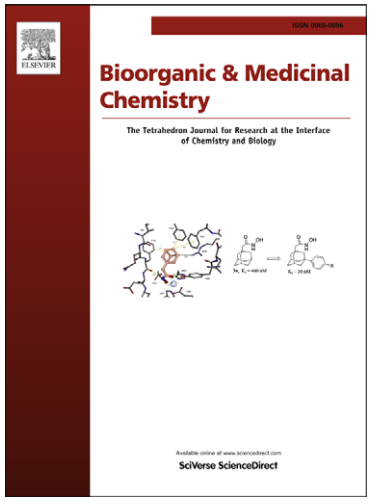

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Design and synthesis of L- and D-phenylalanine derived rhodanines with novel C5-arylidenes as inhibitors of HCV NS5B polymerase

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ABSTRACT: Hepatitis C virus (HCV) NS5B polymerase is a key target for anti-HCV therapeutics development. Herein, we report the synthesis and *in vitro* evaluation of anti-NS5B polymerase activity of a molecular hybrid of our previously reported lead compounds **1** (IC_{50} = 7.7 μM) and **2** (IC_{50} = 10.6 μM) as represented by hybrid compound **27** (IC_{50} = 6.7 μM). We have explored the optimal substituents on the terminal phenyl ring of the 3-phenoxybenzylidene moiety in **27**, by generating a set of six analogs. This resulted in the identification of compound **34** with an IC_{50} of 2.6 μM. To probe the role of stereochemistry towards the observed biological activity, we synthesized and evaluated the D-isomers **41** (IC_{50} = 19.3 μM) and **45** (IC_{50} = 5.4 μM) as enantiomers of the L-isomers **27** and **34**, respectively. The binding site of compounds **32** and **34** was mapped to palm pocket-I (PP-I) of NS5B. The docking models of **34** and **45** within the PP-I of NS5B were investigated to envisage the molecular mechanism of inhibition.

Keywords: L-Phenylalanine, D-Phenylalanine, Rhodanine, Knoevenagel condensation, Ullmann condensation, HCV NS5B polymerase

1. Introduction

Hepatitis C virus (HCV) infection has emerged as one of the most significant disease[1] and an estimated 200 million cases of HCV infections exist worldwide.[2] Of those initially infected with HCV, approximately 80% will progress to develop chronic liver disease and 20%

will eventually progress to liver cirrhosis and hepatocellular carcinoma.[3] Until recently the current therapy for treating HCV infection has been regular injections of pegylated interferon α (PEG-IFN-α) in combination with daily oral administration of ribavirin (RBV). This cumbersome process has found limited patient compliance due to severe adverse effects.[4] Within the past year, two NS3/4 protease inhibitors, telaprevir[5] and boceprevir[6], in combination with PEG-IFN/RBV have been approved for treating HCV genotype 1 infection. Although these combination treatments have shown improved sustained virological response (SVR) in genotype 1 patients;[7,8] they still harbor the severe side effects associated with IFN therapy. Therefore, the search for novel, directly acting antiviral agents that specifically targets HCV and harbors minimal adverse effects on the patient is an urgent medical necessity.

The HCV non-structural protein 5B (NS5B), a 66 kDa RNA-dependent RNA polymerase (RdRp) is an important therapeutic target for its important role in replicating the HCV RNA genome. This target is especially significant from the drug discovery point-of-view since humans lacks its functional equivalent.[9] The combination of crystallographic, biochemical and mutagenesis studies have allowed the identification of at least five nonnucleoside inhibitor (NNI) binding sites on NS5B enzyme.[10] MK-3281 bound to thumb pocket (TP)-I[11] and PF-868554 bound to TP-II[12] of NS5B are located in the thumb domain, whereas acylpyrrolidine bound to palm pocket (PP)-I[13], HCV-796 bound to PP-II[14] and GS9190 bound to PP-III[15] of NS5B are partially overlapped and are located between the thumb and the palm domains, in close proximity to the active site. Several inhibitors targeting these binding sites on NS5B have demonstrated strong efficacy in clinical trials.[10]

Compounds bearing the rhodanine scaffold have been previously reported as HCV NS5B inhibitors, by us[16] and others.[17] Recently, the rhodanine scaffold has been a topic of debate in

regards to its promiscuous nature.[18] While one group of researchers have considered rhodanines as "frequent hitters" that interact with multiple targets and elicit broad inhibitory activity, others believe rhodanines to be "privileged scaffolds". The negative opinion primarily highlights its insufficient selectivity. On the other hand, a more positive view considers rhodanine as a suitable lead compound for tailoring its potency and selectivity at the step of advanced lead optimization.[18] Importantly, clinical success with the rhodanine analog, epalrestat, argues in favor of its safety in humans. As part of our continued efforts towards development of more potent rhodanine derivatives as anti-HCV agents, herein we report on the optimization of our two previous rhodanine leads, compounds **1** and **2**[16], through molecular hybridization, bioisosterism and stereochemical optimization strategies that renders a rhodanine derivative with improved NS5B inhibitory activity (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **23** and **27** was reported by us previously.[19] The benzaldehydes required for the preparation of target compounds were either commercially available or prepared (**3-11, 13-15**) as shown in Scheme 1. The 2-phenoxybenzaldehyde (**3**) and substituted 3-phenoxybenzaldehydes (**4-9**) were synthesized via Ullmann condensation using 2-bromobenzaldehyde and 3-bromobenzaldehyde, respectively.[20] Compounds **10** and **11** were prepared by treating 3-formylphenyl boronic acid with benzyl bromide and bromobenzene, respectively, by following Suzuki coupling procedure.[21] The 3-benzoyl benzaldehyde (**13**) was synthesized through the classical Weinreb amide approach.[22] Weinreb amide was formed by the coupling 3-benzoylbenzoic acid with *N*,*O*-dimethylhydroxylamine hydrochloride in presence of EDC hydrochloride. Weinreb amide was then directly reduced by $LiAlH_4$ to yield benzhydryl

alcohol (**12**) which when subjected to Dess-Martin periodinane oxidation yielded the desired 3-benzoylbenzaldehyde (**13**). Aldehyde intermediates **14** and **15** were synthesized by alkylation of 3-hydroxybenzaldehyde with benzyl bromide and cinnamyl chloride, respectively, in the presence of potassium carbonate. The synthesis of target compounds **17-39** and **41-45** is outlined in Scheme 2. The phenylalanine-derived optically active rhodanine intermediates L-**16** and D-**40** were synthesized, respectively, from the corresponding L- and D-phenylalanine, as per the previously reported procedure.[19] Knoevenagel condensation of L-**16** and D-**40** with various aromatic aldehydes at the nucleophilic C5 active methylene, respectively, resulted in target compounds **17-39 (**L-isomers) and **41-45** (D-isomers) bearing the *Z*-geometry as determined by the chemical shift of the methine proton ranging from 7.7-8.2 ppm as a singlet (chemical shift for corresponding methine proton of *E*-isomer is calculated to be 6.8 ppm).[23,24] Owing to the fact that the optical rotations for the L- and D-isomers were not equally opposite, we presumed that partial racemization may have occurred during rhodanine ring construction and/or Knoevenagel condensation which was further established by enantiomeric excess determination by chiral HPLC methodology.

2.2. Structure-activity relationship

The compounds prepared in this study were evaluated utilizing a biochemical assay against HCV NS5B polymerase as described previously.[16] This assay involves quantification of the amount of radioactive UMP incorporated into nascent RNA products employing homopolymeric poly rA/U_{12} as template-primer and functionally active recombinant HCV NS5BCΔ21 (genotype 1b) protein. The activity of NS5B polymerase in the absence of the inhibitor but containing an equivalent amount of DMSO (control reaction) was set at 100%. The inhibitory activity of synthesized compounds was then quantified relative to this control.

Wedelolactone[25] and aurintricarboxylic acid[26], two validated NS5B inhibitors previously characterized by us, were included as reference standards and yielded IC_{50} values (data not shown) consistent with previously reported values. Depending on the nature of substituents on benzylidene moiety, the tested compounds exhibited IC_{50} values ranging between 2 μM and 50 μM against NS5B polymerase as summarized in Table 1. At the onset, we prepared a set of nine compounds to probe the effect of small electron withdrawing substituents at 3- and 4-positions of the benzylidene moiety. This was important since in our previous studies the electron donating substituents on benzylidene had proven detrimental for the NS5B RdRp activity.[16] Replacement of 2,4-dichlorobenzylidene in compound **2** (IC_{50} = 10.6 μM) with 3-chlorobenzylidene moiety (compound **17**, IC_{50} = 26.6 μM) proved deleterious for the compound's anti-NS5B activity, whereas substitution with 3-bromobenzylidene (compound **18**, IC_{50} = 7.4 μM) exhibited near comparable activity to compound **2**. This suggested that groups with larger van der Waal's radii coupled with high lipophilicity are well-tolerated at the 3-position of the benzylidene moiety for NS5B inhibition. This was also apparent from 3-cyanobenzylidene analog (compound **19**, IC_{50} = 30.7 μM), which exhibited ~3-fold loss of activity. With the objective of investigating the influence of mono-substitutions at the 4-position of benzylidene moiety, we synthesized 4-bromobenzylidene analog (compound **20**, IC_{50} = 11.7 μM), which showed comparable activity to compound **2**. The 4-fluorobenzylidene analog (compound **21**, IC_{50} = 27.7 μM) and the 4-chlorobenzylidene analog (compound **22**, IC_{50} = 22.3 μM) exhibited ~2-3-fold loss in activity. This data suggested that the larger size and lipophilicity of groups at 4-positon may be favorable for NS5B inhibition. We next investigated the influence of disubstitutions on the benzylidene ring. Substituting the 2,4-dichlorobenzylidene moiety in compound **2** with the 3,4-dichlorobenzylidene (compound **23**, IC_{50} = 19.4 μM), 3,4-difluorobenzylidene (compound **24**,

IC_{50} = 16.3 μM), and 3,5-difluorobenzylidene (compound **25**, IC_{50} = 26.0 μM) resulted in 1.5- to 3-fold loss in activity. Thus, smaller substituents at varying positions of the benzylidene moiety were unfavorable compared to lead compound **2**, with the exception of 3-bromo and 4-bromo analogs.

In our earlier work on glycine-derived rhodanines[16], we observed that the 3-phenoxy substitution on the benzylidene moiety enhanced NS5B inhibitory activity. To further explore the role of this substituent, 2-phenoxy (compound **26**, IC_{50} = 13.3 μM), 3-phenoxy (compound **27**, IC_{50} = 6.7 μM), and 4-phenoxy (compound **28**, IC_{50} = 50.7 μM) groups were installed on the benzylidene moiety. This data indicated that the phenoxy substituents are well tolerated at the 2-position, and favor NS5B inhibitory activity at the 3-position of benzylidene. Introduction of 4-phenoxy group on benzylidene; however, resulted in a dramatic loss in activity, suggesting the possibility of a steric restriction in this region of the molecule. Capitalizing on these observations of the importance of a phenoxy substituent at 3-position, we next explored the chemical space around the optimal 3-phenoxy group of compound **27**. Mono substitution with a chloro group (compound **29**, IC_{50} = 4.1 μM) at 3-position of phenoxy ring improved the NS5B inhibitory activity by ~1.5-fold compared to compound **27,** whereas substitution by a fluoro group (compound **30**, IC_{50} = 8.2 μM) resulted in a marginal loss of activity. Analogs with 4-position substituents as the methoxy (compound **31**, IC_{50} = 8.3 μM) or fluoro (compound **33**, IC_{50} = 12.5 μM) groups exhibited 1.5- to 2-fold loss in activity. Interestingly, the 4-chloro substituent (compound **32**, IC_{50} = 3.4 μM) was found to be 2-fold more potent relative to compound **27**. Since the 3-chloro and the 4-chloro substituents demonstrated improved NS5B inhibitory activities, we next synthesized an analog carrying 3,4-dichloro substituent with the expectation that it may further enhance NS5B inhibitory effect. Indeed, compound **34** (IC_{50} =

2.6 μM) bearing 3,4-dichlorophenoxy benzylidene moiety proved to be a better inhibitor than compound **27,** the unsubstituted analog and comparable to compounds **29** and **32** the mono-substituted analogs. Together, these data suggest that the tail groups at 3- and 4-positions of the phenoxy moiety tolerate halogens as well as methoxy group.

Since the concept of bioisosterism has been widely applied in lead optimization program to achieve improved target inhibition as well as desired physicochemical profile, we sought to optimize the heteroatom linkage between the two aryl rings through bioisoteric replacements. Towards this goal, we prepared analogs replacing ether linkage with its classical isostere -CH_2- (compound **35**, IC_{50} = 4.8 μM) that showed comparable activity to compound **27**. Not surprisingly, the non-classical isostere –C(=O)- bearing a carbonyl group (compound **36**, IC_{50} = 11.1 μM) proved detrimental for the activity. From these IC_{50} values, it is apparent that correct positioning of the terminal phenyl ring, rather than the hydrogen bond acceptor ether linkage between the two phenyl rings, may be critical for NS5B inhibition.

We next sought to explore alternate strategies to access putative hydrophobic region in palm pocket-I formed by residues Pro197, Leu384, Met414, Tyr415, Ile447, and Tyr448 by displacing the terminal phenyl ring on the compounds using linkers of varying lengths. To achieve this goal, we prepared a set of three compounds **37**-**39**. These compounds represent no spacer e.g., biphenyl compound **37** (IC_{50} = 16.6 μM); two atom methyleneoxy spacer e.g., compound **38** (IC_{50} = 21.8 μM) and a four atom allyloxy spacer e.g., compound **39** (IC_{50} = 6.4 μM). The ensuing data clearly underscores the importance of a longer spacer, particularly four atoms, between the two phenyl rings for potent NS5B inhibition.

To understand the potential binding interactions of the aforementioned inhibitors within the NNI binding site of HCV NS5B polymerase that could be exploited to improve efficacy of

our compounds, we performed docking studies using Glide v5.0 docking software (Schrodinger, LLC., New York, NY), as previously described.[16] To rule out any bias, each of the five reported NS5B allosteric binding pockets TP-I (PDB ID: 2XWY),[11] TP-II (PDB ID: 3FRZ),[12] PP-I (PDB ID: 3TYV)[27], PP-II (PDB ID: 3FQL),[14] and PP-III, that significantly overlaps with PP-II (large grid box created around PP-II bound HCV-796 to obtain docking pose at PP-III), was examined for inhibitor binding. Analysis of the binding energy data (Glidescores) for all single digit μM inhibitors at each allosteric site revealed that they bind with affinity in the order PP-I>PP-II>PP-III>TP-I>TP-II. We and other groups of researchers have previously shown that rhodanine core containing inhibitors bind within PP-I domain of NS5B.[16,17] Since all of the inhibitors investigated in this report carry one chiral center, we also analyzed the binding models of L- and D-isomers within the PP-I of NS5B. These analyses revealed that both the isomers had comparable binding energy (Glidescore) which prompted us to hypothesize that both isomers would exhibit comparable IC_{50} values. To test this hypothesis, we synthesized and tested representative D-isomers **41** (IC_{50} = 19.3 μM, ee = 84%), **42** (IC_{50} = 4.1 μM, ee = 75%), **43** (IC_{50} = 5.1 μM, ee = 78%), **44** (IC_{50} = 10.3 μM, ee = 85%), and **45** (IC_{50} = 5.4 μM, ee = 93%) as counterparts of compounds **27** (IC_{50} = 6.7 μM, ee = 85%), **29** (IC_{50} = 4.1 μM, ee = 93%), **32** (IC_{50} = 3.4 μM, ee = 86%), **33** (IC_{50} = 12.5 μM, ee = 84%), and **34** (IC_{50} = 2.6 μM, ee = 77%), respectively, starting from the D-phenylalanine-derived rhodanine intermediate (**40**) as depicted in Scheme 2. Taking enantiomeric purity into consideration these investigations indicated rather comparable inhibitory activity of both the isomers. Attempts to correlate Glidescore energy data (kcal/mol) with compound pIC_{50} (-log IC_{50}) values resulted in r^2 value of 0.48 with three outliers (compounds **28**, **37** and **38**). This correlation is reasonable given the fact that there is still no single scoring function that can correctly rank every protein-ligand complex in appropriate order.

2.3. Mapping the inhibitor binding site on NS5B

With the goal of validating the presumed inhibitor binding pocket for the relevant rhodanine derivatives on NS5B, we employed NS5B mutants P495L, M423T and M414T as screens for TP-I, TP-II and PP-I site binders, respectively.[28-30] These residues are critical components of the aforementioned allosteric pockets on NS5B, thus mutations at these sites results in loss of sensitivity to the inhibitor due to decrease in inhibitor binding.[28-30] Consistent with our screening strategy selection for PP-I binders, the inhibitory potency of representative compounds **32** and **34** was impacted by mutation at M414 residue of NS5B, but not with P495L and M423T mutants (Table 2). This was evident from the 16- to18-fold higher IC_{50} values for compounds **32** and **34** against M414T NS5B relative to wild-type NS5B. By contrast, the IC_{50} values of the compounds against NS5B mutants P495L and M423T exhibited ≤1.5 fold change relative to their corresponding wild-type NS5B values. This data thus suggests that the inhibitors bind at PP-I site of NS5B.

2.4. Molecular docking

To gain insight into the molecular mechanism of inhibition, we analyzed the interactions of the docked conformation of compound L-**34** (panel A) and for comparison D-**45** (panel B) within the PP-I of NS5B (PDB ID: 3TYV)[27] as shown in Fig. 2. The phenyl ring of the phenylalanine may enter into cation-pi interaction (4.8 Å and 5.1 Å) with the guanidinium and ε-NH_3 groups of Arg158 and Lys141, respectively. Whereas one of the oxygen atoms of the carboxylate group forms a water-mediated hydrogen bond with the backbone –NH of Ser556 (-COO---H_2O(598)---HN-Ser556), the other forms a water-mediated hydrogen bond with the side chain hydroxyl group of Ser288 (-COO---H_2O(790)---HO-Ser288). The electrophilic benzylidene carbon atom is juxtaposed to initiate covalent chemistry with the nucleophilic –SH

group of Cys366 (-=C---SH-Cys366, 3.4 Å) as previously reported for related benzylidene analog by Powers et al.[17] The nucleophilicity of the –SH group of Cys366 may be enhanced by a potential hydrogen bond with the guanidine group of Arg200 (-SH---N-guanidine-Arg200, 2.6 Å). The first phenyl ring of the benzylidene moiety is extensively stabilized by the hydrophobic residues Pro197, Tyr448, Leu384, Met414, and the methylene groups of Arg200. The 3,4-dichlorophenoxy ring is also stabilized through hydrophobic interactions with the side chains of Met414, Tyr415, and Ile447. Whereas the 3-chloro group enter into a dipole interaction with the side chain amide group of Asn411 (3-Cl---C=O, 3.4 Å), the 4-chloro group enter into two water-mediated hydrogen bonding interactions with the amide group of Asn411 (4-Cl---HOH(1202)---$OH_2$677---O=C(NH_2)-Asn411). The rhodanine ring acting as a scaffold for correct positioning of the pharmacophore groups (the phenylalanine and the benzylidene moiety) is mainly stabilized by Phe193 and Tyr448. The proposed binding mode is consistent with the observed SAR: a) the requirement for correct positioning of the terminal phenyl ring, and b) the ability to tolerate a variety of electron withdrawing hydrophobic substituents at the terminal phenyl ring.

The binding mode of D-**45** was found to be relatively similar to that observed for L-**34** with the subtle variations as described below. The phenyl ring of the phenylalanine portion is 5.6 and 6.3 Å away from the guanidinium and ε-NH_3 groups of Arg158 and Lys141, respectively. The electrophilic benzylidene carbon atom is located 3.5 Å away from the nucleophilic –SH group of Cys366. Based on above observations it seems that cation-pi interaction may play a positive role in NS5B inhibitory activity of the L-isomer.

Of the 28 rhodanine derivatives reported here, compound **35** seems to be the most promising for futher optimization studies, as it exhibited no cellular toxicity and limited antiviral activity (~30% inhibition of HCV RNA replication) at 100 μM concentration against HCV

replicon bearing hepatoma cells. We speculate that its ionizable carboxyl group may limit its membrane permeability and thus its antiviral efficacy. We therefore propose to explore various substitutions at the benzyl moiety of compound **35** for future optimization. Another possibility would be replacement of the thiazolidinone core with alternate five membered scaffolds to eliminate promiscuous nature of rhodanine ring. In addition, replacement of the carboxyl group with ester and bioisosteric tetrazole ring may help improve cellular permeability and antiviral potency. These proposed modifications would be guided by molecular docking investigations.

3. Conclusions

We have disclosed that properly functionalized benzylidene moiety at the C5-position of the phenylalanine-derived rhodanine scaffold provides several single digit micromolar NS5B inhibitors. Docking experiments have facilitated the interpretation of the inhibitory activity of these rhodanines by predicting the molecular interactions of the compounds with NS5B. We have also mapped the inhibitor binding site to PP-I of NS5B by counter screening against PP-I site mutant, M414T NS5B. Recognizing the feasibility of improving the activity of 3-phenoxy group by replacing it with 3-benzyl moiety and the preference for L-isomer, further SAR efforts will be directed in future to explore the most optimal substituents at the benzyl moiety of the L-isomer.

4. Experimental

4.1. General

Melting points (m.p.) were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The reagents for organic synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI), TCI America (Portland, OR), Alfa Aesar (Ward Hill, MA), and Acros Organics (Antwerp, Belgium) and were used as received. All compounds were checked

for homogeneity by TLC using silica gel as a stationary phase. NMR spectra were recorded on a Bruker 400 Avance DPX spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) outfitted with a z-axis gradient probe. The chemical shifts for ^{1}H and ^{13}C are reported in parts per million (δ ppm) downfield from tetramethylsilane (TMS) as an internal standard. The ^{1}H NMR data are reported as follows: chemical shift, multiplicity (s) singlet, (d) doublet, (dd) doublet of doublets, (t) triplet, (dt) doublet of triplets and (m) multiplet, respectively. Optical rotation of the chiral compounds was measured using PerkinElmer 241 Polarimeter with ethyl acetate as the solvent, concentrations *c* are expressed as g/100 mL. The C, H, and N analyses were performed by Atlantic Microlabs, Inc., (Norcross, GA) and the observed values were within ±0.4% of calculated values.

Chiral HPLC analysis

Chiral HPLC analysis was performed using Dionex Ultimate 3000 Series instrument. The compounds were dissolved in ethyl acetate and injected (20 μL) into the chiralpak 1B column (Daicel Corp., Fort Lee, NJ) with stationary phase as cellulose tris(3,5-dimethylphenylcarbamate) immobilized on 5 μm silica-gel. Optimum resolution of the enantiomers was achieved using an isocratic mobile phase (75:25 Hexane:Ethyl acetate with 0.1% TFA) eluting at a flow rate of 1 mL/min. The elutions were monitored at UV 370 nm in form of major and minor peaks representing the respective enantiomers. The retention times (t_R) for major and minor peaks are given in minutes. The enantiomeric excess (ee) values were calculated based on the area under peak for each enantiomers.

4.2. Synthesis

4.2.1. General procedure for preparation of phenoxybenzaldehydes (3-9)

Intermediates **3-9** were prepared following the procedure reported by Sasaki et al.[20]

4.2.2. 2-Phenoxybenzaldehyde (3)

Starting with 2-bromobenzaldehyde (2.0 g, 10.81 mmol) and phenol (1.11 g, 11.89 mmol), compound **3** (1.30 g, 61%) was obtained as colorless oil; R_f = 0.42 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 10.52 (1H, s), 7.94 (1H, dd, *J* = 7.7 Hz, 1.6 Hz), 7.51 (1H, t, *J* = 7.7 Hz), 7.39 (2H, m), 7.19 (2H, t, *J* = 7.4 Hz), 7.07 (2H, d, *J* = 8.1 Hz), 6.89 (1H, d, *J* = 8.3 Hz).

4.2.3. 3-(3-Chlorophenoxy)benzaldehyde (4)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 3-chlorophenol (1.53 g, 11.89 mmol), compound **4** (1.12 g, 45%) was obtained as yellow oil; R_f = 0.36 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.98 (1H, s), 7.65 (1H, d, *J* = 7.5 Hz), 7.53 (1H, t, *J* = 7.8 Hz), 7.48 (1H, s), 7.27-7.31 (2H, m), 7.14 (1H, d, *J* = 8.1 Hz), 7.01-7.02 (1H, m), 6.92 (1H, d, *J* = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 191.45, 157.48, 157.21, 138.15, 135.27, 130.80, 130.69, 125.54, 125.05, 124.19, 119.49, 118.54, 117.31.

4.2.4. 3-(3-Fluorophenoxy)benzaldehyde (5)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 3-fluorophenol (1.33 g, 11.89 mmol), compound **5** (0.92 g, 39%) was obtained as yellow oil; R_f = 0.35 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.96 (1H, s), 7.65 (1H, d, *J* = 7.6 Hz), 7.49-7.54 (2H, m), 7.27-7.33 (2H, m), 6.79-6.87 (2H, m), 6.73 (1H, dt, *J* = 9.9 Hz, 2.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 191.43, 171.21, 163.54 (d, *J* = 247.4 Hz), 157.77, 157.51, 138.24, 130.83, 130.68, 125.33, 118.75, 114.65, 110.90 (d, *J* = 21.2 Hz), 106.84 (d, *J* = 24.6 Hz).

4.2.5. 3-(4-Methoxyphenoxy)benzaldehyde (6)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 4-methoxyphenol (1.47 g, 11.89 mmol), compound **6** (1.64 g, 67%) was obtained as yellow oil; R_f = 0.31 (n-hexane:ethyl acetate

95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.93 (1H, s), 7.54 (1H, d, *J* = 7.5 Hz), 7.46 (1H, t, *J* = 7.8 Hz), 7.37 (1H, s), 7.23 (1H, d, *J* = 8.1 Hz), 7.00 (2H, d, *J* = 9.0 Hz), 6.91 (2H, d, *J* = 9.0 Hz), 3.82 (3H, s).

4.2.6. 3-(4-Chlorophenoxy)benzaldehyde (7)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 4-chlorophenol (1.53 g, 11.89 mmol), compound **7** (0.90 g, 36%) was obtained as yellow oil; R_f = 0.36 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.95 (1H, s), 7.62 (1H, d, *J* = 7.5 Hz), 7.51 (1H, t, *J* = 7.8 Hz), 7.44 (1H, s), 7.26-7.34 (3H, m), 6.96-6.98 (2H, m).

4.2.7. 3-(4-Fluorophenoxy)benzaldehyde (8)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 4-fluorophenol (1.33 g, 11.89 mmol), compound **8** (0.83 g, 36%) was obtained as yellow oil; R_f = 0.34 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.93 (1H, s), 7.58 (1H, d, *J* = 7.6 Hz), 7.48 (1H, t, *J* = 7.8 Hz), 7.40 (1H, s), 7.24 (1H, d, *J* = 8.1 Hz), 6.99-7.08 (4H, m).

4.2.8. 3-(3,4-Dichlorophenoxy)benzaldehyde (9)

Starting with 3-bromobenzaldehyde (2.0 g, 10.81 mmol) and 3,4-dichlorophenol (1.94 g, 11.89 mmol), compound **9** (0.82 g, 28%) was obtained as yellow oil; R_f = 0.34 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.95 (1H, s), 7.65 (1H, d, *J* = 7.5 Hz), 7.53 (1H, t, *J* = 7.8 Hz), 7.47 (1H, s), 7.39 (1H, d, *J* =8.7 Hz), 7.28 (1H, d, *J* = 8.0 Hz), 7.10 (1H, s), 6.88 (1H, d, *J* = 8.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 191.64, 157.16, 155.45, 138.06, 133.43, 131.26, 130.80, 127.47, 125.91, 125.09, 120.96, 118.51, 118.49.

4.2.9. 3-Benzylbenzaldehyde (10)

Following the reported procedure[21], starting with 3-formylphenyl boronic acid (1.75 g, 11.69 mmol) and benzyl bromide (2.0 g, 11.69 mmol), compound **10** (1.84 g, 80%) was prepared as

colorless oil; R_f = 0.50 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; DMSO-d_6; TMS) δ 1H NMR (400 MHz; DMSO-d6; TMS) δ 9.99 (1H, s), 7.79 (1H, s), 7.76 (1H, d, *J* = 7.5 Hz), 7.58 (1H, d, *J* = 7.6 Hz), 7.51 (1H, t, *J* = 7.5 Hz), 7.26-7.32 (4H, m), 7.20 (1H, t, *J* = 6.8 Hz), 4.04 (2H, s).

4.2.10. 3-Phenylbenzaldehyde (11)

Following the reported procedure[21], starting with 3-formylphenyl boronic acid (1.75 g, 11.69 mmol) and bromobenzene (1.83 g, 11.69 mmol), compound **11** (1.78 g, 84%) was prepared as colorless oil; R_f = 0.40 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 10.12 (1H, s), 8.14 (1H, s), 7.89 (2H, dd, *J* = 7.6 Hz, 1.7 Hz), 7.64-7.68 (3H, m), 7.51-7.41 (3H, m).

4.2.11. 3-Benzoylbenzaldehyde (13)

Intermediate **13** was prepared following a diminutive variation in the literature procedure.[22] *N,O*-dimethyl hydroxylamine HCl (1.36 g, 13.92 mmol), EDC.HCl (2.67 g, 13.92 mmol) and Et_3N (1.41 g, 13.92 mmol) were sequentially added to the solution of 3-benzoylbenzoic acid (3.0 g, 13.26 mmol) in DMF and stirred overnight at room temperature. Upon completion, the reaction mass was diluted with ethyl acetate and washed thrice, each time with 10% citric acid, 10% sodium bicarbonate and brine. The collected organic layers were dried over magnesium sulfate and the solvent was evaporated under reduced pressure to give colorless viscous oil. The colorless oil was further dissolved in anhydrous THF under nitrogen atmosphere. To it was added $LiAlH_4$ (0.23 g, 6.14 mmol) portion wise at -78°C. The mixture was stirred for 3 hours at the same temperature. Upon completion, it was quenched with water. The quenched reaction was diluted with ethyl acetate and filtered to remove the inorganic solid mass. The filtrate was washed with brine, dried over magnesium sulfate and the solvent was removed in vacuo to give crude product which was purified through flash chromatography (n-hexane:ethyl acetate 90:10)

to yield 3-(hydroxy(phenyl)methyl)benzaldehyde (**12**) (1.93 g, 69%) as colorless oil; R_f = 0.50 (n-hexane:ethyl acetate 90:10); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.95 (1H, s), 7.91 (1H, s), 7.76 (1H, d, *J* = 7.6 Hz), 7.65 (1H, d, *J* = 7.7 Hz), 7.48 (1H, t, *J* = 7.7 Hz), 7.27-7.37 (5H, m), 5.89 (1H, s), 2.83 (1H, s).

Intermediate **12** (1.0 g, 4.71 mmol) was dissolved in THF followed by addition of Dess-Martin periodinane (3.0 g, 7.07 mmol) and stirred for 4 hours at room temperature and, upon completion of the reaction, it was quenched by saturated $NaHCO_3$ and saturated $Na_2S_2O_3$. The quenched reaction was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine, dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography (n-hexane:ethyl acetate 95:5) to yield **13** (0.8 g, 81%) as colorless oil; R_f = 0.45 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 10.09 (1H, s), 8.28 (1H, t, *J* = 1.6 Hz), 8.12 (1H, dt, *J* = 7.6 Hz, *J* = 1.3 Hz), 8.08 (1H, dt, *J* = 7.8 Hz, *J* = 1.5 Hz), 7.80 (2H, d, *J* = 7.9 Hz), 7.68 (1H, t, *J* = 7.7 Hz), 7.63 (1H, t, *J* = 7.4 Hz), 7.50-7.53 (2H, m).

4.2.12. 3-Benzyloxybenzaldehyde (14)

To the solution of the 3-hydroxybenzaldehyde (1.43 g, 11.69 mmol) in acetonitrile were added benzyl bromide (2.0 g, 11.69 mmol) and potassium carbonate (3.23 g, 23.38 mmol) and stirred for 14 hours at room temperature. After the completion of reaction, as indicated by TLC, solvent was removed in vacuo, the residue was diluted with water and extracted with ethyl acetate (3 x 20 mL). The separated organic layers were dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The residual oil was purified by flash chromatography (n-hexane:ethyl acetate 95:5) to yield compound **14** (2.2 g, 89%) as white solid; R_f = 0.54 (n-

hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.97 (1H, s), 7.32-7.48 (8H, m), 7.23-7.26 (1H, m), 5.12 (2H, s).

4.2.13. 3-Cinnamyloxybenzaldehyde (15)

Following the procedure as described for **14**, starting with 3-hydroxybenzaldehyde (1.60 g, 13.10 mmol) and cinnamyl chloride (2.0 g, 13.10 mmol), compound **15** (2.46 g, 79%) was obtained as pale yellow solid; R_f = 0.62 (n-hexane:ethyl acetate 95:5); ^{1}H NMR (400 MHz; $CDCl_3$; TMS) δ 9.98 (1H, s), 7.41-7.48 (6H, m), 7.34 (3H, m), 6.76 (1H, d, *J* = 15.9 Hz), 6.42 (1H, dt, *J* = 16.1 Hz, 5.8 Hz), 4.77 (2H, d, *J* = 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 192.22, 159.14, 137.76, 136.21, 133.49, 130.13, 128.62, 128.05, 126.60, 123.68, 123.64, 122.15, 113.12, 68.64.

4.2.14. Synthesis of 2-(5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid derivatives (17-39)

Intermediate L-**16** ($[\alpha]^{25}_{D}$ –80.79° (*c* 0.1) was prepared and subjected to Knoevenagel condensation with aromatic aldehydes adhering to the procedures described in our previous reports;[16,19] to obtain target compounds **17-39**.

4.2.15. (L,Z)-2-(5-(3-Chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (17)[31]

Starting with L-**16** (0.30 g, 1.07 mmol) and 3-chlorobenzaldehyde (0.15 g, 1.07 mmol), compound **17** (0.30 g, 72%) was obtained as brown solid; m.p. 146-148 °C; R_f = 0.60 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.51 (1H, s), 7.81 (1H, s), 7.74 (1H, s), 7.54-7.58 (3H, m), 7.16-7.20 (5H, m), 5.88 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.03, 169.12, 166.78, 136.95, 135.24, 134.57, 132.74, 131.82, 131.27, 129.46, 128.92, 128.84, 128.79, 127.25, 58.68, 33.52; Anal. Calcd. for $C_{19}H_{14}ClNO_3S_2 \cdot 1/4C_6H_{14}$: C, 57.87; H, 4.15; N, 3.29. Found: C, 58.13; H, 4.29; N, 3.66.

4.2.16. (L,Z)-2-(5-(3-Bromobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (18)

Starting with L-**16** (0.30 g, 1.07 mmol) and 3-bromobenzaldehyde (0.19 g, 1.07 mmol), compound **18** (0.23 g, 48%) was obtained as brown solid; m.p. 128-130 °C; R_f = 0.64 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.51 (1H, s), 7.88 (1H, s), 7.81 (1H, s), 7.72 (1H, d, *J* = 7.7 Hz), 7.58 (1H, d, *J* = 7.4 Hz), 7.50 (1H, t, *J* = 7.7 Hz), 7.15-7.23 (5H, m), 5.89 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.06, 169.10, 166.77, 136.98, 135.52, 134.13, 132.68, 132.02, 129.46, 129.17, 128.79, 127.24, 123.07, 122.89, 58.72, 33.56; Anal. Calcd. for $C_{19}H_{14}BrNO_3S_2$·1/10H_2O: C, 50.69; H, 3.18; N, 3.11. Found: C, 50.83; H, 3.56; N, 3.15.

4.2.17. (L,Z)-2-(5-(3-Cyanobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (19)

Starting with L-**16** (0.30 g, 1.07 mmol) and 3-cyanobenzaldehyde (0.14 g, 1.07 mmol), compound **19** (0.12 g, 29%) was obtained as yellow solid; m.p. 103-105 °C; R_f = 0.67 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.53 (1H, s), 8.12 (1H, s), 7.96 (1H, d, *J* = 7.7 Hz), 7.88 (1H, d, *J* = 7.8 Hz), 7.84 (1H, s), 7.73 (1H, t, *J* = 7.8 Hz), 7.14-7.25 (5H, m), 5.89 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 192.97, 169.09, 166.78, 136.93, 135.14, 134.55, 134.49, 134.30, 132.00, 131.16, 129.46, 128.79, 127.26, 123.63, 118.48, 113.10, 58.72, 33.52; Anal. Calcd. for $C_{20}H_{14}N_2O_3S_2$.1/10H_2O: C, 60.62; H, 3.61; N, 7.07. Found: C, 60.49; H, 3.47; N, 7.09.

4.2.18. (L,Z)-2-(5-(4-Bromobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (20)[32]

Starting with L-**16** (0.30 g, 1.07 mmol) and 4-bromobenzaldehyde (0.19 g, 1.07 mmol), compound **20** (0.38 g, 79%) was obtained as yellow solid; m.p. 175-180 °C; R_f = 0.62 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.49 (1H, s), 7.79 (1H, s), 7.74 (2H, d, *J* = 8.5 Hz), 7.56 (2H, d, *J* = 8.5 Hz), 7.15-7.23 (5H, m), 5.88 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.20, 169.12, 166.88, 136.95, 133.14, 133.01, 132.33, 129.45, 128.76, 127.23, 125.56, 125.44, 121.90, 58.66, 33.79; Anal. Calcd. for $C_{19}H_{14}BrNO_3S_2 \cdot 1/4H_2O$: C, 50.39; H, 3.23; N, 3.09. Found: C, 50.11; H, 3.63; N, 3.07.

4.2.19. (L,Z)-2-(5-(4-Fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (21)

Starting with L-**16** (0.30 g, 1.07 mmol), and 4-fluorobenzaldehyde (0.13 g, 1.07 mmol), compound **21** (0.34 g, 83%) was obtained as brown solid; m.p. 146-148 °C; R_f = 0.60 (DCM:MeOH 95:5), ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.43 (1H, s), 7.83 (1H, s), 7.69-7.73 (2H, m), 7.38-7.42 (2H, m), 7.14-7.22 (5H, m), 5.88 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.79, 169.14, 166.91, 163.77 (d, *J* = 251.8 Hz), 136.97, 133.91, 133.31, 129.87, 129.45, 128.75, 127.21, 120.81, 117.24 (d, *J* = 22.2 Hz), 58.65, 33.54; Anal. Calcd. for $C_{19}H_{14}FNO_3S_2$: C, 58.90; H, 3.64; N, 3.62. Found: C, 59.18; H, 3.70; N, 3.60.

4.2.20. (L,Z)-2-(5-(4-Chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (22)

Starting with L-**16** (0.30 g, 1.07 mmol) and 4-chlorobenzaldehyde (0.15 g, 1.07 mmol), compound **22** (0.22 g, 51%) was obtained as yellow solid; m.p. 173-176 °C; R_f = 0.60 (DCM:MeOH 95:5), ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.63 (1H, s), 7.81 (1H, s), 7.60-7.66 (4H, m), 7.14-7.22 (5H, m), 5.88 (1H, s), 3.52 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.13, 169.21, 166.94, 137.13, 136.38, 132.97, 132.92, 132.04, 130.08, 129.44, 128.76,

127.20, 121.89, 58.84, 33.58; Anal. Calcd. for $C_{19}H_{14}ClNO_3S_2$: C, 56.50; H, 3.49; N, 3.47. Found: C, 56.42; H, 3.42; N, 3.54.

4.2.21. (L,Z)-2-(5-(3,4-Dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (23)

Experimental procedure and spectral characterization data is same as reported previously.[19]

4.2.22. (L,Z)-2-(5-(3,4-Difluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (24)

Starting with L-**16** (0.30 g, 1.07 mmol) and 3,4-difluorobenzaldehyde (0.15 g, 1.07 mmol), compound **24** (0.31 g, 72%) was obtained as yellow solid; m.p. 158-162 °C; R_f = 0.52 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.53 (1H, s), 7.74-7.79 (2H, m), 7.58-7.65 (1H, m), 7.46-7.48 (1H, m), 7.14-7.22 (5H, m), 5.88 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.20, 169.01, 166.79, 151.21 (dd, J = 253.6, 12.96 Hz), 150.11 (dd, J = 248.9, 12.96 Hz), 136.96, 132.10, 130.90, 129.43, 128.76, 128.19, 127.22, 122.47, 120.46 (d, J = 17.9 Hz), 119.26 (d, J = 17.9 Hz), 58.72, 33.54; Anal. Calcd. for $C_{19}H_{13}F_2NO_3S_2 \cdot CH_2Cl_2$: C, 48.99; H, 3.08; N, 2.86. Found: C, 48.79; H, 2.78; N, 3.07.

4.2.23. (L,Z)-2-(5-(3,5-Difluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (25)

Starting with L-**16** (0.30 g, 1.07 mmol) and 3,5-difluorobenzaldehyde (0.15 g, 1.07 mmol), compound **25** (0.29 g, 67%) was obtained as yellow solid; m.p. 156-160 °C; R_f = 0.50 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.53 (1H, s), 7.80 (1H, s), 7.45 (1H, tt, J = 9.1 Hz, 2.3 Hz), 7.35 (2H, d, J = 6.6 Hz), 7.15-7.23 (5H, m), 5.89 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 192.94, 169.04, 166.72, 162.99 (dd, J = 247.7 Hz, 13.2 Hz), 161.79, 136.92, 136.35, 131.62, 129.43, 128.78, 127.24, 124.33, 113.86 (d, J = 26.5

Hz), 106.75 (d, *J* = 20.2 Hz), 58.74, 33.53; Anal. Calcd. for $C_{19}H_{13}F_2NO_3S_2$: C, 56.29; H, 3.23; N, 3.45. Found: C, 56.09; H, 3.26; N, 3.36.

4.2.24. (L,Z)-2-(5-(2-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (26)

Starting with L-**16** (0.30 g, 1.07 mmol) and **3** (0.21 g, 1.07 mmol), compound **26** (0.16 g, 33%) was obtained as yellow solid; m.p. 65-70 °C; R_f = 0.62 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.51 (1H, s), 7.91 (1H, s), 7.57 (1H, d, *J* = 7.8 Hz), 7.44-7.53 (3H, m), 7.24-7.32 (2H, m), 7.09-7.22 (7H, m), 6.90 (1H, d, *J* = 8.3 Hz), 5.87 (1H, s), 3.49 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) 193.64, 169.14, 166.99, 156.70, 156.13, 137.03, 133.88, 130.87, 129.45, 128.75, 128.31, 127.19, 125.01, 124.74, 123.91, 123.40, 122.44, 119.76 , 118.76, 58.74, 33.56; Anal. Calcd. for $C_{25}H_{19}NO_4S_2 \cdot 1/10H_2O$: C, 64.80; H, 4.18; N, 3.02. Found: C, 64.57; H, 4.04; N, 2.95.

4.2.25. (L,Z)-2-(5-(3-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (27)

Experimental procedure and spectral characterization data is same as reported previously[19] except the new information provided in regards to specific rotation: $[\alpha]^{25}_D$ -155.09° (*c* 10); ee = 85% (t_R (major) = 13.58, t_R (minor) = 11.32).

4.2.26. (L,Z)-2-(5-(4-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (28)

Starting with L-**16** (0.30 g, 1.07 mmol) and 4-phenoxybenzaldehyde (0.21 g, 1.07 mmol), compound **28** (0.42 g, 86%) was obtained as yellow solid; m.p. 137-139 °C; R_f = 0.8 (DCM:MeOH 95:5), ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.48 (1H, s), 7.79 (1H, s), 7.64 (2H, d, *J* = 8.4 Hz), 7.46 (2H, t, *J* = 7.6 Hz), 7.08-7.27 (10H, m), 5.88 (1H, s), 3.52 (2H, s); ^{13}C

NMR (100 MHz, DMSO-d_6, TMS) δ 193.22, 169.21, 166.99, 160.17, 155.35, 137.01, 133.89, 133.75, 130.83, 129.45, 128.74, 127.80, 127.19, 125.31, 120.47, 119.29, 118.75, 58.61, 33.56; Anal. Calcd. for $C_{25}H_{19}NO_4S_2 \cdot 1/6C_6H_{14}$: C, 65.62; H, 4.52; N, 2.94. Found: C, 65.48; H, 4.89; N, 2.79.

4.2.27. (L,Z)-2-(5-(3-(3-Chlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (29)

Starting with L-**16** (0.30 g, 1.07 mmol) and **4** (0.25 g, 1.07 mmol), compound **29** (0.32 g, 60%) was obtained as yellow solid, m.p. 55-58 °C; R_f = 0.50 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ -171.06° (*c* 10); ee = 93% (t_R (major) = 14.54, t_R (minor) = 11.99); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.52 (1H, s), 7.81 (1H, s), 7.57 (1H , t, *J* = 7.8 Hz), 7.39-7.45 (2H, m), 7.31 (1H, s), 7.15-7.26 (8H, m), 7.04 (1H, d, *J* = 7.8 Hz), 5.87 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.14, 169.12, 166.81, 157.57, 157.24, 137.03, 135.19, 134.63, 133.55, 132.15, 131.91, 129.44, 128.76, 127.23, 126.31, 124.50, 122.25, 121.98, 121.30, 119.46, 118.10, 58.73, 33.53; Anal. Calcd. for $C_{25}H_{18}ClNO_4S_2$: C, 60.54; H, 3.66; N, 2.82. Found: C, 60.29; H, 3.68; N, 2.81.

4.2.28. (L,Z)-2-(5-(3-(3-Fluorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (30)

Starting with L-**16** (0.30 g, 1.07 mmol) and **5** (0.23 g, 1.07 mmol), compound **30** (0.40 g, 78%) was obtained as yellow solid; m.p. 58-60 °C; R_f = 0.45 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.54 (1H, s), 7.82 (1H, s), 7.58 (1H, t, *J* = 8.0 Hz), 7.39-7.47 (2H , m), 7.31 (1H, s), 7.15-7.23 (6H, m), 7.03 (1H, t, *J* = 8.4 Hz), 6.98 (1H, d, *J* = 10.4 Hz), 6.90 (1H, d, *J* = 8.3 Hz), 5.87 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.20, 169.13, 166.81, 163.40 (d, *J* = 245.3 Hz), 157.84, 157.19, 137.00, 135.15, 133.55, 132.01, 131.88, 129.44, 128.76, 127.21, 126.24, 122.21, 121.97, 121.21, 115.29, 111.26 (d, *J* = 20.9 Hz), 107.05

(d, J = 24.5 Hz), 58.73, 33.53; Anal. Calcd. for $C_{25}H_{18}FNO_4S_2.4/5H_2O$: C, 60.79; H, 4.00; N, 2.84. Found: C, 60.41; H, 3.73; N, 2.83.

4.2.29. (L,Z)-2-(5-(3-(4-Methoxyphenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (31)

Starting with L-**16** (0.30 g, 1.07 mmol) and **6** (0.24 g, 1.07 mmol), compound **31** (0.38 g, 73%) was obtained as yellow solid; m.p. 68-72 °C; R_f = 0.48 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.74 (1H, s), 7.76 (1H, s), 7.50 (1H, t, J = 8.0 Hz), 7.29 (1H, d, J = 7.8 Hz), 7.17-7.20 (2H, m), 7.11-7.14 (4H, m), 7.05-7.07 (3H, m), 6.98-7.00 (2H, m), 5.82 (1H, s), 3.75 (3H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.33, 166.92, 159.40, 156.59, 148.95, 137.44, 134.89, 133.54, 131.57, 129.38, 128.74, 127.16, 125.11, 122.10, 121.73, 120.20, 119.02, 116.02, 115.74, 59.35, 55.92, 33.73; Anal. Calcd. for $C_{26}H_{21}NO_5S_2 \cdot 2/5H_2O$: C, 62.61; H, 4.41; N, 2.81. Found: C, 62.22; H, 4.16; N, 2.82.

4.2.30. (L,Z)-2-(5-(3-(4-Chlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (32)

Starting with L-**16** (0.30 g, 1.07 mmol) and **7** (0.25 g, 1.07 mmol), compound **32** (0.42 g, 79%) was obtained as yellow solid; m.p. 132-135 °C; R_f = 0.48 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ -159.16° (*c* 10); ee = 86% (t_R (major) = 13.88, t_R (minor) = 11.44); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.53 (1H, s), 7.80 (1H, s), 7.56 (1H, t, J = 8.0 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.38 (1H, d, J = 7.6 Hz), 7.28 (1H, s), 7.09-7.19 (8H, m), 5.87 (1H, s), 3.49 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.13, 169.12, 166.81, 157.62, 155.32, 137.00, 135.12, 133.57, 131.82, 130.54, 129.44, 128.76, 128.37, 127.21, 125.92, 122.16, 121.64, 121.33, 120.93, 58.71, 33.55; Anal. Calcd. for $C_{25}H_{18}ClNO_4S_2$: C, 60.54; H, 3.66; N, 2.82. Found: C, 60.33; H, 3.44; N, 2.65.

4.2.31. (L,Z)-2-(5-(3-(4-Fluorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (33)

Starting with L-**16** (0.30 g, 1.07 mmol) and **8** (0.23 g, 1.07 mmol), compound **33** (0.35 g, 69%) was obtained as yellow solid; m.p. 58-62 °C; R_f = 0.42 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ -168.45° (*c* 9); ee = 84% (t_R (major) = 14.11, t_R (minor) = 11.43); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.55 (1H, s), 7.79 (1H, s), 7.53 (1H, t, *J* = 8.0 Hz), 7.34 (1H, d, *J* = 7.6 Hz), 7.27 (2H, t, *J* = 8.7 Hz), 7.13-7.19 (9H, m), 5.87 (1H, s), 3.49 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.14, 169.15, 166.84, 159.05 (d, *J* = 239.9 Hz), 158.52, , 152.23, 137.03, 135.03, 133.72, 131.76, 129.51, 128.77, 127.23, 125.64, 122.05, 121.90, 120.92, 119.99, 117.30 (d, *J* = 23.8 Hz), 58.72, 33.53; Anal. Calcd. for $C_{25}H_{18}FNO_4S_2$: C, 62.62; H, 3.78; N, 2.92. Found: C, 62.35; H, 3.85; N, 2.90.

4.2.32. (L,Z)-2-(5-(3-(3,4-dichlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (34)

Starting with L-**16** (0.30 g, 1.07 mmol) and **9** (0.29 g, 1.07 mmol), compound **34** (0.30 g, 53%) was obtained as yellow solid; m.p. 182-185 °C; R_f = 0.49 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ -144.69° (*c* 10); ee = 77% (t_R (major) = 15.55, t_R (minor) = 13.03); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.54 (1H, s), 7.82 (1H, s), 7.66 (1H, d, *J* = 8.9 Hz), 7.58 (1H, t, *J* = 8.0 Hz), 7.39-7.41 (2H, m), 7.35 (1H, s), 7.15-7.26 (6H, m), 7.08 (1H, dd, *J* = 8.8 Hz, 2.6 Hz), 5.87 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.21, 169.21, 166.86, 157.01, 156.04, 137.10, 135.25, 133.43, 132.62, 132.18, 131.91, 129.42, 128.75, 127.19, 126.53, 126.25, 122.35, 121.96, 121.48, 121.42, 119.76, 58.83, 33.63; Anal. Calcd. for $C_{25}H_{17}Cl_2NO_4S_2$: C, 56.61; H, 3.23; N, 2.64. Found: C, 56.51; H, 3.14; N, 2.69.

4.2.33. (L,Z)-2-(5-(3-Benzylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (35)

Starting with L-**16** (0.30 g, 1.07 mmol) and **10** (0.21 g, 1.07 mmol), compound **35** (0.19 g, 39%) was obtained as yellow solid; m.p. 122-125 °C; R_f = 0.54 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.54 (1H, s), 7.75 (1H, s), 7.44-7.46 (3H, m), 7.40 (1H, s), 7.25-7.32 (4H, m), 7.13-7.21 (6H, m), 5.87 (1H, s), 4.01 (2H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.36, 169.16, 166.90, 143.33, 141.10, 137.03, 134.31, 133.32, 132.19, 131.14, 130.17, 129.44, 129.23, 129.03, 128.75, 127.19, 126.66, 121.14, 58.71, 41.10, 33.55; Anal. Calcd. for $C_{26}H_{21}NO_3S_2$: C, 67.95; H, 4.61; N, 3.05. Found: C, 68.08; H, 4.60; N, 2.99.

4.2.34. (L,Z)-2-(5-(3-Benzoylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (36)

Starting with L-**16** (0.30 g, 1.07 mmol) and **13** (0.22 g, 1.07 mmol), compound **36** (0.17 g, 34%) was obtained as yellow solid; m.p. 70-74 °C; R_f = 0.46 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.53 (1H, s), 7.90-7.94 (3H, m), 7.86 (1H, d, J = 7.6 Hz), 7.78 (2H, d, J = 7.8 Hz), 7.70-7.75 (2H, m), 7.57-7.60 (2H, m), 7.15-7.20 (5H, m), 5.88 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 195.37, 193.12, 169.10, 166.78, 138.47, 137.16, 137.02, 136.87, 134.69, 133.62, 133.46, 132.23, 132.06, 130.41, 130.29, 129.45, 129.17, 128.77, 127.23, 122.51, 58.76, 33.56; Anal. Calcd. for $C_{26}H_{19}NO_4S_2 \cdot 1/3H_2O$: C, 65.12; H, 4.13; N, 2.92. Found: C, 64.98; H, 3.99; N, 2.93.

4.2.35. (L,Z)-2-(5-(3-Phenylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (37)

Starting with L-**16** (0.30 g, 1.07 mmol) and **11** (0.19 g, 1.07 mmol), compound **37** (0.24 g, 51%) was obtained as yellow solid; m.p. 75-77 °C; R_f = 0.42 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz,

DMSO-d_6, TMS) δ 13.64 (1H, s), 7.91 (1H, s), 7.83 (1H, d, *J* = 7.6 Hz), 7.73 (2H, d, *J* = 7.7 Hz), 7.64 (1H, t, *J* = 7.7 Hz), 7.59 (1H, d, *J* = 7.7 Hz), 7.51 (2H, t, *J* = 7.5 Hz), 7.42 (1H, t, *J* = 7.3 Hz), 7.15-7.23 (6H, m), 5.87 (1H, s), 3.53 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.38, 169.40, 166.97, 141.73, 139.41, 137.32, 134.27, 133.90, 130.67, 129.93, 129.90, 129.59, 129.43, 128.76, 128.68, 128.55, 127.16, 127.31, 121.72, 59.05, 33.70; Anal. Calcd. for $C_{25}H_{19}NO_3S_2.4/5H_2O$: C, 65.28; H, 4.51; N, 3.05. Found: C, 64.97; H, 4.39; N, 3.12.

4.2.36. (L,Z)-2-(5-(3-Benzyloxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (38)

Starting with L-**16** (0.30 g, 1.07 mmol) and **14** (0.23 g, 1.07 mmol), compound **38** (0.22 g, 43%) was obtained as yellow solid; m.p. 70-73 °C; R_f = 0.45 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.78 (1H, s), 7.76 (1H, s), 7.46-7.48 (3H, m), 7.40 (2H, t, *J* = 7.2 Hz), 7.35 (1H, d, *J* = 6.8 Hz), 7.16-7.24 (8H, m), 5.83 (1H, s), 5.17 (2H, s), 3.52 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.60, 167.00, 159.29, 137.46, 137.09, 134.55, 133.94, 131.17, 129.39, 128.97, 128.74, 128.45, 128.24, 127.11, 123.46, 121.74, 118.63, 117.05, 69.87, 59.31, 33.79; Anal. Calcd. for $C_{26}H_{21}NO_4S_2.2/5H_2O$: C, 64.68; H, 4.55; N, 2.90. Found: C, 64.57; H, 4.51; N, 2.88.

4.2.37. (L,Z)-2-(5-(3-Cinnamyloxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (39)

Starting with L-**16** (0.30 g, 1.07 mmol) and **15** (0.25 g, 1.07 mmol), compound **39** (0.30 g, 56%) was obtained as yellow solid; m.p. 137-140 °C; R_f = 0.58 (DCM:MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.62 (1H, s), 7.79 (1H, s), 7.45-7.50 (3H, m), 7.35 (2H, t, *J* = 7.4 Hz), 7.14-7.29 (9H, m), 6.80 (1H, d, *J* = 16.0 Hz), 6.52 (1H, dt, *J* = 15.95 Hz, 5.83 Hz), 5.88 (1H, s), 4.80 (2H, d, *J* = 5.6 Hz), 3.52 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.37, 169.29,

166.93, 159.18, 137.14, 136.50, 134.51, 134.26, 133.28, 131.17, 129.43 129.15, 128.75, 128.42, 127.18, 126.99, 125.00, 123.45, 121.57, 118.65, 116.92, 68.79, 58.86, 33.62; Anal. Calcd. for $C_{28}H_{23}NO_4S_2$. 1/4H_2O: C, 66.45; H, 4.68; N, 2.77. Found: C, 66.09; H, 4.63; N, 2.70.

4.2.38. (D)-2-(4-Oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (40)

It was prepared following the same procedure as for compound L-**16**, except substituting D-phenylalanine for L-phenylalanine; obtained as yellow oil (3 g, 35%); R_f = 0.70 (DCM:MeOH 95:5) $[\alpha]^{25}_D$ +60.77° (*c* 10); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.32 (1H, s), 7.15-7.25 (5H, m), 5.68 (1H, s), 4.21-4.36 (2H, m), 3.42-3.47 (2H, m).

4.2.39. (D,Z)-2-(5-(3-Phenoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (41)

Starting with D-**40** (0.30 g, 1.07 mmol) and 3-phenoxybenzaldehyde (0.21 g, 1.07 mmol), compound **41** (0.17 g, 35%) was obtained as yellow solid with slight variations from the reported procedure[33]; m.p. 70-75 °C; R_f = 0.60 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ +166.83° (*c* 10); ee = 84% (t_R (major) = 11.09, t_R (minor) = 13.34); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.70 (1H, s), 7.77 (1H, s), 7.54 (1H, t, *J* = 7.9 Hz), 7.44 (2H, t, *J* = 7.8 Hz), 7.35 (1H, d, *J* = 7.7 Hz), 7.15-7.22 (8H, m), 7.08 (2H, d, *J* = 7.8 Hz), 5.81 (1H, s), 3.52 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 192.66, 168.70, 166.36, 157.63, 155.79, 140.76, 136.52, 134.54, 133.26, 131.74, 130.74, 129.00, 128.32, 126.78, 125.35, 124.23, 123.03, 121.64, 121.06, 119.91, 119.32, 58.22, 33.10; Anal. Calcd. for $C_{25}H_{19}NO_4S_2$: C, 65.06; H, 4.15; N, 3.03. Found: C, 64.78; H, 4.38; N, 2.96.

4.2.40. (D,Z)-2-(5-(3-(3-Chlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (42)

Starting with D-**40** (0.30 g, 1.07 mmol) and **4** (0.25 g, 1.07 mmol), compound **42** (0.17 g, 32%) was obtained as yellow solid; m.p. 55-57 °C; R_f = 0.50 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ +126.61° (*c*

10); ee = 75% (t_R (major) = 11.97, t_R (minor) = 14.51); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.69 (1H, s), 7.80 (1H, s), 7.58 (1H, t, *J* = 7.6 Hz), 7.39-7.46 (2H, m), 7.31 (1H, s), 7.15-7.27 (8H, m), 7.04 (1H, d, *J* = 7.9 Hz), 5.82 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.16, 169.21, 166.83, 157.52, 157.18, 137.08, 135.20, 134.60, 133.49, 132.09, 131.88, 129.45, 128.76, 127.20, 126.27, 124.46, 122.28, 121.98, 121.23, 119.46, 118.04, 58.82, 33.59; Anal. Calcd. for $C_{25}H_{18}ClNO_4S_2.1/4H_2O$: C, 59.99; H, 3.73; N, 2.80. Found: C, 59.60; H, 3.72; N, 2.86.

4.2.41. (D,Z)-2-(5-(3-(4-Chlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (43)

Starting with D-**40** (0.30 g, 1.07 mmol) and **7** (0.25 g, 1.07 mmol), compound **43** (0.19 g, 36%) was obtained as yellow solid; m.p. 68-72 °C; R_f = 0.48 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ +139.20° (*c* 10); ee = 78% (t_R (major) = 11.47, t_R (minor) = 13.69); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.62 (1H, s), 7.80 (1H, s), 7.57 (1H, t, *J* = 8.0 Hz), 7.47 (2H, d, *J* = 8.7 Hz), 7.38 (1H, d, *J* = 7.7 Hz), 7.28 (1H, s), 7.10-7.22 (8H, m), 5.83 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.19, 169.10, 166.86, 157.63, 155.32, 137.06, 135.13, 133.54, 131.83, 130.55, 129.43, 128.76, 128.37, 127.20, 125.92, 122.21, 121.64, 121.34, 120.93, 58.84, 33.57; Anal. Calcd. for $C_{25}H_{18}ClNO_4S_2$: C, 60.54; H, 3.66; N, 2.82. Found: C, 60.26; H, 3.84; N, 2.87.

4.2.42. (D,Z)-2-(5-(3-(4-Fluorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (44)

Starting with D-**40** (0.30 g, 1.07 mmol) and **8** (0.23 g, 1.07 mmol), compound **44** (0.15 g, 29%) was obtained as yellow solid; m.p. 73-76 °C; R_f = 0.42 (DCM:MeOH 95:5); $[\alpha]^{25}_D$ +144.59° (*c* 9); ee = 85% (t_R (major) = 11.28, t_R (minor) = 13.47); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.67 (1H, s), 7.77 (1H, s), 7.54 (1H, t, *J* = 8.0 Hz), 7.35 (1H, d, *J* = 7.7 Hz), 7.28 (2H, t, *J* = 8.7

Hz), 7.14-7.20 (9H, m), 5.80 (1H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ 193.16, 169.20, 166.85, 159.07 (d, J = 239.7 Hz), 158.53, , 152.18, 137.17, 135.04, 133.58, 131.75, 129.44, 128.79, 127.21, 125.58, 122.10, 121.90, 120.95, 119.98, 117.28 (d, J = 23.4 Hz), 58.89, 33.63; Anal. Calcd. for $C_{25}H_{18}FNO_4S_2.4/5H_2O$: C, 60.79; H, 4.00; N, 2.84. Found: C, 60.83; H, 3.75; N, 2.79.

4.2.43. (D,Z)-2-(5-(3-(3,4-Dichlorophenoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-3-phenylpropanoic acid (45)

Starting with D-**40** (0.30 g, 1.07 mmol) and **9** (0.29 g, 1.07 mmol), compound **45** (0.23 g, 40%) was obtained as yellow solid with slight variations from the reported procedure[33]; m.p. 175-180 °C; R_f = 0.49 (DCM:MeOH 95:5); $[\alpha]^{25}_{D}$ +157.54° (*c* 10); ee = 93% (t_R (major) = 12.89, t_R (minor) = 15.24); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 13.60 (1H, s), 7.81 (1H, s), 7.66 (1H, d, J = 8.9 Hz), 7.59 (1H, t, J = 8.0 Hz), 7.39-7.41 (2H, m), 7.34 (1H, s), 7.15-7.26 (6H, m), 7.08 (1H, dd, J = 8.8 Hz, 2.6 Hz), 5.86 (1H, s), 3.51 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) 193.13, 169.15, 166.82, 157.04, 156.05, 136.98, 135.25, 133.53, 132.64, 132.20, 131.94, 129.46, 128.78, 127.24, 126.55, 126.27, 122.33, 122.00, 121.52, 121.45, 119.80, 58.69, 33.54; Anal. Calcd. for $C_{25}H_{17}Cl_2NO_4S_2$: C, 56.61; H, 3.23; N, 2.64. Found: C, 56.89; H, 3.21; N, 2.61.

4.3. Determination of NS5B inhibitory activity

HCV NS5B polymerase inhibition was evaluated essentially as reported previously.[16]

4.4. Mutant counter screen assay

The binding site of the inhibitors on NS5B was investigated employing a mutant counter-screen assay as described previously.[28-30] Recombinant NS5BCΔ21 mutant proteins P495L, M423T and M414T were employed to screen for TP-I, TP-II and PP-I site binders, respectively.

NS5B inhibition assay with the mutants was essentially carried out as described for the wild-type NS5B.

4.5. Molecular modeling

Molecular docking computations were carried out on a Dell Precision 470n workstation with the RHEL 4.0 operating system using Glide 5.0 (Schrodinger, LLC, New York, NY). 3D Structures of target compounds **17-39** and **41**-**45** were constructed using the fragment dictionary of Maestro 9.0 (Schrodinger, LLC, New York, NY) and geometry was optimized by Macromodel program v9.5 using the OPLS-AA force field with the steepest descent followed by truncated Newton conjugate gradient protocol. The X-ray crystal structure of NS5B polymerase in complex with MK-3281 (PDB ID: 2XWY)[11], with PF-868554 (PDB ID: 3FRZ)[12], with indole C2-acyl sulfonamide (PDB ID: 3TYV)[27], with HCV-796 (PDB ID: 3FQL)[14] and palm pocket (PP)-III, that significantly overlaps with PP-II (large grid box created around PP-II bound HCV-796 to obtain docking pose at PP-III) representing thumb pocket (TP)-I, TP-II, palm pocket (PP)-I, PP-II and PP-III pockets, respectively, obtained from the RCSB Protein Data Bank (PDB), were used in this study. The protein was optimized for docking using the "Protein Preparation Wizard" and "Prime-Refinement Utility" of Maestro 9.0. The grids were generated using bound inhibitor with the default parameters. The detailed docking parameters were from our previous studies.[16]

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Figure 1. Molecular hybridization of lead compounds **1** and **2** to create a new template.

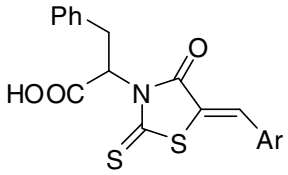
Figure 2. SP-Glide predicted binding model of compounds L-**34** (panel A) and D-**45** (panel B) within PP-I of NS5B polymerase. Key amino acids are depicted as stick model with the atoms colored as carbon – green, hydrogen – white, nitrogen – blue, oxygen – red and sulfur – yellow. Compounds are shown as ball and stick model with the same color scheme as above except carbon atoms are represented in orange and the chloro atoms in dark green. The dotted black line indicates hydrogen bonding interaction whereas the dotted red line indicates potential electrostatic contacts with distances in Å.

Scheme 1. Reagents and conditions: (a) RPhOH, CuO, K_2CO_3, pyridine, quinoline, 170 °C; (b) $Pd(PPh_3)_4$, K_2CO_3, THF, 80 °C; (c) *N*,*O*-dimethyl hydroxylamine·HCl, EDC·HCl, Et_3N, DMF, rt; (d) $LiAlH_4$, THF, -78 °C ; (e) Dess-Martin periodinane, THF, rt; (f) K_2CO_3, CH_3CN, rt.

Scheme 2. Reagents and conditions: (a) ArCHO, ammonium acetate, toluene, reflux, 4-6 hours.

Table 1

Chemical structure and IC_{50} values of compounds **17**-**39** and **41**-**45**



Compd	Ar	IC_{50} (μM)[a]	Gscore[c]
2	2,4-di-Cl-Ph	10.6 ± 1.5	-6.82
17	3-Cl-Ph	26.6 ± 0.6	-7.87
18	3-Br-Ph	7.4 ± 1.4	-7.80
19	3-CN-Ph	30.7 ± 1.3	-8.00
20	4-Br-Ph	11.7 ± 1.2	-7.73
21	4- F-Ph	27.7 ± 1.2	-8.1
22	4-Cl-Ph	22.3 ± 3.8	-7.91
23	3,4-di-Cl-Ph[19]	19.4 ± 1.2	-8.14
24	3,4-di-F-Ph	16.3 ± 1.3	-8.36
25	3,5-di-F-Ph	26.0 ± 1.2	-7.96
26	2-OPhe-Phe	13.3 ± 1.4	-[d]
27	3-OPhe-Phe[19]	6.7 ± 1.3 (ee = 85%)[b]	-6.60
28	4-OPh-Phe	50.7 ± 5.9	-5.84
29	3-(3-Cl)OPh-Phe	4.1 ± 1.1 (ee = 93%)[b]	-6.9
30	3-(3-F)OPh-Phe	8.2 ± 2.1	-6.76
31	3-(4-OMe)OPh-Phe	8.3 ± 0.3	-6.74
32	3-(4-Cl)OPh-Phe	3.4 ± 0.5 (ee = 86%)[b]	-7.1
33	3-(4-F)OPh-Phe	12.5 ± 1.7 (ee = 84%)[b]	-7.45
34	3-(3,4-di-Cl)OPh-Phe	2.6 ± 0.3 (ee = 77%)[b]	-6.92
35	3-benzylphenyl	4.8 ± 0.4	-6.88
36	3-benzoylphenyl	11.1 ± 0.6	-7.76
37	3-biphenyl	16.6 ± 2.8	-6.65
38	3-benzyloxyphenyl	21.8 ± 0.8	-6.79

39	3-cinnamyloxyphenyl	6.4 ± 0.1	-5.96
41	3-OPhe-Phe	19.3 ± 1.5 (ee = 84%)[b]	-7.38
42	3-(3-Cl)OPh-Phe	4.1 ± 1.0 (ee = 75%)[b]	-6.96
43	3-(4-Cl)OPh-Phe	5.1 ± 0.1 (ee = 78%)[b]	-7.01
44	3-(4-F)OPh-Phe	10.3 ± 1.0 (ee = 85%)[b]	-6.97
45	3-(3,4-di-Cl)OPh-Phe	5.4 ± 0.2 (ee = 93%)	-7.53

[a] The IC_{50} values of the compounds were determined from dose–response curves using 8-10 concentrations of the compound in duplicate in two independent experiments. Curves were fitted to data points using nonlinear regression analysis and IC_{50} values were interpolated from the resulting curves using GraphPad Prism 3.03 software. The IC_{50} values are expressed as the mean ± SD from two independent experiments in duplicate.
[b] % enantiomeric excess.
[c] Gscore (Glidescore) in kcal/mol.
[d] No docking pose resulted from Glide docking calculations.

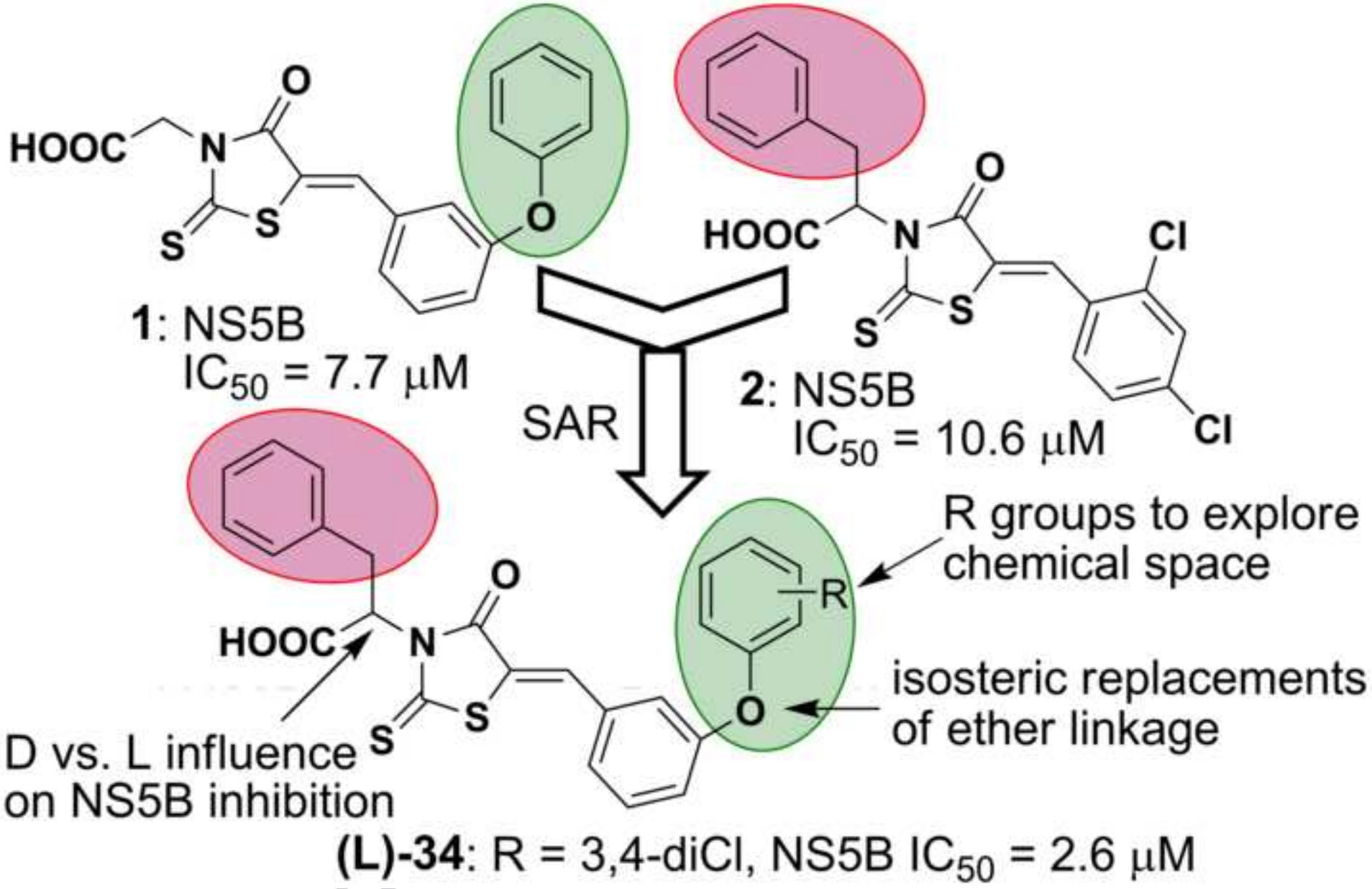
Table 2

Activity of inhibitors on NS5B mutants

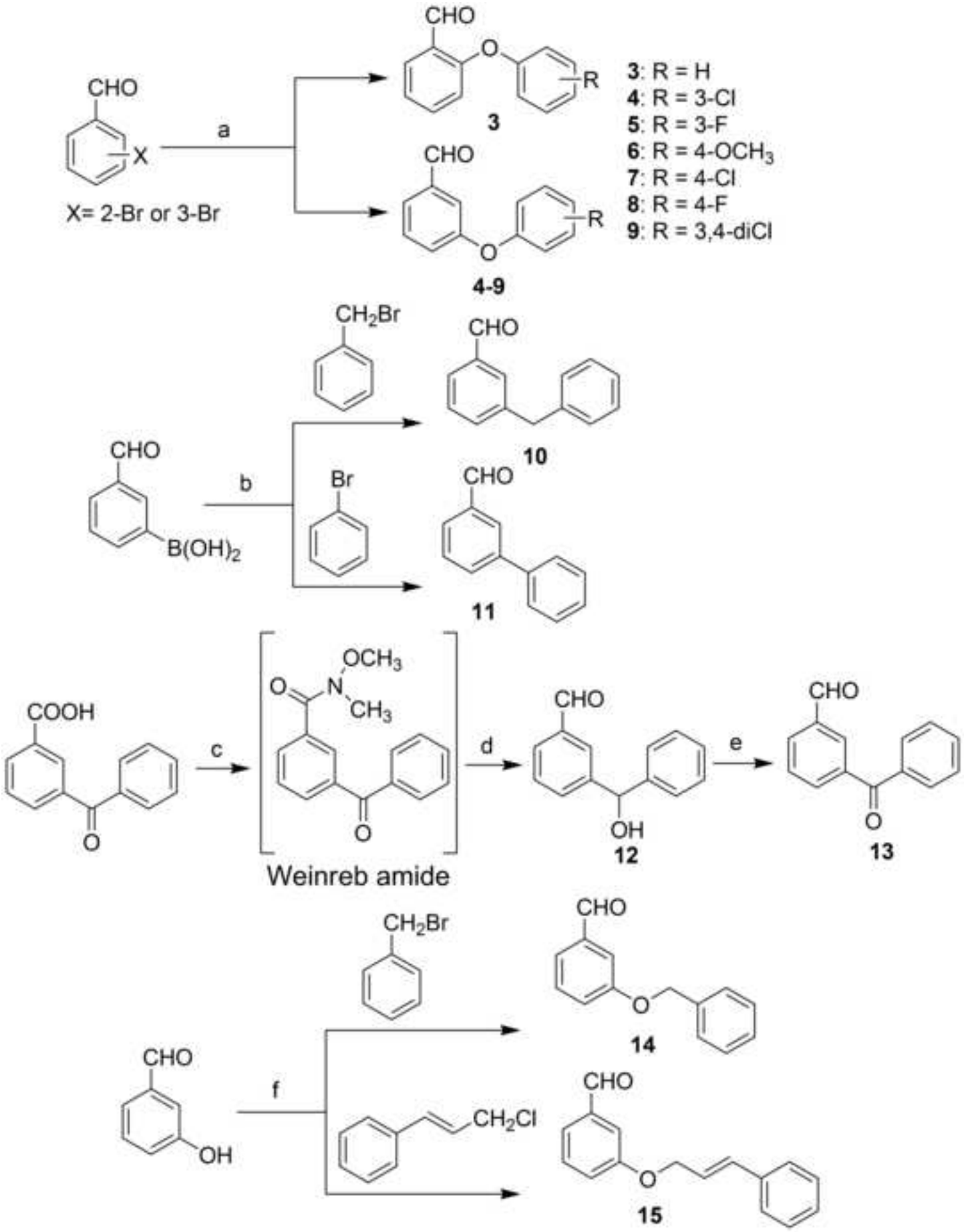
Compound	NS5B-P495L IC_{50} (μM)	NS5B-M423T IC_{50} (μM)	NS5B-M414T IC_{50} (μM)
32	3.6 ± 0.2	4.2 ± 0.2	55.7 ± 1.2
34	2.8 ± 0.3	3.0 ± 0.5	47.8 ± 2.5

The IC_{50} values of the compounds against the indicated NS5B mutants were determined from dose-response curves as described for the wild-type NS5B. The values represent an average ± SD from at least two independent experiments in duplicate.

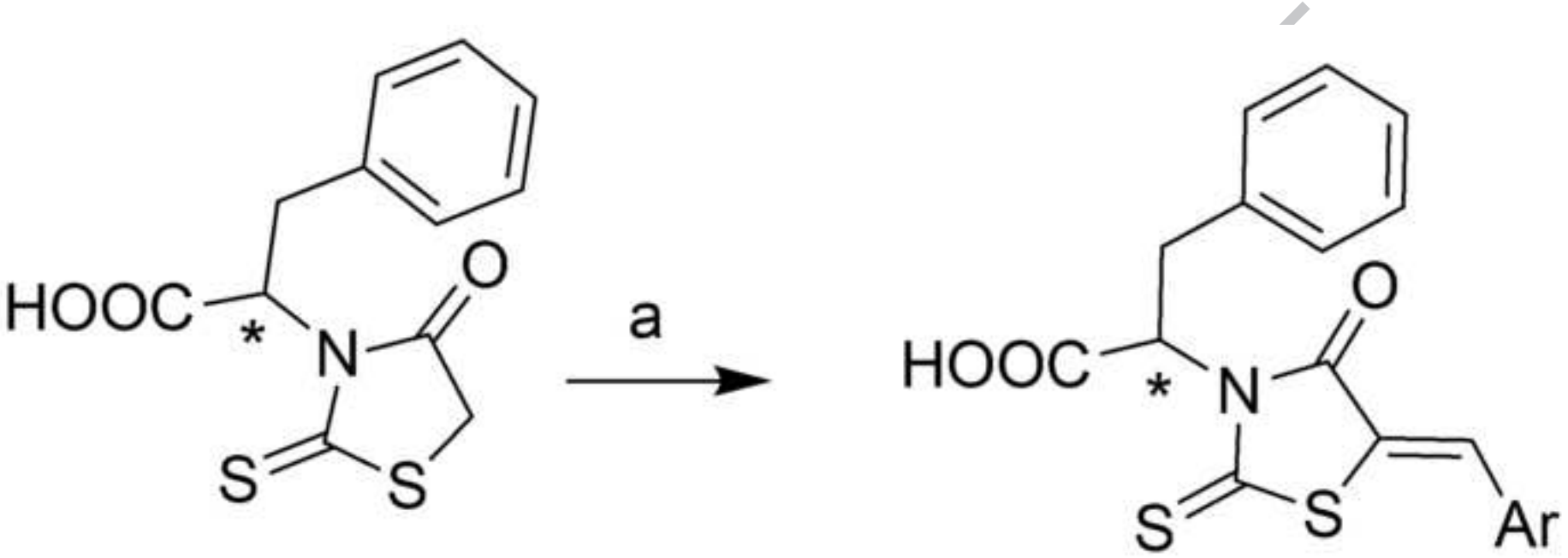
Figure 1



Scheme 1



Scheme 2



16: * L-configuration
40: * D-configuration

17-39: * L configuration
41-45: * D configuration

Figure 2A

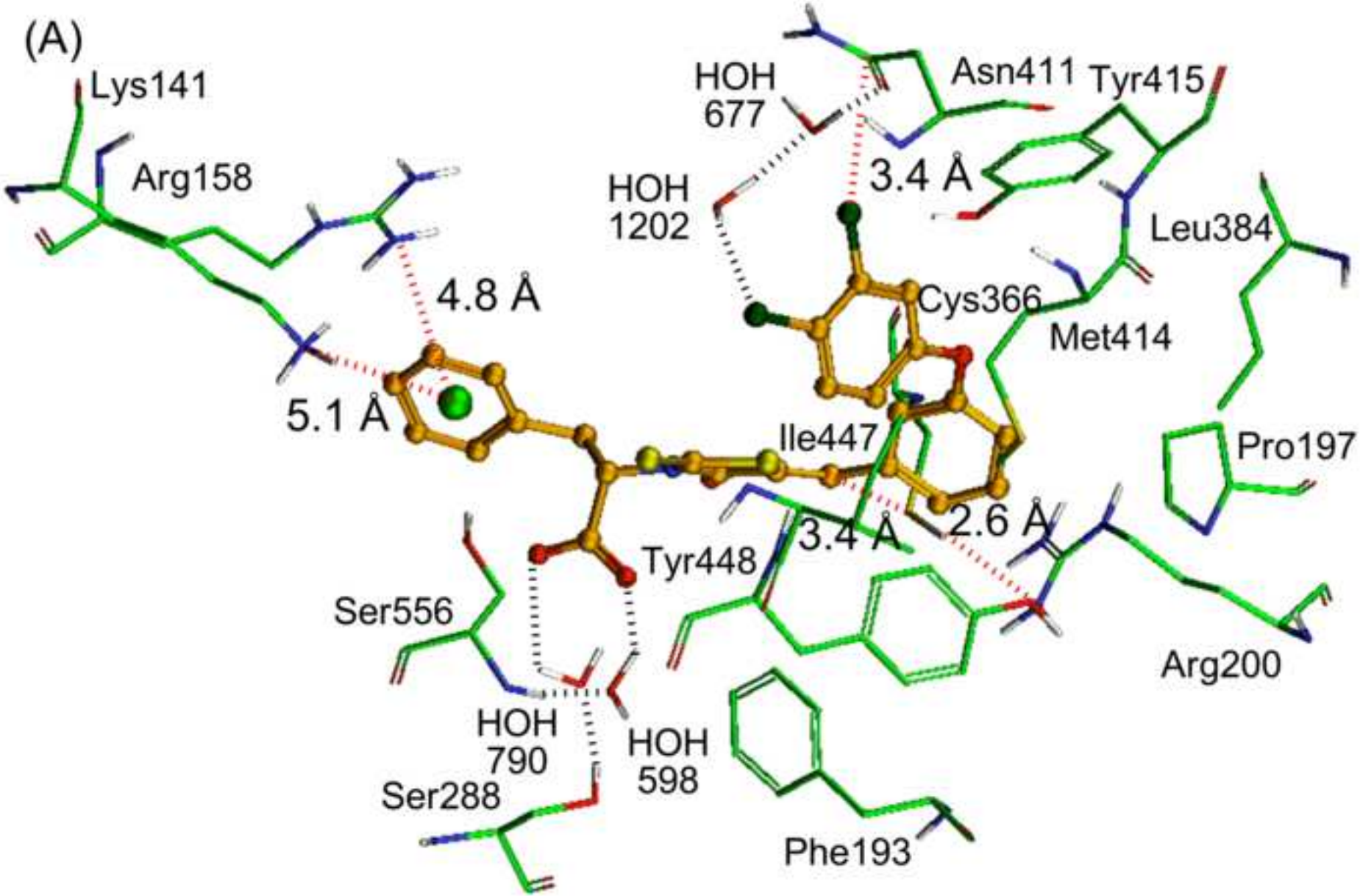
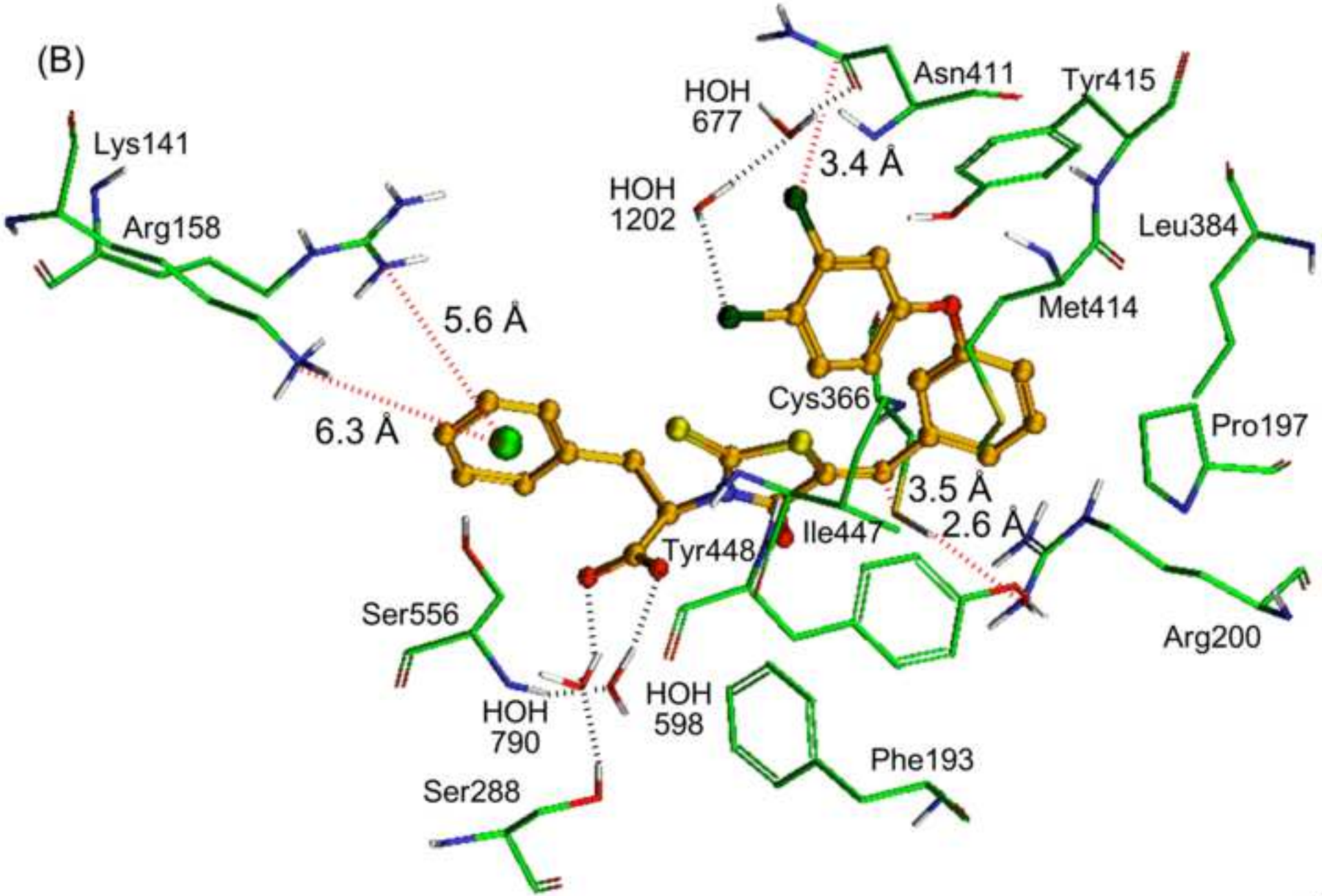


Figure 2B



*Graphical Abstract (for review)