A. In this figure, the TZD carbonyl group of molecule 44 displayed chelation with \(\text{Zn}^{2+}\) while the terminal phenyl showed hydrophobic interactions with Ala197, Phe198 and Leu265 amino acid residues of the active site. The corroborated information of crystal structure and docking analysis is maneuvered to propose the SAR for TZD inhibitors of HDAC1 (Fig. 14B).

4. Pharmacokinetic properties of TZDs

The major concern of any drug development process is how much drug enters into the bloodstream, reach to the target of choice, metabolize, distribute and excrete out of the body, that is, pharmacokinetic properties of drug. Thus, to extend the scope of this article for the researchers, we reviewed the pharmacokinetic behavior of various TZD derivatives reported in the literature.

The TZD based PPAR\(\gamma\) agonists such as troglitazone, pioglitazone, rosiglitazone and lobeglitazone commonly metabolize through the hepatic route; however, the required cytochrome enzymes are specific for these drug molecules. For instance,
CYP3A4/2C8 is required for the metabolism of troglitazone and pioglitazone, CYP2C8/2C9 for rosiglitazone and CYP1A2/2C9/2C19 for lobeglitazone. Outlines of metabolism of troglitazone and rosiglitazone are displayed in Figures 15 and 16, respectively. The oxidation of troglitazone cleave the TZD ring to yield reactive metabolites (I–VI) as well as the quinone metabolites (VII–X).\textsuperscript{166} The metabolism of troglitazone also occurs through sulfation (metabolite XI) and glucuronidation.\textsuperscript{167–169} On the other hand,
rosiglitazone metabolism occurs through N-demethylation, hydroxylation and subsequent conjugation (I–XI).  

The partition coefficient (Log P) of these molecules, that is, 2.95, 3.17, 4.16 and 4.20 for rosiglitazone, pioglitazone, troglitazone and lobeglitazone, respectively, confer hydrophobic nature that helps to achieve high extent of oral absorption, that is, 95%, 83%, 50% and 72% for mentioned molecules, respectively.  

5. Bioisosteric replacements for TZD nucleus  

The electrophilic character and the hydrogen bonding rich features of TZD nucleus can be bioisostERICally replaced with the substitution of oxazolidine-2,4-dione, isoAxazolidine-3,5-dione and succinimide rings. The bioisostERIC replacement of TZD with mentioned ring systems tend to develop potent and safe molecules.  

The partition coefficient (log $P_{\text{ow}}$) of TZD compound showed very good anti-hyperglycemic activity as of TZD derivatives significantly contributed to the field of medicinal chemistry and drug design. Thus, the understanding of SAR of TZD derivatives for different protein targets would certainly help the researchers involved in the area of TZD based novel drug design.

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References and notes
