

Results and Discussion

4. RESULTS AND DISCUSSION

The work carried out towards achieving the proposed plan has been discussed under the following headings:

4.1 Molecular Modeling Studies

4.2 Chemical Studies

4.3 Biological Studies

4.1. Molecular Modeling Studies

4.1.1 Docking Studies

For docking studies of final compounds, protein structure of human PTP1B having PDB Id: 1XBO was selected. In the structure 1XBO the WPD loop is in closed form and the main axis of ligand reaches deeper into the secondary pocket lined by residues Arg24 to Lys36. The selected protein structure was processed in Maestro (Schrodinger) using the Protein Preparation Wizard. All water molecules were removed from the protein structure. The induced Fit Docking protocol developed by Schrodinger was used for docking our compounds. The approach seems reasonable because in PTP1B, flexible side chains of multiple residues reach into the binding site. All other values of the IFD protocol were kept at their default values. All compounds were drawn and minimized using Maestro structure builder. Docking in 1XBO resulted in poses with the carboxylate group positioned at the phosphate binding region with establishment of multiple hydrogen bonds with the side chain of Ser216 & Arg221 and distal group stretching into the secondary non-catalytic hydrophobic pocket. The developed model was validated (RMSD = 0.320) and used for docking study.

Initial molecular docking experiments were performed on 4-[[4-oxo-3-phenyl-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid to confirm the orientation of the molecule at active site and its interaction with Ser216 and Arg221 at the active site of the PTP1B enzyme and also the hydrophobic interactions. Results obtained confirmed the desired interactions and orientation of ligand in the active site of

enzyme and on this basis it was proceeded to synthesize derivatives of 2-iminothiazolidin-4-one.

In pursue of our hypothesis based on literature studies and initial docking studies for the synthesized compounds, molecular docking experiments have been performed to study the interaction of the ligands with the PTP1B enzyme. The results of the docking experiment show GLIDE score in the range of -7.83 to -3.94.

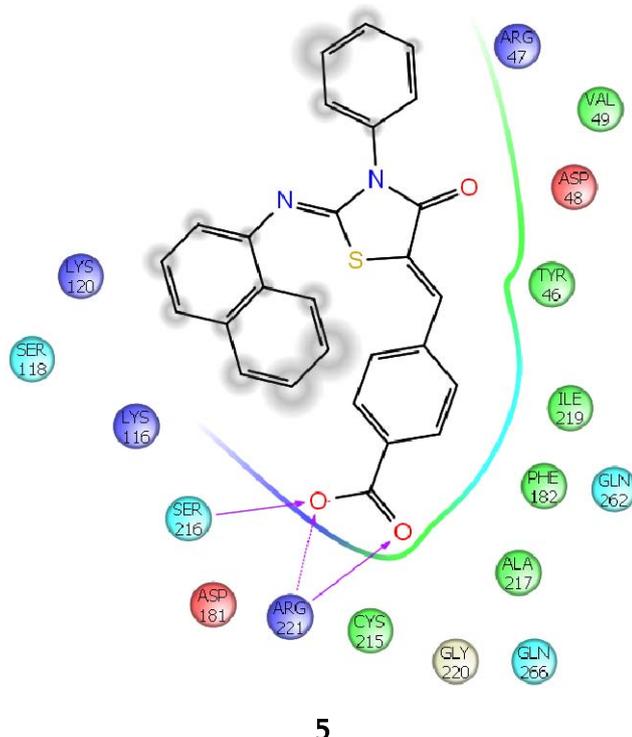
A structural feature that is highly conserved among PTPs is the catalytic, or PTP loop (also known as the signature motif). This PTP loop comprises of 11 residues (I/V)HCXAGXXR(S/T)G. Cys 215 and Arg 221 residues in the PTP loop are vital for catalysis. Another conserved loop, the recognition loop, plays an important role in substrate recognition. The residues Val 49 and Tyr 46 assist the substrate's insertion into catalytic site. Ser 216 of the PTP loop forms a hydrogen bond with the recognition loop, stabilizing the active site cleft. A third conserved loop is the WPD loop, on and near the WPD loop are key residues that function in PTP1B catalysis. Asp 181 and Gln 262 become especially important in the second part of the reaction.

Based on *in vitro* PTP1B inhibition studies we have selected the top ten most active compounds for further analysis with regards to the docking studies and their poses in the active site of PTP1B. Compound **5** ($IC_{50} = 0.21 \mu\text{g/mL}$) was the most active compound among the series but surprisingly docking experiments indicated the GLIDE score of -4.64 (**Table-3**) which is one of the least scorer in the series. Further analysis of the docked pose of compound **5** (**Figure-8**) shows all the required interactions in the binding pocket of PTP1B and it was observed that compounds having a spacer between the carboxylic group and phenyl ring are among the top scorers in the series which is due to a flexible carboxylic group available at the active site which was not the case with compound **5** hence, among the least GLIDE scorers. Compound **60, 63, 79, 90, 92, 102, 110, 114** and **128** GLIDE score were obtained between -6.83 to -3.94 (**Table-3**).

Table-3: Glide score of the most potent PTP1B inhibitor

Sl. No.	Compound	Glide Score	Sl. No.	Compound	Glide Score
1	5	-4.64701	6	92	-6.83832
2	60	-6.72988	7	102	-6.34951
3	63	-6.53854	8	110	-4.92129
4	79	-6.28782	9	114	-5.81469
5	90	-6.67712	10	128	-3.94045

Interaction study of a representative ligand, compound **5** (**Figure 8**) indicated perfect orientation of the carboxylic group of **5** towards the active site region. Two strong interactions were observed. One interaction was between Arg221 and oxygen of carboxylic group and other interaction between Ser216 and oxygen of carboxylic group. Naphthalene ring is oriented towards Lys120, Ser118 and Lys116. Phenylimino group of thiazolidine ring oriented towards Arg47. Thiazolidine ring comfortably occupied its position in the nearby region of Tyr46, Asp48 and Val49. Thus, compound **5** is totally aligned towards the active site and because of its ability to form strong hydrogen bonds with Ser216 and Arg221 it may be giving stability to the compound and this could be one of the reasons for its best activity among the series.

**Figure 8:** Docking pose of compound **5** in the active site of PTP1B

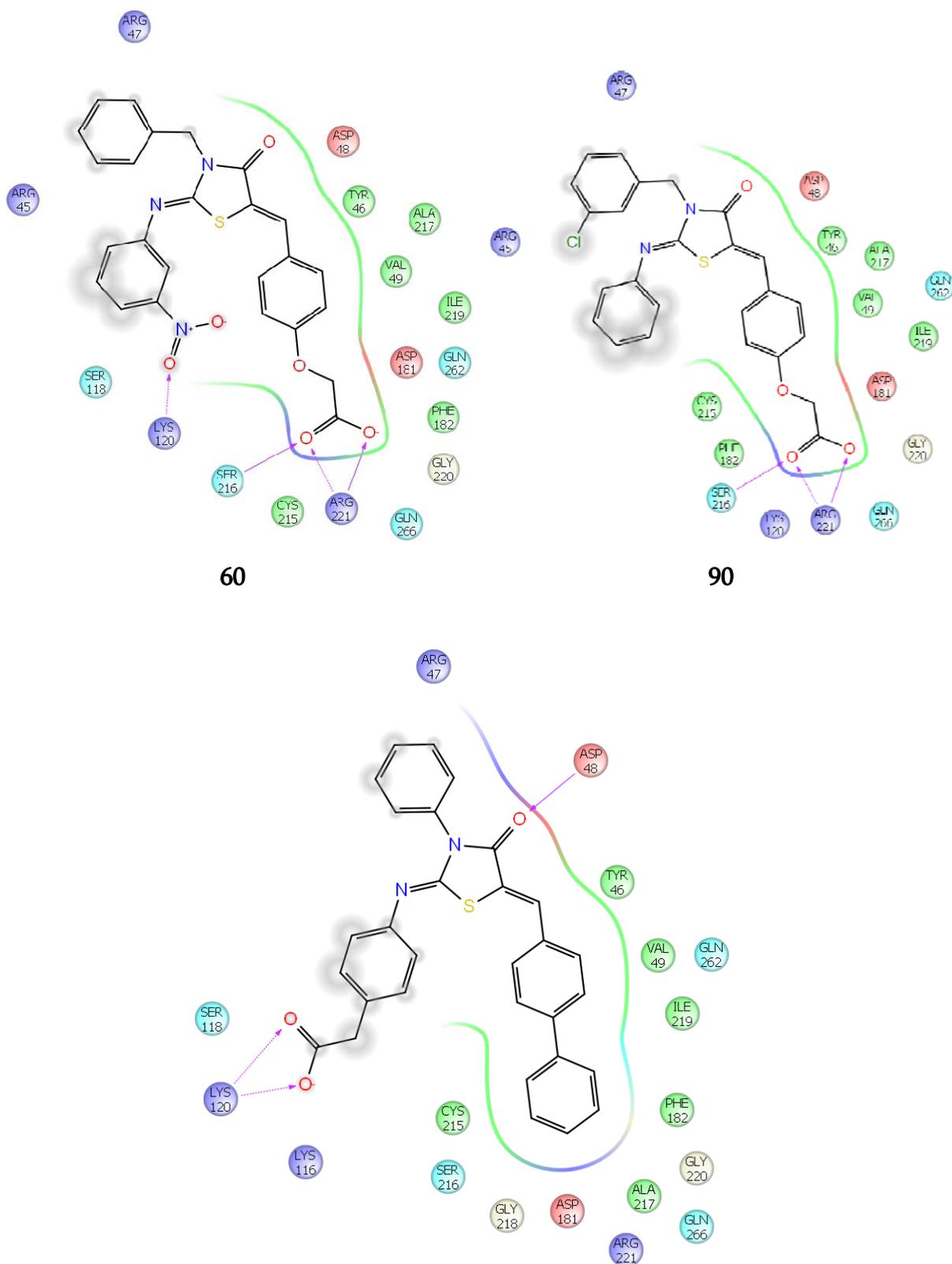


Figure 9: Docking pose of compound 60, 90 and 128 in the active site of PTP1B

Similar interactions were also observed for other compounds except **128** ($IC_{50} = 3.51 \mu\text{g/mL}$) which showed GLIDE score -3.94 (**Figure 9**) and after analysing the docking pose it was observed that polar region of compound **128**, the phenylacetic acid is oriented towards Ser118, Lys120 and Lys116 and biphenyl ring is oriented towards active site region having amino acids Cys215, Ser216, Gly218, Asp181, Ala217, Phe182, Ile219 and Val49.

After performing the *in vitro* selectivity study over TCPTP, SHP-1, LAR and CDC25B (**Table-4**) it was observed that compound **5** which is most potent among the series have shows 3.6 fold selectivity over homologous TCPTP was also found a potent inhibitor of CDC25B ($IC_{50} = 0.13 \mu\text{g/mL}$).

Compound **90** ($IC_{50} = 1.45 \mu\text{g/mL}$) and compound **60** ($IC_{50} = 2.38 \mu\text{g/mL}$) showed highest selectivity towards PTP1B enzyme. A similar type of observation was also made through docking experiments since both the compounds showed higher GLIDE score i.e. -6.67 and -6.72 respectively as compared to compound **5** (-4.64). After studying the docked pose of compound **60** it is observed that the oxygen of the nitro group has shown additional hydrogen bond interactions with Lys120 (**Figure-9**) which could be the possible reason of giving the highest selectivity among the series.

Table-4: Selectivity profile of compound **5**, **60**, **63**, **79**, **90** and **92**

S. No.	Compound No.	IC_{50}				
		PTP1B	TCPTP	SHP-1	LAR	CDC25B
1	5	0.21	0.76	2.15	6.86%	0.13
2	60	2.38	5.56%	7.13%	15.15%	3.03%
3	63	2.28	12.71	39.54%	14.76%	0.93
4	79	2.67	14.31	9.44%	2.32%	2.34
5	90	1.45	3.76	8.41	9.64%	2.57
6	92	2.67	4.42	9.79	13.61%	0.89

4.2. Chemical Studies

To synthesize the envisaged compounds research schemes were planned and discussed under the following headings:

4.2.1. Synthesis of substituted 4-[[4-oxo-3-phenyl-2-(imino)thiazolidin-5-ylidene]methyl]benzoic acid (1-48)

4.2.1.1. Synthesis of substituted 2-imino-thiazolidin-4-ones (A1-A53)

4.2.1.2. Synthesis of derivatives of 4-[[4-oxo-3-phenyl-2-(imino)thiazolidin-5-ylidene]methyl]benzoic acid

4.2.2. Synthesis of substituted 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (49-96).

4.2.2.1. Synthesis of substituted 5-(4-hydroxybenzylidene)-2-imino-3-phenylthiazolidin-4-one (B1-B53)

4.2.2.2. Synthesis of substituted ethyl 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetate (C1-C53)

4.2.2.3. Synthesis of derivatives of 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (49-96)

4.2.3. Synthesis of substituted 4-[[5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid

4.2.3.1. Synthesis of 4-[[4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (D)

4.2.3.2. Synthesis of derivatives of 4-[[5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (97-114)

4.2.4. Synthesis of substituted 2-{4-[(5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (115-132)

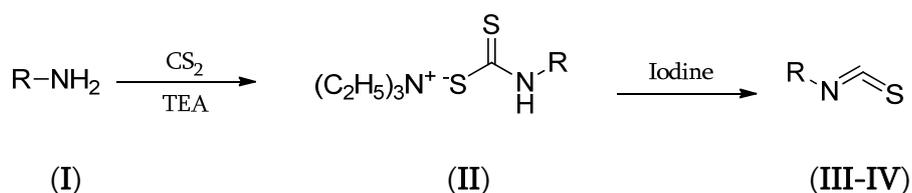
4.2.4.1. Synthesis of 2-{4-[(4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl} acetic acid (E)

4.2.4.2. Synthesis of derivatives of 2-{4-[(5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (115-132)

4.2.1. Synthesis of substituted 4-[[4-oxo-3-phenyl-2-(imino)thiazolidin-5-ylidene]methyl]benzoic acid (1-48).

4.2.1.1. Synthesis of substituted 2-Imino-thiazolidin-4-ones (A1-A53).

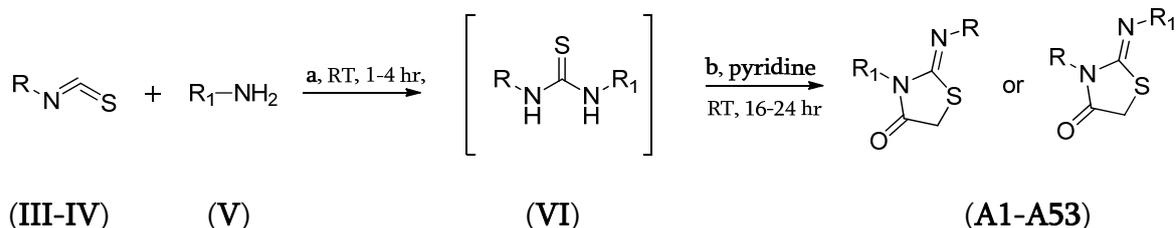
Dithiocarbamate salts that are generated *in situ* by treatment of aryl amines with carbon disulfide and triethylamine was decomposed with iodine to obtain desired aryl isothiocyanate (III-IV) which was (Scheme-I) used for the preparation of corresponding thiourea derivatives (VI).



R = Phenyl (III), Benzyl (IV)

Scheme-I

Aryl isothiocyanate (III-IV) was stirred with substituted amines (V) in methanol or methylene chloride to afford corresponding thiourea derivatives, which was directly cyclised to the iminothiazolidinone by treating this intermediate (VI) with ethyl chloroacetate or bromoacetyl bromide in presence of pyridine (Scheme-II). The regioselectivity of the cyclization was influenced by the reaction conditions and choice of the halo acetic acid derivatives.

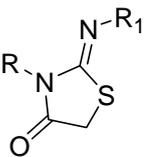
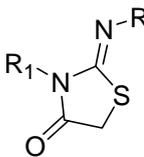


a = MeOH/MDC,

b = Ethyl chloroacetate/bromoacetyl bromide.

Scheme II

Table- 5: Substitutions

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>(a)</p> </div> <div style="text-align: center;">or</div> <div style="text-align: center;">  <p>(b)</p> </div> </div>					
A1-A53					
S. No.	Code	R ₁	R	Regioisomer	Cyclising agent*
1	A1	2-methoxyphenyl	Phenyl	a	d
2	A2	2-methoxyphenyl	Benzyl	a	d
3	A3	4-methoxyphenyl	Phenyl	a	c
4	A4	4-methoxyphenyl	Benzyl	a	c
5	A5	1-Naphthyl	Phenyl	a	c
6	A6	1-Naphthyl	Benzyl	a	c
7	A7	4-Fluorophenyl	Phenyl	a	c
8	A8	4-Fluorophenyl	Benzyl	a	c
9	A9	4-Methylphenyl	Phenyl	a	c
10	A10	4-Methylphenyl	Benzyl	a	c
11	A11	3-Nitrophenyl	Phenyl	a	c
12	A12	3-Nitrophenyl	Benzyl	a	c
13	A13	3-Chlorophenyl	Phenyl	a	c
14	A14	3-Chlorophenyl	Benzyl	a	c
15	A15	3-Bromophenyl	Phenyl	a	c
16	A16	3-Bromophenyl	Benzyl	a	c
17	A17	Cyclohexyl	Phenyl	a	c
18	A18	Cyclohexyl	Benzyl	a	c
19	A19	Benzyl	Phenyl	b	c
20	A20	Phenylethyl	Phenyl	b	c
21	A21	4-Ethyl-morpholine	Phenyl	b	c
22	A22	4-Ethyl-morpholine	Benzyl	b	c
23	A23	3-Pyridine	Phenyl	a	c
24	A24	3-Pyridine	Benzyl	a	c
25	A25	<i>n</i> -Propyl	Phenyl	a	d
26	A26	<i>n</i> -Propyl	Benzyl	a	d
27	A27	<i>iso</i> -Propyl	Phenyl	a	d

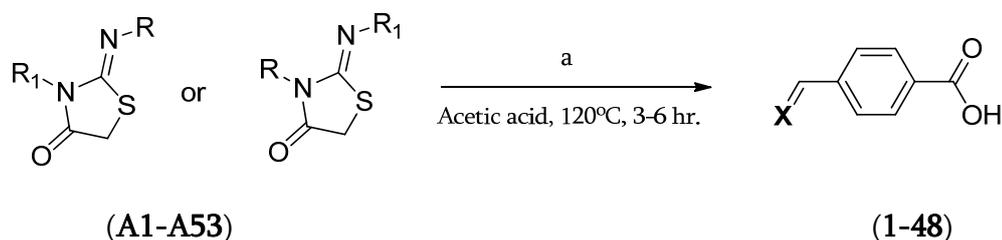
S. No.	Code	R ₁	R	Regioisomer	Cyclising agent*
28	A28	<i>iso</i> -Propyl	Benzyl	a	d
29	A29	Cyclopropyl	Phenyl	a	d
30	A30	Cyclopropyl	Benzyl	a	d
31	A31	n-Butyl	Phenyl	a	d
32	A32	n-Butyl	Benzyl	a	d
33	A33	4-Methoxybenzyl	Phenyl	b	c
34	A34	4-Methoxybenzyl	Benzyl	b	c
35	A35	2-Chlorobenzyl	Phenyl	b	d
36	A36	2-Chlorobenzyl	Benzyl	b	d
37	A37	3-Picolyl	Phenyl	b	d
38	A38	3-Picolyl	Benzyl	b	d
39	A39	2-methylphenyl	Phenyl	b	c
40	A40	2-methylphenyl	Benzyl	b	c
41	A41	4-ethylphenyl	Phenyl	a	c
42	A42	4-ethylphenyl	Benzyl	a	c
43	A43	Phenyl-acetic acid methyl ester	Phenyl	a	c
44	A44	Phenyl-acetic acid methyl ester	Benzyl	a	c
45	A45	3-(Phenyl)-acrylic acid methyl ester	Phenyl	a	c
46	A46	3-(Phenyl)-acrylic acid methyl ester	Benzyl	a	c
47	A47	3-Chlorobenzyl	Phenyl	b	d
48	A48	3-Chlorobenzyl	Benzyl	b	d
49	A49	4-Chlorobenzyl	Phenyl	b	d
50	A50	4-Chlorobenzyl	Benzyl	b	d
51	A51	4-Fluorobenzyl	Phenyl	b	d
52	A52	4-Fluorobenzyl	Benzyl	b	d
53	A53	3,4-Dimethoxybenzyl	Benzyl	b	c

* c = ethyl chloroacetate, d = bromoacetyl bromide

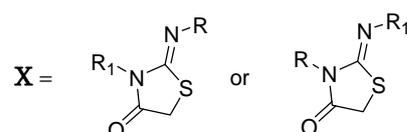
4.2.1.2. Synthesis of derivatives of 4-[[4-oxo-3-phenyl-2-(imino)thiazolidin-5-ylidene]methyl]benzoic acid (1-48).

The condensation of substituted 2-imino-thiazolidin-4-one derivatives (A1-A53) and commercially available 4-formylbenzoic acid was carried out in refluxing acetic acid for 6 hr. in presence of β -alanine (Scheme-III) to yield [[4-oxo-2-(imino)-1,3-thiazolidin-5-ylidene]methyl]benzoic acid (1-48). The reaction was tried with different reported conditions out of which use of β -alanine as base, significantly

reduced reaction time. The pure compounds were obtained by giving repeated washing with cold acetic acid followed by water.

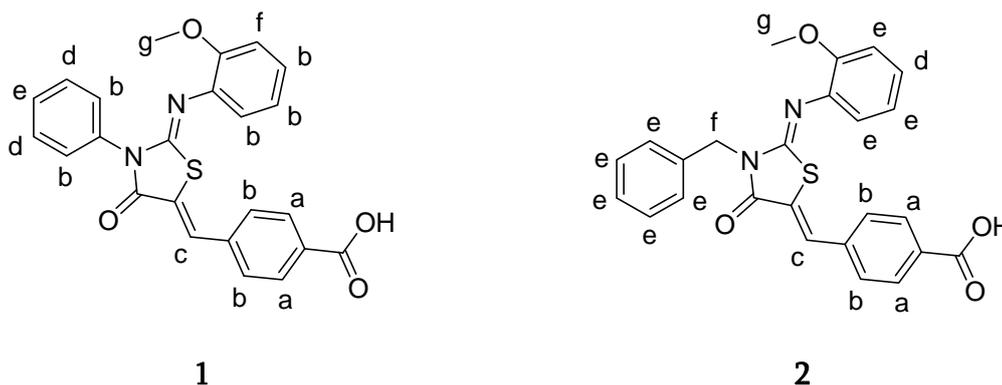


a = 4-Formylbenzoic acid, β -alanine.



Scheme III

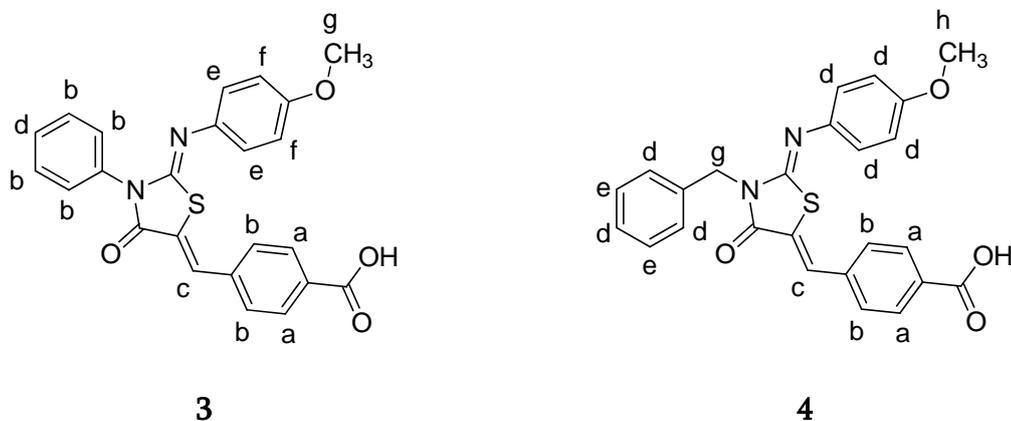
4-[[2-((2-methoxyphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**1**) was prepared by condensation of 3-(2-Methoxyphenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A1**) with 4-formylbenzoic acid. Compound (**1**) showed characteristic IR peaks at 3024 cm^{-1} (OH str), 1719 & 1692 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1601 cm^{-1} (C=C str). It offered signals at δ 8.02-8.04 (*d*, 2H, ArH_a), 7.81 (*s*, 1H, CH_c), 7.36-7.63 (*m*, 7H, ArH_b), 7.10-7.19 (*m*, 2H, ArH_d), 6.90-7.01 (*m*, 2H, ArH_e), and 3.86 (*s*, 3H, ArH_g) in its $^1\text{H-NMR}$ spectrum.



4-[[3-benzyl-2-((2-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**2**) was prepared by condensation of 3-Benzyl-2-(2-methoxyphenylimino)-thiazolidin-4-one (**A2**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3441 cm^{-1} (OH str), 1719 & 1690 cm^{-1} (C=O str), 1647 cm^{-1} (C=N) and 1606 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.10-8.12

(*d*, 2H, *ArH_a*), 7.77 (*s*, 1H, *CH_c*), 7.64-7.66 (*d*, 2H, *ArH_b*), 7.44 (*t*, 1H, *ArH_d*), 7.04-7.31 (*m*, 8H, *ArH_e*), 4.61 (*s*, 2H, *CH_{2f}*), and 3.82 (*s*, 3H, *CH_{3g}*).

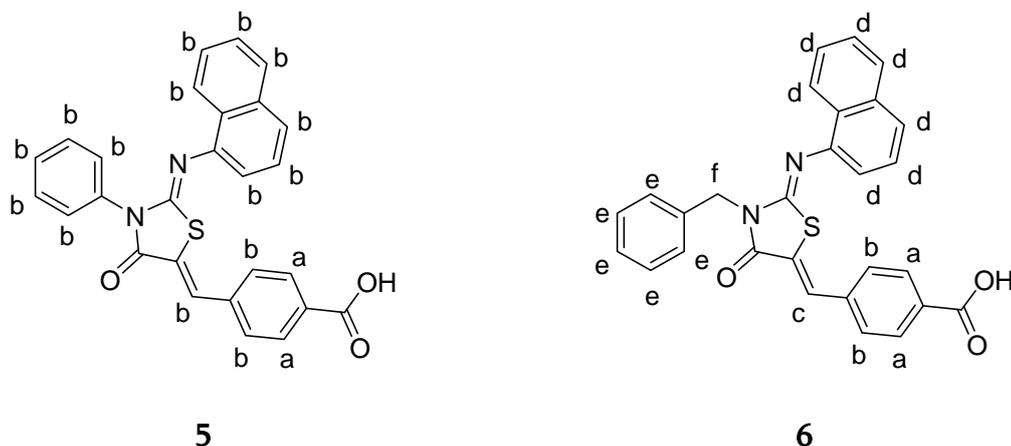
4-[[2-((4-methoxyphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl] benzoic acid (**3**) was prepared by condensation of 3-(4-Methoxyphenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A3**) with 4-formylbenzoic acid. Compound (**3**) showed characteristic IR peaks at 3008 cm^{-1} (OH str), 1726 ($\text{C}=\text{O}$ str), 1625 cm^{-1} ($\text{C}=\text{N}$ str) and 1590 ($\text{C}=\text{C}$ str). It offered signals at δ 8.03-8.04 (*d*, 2H, *ArH_a*), 7.81 (*s*, 2H, *CH_c*), 7.35-7.63 (*m*, 6H, *ArH_b*), 7.16 (*t*, 1H, *ArH_d*), 7.06-7.08 (*d*, 2H, *ArH_e*), 6.88-6.95 (*m*, 2H, *ArH_f*) and 3.85 (*s*, 3H, *ArH_g*) in its ^1H -NMR spectrum.



4-[[3-benzyl-2-((4-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene]methyl] benzoic acid (**4**) was prepared by condensation of 3-Benzyl-2-(4-methoxyphenylimino)-thiazolidin-4-one (**A4**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3000 cm^{-1} (OH str), 1692 cm^{-1} ($\text{C}=\text{O}$ str), 1650 cm^{-1} ($\text{C}=\text{N}$ str), 1606 cm^{-1} ($\text{C}=\text{C}$ str) while its ^1H -NMR spectrum displayed peaks at δ 8.07-8.10 (*m*, 2H, *ArH_a*), 7.78 (*s*, 1H, *CH_c*), 7.65-7.69 (*m*, 2H, *ArH_b*), 7.21-7.31 (*m*, 7H, *ArH_d*), 7.00-7.04 (*t*, 2H, *ArH_e*), 4.62 (*s*, 2H, *CH_{2g}*), and 3.82 (*s*, 3H, *CH_{3h}*).

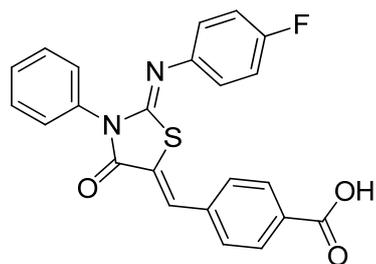
4-[[2-(naphthalen-1-ylimino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl] benzoic acid (**5**) was prepared by condensation of 3-(Naphthalen-1-yl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A5**) which displayed ^1H -NMR spectrum peaks at δ 8.12-8.14 (*d*, 1H, *ArH*), 8.03-8.05 (*d*, 1H, *ArH*), 7.85-7.87 (*d*, 1H, *ArH*), 7.42- 7.58 (*m*, 6H, *ArH*), 7.25-7.29(*t*, 2H, *ArH*), 6.95-6.99 (*t*, 1H, *ArH*) and 3.7 (*s*, 2H, *CH₂*) with

4-formylbenzoic acid. Compound (**5**) showed characteristic IR peaks at 3136 cm^{-1} (OH str), 1697 cm^{-1} (C=O str) and 1609 cm^{-1} (C=N str). It offered signals at δ 11.2-13.8 (*br*, OH), 8.13-8.15 (*d*, 2H, *ArH_a*), 6.98-7.99 (*m*, 15H, *ArH_b*), in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 451 (M^+).

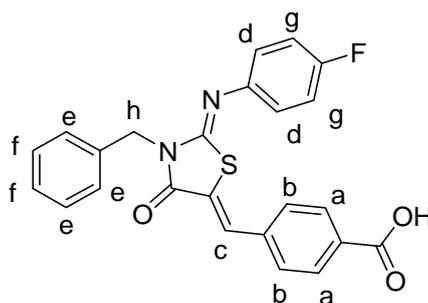


4-[[3-benzyl-2-(naphthalen-1-ylimino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**6**) was prepared by condensation of 3-Benzyl-2-(naphthalen-1-ylimino)-thiazolidin-4-one (**A6**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3433 cm^{-1} (OH str), 1716 cm^{-1} (C=O str), 1651 cm^{-1} (C=N str), 1607 cm^{-1} (C=C str). While its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.13-8.17 (*d*, 2H, *ArH_a*), 7.97-8.02 (*m*, 2H, *ArH_b*), 7.83 (*s*, 1H, *CH_c*), 7.39-7.72 (*m*, 7H, *ArH_d*), 7.09-7.23 (*m*, 5H, *ArH_e*) and 4.62 (*s*, 2H, *CH_{2f}*). Mass spectrum showed molecular ion peak at m/z 465 (M^+).

4-[[2-((4-fluorophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**7**) was prepared by condensation of 3-(4-Fluorophenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A7**) with 4-formylbenzoic acid. Compound (**7**) showed characteristic IR peaks at 3114 cm^{-1} (OH str), 1694 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 418 (M^+), 419 ($\text{M}+1$) and 420 ($\text{M}+2$).



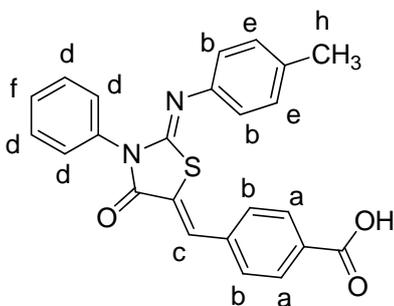
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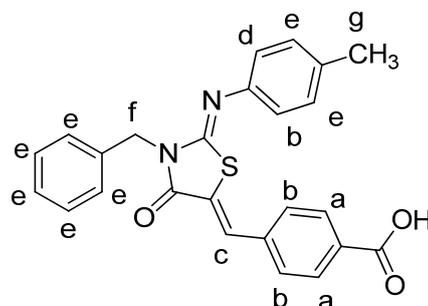
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4-[[3-benzyl-2-((4-fluorophenyl)imino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**8**) was prepared by condensation of 3-Benzyl-2-(4-fluoro-phenylimino)-thiazolidin-4-one (**A8**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3103 cm^{-1} (OH str), 1692 cm^{-1} (C=O str), 1641 cm^{-1} (C=N str), 1605 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.00-8.02 (*d*, 2H, ArH_a), 7.80 (*s*, 1H, CH_c), 7.59-7.57 (*d*, 2H, ArH_b), 7.43-7.45 (*d*, 2H, ArH_d), 7.27-7.37 (*m*, 3H, ArH_e), 7.13-7.17 (*m*, 2H, ArH_f), 6.97-7.01 (*m*, 2H, ArH_g) and 5.13 (*s*, 2H, CH_{2h}).

4-[[4-oxo-3-phenyl-2-(*p*-tolylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**9**) was prepared by condensation of 3-(4-Methylphenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A9**) with 4-formylbenzoic acid. Compound (**9**) showed characteristic IR peaks at 3136 cm^{-1} (OH str), 1687 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1603 cm^{-1} (C=C str). It offered signals at δ 8.02-8.04 (*d*, 2H, ArH_a), 7.81 (*s*, 1H, CH_c), 7.47-7.61 (*m*, 4H, ArH_b), 7.33-7.39 (*m*, 4H, ArH_d), 7.16-7.18 (*m*, 2H, ArH_e), 6.82-6.85 (*m*, 1H, ArH_f), and 2.54 (*s*, 3H, ArH_g) in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 415 (M^+).



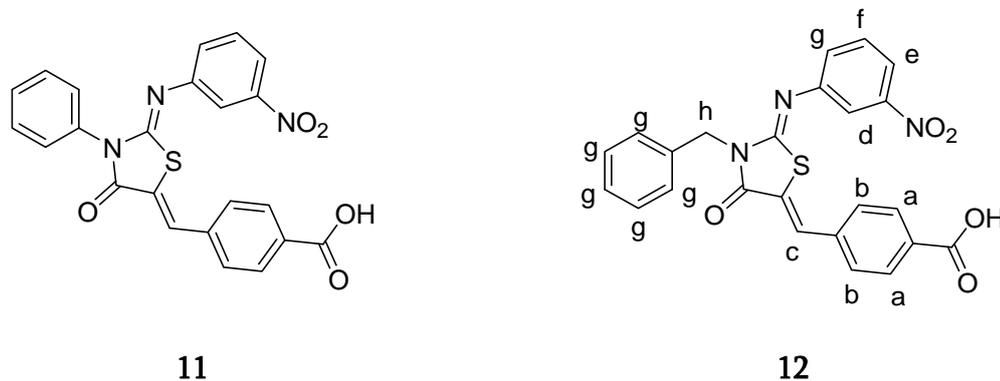
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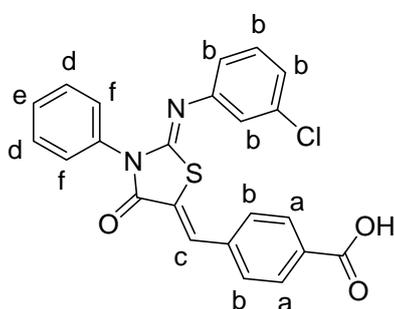
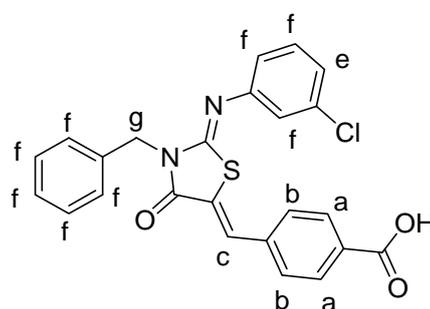
4-[[3-benzyl-4-oxo-2-(p-tolylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**10**) was prepared by condensation of 3-Benzyl-2-(3-nitro-phenylimino)-thiazolidin-4-one (**A10**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3017 cm^{-1} (OH str), 1692 & 1648 cm^{-1} (C=O str), 1605 cm^{-1} (C=N str), 1564 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.06-8.10 (*m*, 2H, ArH_a), 7.99 (*s*, 1H, CH_c), 7.72-7.81 (*m*, 3H, ArH_b), 7.58-7.60 (*d*, 1H, ArH_a), 7.18-7.44 (*m*, 7H, ArH_e), 4.61 (*s*, 2H, CH_{2f}) and 2.38 (*s*, 3H, CH_{3g}).

4-[[2-((3-nitrophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**11**) was prepared by condensation of 3-(3-nitrophenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A11**) with 4-formylbenzoic acid. Compound (**11**) showed characteristic IR peaks at 3428 cm^{-1} (OH str), 1716 & 1695 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1610 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 445.7 (M⁺).



4-[[3-benzyl-2-((3-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**12**) was prepared by condensation of 3-Benzyl-2-(3-nitrophenyl-phenylimino)-thiazolidin-4-one (**A12**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3413 cm^{-1} (OH str), 1695 cm^{-1} (C=O str), 1643 cm^{-1} (C=N str), 1531 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.04-8.07 (*m*, 1H, ArH_a), 7.99-8.01 (*d*, 2H, ArH_a), 7.86 (*s*, 1H, CH_c), 7.79-7.80 (*m*, 1H, ArH_e), 7.66-7.70 (*t*, 1H, ArH_f), 7.58-7.61 (*m*, 2H, ArH_b), 7.29-7.47 (*m*, 6H, ArH_g) and 5.15 (*s*, 2H, CH_{2h}).

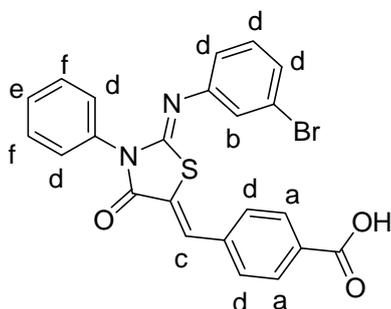
4-[[2-((3-chlorophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl] benzoic acid (**13**) was prepared by condensation of 3-(3-chlorophenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A13**) with 4-formylbenzoic acid. Compound (**13**) showed characteristic IR peaks at 3413 cm^{-1} (OH str), 1694 cm^{-1} (C=O str), 1646 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). It offered signals at δ 11-15 (*br*, 1H, *OH*), 8.02-8.04 (*d*, 2H, *ArH_a*), 7.83 (*s*, 1H, *CH_c*), 7.49-7.62 (*m*, 6H, *ArH_b*), 7.36-7.40 (*t*, 2H, *ArH_d*), 7.16-7.20 (*t*, 1H, *ArH_e*) and 6.96-6.99 (*m*, 2H, *ArH_f*) in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 434.8 (M^+) and 436.9 ($M+2$).

**13****14**

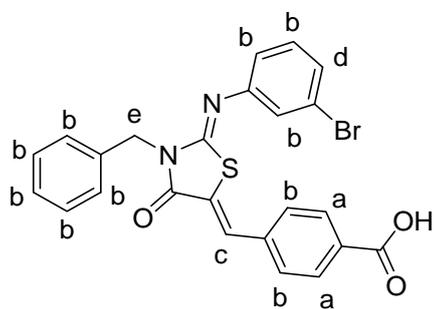
4-[[3-benzyl-2-((3-chlorophenyl)imino)-4-oxothiazolidin-5-ylidene]methyl] benzoic acid (**14**) was prepared by condensation of 3-Benzyl-2-(3-chlorophenyl-phenylimino)-thiazolidin-4-one (**A14**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3115 cm^{-1} (OH str), 1693 cm^{-1} (C=O str), 1643 cm^{-1} (C=N str), 1607 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.09-8.11 (*d*, 2H, *ArH_a*), 8.02-8.04 (*d*, 1H, *ArH_e*), 7.82 (*s*, 1H, *CH_c*), 7.68-7.70 (*d*, 2H, *ArH_b*), 7.17-7.55 (*m*, 8H, *ArH_f*), 4.64 (*s*, 2H, *CH_g*). Mass spectrum showed molecular ion peak at m/z 448.9 (M^+), 450 ($M+1$) and 451 ($M+2$).

4-[[2-((3-bromophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl] benzoic acid (**15**) was prepared by condensation of 3-(3-bromophenyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A15**) with 4-formylbenzoic acid. Compound (**15**) showed characteristic IR peaks at 3105 cm^{-1} (OH str), $1718\& 1689\text{ cm}^{-1}$ (C=O str), 1641 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). It offered signals at δ 8.06-8.10 (*d*, 2H, *ArH_a*),

7.83 (*s*, 1H, *ArH_b*), 7.76 (*s*, 1H, *CH_c*), 7.30-7.66 (*m*, 8H, *ArH_a*), 7.14-7.20 (*m*, 1H, *ArH_e*) and 6.94-6.98 (*m*, 2H, *ArH_f*) in its ¹H-NMR spectrum.



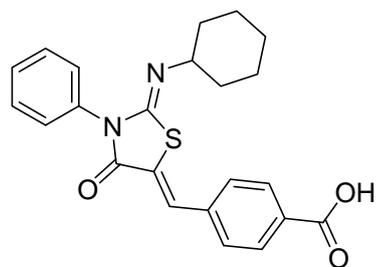
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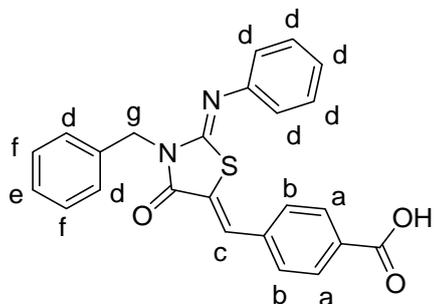
16

4-[[3-benzyl-2-((3-bromophenyl)imino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**16**) was prepared by condensation of 3-Benzyl-2-(3-bromophenyl-phenylimino)-thiazolidin-4-one (**A16**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3088 cm^{-1} (OH str), 1694 cm^{-1} (C=O str), 1657 cm^{-1} (C=N str), 1604 cm^{-1} (C=C str) while its ¹H-NMR spectrum displayed peaks at δ 8.09-8.11 (*d*, 2H, *ArH_a*), 8.02-8.04 (*d*, 1H, *ArH_d*), 7.82 (*s*, 1H, *CH_c*), 7.22-7.71 (*m*, 10H, *ArH_b*) and 4.64 (*s*, 2H, *CH_e*). Mass spectrum showed molecular ion peak at m/z 494.9 (M^+) and 495.8 ($M+1$).

4-[[2-(cyclohexylimino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**17**) was prepared by condensation of 3-Cyclohexyl-2-(phenylimino)-1,3-thiazolidin-4-one (**A17**) with 4-formylbenzoic acid. Compound (**17**) showed characteristic IR peaks at 3105 cm^{-1} (OH str), 1691 cm^{-1} (C=O str), 1647 cm^{-1} (C=N str) and 1605 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 407.2 (M^+).



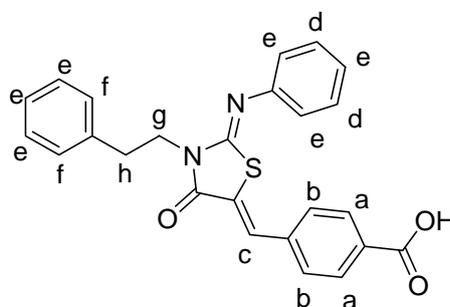
17



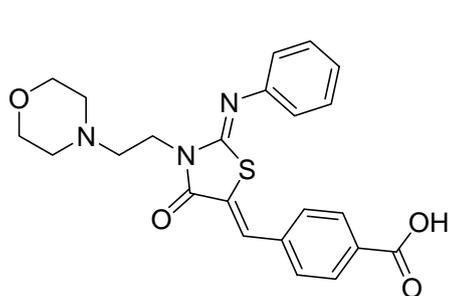
18

4-[[3-benzyl-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**18**) was prepared by condensation of 3-Benzyl-2-(phenylimino)-1,3-thiazolidin-4-one (**A19**) with 4-formylbenzoic acid. Compound (**18**) showed characteristic IR peaks at 3101 cm^{-1} (OH str), 1702 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1601 cm^{-1} (C=C str). It offered signals at δ 7.99-8.01 (*d*, 2H, ArH_a), 7.80 (*s*, 1H, CH_c), 7.57-7.59 (*d*, 2H, ArH_b), 7.28-7.46 (*m*, 7H, ArH_d), 7.17-7.21 (*t*, 1H, ArH_e), 6.97-6.99 (*m*, 2H, ArH_f) and 5.13 (*s*, 2H, CH_{2g}) in its ¹H-NMR spectrum.

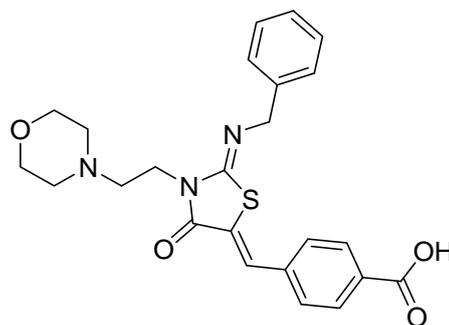
4-[[4-oxo-3-phenethyl-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**19**) was prepared by condensation of 3-(2-Phenylethyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A20**) with 4-formylbenzoic acid. Compound (**19**) showed characteristic IR peaks at 3413 cm^{-1} (OH str), 1690 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1600 cm^{-1} (C=C str). It offered signals at δ 7.99-8.01 (*d*, 2H, ArH_a), 7.73 (*s*, 1H, CH_c), 7.54-7.56 (*d*, 2H, ArH_b), 7.36-7.40 (*t*, 2H, ArH_d), 7.16-7.36 (*m*, 6H, ArH_e), 6.88-6.91 (*dd*, 2H, ArH_f), 4.17-4.21 (*t*, 2H, CH_{2g}) and 3.05-3.09 (*t*, 2H, CH_{2h}) in its ¹H-NMR spectrum. Mass spectrum showed molecular ion peak at m/z 429 (M⁺).

**19**

4-[[3-(2-morpholinoethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**20**) was prepared by condensation of 3-(2-Morpholinoethyl)-2-(phenylimino)-1,3-thiazolidin-4-one (**A21**) with 4-formylbenzoic acid. Compound (**20**) showed characteristic IR peaks at 3409 cm^{-1} (OH str), 1711 cm^{-1} (C=O str), 1644 cm^{-1} (C=N str) and 1593 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 438.1 (M⁺).



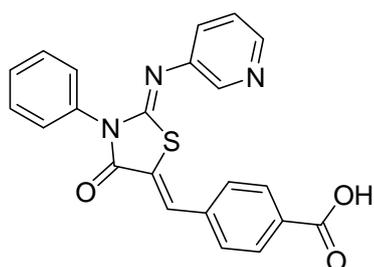
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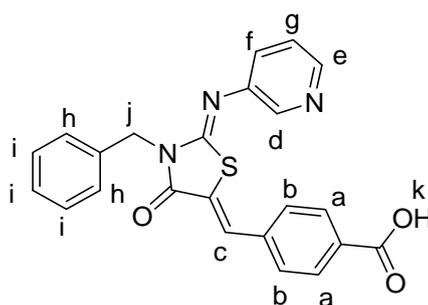
21

4-[[2-(benzylimino)-3-(2-morpholinoethyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**21**) was prepared by condensation of 3-Benzyl-2-(2-morpholinoethylimino)-thiazolidin-4-one (**A22**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3126 cm^{-1} (OH str), 1728 & 1704 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str), 1595 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 450.2 (M-1).

4-[[4-oxo-3-phenyl-2-(pyridin-3-ylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**22**) was prepared by condensation of 3-Phenyl-2-(pyridin-3-ylimino)-thiazolidin-4-one (**A23**) with 4-formylbenzoic acid. Compound (**22**) showed characteristic IR peaks at 3397 cm^{-1} (OH str), 1712 cm^{-1} (C=O str), 1734 cm^{-1} (C=N str) and 1610 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 402.2 (M⁺)



22

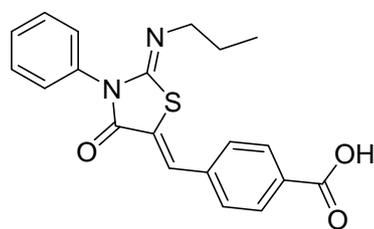
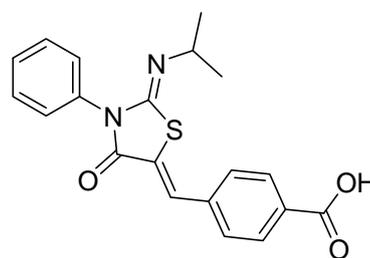


23

4-[[3-benzyl-4-oxo-2-(pyridin-3-ylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**23**) was prepared by 3-Benzyl-2-(pyridin-3-ylimino)-thiazolidin-4-one (**A24**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3111 cm^{-1} (OH str), 1710 cm^{-1} (C=O str), 1654 cm^{-1} (C=N str), 1603 cm^{-1} (C=C str) while its ¹H-NMR

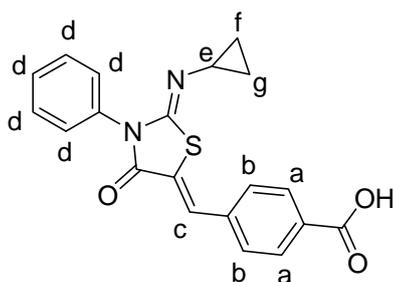
spectrum displayed peaks at δ 12.75 (*bs*, 1H, *OH_k*), 8.680-8.686 (*d*, 1H, *ArH_d*), 8.61-8.63 (*dd*, 1H, *ArH_e*), 8.07-8.14 (*d*, 2H, *ArH_a*), 7.90-7.93 (*d*, 1H, *ArH_f*), 7.85 (*s*, 1H, *CH_c*), 7.74-7.76 (*d*, 2H, *ArCH_b*), 7.54-7.57 (*m*, 1H, *ArH_g*), 7.20-7.32 (*m*, 5H, *ArH_i*) and 4.63 (*s*, 2H, *CH_j*).

4-[[4-oxo-3-phenyl-2-(propylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**24**) was prepared by condensation of 3-Phenyl-2-(propylimino)-thiazolidin-4-one (**A25**) with 4-formylbenzoic acid. Compound (**24**) showed characteristic IR peaks at 3112 cm^{-1} (OH str), 1693 cm^{-1} (C=O str), 1648 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 366.9 (M^+)

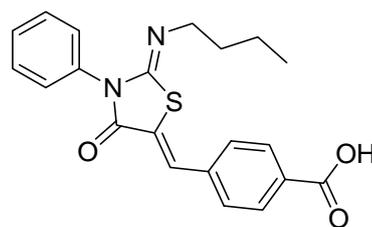
**24****25**

4-[[2-(isopropylimino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**25**) was prepared by condensation of 3-Phenyl-2-(isopropylimino)-thiazolidin-4-one (**A27**) with 4-formylbenzoic acid. Compound (**25**) showed characteristic IR peaks at 3111 cm^{-1} (OH str), 1689 cm^{-1} (C=O str), 1644 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at m/z 367 (M^+)

4-[[2-(cyclopropylimino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**26**) was prepared by condensation of 3-Phenyl-2-(cyclopropylimino)-thiazolidin-4-one (**A29**) with 4-formylbenzoic acid. Compound (**26**) showed characteristic IR peaks at 3109 cm^{-1} (OH str), 1709 & 1690 cm^{-1} (C=O str), 1650 cm^{-1} (C=N str) and 1601 cm^{-1} (C=C str). It offered signals at δ 8.04-8.06 (*d*, 2H, *ArH_a*), 7.74 (*s*, 1H, *CH_c*), 7.70-7.72 (*d*, 2H, *ArH_b*), 7.30-7.48 (*m*, 5H, *ArH_d*), 2.77-2.80 (*m*, 1H, *CH_e*), 0.81-0.84 (*m*, 2H, *CH_x*) and 0.55-0.59 (*m*, 2H, *CH_{2g}*) in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 365 (M^+)



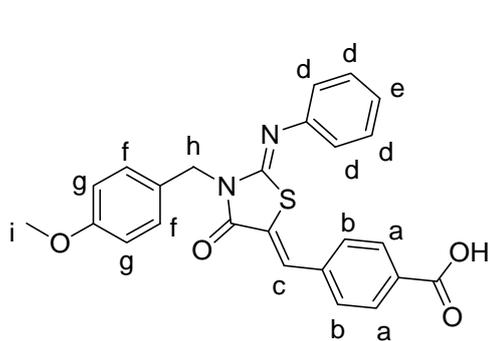
26



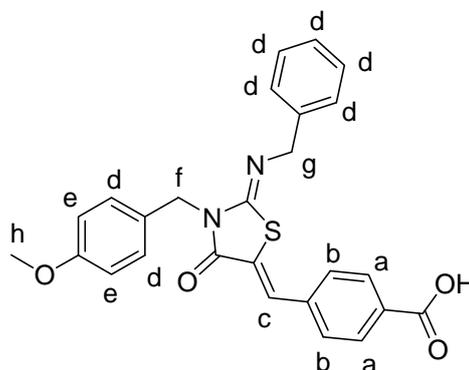
27

4-[[2-(butylimino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**27**) was prepared by condensation of 3-Phenyl-2-(butylimino)-thiazolidin-4-one (**A31**) with 4-formylbenzoic acid. Compound (**27**) showed characteristic IR peaks at 3076 cm^{-1} (OH str), 1692 cm^{-1} (C=O str), 1649 cm^{-1} (C=N str) and 1603 cm^{-1} (C=C str). Mass spectrum showed molecular ion peak at $m/z\ 380.9$ (M^+)

4-[[3-(4-methoxybenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**28**) was prepared by condensation of 3-[(4-methoxyphenyl)methyl]-2-(phenyl imino)-1,3-thiazolidin-4-one (**A33**) with 4-formylbenzoic acid. Compound (**28**) showed characteristic IR peaks at 3116 cm^{-1} (OH str), 1691 cm^{-1} (C=O str), 1641 cm^{-1} (C=N str) and 1609 cm^{-1} (C=C str). It offered signals at $\delta\ 7.99\text{--}8.01$ (*d*, 2H, ArH_a), 7.77 (*s*, 1H, CH_c), $7.53\text{--}7.55$ (*d*, 2H, ArH_b), $7.37\text{--}7.44$ (*m*, 4H, ArH_d), $7.17\text{--}7.21$ (*t*, 1H, ArH_e), $6.98\text{--}7.00$ (*d*, 2H, ArH_f), $6.86\text{--}6.89$ (*d*, 2H, ArH_g), 5.07 (*s*, 2H, CH_{2h}) and 3.77 (*s*, 3H, CH_{3i}) in its $^1\text{H-NMR}$ spectrum.



28

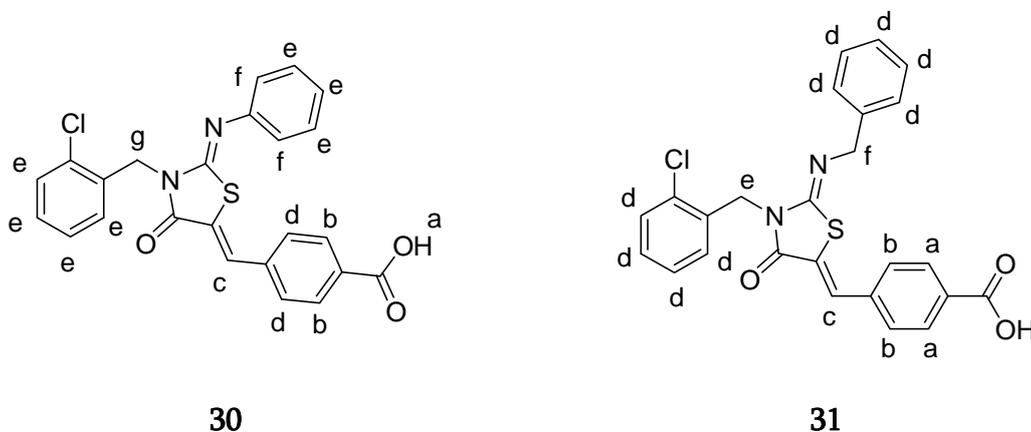


29

4-[[2-(benzylimino)-3-(4-methoxybenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**29**) was prepared by 3-Benzyl-2-(butylimino)-thiazolidin-4-one (**A34**)

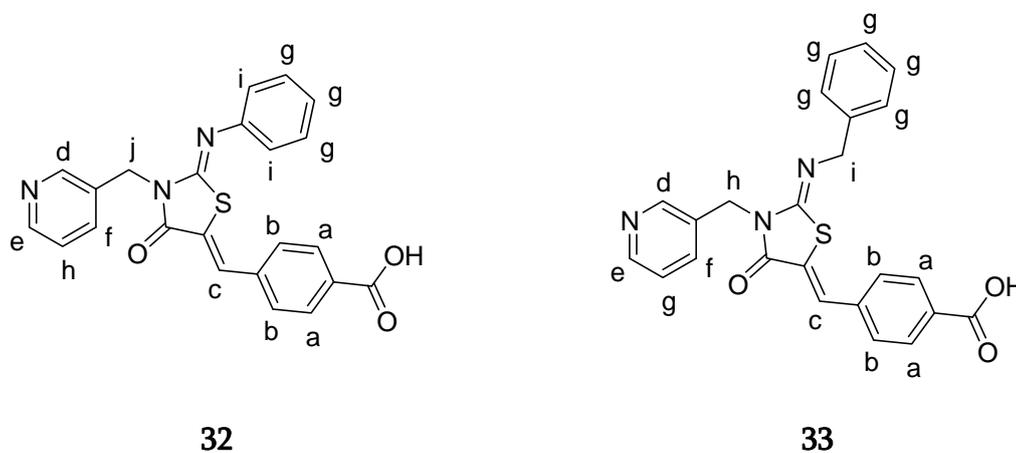
with 4-formylbenzoic acid. It showed characteristic IR peaks at 3455 cm^{-1} (OH str), 1701 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str), 1600 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.04-8.06 (*d*, 2H, Ar*H*_a), 7.77 (*s*, 1H, CH_c), 7.67-7.69 (*d*, 2H, Ar*H*_b), 7.17-7.37 (*m*, 7H, Ar*H*_d), 6.83-6.85 (*d*, 2H, Ar*H*_e), 4.96 (*s*, 2H, CH_{2g}), 4.63 (*s*, 2H, CH_{2f}) and 3.73 (*s*, 3H, CH_{3h}).

4-[[3-(2-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**30**) was prepared by condensation of 3-[(2-chlorophenyl)methyl]-2-(phenyl imino)-1,3-thiazolidin-4-one (**A35**) with 4-formylbenzoic acid. Compound (**30**) showed characteristic IR peaks at 3066 cm^{-1} (OH str), 1722 & 1690 cm^{-1} (C=O str), 1643 cm^{-1} (C=N str) and 1611 cm^{-1} (C=C str). It offered signals at δ 12.92 (bs, 1H, OH_a), 8.02-8.04 (*d*, 2H, Ar*H*_b), 7.83 (*s*, 1H, CH_c), 7.59-7.61 (*d*, 2H, Ar*H*_d), 7.16-7.46 (*m*, 7H, Ar*H*_e), 6.93-6.95 (*d*, 2H, Ar*H*_f), and 5.22 (*s*, 2H, CH_{2g}) in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 448.41 (M^+).



4-[[2-(benzylimino)-3-(2-chlorobenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**31**) was prepared by 3-Benzyl-2-(2-chloro-benzylimino)-thiazolidin-4-one (**A36**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3414 cm^{-1} (OH str), 1692 cm^{-1} (C=O str), 1639 cm^{-1} (C=N str), 1604 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 8.08-8.10 (*d*, 2H, Ar*H*_a), 7.79 (*s*, 1H, CH_c), 7.64-7.67 (*d*, 2H, Ar*H*_b), 7.08-7.33 (*m*, 9H, Ar*H*_d), 5.16 (*s*, 2H, NCH_{2f}) and 4.65 (*s*, 2H, CH_{2e}). Mass spectrum showed molecular ion peak at m/z 462.9 (M^+) and 464.9 ($\text{M}+2$).

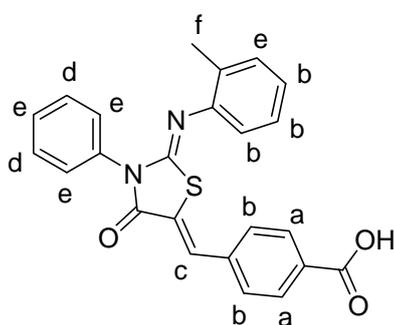
4-[[4-oxo-2-(phenylimino)-3-(pyridin-3-ylmethyl)thiazolidin-5-ylidene]methyl]benzoic acid (**32**) was prepared by condensation of 2-(phenylimino)-3-((pyridin-3-yl)methyl)-1,3-thiazolidin-4-one (**A37**) with 4-formylbenzoic acid. Compound (**32**) showed characteristic IR peaks at 3126 cm^{-1} (OH str), 1708 cm^{-1} (C=O str), 1649 cm^{-1} (C=N str) and 1598 cm^{-1} (C=C str). It offered signals at δ 8.69 (*s*, 1H, ArH_d), 8.50-8.51 (*d*, 1H, ArH_e), 7.97-7.99 (*d*, 1H, ArH_f), 7.85-7.87 (*d*, 2H, ArH_a), 7.80 (*s*, 1H, CH_c), 7.55-7.57 (*d*, 2H, ArH_b), 7.36-7.40 (*m*, 3H, ArH_g), 7.16-7.19 (*t*, 1H, ArH_h), 6.96-6.98 (*d*, 2H, ArH_i) and 5.14 (*s*, 2H, CH_{2j}) in its ¹H-NMR spectrum. Mass spectrum showed molecular ion peak at *m/z* 415.9 (M⁺).



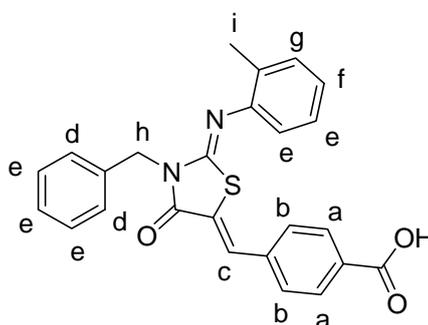
4-[[2-(benzylimino)-4-oxo-3-(pyridin-3-ylmethyl)thiazolidin-5-ylidene]methyl]benzoic acid (**33**) was prepared by 2-Benzylimino-3-pyridin-3-ylmethyl-thiazolidin-4-one (**A38**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3397 cm^{-1} (OH str), 1708 cm^{-1} (C=O str), 1649 cm^{-1} (C=N str), 1597 cm^{-1} (C=C str) while its ¹H-NMR spectrum displayed peaks at δ 8.63 (*s*, 1H, ArH_d), 8.47-8.49 (*d*, 1H, ArH_e), 8.04-8.06 (*d*, 2H, ArH_a), 7.79 (*s*, 1H, CH_c), 7.74-7.75 (*d*, 1H, ArH_f), 7.69-7.71 (*d*, 2H, ArH_b), 7.22-7.37 (*m*, 6H, ArH_g), 5.07 (*s*, 2H, NCH_{2i}) and 4.64 (*s*, 2H, CH_{2h}).

4-[[4-oxo-3-phenyl-2-(o-tolylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**34**) was prepared by condensation of 2-[(2-methylphenyl)imino]-3-phenyl-1,3-thiazolidin-4-one (**A39**) with 4-formylbenzoic acid. Compound (**34**) showed characteristic IR peaks at 3123 cm^{-1} (OH str), 1717 and 1691 cm^{-1} (C=O str), 1643 cm^{-1} (C=N str) and 1601 cm^{-1} (C=C str). It offered signals at δ 7.97-7.99 (*d*, 2H, ArH_a), 7.76

(*s*, 1H, *CH_c*), 7.43-7.53 (*m*, 5H, *ArH_b*), 7.29-7.35 (*m*, 2H, *ArH_d*), 6.79-7.17 (*m*, 4H, *ArH_e*), and 2.07 (*s*, 3H, *CH_{3f}*) in its ¹H-NMR spectrum.



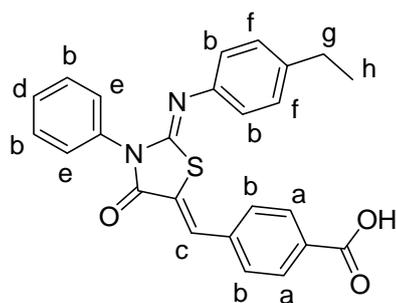
34



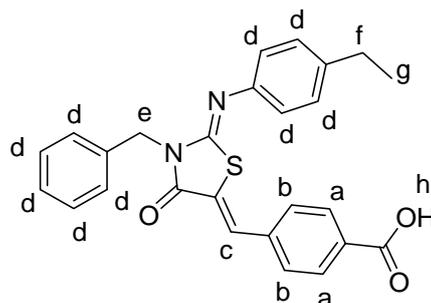
35

4-[[3-benzyl-4-oxo-2-(o-tolylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**35**) was prepared by 3-Benzyl-2-(2-methyl-benzylimino)-thiazolidin-4-one (**A40**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3111 cm^{-1} (OH str), 1717 and 1691 cm^{-1} (C=O str), 1643 cm^{-1} (C=N str), 1601 cm^{-1} (C=C str) while its ¹H-NMR spectrum displayed peaks at δ 7.98-8.01 (*d*, 2H, *ArH_a*), 7.83 (*s*, 1H, *CH_c*), 7.59-7.61 (*d*, 1H, *ArH_b*), 7.41-7.43 (*d*, 2H, *ArH_d*), 7.18-7.38 (*m*, 5H, *ArH_e*), 7.06-7.10 (*t*, 1H, *ArH_f*), 6.86-6.88 (*d*, 1H, *ArH_g*), 5.14 (*s*, 2H, *CH_{2h}*) and 1.91 (*s*, 3H, *CH_{3i}*).

4-[[2-((4-ethylphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**36**) was prepared by condensation of 2-[(4-ethylphenyl)imino]-3-phenyl-1,3-thiazolidin-4-one (**A41**) with 4-formylbenzoic acid. Compound (**36**) showed characteristic IR peaks at 3421 cm^{-1} (OH str), 1717 and 1690 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). It offered signals at δ 8.03-8.05 (*d*, 2H, *ArH_a*), 7.80 (*s*, 1H, *CH_c*), 7.35-7.58 (*m*, 7H, *ArH_b*), 7.17-7.20 (*t*, 1H, *ArH_d*), 6.93-6.95 (*d*, 2H, *ArH_e*), 6.86-6.88 (*d*, 1H, *ArH_f*), 2.70-2.76 (*m*, 2H, *CH_{2g}*) and 1.23-1.31 (*m*, 3H, *CH_{3h}*) in its ¹H-NMR spectrum.



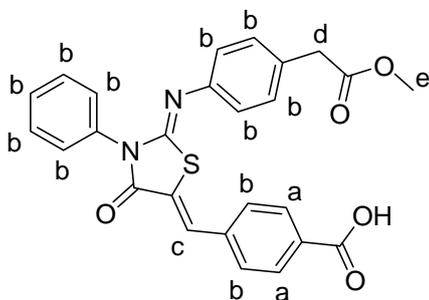
36



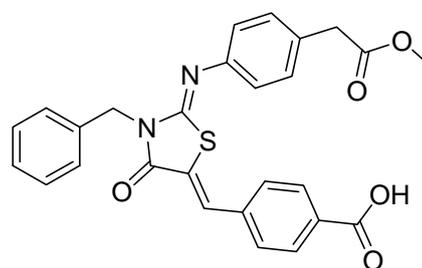
37

4-[[3-benzyl-2-((4-ethylphenyl)imino)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**37**) was prepared by 3-Benzyl-2-(4-ethylphenylimino)-thiazolidin-4-one (**A42**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3017 cm^{-1} (OH str), 1715 and 1691 cm^{-1} (C=O str), 1653 cm^{-1} (C=N str), 1606 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 13.00 (bs, 1H, OH_h), 8.06-8.08 (*d*, 2H, ArH_a), 7.81 (*s*, 1H, CH_c), 7.73-7.75 (*d*, 2H, ArH_b), 7.19-7.35 (*m*, 9H, ArH_d), 4.62 (*s*, 2H, CH_{2e}), 2.65-2.71 (*q*, 2H, CH_{2f}) and 1.19-1.26 (*t*, 3H, CH_{3g}).

4-[[2-((4-(2-methoxy-2-oxoethyl)phenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**38**) was prepared by condensation of Methyl 2-(4-[[4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]amino]phenyl)acetate (**A43**) with 4-formylbenzoic acid. Compound (**38**) showed characteristic IR peaks at 3415 cm^{-1} (OH str), 1736 , 1720 and 1691 cm^{-1} (C=O str), 1647 cm^{-1} (C=N str) and 1608 cm^{-1} (C=C str). It offered signals at δ 7.85-7.90 (*m*, 2H, ArH_a), 7.75 (*s*, 1H, CH_c), 6.74-7.53 (*m*, 11H, ArH_b), 3.53 (*s*, 2H, CH_{2d}) and 2.40 (*s*, 3H, CH_{3e}) in its $^1\text{H-NMR}$ spectrum.



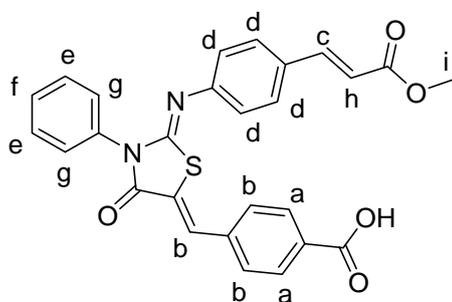
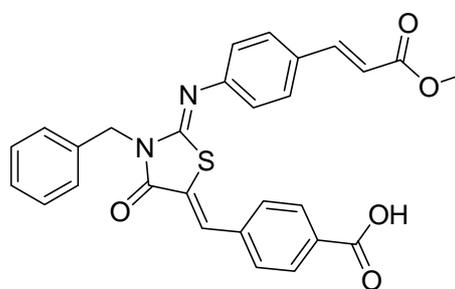
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39

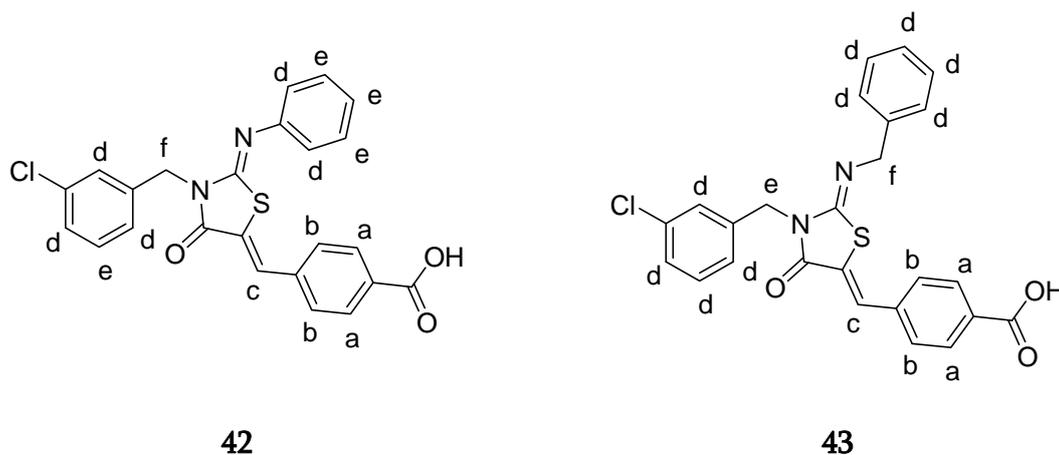
4-[[3-benzyl-2-((4-(2-methoxy-2-oxoethyl)phenyl)imino)-4-oxothiazolidin-5-ylidene] methyl]benzoic acid (**39**) was prepared by 3-Benzyl-2-(4-ethylphenylimino)-thiazolidin-4-one (**A44**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3129 cm^{-1} (OH str), 1736 , 1720 and 1691 cm^{-1} (C=O str), 1647 cm^{-1} (C=N str) and 1608 cm^{-1} (C=C str) while its Mass spectrum showed molecular ion peak at m/z 485.23 (M-1).

4-[[2-((4-(3-methoxy-3-oxoprop-1-en-1-yl)phenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene] methyl]benzoic acid (**40**) was prepared by condensation of Methyl-3-(4-[[4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]amino]phenyl)prop-2-enoate (**A45**) with 4-formylbenzoic acid. Compound (**40**) showed characteristic IR peaks at 3116 cm^{-1} (OH str), 1717 and 1690 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str). It offered signals at δ 7.96-7.98 (*d*, 2H, ArH_a), 7.75-7.78 (*m*, 3H, ArH_b), 7.64-7.68 (*d*, 1H, CH_c), 7.51-7.55 (*m*, 4H, ArH_d), 7.29-7.33 (*t*, 2H, ArH_e), 7.09-7.13 (*t*, 1H, ArH_f), 6.88-6.90 (*d*, 2H, ArH_g), 6.54-6.58 (*d*, 1H, CH_h), and 3.71 (*s*, 3H, CH_{3i}) in its $^1\text{H-NMR}$ spectrum.

**40****41**

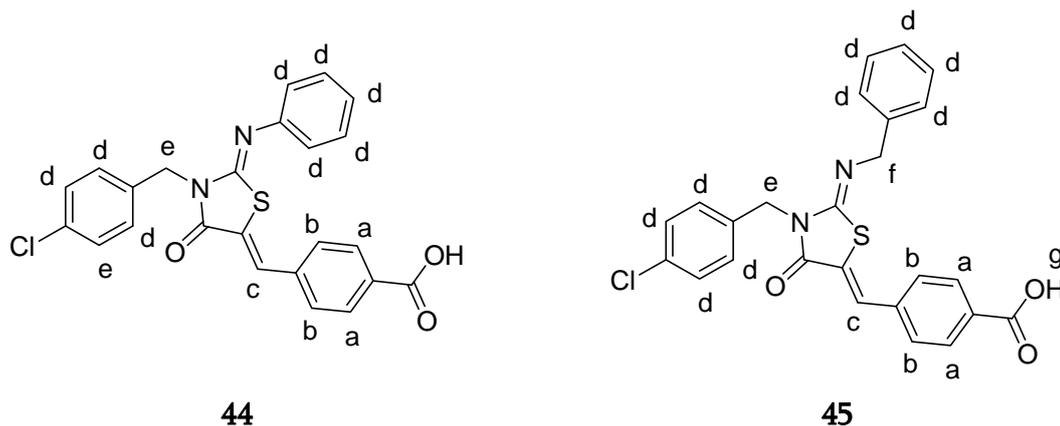
4-[[3-benzyl-2-((4-(3-methoxy-3-oxoprop-1-en-1-yl)phenyl)imino)-4-oxothiazolidin-5-ylidene] methyl]benzoic acid (**41**) was prepared by 3-Benzyl-2-(4-(2-methoxycarbonyl-vinyl)phenylimino)-thiazolidin-4-one (**A46**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3119 cm^{-1} (OH str), 1717 and 1690 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1604 cm^{-1} (C=C str) while its Mass spectrum showed molecular ion peak at m/z 499.13 (M^+).

4-[[3-(3-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**42**) was prepared by condensation of 3-[(3-chlorophenyl)methyl]-2-(phenylimino)-1,3-thiazolidin-4-one (**A47**) with 4-formylbenzoic acid. Compound (**42**) showed characteristic IR peaks at 3056 cm^{-1} (OH str), 1719 and 1693 cm^{-1} (C=O str), 1658 cm^{-1} (C=N str) and 1611 cm^{-1} (C=C str). It offered signals at δ 8.11-8.13 (*d*, 2H, ArH_a), 7.82 (*s*, 1H, CH_c), 7.66-7.68 (*d*, 2H, ArH_b), 7.39-7.55 (*m*, 5H, ArH_d), 7.15-7.28 (*m*, 4H, ArH_e), and 4.63 (*s*, 2H, CH_{2f}) in its ¹H-NMR spectrum.



4-[[2-(benzylimino)-3-(3-chlorobenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**43**) was prepared by 3-(3-Chloro-benzyl)-2-phenylimino-thiazolidin-4-one (**A48**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3104 cm^{-1} (OH str), 1686 cm^{-1} (C=O str), 1647 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C str) while its ¹H-NMR spectrum displayed peaks at δ 13.11 (*brs*, 1H, OH_g), 8.04-8.06 (*d*, 2H, ArH_a), 7.80 (*s*, 1H, CH_c), 7.68-7.70 (*d*, 2H, ArH_b), 7.19-7.42 (*m*, 9H, ArH_d), 5.03-5.05 (*d*, 2H, NCH_{2f}) and 4.63 (*s*, 2H, CH_{2e}).

4-[[3-(4-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**44**) was prepared by condensation of 3-[(4-chlorophenyl)methyl]-2-(phenylimino)-1,3-thiazolidin-4-one (**A49**) with 4-formylbenzoic acid. Compound (**44**) showed characteristic IR peaks at 3430 cm^{-1} (OH str), 1686 cm^{-1} (C=O str), 1641 cm^{-1} (C=N str) and 1585 cm^{-1} (C=C str). It offered signals at δ 8.08-8.10 (*d*, 2H, ArH_a), 7.82 (*s*, 1H, CH_c), 7.70-7.72 (*d*, 2H, ArH_b), 7.22-7.54 (*m*, 9H, ArH_d), and 4.61 (*s*, 2H, CH_{2f}) in its ¹H-NMR spectrum.

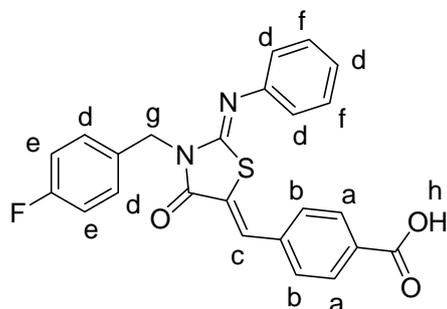


4-[[2-(benzylimino)-3-(4-chlorobenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**45**) was prepared by 3-(4-Chloro-benzyl)-2-phenylimino-thiazolidin-4-one (**A50**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3107 cm^{-1} (OH str), 1685 cm^{-1} (C=O str), 1644 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 13.11 (*brs*, 1H, OH_g), 8.04-8.06 (*d*, 2H, ArH_a), 7.80 (*s*, 1H, CH_c), 7.70-7.72 (*d*, 2H, ArH_b), 7.25-7.38 (*m*, 9H, ArH_d), 5.03 (*s*, 2H, NCH_2f) and 4.62 (*s*, 2H, CH_2e).

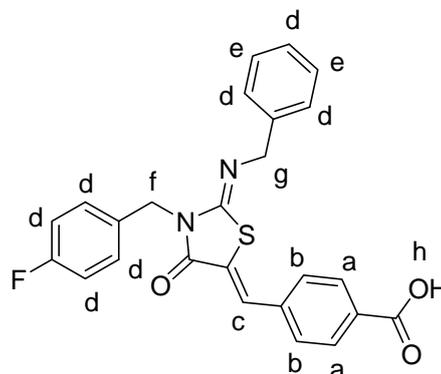
4-[[3-(4-fluorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene]methyl]benzoic acid (**46**) was prepared by condensation of 3-[(4-fluorophenyl)methyl]-2-(phenylimino)-1,3-thiazolidin-4-one (**A51**) with 4-formylbenzoic acid. Compound (**46**) showed characteristic IR peaks at 3412 cm^{-1} (OH str), 1714 and 1684 cm^{-1} (C=O str), 1652 cm^{-1} (C=N str) and 1599 cm^{-1} (C=C str). It offered signals at δ 12.97 (*bs*, 1H, OH_h), 8.08-8.10 (*d*, 2H, ArH_a), 7.82 (*s*, 1H, CH_c), 7.71-7.73 (*d*, 2H, ArH_b), 7.40-7.54 (*m*, 5H, ArH_d), 7.23-7.27 (*m*, 2H, ArH_e), 7.00-7.05 (*t*, 2H, ArH_f), and 4.60 (*s*, 2H, CH_2g) in its $^1\text{H-NMR}$ spectrum.

4-[[2-(benzylimino)-3-(4-fluorobenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**47**) was prepared by 3-(4-fluoro-benzyl)-2-phenylimino-thiazolidin-4-one (**A52**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3112 cm^{-1} (OH str), 1685 cm^{-1} (C=O str), 1644 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 12.76 (*brs*, 1H, OH_h), 7.98-8.00 (*d*, 2H, ArH_a), 7.17

(*s*, 1H, *CH_c*), 7.60-7.62 (*d*, 2H, *ArH_b*), 7.16-7.37 (*m*, 7H, *ArH_a*), 6.96-7.00 (*m*, 2H, *ArH_e*), 4.97 (*s*, 2H, *NCH_{2g}*) and 4.57 (*s*, 2H, *CH_{2f}*).

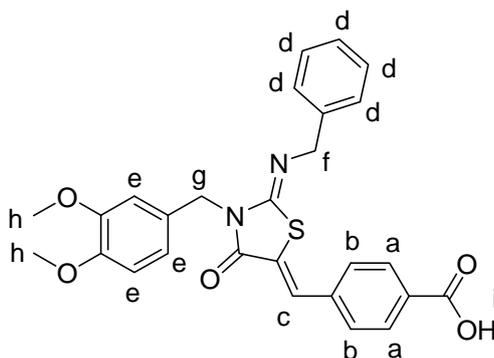


46



47

4-[[2-(benzylimino)-3-(3,4-dimethoxybenzyl)-4-oxothiazolidin-5-ylidene]methyl]benzoic acid (**48**) was prepared by 3-(3,4-dimethoxybenzyl)-2-phenyliminothiazolidin-4-one (**A53**) with 4-formylbenzoic acid. It showed characteristic IR peaks at 3417 cm^{-1} (OH str), 1691 cm^{-1} (C=O str), 1645 cm^{-1} (C=N str) and 1611 cm^{-1} (C=C str) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 12.98 (*br*, 1H, *OH*), 8.03-8.05 (*d*, 2H, *ArH_a*), 7.78 (*s*, 1H, *CH_c*), 7.69-7.71 (*d*, 2H, *ArH_b*), 7.23-7.36 (*m*, 5H, *ArH_a*), 6.77-6.97 (*m*, 3H, *ArH_e*), 5.03 (*s*, 2H, *NCH_{2g}*), 4.63 (*s*, 2H, *CH_{2f}*) and 3.72-3.75 (*s*, 6H, *CH_{3h}*).

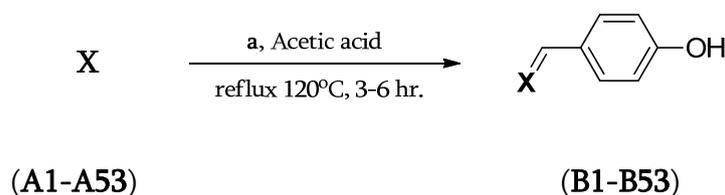


48

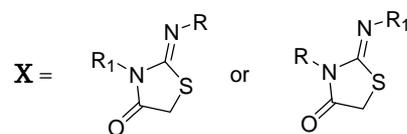
4.2.2 Synthesis of substituted 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (49-96).

4.2.2.1. Synthesis of substituted 5-(4-hydroxybenzylidene)-2-imino-3-phenylthiazolidin-4-one (B1-B53)

Substituted 5-(4-hydroxybenzylidene)-2-imino-3-phenylthiazolidin-4-one (**B1-B53**) (**Scheme-IV**) were also prepared in a similar way with conditions mentioned in the Scheme-III by reacting the 4-hydroxybenzaldehyde with substituted 2-imino-thiazolidin-4-one derivatives (**A1-A53**). Synthesized derivatives were given wash with cold acetic acid followed by water and confirmed by IR, which shows appearance of characteristic peak of hydroxyl (-OH str) between 3347-3395 cm^{-1} .



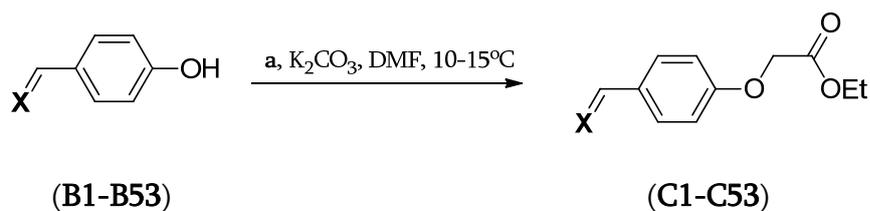
a = 4-Hydroxybenzaldehyde, β -alanine.



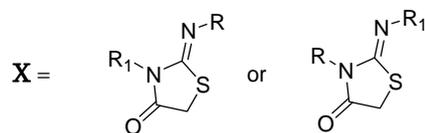
Scheme IV

4.2.2.2. Synthesis of substituted Ethyl 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetate (C1-C53).

The corresponding Ethyl 2-{4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetate (**C1-C53**) were synthesized by controlled addition of ethyl bromoacetate, dropwise to a suspension of substituted 5-(4-hydroxybenzylidene)-2-imino-3-phenylthiazolidin-4-one (**B1-B53**) and anhydrous potassium carbonate in dimethyl formamide at 10-15 $^{\circ}\text{C}$ (**Scheme-V**). The pure compounds were obtained by giving repeated washing with cold hexane. All the synthesized derivatives (**C1-C53**) were characterized on the basis of their IR spectra. The purity of the derivatives was checked by TLC in different solvent systems.



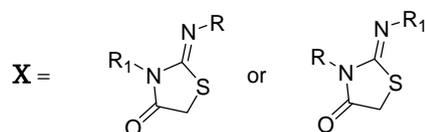
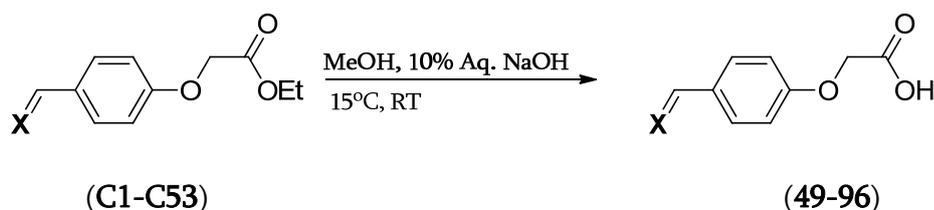
a = Ethylbromoacetate.



Scheme V

4.2.2.3. Synthesis of derivatives 2-[4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (49-96).

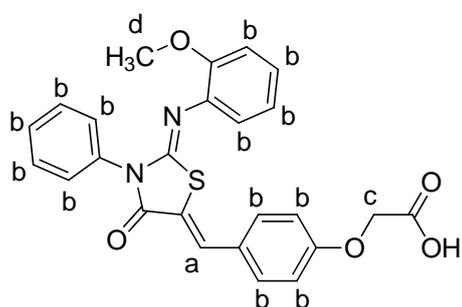
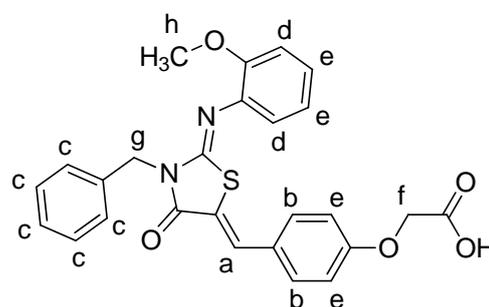
The Ethyl 2-[4-[(2-imino-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetate derivatives (C1-C53) were hydrolysed in alkaline medium using 10% aqueous potassium hydroxide solution. The corresponding acid derivatives (49-96) were characterized on the basis of their IR spectra which showed shifting of characteristic peak of C=O from 1750 to 1610 cm^{-1} and the appearance of peak of carboxylic -OH at 3300 cm^{-1} . Purity of all the synthesized compounds was checked by TLC in different solvent systems and further confirmed by NMR and Mass spectra.



Scheme VI

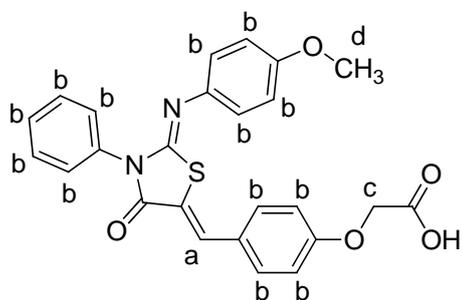
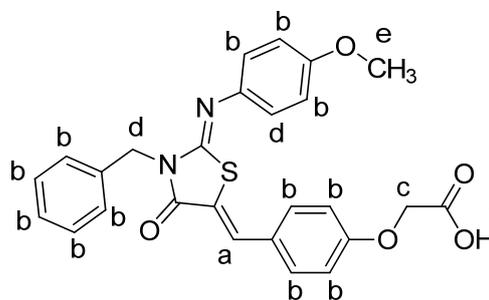
2-[4-[(2-((2-methoxyphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (49) was synthesized by hydrolysis of compound (C1). Compound (49) displayed the IR peaks at 3414 cm^{-1} (OH str), 1718 cm^{-1} (C=O str), 1640 cm^{-1} (C=N)

and 1594 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**49**) appeared at δ 7.63 (*s*, 1H, CH_a), 6.92-7.54 (*m*, 13H, ArH_b), 4.67 (*s*, 2H, CH_{2c}) and 3.87 (*s*, 3H, CH_{3d}).

**49****50**

2-[4-[(3-benzyl-2-((2-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**50**) was obtained by hydrolysis of compound (**C2**) showed IR peaks at 3350 cm^{-1} (OH str), 1700 cm^{-1} (C=O str), 1642 cm^{-1} (C=N) and 1601 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 12.61 (*bs*, 1H, OH_i), 7.63 (*s*, 1H, CH_a), 7.57-7.59 (*d*, 2H, ArH_b), 7.16-7.27 (*m*, 5H, ArH_c), 6.91-6.93 (*d*, 2H, ArH_d), 6.74-6.86 (*m*, 4H, ArH_e), 4.67 (*s*, 2H, CH_{2f}), 4.25 (*s*, 2H, CH_{2g}) and 3.78 (*s*, 3H, CH_{3h}).

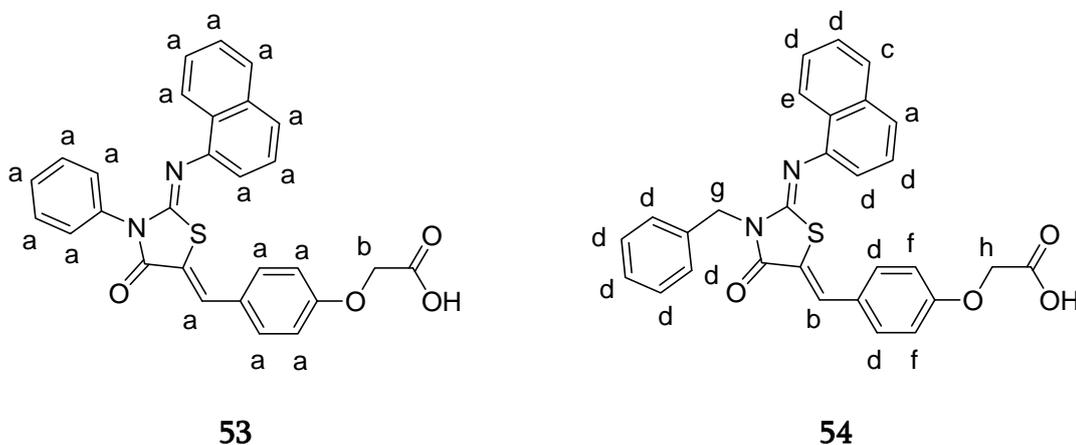
2-[4-[(2-((4-methoxyphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**51**) was synthesized by hydrolysis of compound (**C3**). Compound (**51**) displayed the IR peaks at 3117 cm^{-1} (OH str), 1708 cm^{-1} (C=O str), 1630 cm^{-1} (C=N) and 1597 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**51**) appeared at δ 7.72 (*s*, 1H, CH_a), 6.80-7.40 (*m*, 13H, ArH_b), 4.58 (*s*, 2H, CH_{2c}) and 3.85 (*s*, 3H, CH_{3d}).

**51****52**

2-[4-[(3-benzyl-2-((4-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**52**) was obtained by hydrolysis of compound (**C4**) showed IR

peaks at 3471 cm^{-1} (OH str), 1704 cm^{-1} (C=O str), 1634 cm^{-1} (C=N) and 1599 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 13.00 (*bs*, 1H, OH_f), 7.63 (*s*, 1H, CH_a), 6.77-7.82 (*m*, 13H, ArH_b), 5.10 (*s*, 2H, CH_{2c}), 4.70 (*s*, 2H, CH_{2d}) and 3.78 (*s*, 3H, CH_{3e}).

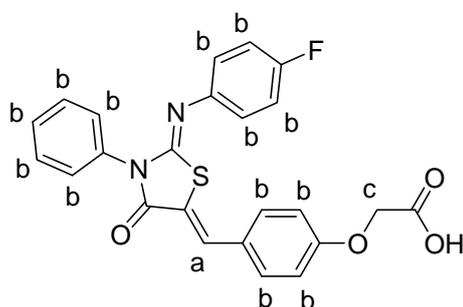
2-{4-[(2-(naphthalen-1-ylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**53**) was synthesized by hydrolysis of compound (**C5**). Compound (**53**) displayed the IR peaks at 3410 cm^{-1} (OH str), 1713 cm^{-1} (C=O str), 1639 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**53**) appeared at δ 6.69-7.97 (*m*, 17H, ArH_a) and 4.44 (*s*, 2H, CH_{2b}). Mass spectrum showed molecular ion peak at m/z 480.6 (M^+) and 494.9 ($M+23$).



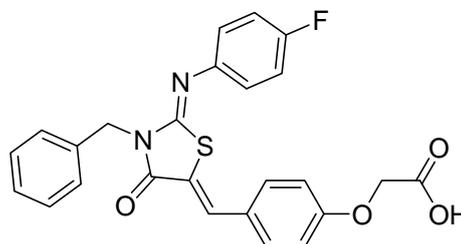
2-{4-[(3-benzyl-2-(naphthalen-1-ylimino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**54**) was obtained by hydrolysis of compound (**C6**) showed IR peaks at 3300 cm^{-1} (OH str), 1727 cm^{-1} (C=O str), 1694 cm^{-1} (C=N) and 1628 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.86-7.88 (*d*, 1H, ArH_a), 7.76 (*s*, 1H, CH_b), 7.69-7.71 (*d*, 1H, ArH_c), 7.31-7.55 (*m*, 11H, ArH_d), 7.07-7.09 (*d*, 1H, ArH_e), 6.94-6.97 (*d*, 2H, ArH_f) 5.26 (*s*, 2H, CH_{2g}) and 4.70 (*s*, 2H, CH_{2h}).

2-{4-[(2-((4-fluorophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**55**) was synthesized by hydrolysis of compound (**C7**). Compound (**55**) displayed the IR peaks at 3121 cm^{-1} (OH str), 1719 cm^{-1} (C=O str), 1641 cm^{-1} (C=N) and 1594 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**55**) appeared at δ 7.74 (*s*, 1H,

CH_a), 6.64-7.56 (*m*, 13H, ArH_b) and 4.64 (*s*, 2H, CH_{2c}). Mass spectrum showed molecular ion peak at m/z 448.9 (M^+) and 446.9 ($M-1$).



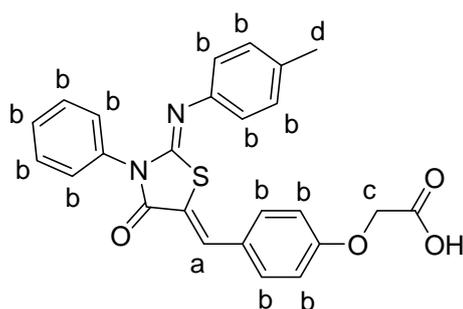
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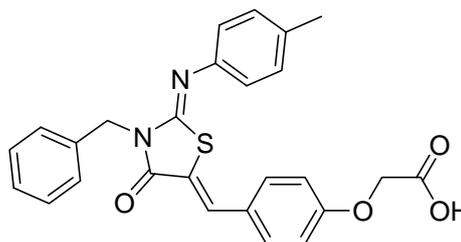
56

2-{4-[(3-benzyl-2-((4-fluorophenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**56**) was obtained by hydrolysis of compound (**C8**) showed IR peaks at 3382 cm^{-1} (OH str), 1711 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1597 cm^{-1} (C=C). Mass spectrum showed molecular ion peak at m/z 464.13 ($M+2$).

2-{4-[(4-oxo-3-phenyl-2-(*p*-tolylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**57**) was synthesized by hydrolysis of compound (**C9**). Compound (**57**) displayed the IR peaks at 3415 cm^{-1} (OH str), 1710 cm^{-1} (C=O str), 1629 cm^{-1} (C=N) and 1596 cm^{-1} (C=C). 1H -NMR signals for the compound (**57**) appeared at δ 7.80 (*s*, 1H, CH_a), 6.82-7.77 (*m*, 13H, ArH_b), 4.66 (*s*, 2H, CH_{2c}) and 2.56 (*s*, 3H, CH_{3d}).



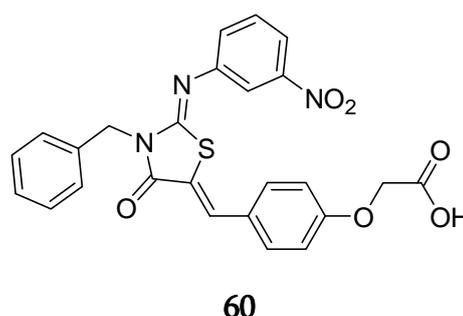
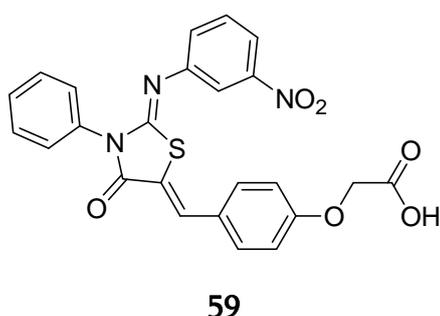
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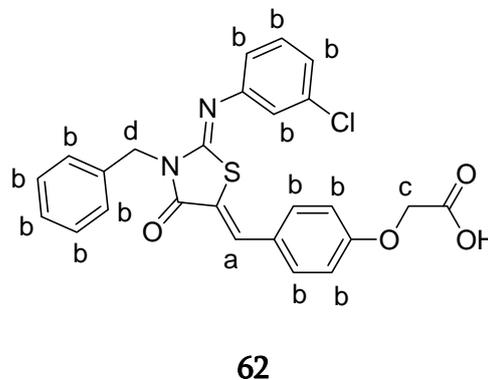
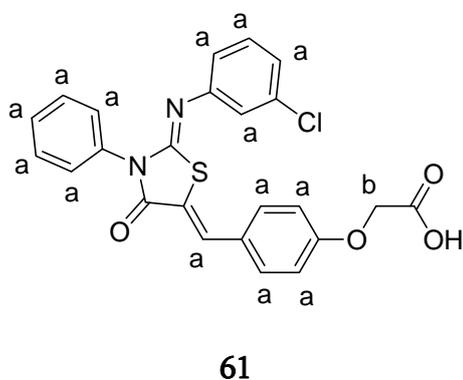
2-{4-[(3-benzyl-4-oxo-2-(*p*-tolylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**58**) was obtained by hydrolysis of compound (**C10**) showed IR peaks at 3310 cm^{-1} (OH str), 1728 cm^{-1} (C=O str), 1635 cm^{-1} (C=N) and 1595 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at m/z 457.3 ($M-1$).

2-{4-[(2-((3-nitrophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**59**) was synthesized by hydrolysis of compound (**C11**). Compound (**59**) displayed the IR peaks at 3391 cm^{-1} (OH str), 1710 cm^{-1} (C=O str), 1640 cm^{-1} (C=N) and 1596 cm^{-1} (C=C). Mass spectrum showed molecular ion peak at m/z 475.9 (M^+).



2-{4-[(3-benzyl-2-((3-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**60**) was obtained by hydrolysis of compound (**C12**) showed IR peaks at 3473 cm^{-1} (OH str), 1714 cm^{-1} (C=O str), 1639 cm^{-1} (C=N) and 1602 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at m/z 489.30 (M^+).

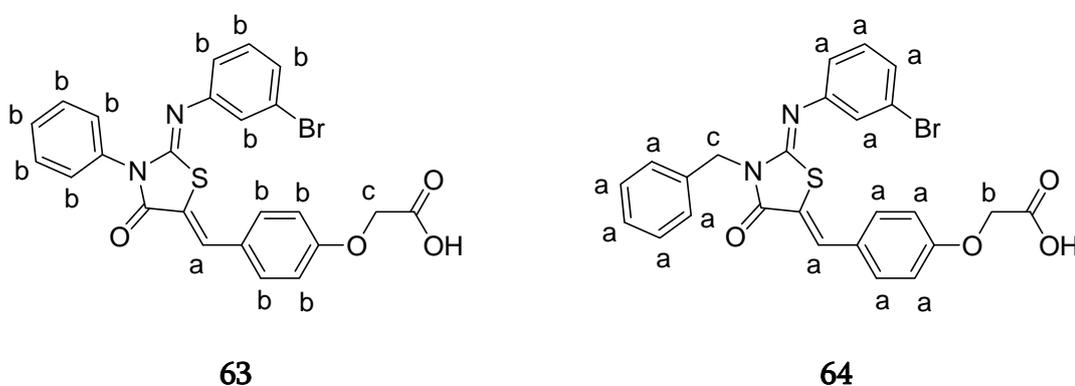
2-{4-[(2-((3-chlorophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**61**) was synthesized by hydrolysis of compound (**C13**). Compound (**61**) displayed the IR peaks at 3115 cm^{-1} (OH str), 1710 cm^{-1} (C=O str), 1637 cm^{-1} (C=N) and 1596 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**61**) appeared at δ 6.76-7.81 (*m*, 14H, ArH_a) and 4.68 (*s*, 2H, CH_{2b}).



2-{4-[(3-benzyl-2-((3-chlorophenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**62**) was obtained by hydrolysis of compound (**C14**) showed IR

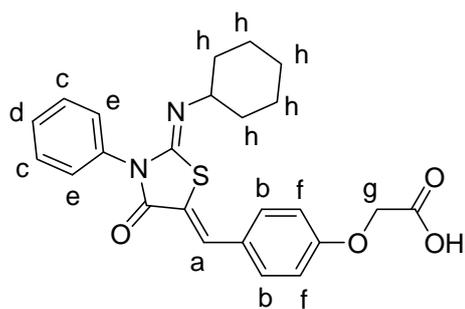
peaks at 3473 cm^{-1} (OH str), 1714 cm^{-1} (C=O str), 1639 cm^{-1} (C=N) and 1602 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.74 (*s*, 1H, CH_a), 6.88-7.48 (*m*, 13H, ArH_b), 5.09 (*s*, 2H, CH_{2c}) and 4.62 (*s*, 2H, CH_{2d}).

2-{4-[(2-((3-bromophenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**63**) was synthesized by hydrolysis of compound (**C15**). Compound (**63**) displayed the IR peaks at 3122 cm^{-1} (OH str), 1716 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1591 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**63**) appeared at δ 7.76 (*s*, 1H, CH_a), 6.92-7.57 (*m*, 13H, ArH_b) and 4.66 (*s*, 2H, CH_{2b}).

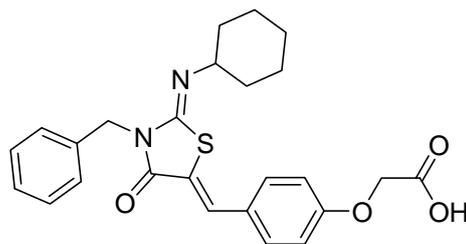


2-{4-[(3-benzyl-2-((3-bromophenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**64**) was obtained by hydrolysis of compound (**C16**) showed IR peaks at 3319 cm^{-1} (OH str), 1708 cm^{-1} (C=O str), 1632 cm^{-1} (C=N) and 1590 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 6.62-7.84 (*m*, 14H, ArH_a), 5.61 (*s*, 2H, CH_{2b}) and 4.34 (*s*, 2H, CH_{2c}). Mass spectrum showed molecular ion peak at m/z 524.8 (M+1).

2-{4-[(2-(cyclohexylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**65**) was synthesized by hydrolysis of compound (**C17**). Compound (**65**) displayed the IR peaks at 3121 cm^{-1} (OH str), 1716 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1591 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**65**) appeared at δ 7.67 (*s*, 1H, CH_a), 7.59-7.60 (*d*, 2H, ArH_b), 7.47-7.50 (*t*, 2H, ArH_c), 7.39-7.42 (*m*, 1H, ArH_d), 7.33-7.35 (*d*, 2H, ArH_e), 7.05-7.07 (*d*, 2H, ArH_f), 4.71 (*s*, 2H, CH_{2g}) and 1.19-1.72 (*m*, 10H, CH_{2h}).



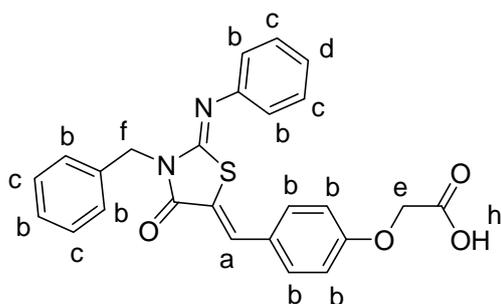
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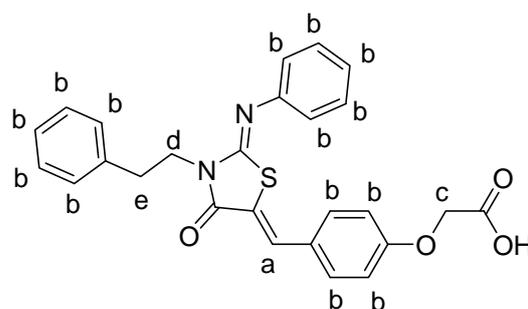
66

2-{4-[(3-benzyl-2-(cyclohexylimino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy} acetic acid (**66**) was obtained by hydrolysis of compound (**C18**) showed IR peaks at 3315 cm^{-1} (OH str), 1715 cm^{-1} (C=O str), 1632 cm^{-1} (C=N) and 1597 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at $m/z\ 451.1(M^+)$.

2-{4-[(3-benzyl-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy} acetic acid (**67**) was synthesized by hydrolysis of compound (**C19**). Compound (**67**) displayed the IR peaks at 3382 cm^{-1} (OH str), 1707 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1594 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**67**) appeared at $\delta\ 13.05$ (*bs*, 1H, OH_g), 7.70 (*s*, 1H, CH_a), $7.26\text{--}7.45$ (*m*, 9H, ArH_b), $7.15\text{--}7.19$ (*t*, 1H, ArH_c), $6.96\text{--}6.99$ (*t*, 4H, ArH_d), 5.12 (*s*, 2H, CH_{2e}) and 4.66 (*s*, 2H, CH_{2f}).



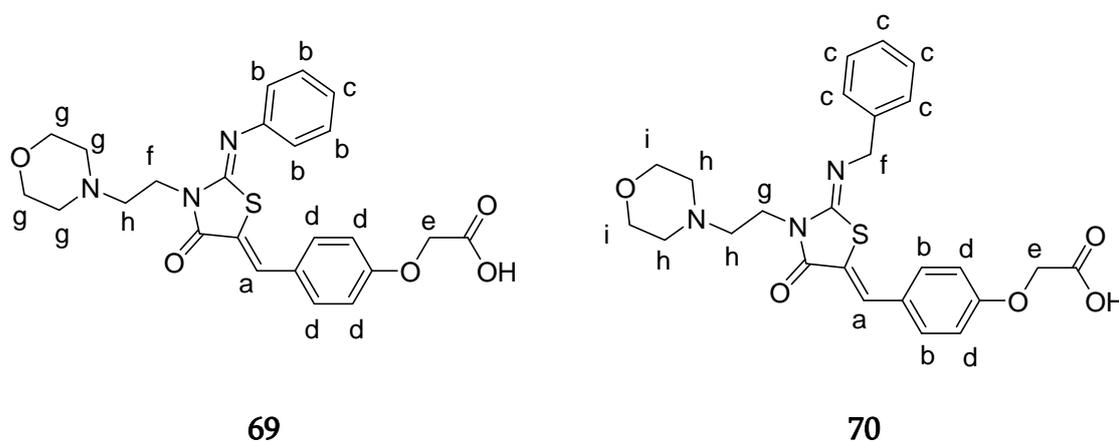
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68

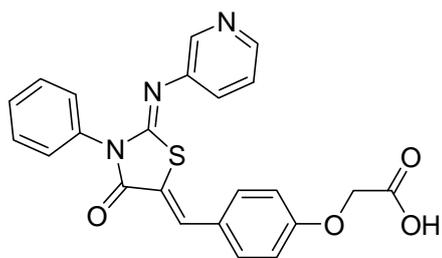
2-{4-[(4-oxo-3-phenethyl-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy} acetic acid (**68**) was obtained by hydrolysis of compound (**C20**) showed IR peaks at 3422 cm^{-1} (OH str), 1702 cm^{-1} (C=O str), 1635 cm^{-1} (C=N) and 1570 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at $\delta\ 7.64$ (*s*, 1H, CH_a), $6.90\text{--}7.43$ (*m*, 14H, ArH_b), 4.63 (*s*, 2H, CH_{2c}), 4.17 (*t*, 2H, CH_{2d}) and 3.06 (*t*, 2H, CH_{2e}).

2-{4-[(3-(2-morpholinoethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**69**) was synthesized by hydrolysis of compound (**C21**). Compound (**69**) displayed the IR peaks at 3416 cm^{-1} (OH str), 1701 cm^{-1} (C=O str), 1643 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**69**) appeared at δ 7.67 (*s*, 1H, CH_a), 7.38-7.45 (*m*, 4H, ArH_b), 7.16-7.20 (*t*, 1H, ArH_c), 6.97-6.99 (*m*, 4H, ArH_d), 4.66 (*s*, 2H, CH_{2e}), 4.04-4.07 (*t*, 2H, CH_{2f}), 3.57-3.59 (*m*, 8H, CH_{2g}) and 2.70-2.73 (*t*, 2H, CH_{2h}).

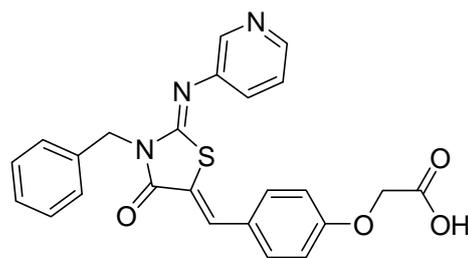


2-{4-[(2-(benzylimino)-3-(2-morpholinoethyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**70**) was obtained by hydrolysis of compound (**C22**) showed IR peaks at 3496 cm^{-1} (OH str), 1728 cm^{-1} (C=O str), 1645 cm^{-1} (C=N) and 1595 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.63 (*s*, 1H, CH_a), 7.50-7.52 (*d*, 2H, ArH_b), 7.19-7.32 (*m*, 5H, ArH_c), 6.98-7.00 (*d*, 2H, ArH_d), 4.63 (*s*, 2H, CH_{2e}), 4.55 (*s*, 2H, CH_{2f}), 4.10 (*bs*, 2H, CH_{2g}), 3.62-3.69 (*bs*, 6H, CH_{2h}) and 3.30 (*s*, 4H, CH_{2i}).

2-{4-[(4-oxo-3-phenyl-2-(pyridin-3-ylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**71**) was synthesized by hydrolysis of compound (**C23**). Compound (**71**) displayed the IR peaks at 3396 cm^{-1} (OH str), 1705 cm^{-1} (C=O str), 1648 cm^{-1} (C=N) and 1618 cm^{-1} (C=C). Mass spectrum showed molecular ion peak at m/z 431.80 (M^+).



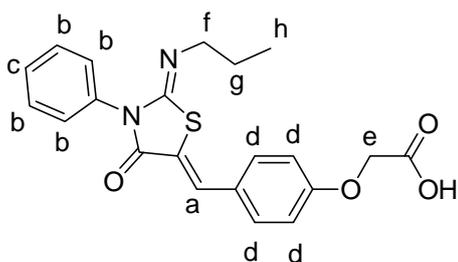
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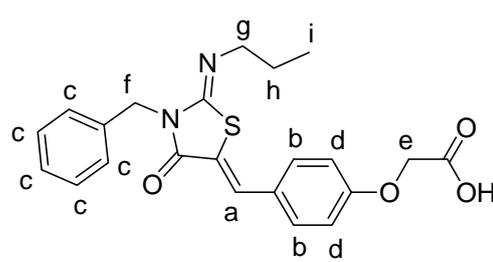
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2-[4-[(3-benzyl-4-oxo-2-(pyridin-3-ylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**72**) was obtained by hydrolysis of compound (**C24**) showed IR peaks at 3360 cm^{-1} (OH str), 1709 cm^{-1} (C=O str), 1642 cm^{-1} (C=N) and 1600 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at 444.1 (M-1).

2-[4-[(4-oxo-3-phenyl-2-(propylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**73**) was synthesized by hydrolysis of compound (**C25**). Compound (**73**) displayed the IR peaks at 3396 cm^{-1} (OH str), 1707 cm^{-1} (C=O str), 1678 cm^{-1} (C=N) and 1640 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**73**) appeared at δ 7.64 (*s*, 1H, CH_a), 7.37-7.41 (*m*, 4H, ArH_b), 7.16-7.19 (*m*, 1H, ArH_c), 6.92-6.99 (*m*, 4H, ArH_d), 4.42 (*s*, 2H, CH_{2e}), 3.87-3.90 (*t*, 2H, CH_{2f}), 1.76-1.78 (*m*, 2H, CH_{2g}) and 0.95-0.99 (*t*, 3H, CH_{3h}).



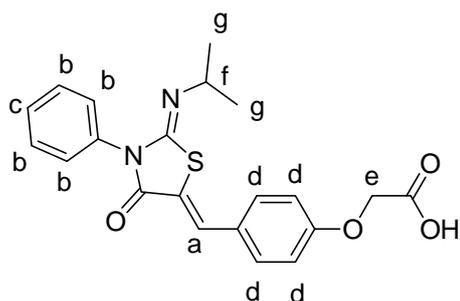
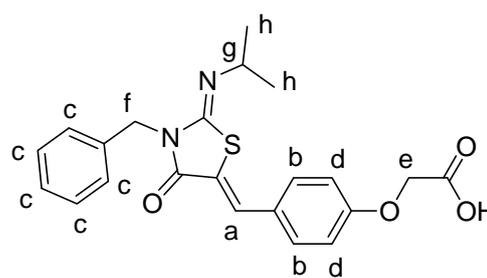
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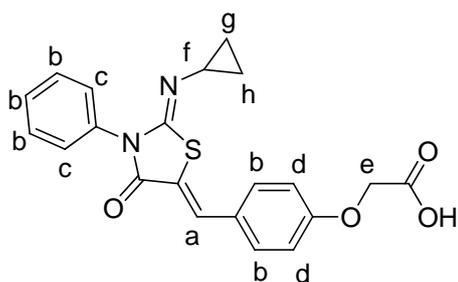
2-[4-[(3-benzyl-4-oxo-2-(propylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**74**) was obtained by hydrolysis of compound (**C26**) showed IR peaks at 3112 cm^{-1} (OH str), 1702 cm^{-1} (C=O str), 1646 cm^{-1} (C=N) and 1599 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.65 (*s*, 1H, CH_a), 7.56-7.58 (*d*, 2H, ArH_b), 7.22-7.36 (*m*, 5H, ArH_c), 7.03-7.05 (*d*, 2H, ArH_d), 4.72 (*s*, 2H, CH_{2e}), 4.61 (*s*, 2H, CH_{2f}), 3.79-3.82 (*t*, 2H, CH_{2g}), 1.61-1.72 (*m*, 2H, CH_{2h}) and 0.88-0.92 (*m*, 3H, CH_{3i}).

2-[4-[(2-(isopropylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**75**) was synthesized by hydrolysis of compound (**C27**). Compound (**75**) displayed the IR peaks at 3130 cm^{-1} (OH str), 1714 cm^{-1} (C=O str), 1644 cm^{-1} (C=N) and 1598 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**75**) appeared at δ 7.66 (*s*, 1H, CH_a), 7.39-7.41 (*m*, 4H, ArH_b), 7.16-7.19 (*m*, 1H, ArH_c), 6.93-6.99 (*m*, 4H, ArH_d), 4.43 (*s*, 2H, CH_{2e}), 1.74-1.79 (*m*, 1H, CH_{2f}) and 0.95-0.98 (*d*, 6H, CH_{3g}).

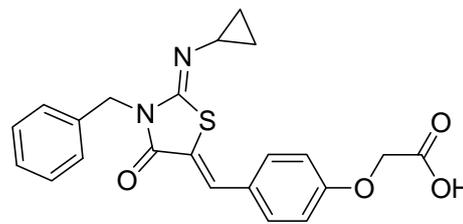
**75****76**

2-[4-[(3-benzyl-2-(isopropylimino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**76**) was obtained by hydrolysis of compound (**C28**) showed IR peaks at 3396 cm^{-1} (OH str), 1706 cm^{-1} (C=O str), 1641 cm^{-1} (C=N) and 1598 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.61 (*s*, 1H, CH_a), 7.50-7.52 (*d*, 2H, ArH_b), 7.22-7.38 (*m*, 5H, ArH_c), 7.00-7.02 (*d*, 2H, ArH_d), 4.94 (*s*, 2H, CH_{2e}), 4.64 (*s*, 2H, CH_{2f}), 3.51-3.57 (*m*, 1H, CH_g) and 1.18-1.20 (*d*, 6H, CH_{3h}).

2-[4-[(2-(cyclopropylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**77**) was synthesized by hydrolysis of compound (**C29**). Compound (**77**) displayed the IR peaks at 3127 cm^{-1} (OH str), 1711 cm^{-1} (C=O str), 1641 cm^{-1} (C=N) and 1599 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**77**) appeared at δ 7.63 (*s*, 1H, CH_a), 7.34-7.55 (*m*, 5H, ArH_b), 7.25-7.27 (*d*, 2H, ArH_c), 6.99-7.02 (*d*, 2H, ArH_d), 4.66 (*s*, 2H, CH_{2e}), 2.72-2.75 (*m*, 1H, CH_f), 0.77-0.81 (*m*, 2H, CH_{2g}) and 0.54-0.57 (*m*, 2H, CH_{2h}). Mass spectrum showed molecular ion peak at m/z 395.2 (M^+).



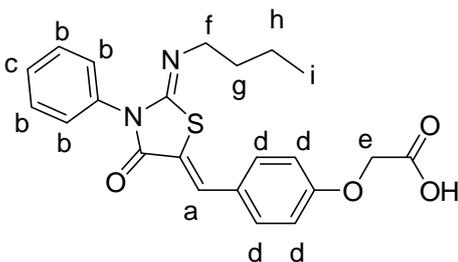
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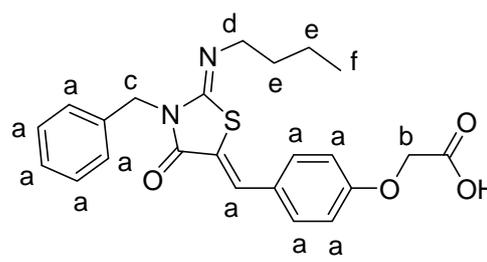
78

2-[4-[(3-benzyl-2-(cyclopropylimino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**78**) was obtained by hydrolysis of compound (**C30**) showed IR peaks at 3400 cm^{-1} (OH str), 1706 cm^{-1} (C=O str), 1641 cm^{-1} (C=N) and 1598 cm^{-1} (C=C) while its mass spectrum showed molecular ion peak at m/z 406.99 (M-1).

2-[4-[(2-(butylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**79**) was synthesized by hydrolysis of compound (**C31**). Compound (**79**) displayed the IR peaks at 3126 cm^{-1} (OH str), 1703 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1597 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**79**) appeared at δ 7.66 (*s*, 1H, CH_a), 7.37-7.45 (*m*, 4H, ArH_b), 7.16-7.19 (*m*, 1H, ArH_c), 6.98-7.00 (*m*, 4H, ArH_d), 4.67 (*s*, 2H, CH_{2e}), 3.91-3.94 (*t*, 2H, CH_{2f}), 1.71-1.74 (*m*, 2H, CH_{2g}), 1.37-1.42 (*m*, 2H, CH_{2h}) and 0.95-0.99 (*t*, 3H, CH_{3i}).



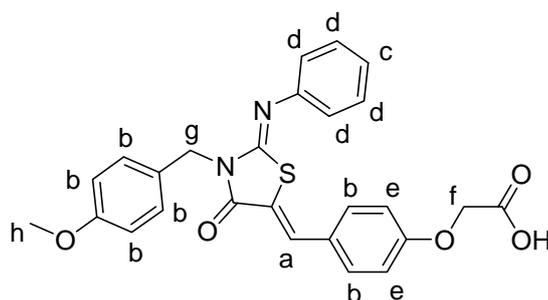
79



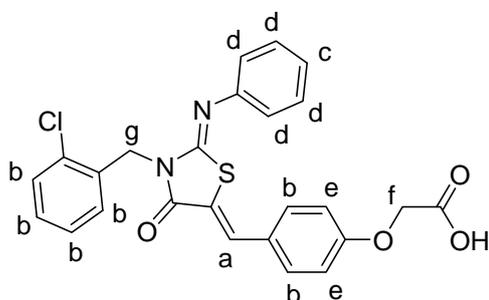
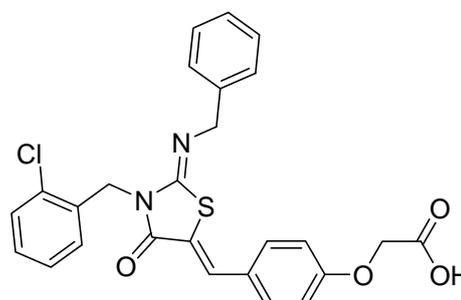
80

2-[4-[(3-benzyl-2-(butylimino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**80**) was obtained by hydrolysis of compound (**C32**) showed IR peaks 3120 cm^{-1} (OH str), 1701 cm^{-1} (C=O str), 1636 cm^{-1} (C=N) and 1600 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 6.76-7.81 (*m*, 10H, CH_a), 4.57 (*s*, 2H, CH_{2b}), 4.23 (*s*, 2H, CH_{2c}), 3.02-3.07 (*m*, 2H, CH_d), 1.24-1.41 (*m*, 4H, CH_{2e}) and 0.87-0.94 (*m*, 3H, CH_{3f}).

2-[4-[(3-(4-methoxybenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**81**) was synthesized by hydrolysis of compound (**C33**). Compound (**81**) displayed the IR peaks at 3124 cm^{-1} (OH str), 1708 cm^{-1} (C=O str), 1639 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**81**) appeared at δ 7.59 (*s*, 1H, CH_a), 7.29-7.38 (*m*, 6H, ArH_b), 7.11-7.12 (*t*, 1H, ArH_c), 6.87-6.92 (*m*, 4H, ArH_d), 6.78-6.80 (*d*, 2H, ArH_e), 4.99 (*s*, 2H, CH_{2f}), 4.55 (*s*, 2H, CH_{2g}) and 3.70 (*s*, 3H, CH_{3h}).

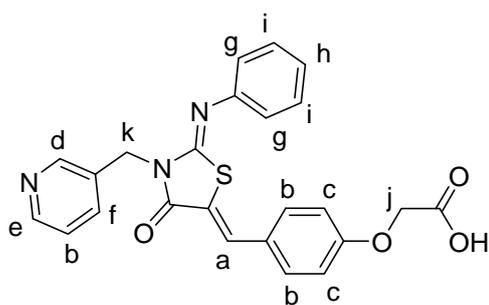
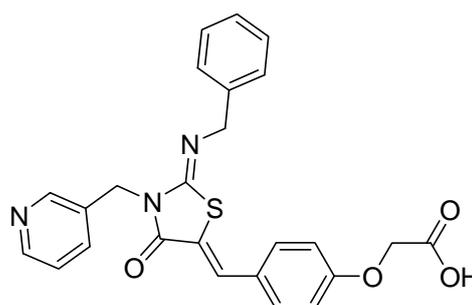
**81**

2-[4-[(3-(2-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**82**) was synthesized by hydrolysis of compound (**C35**). Compound (**82**) displayed the IR peaks at 3310 cm^{-1} (OH str), 1709 cm^{-1} (C=O str), 1641 cm^{-1} (C=N) and 1594 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**82**) appeared at δ 7.59 (*s*, 1H, CH_a), 7.29-7.38 (*m*, 6H, ArH_b), 7.11-7.12 (*t*, 1H, ArH_c), 6.87-6.92 (*m*, 4H, ArH_d), 6.78-6.80 (*d*, 2H, ArH_e), 4.99 (*s*, 2H, CH_{2f}), 4.55 (*s*, 2H, CH_{2g}) and 3.70 (*s*, 3H, CH_{3h}).

**82****83**

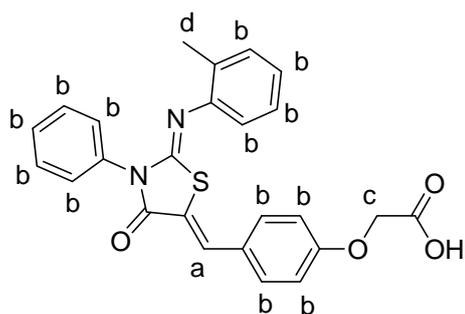
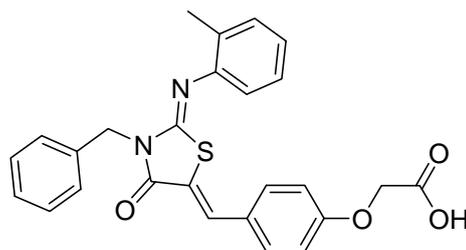
2-{4-[(2-(benzylimino)-3-(2-chlorobenzyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**83**) was obtained by hydrolysis of compound (**C36**) showed IR peaks 3310 cm^{-1} (OH str), 1709 cm^{-1} (C=O str), 1623 cm^{-1} (C=N) and 1598 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at m/z 495 (M^+) and 517 ($M+\text{Na}$).

2-{4-[(4-oxo-2-(phenylimino)-3-(pyridin-3-ylmethyl)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**84**) was synthesized by hydrolysis of compound (**C37**). Compound (**84**) displayed the IR peaks at 3151 cm^{-1} (OH str), 1705 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1594 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**84**) appeared at δ 8.66 (*s*, 1H, ArH_d), 8.43-8.44 (*d*, 1H, ArH_e), 7.78-7.79 (*d*, 1H, ArH_f), 7.50 (*s*, 1H, CH_a), 7.22-7.28 (*m*, 3H, ArH_b), 7.13-7.15 (*d*, 2H, ArH_g), 7.04-7.08 (*t*, 1H, ArH_h), 6.86-6.88 (*d*, 2H, ArH_i), 6.77-6.79 (*d*, 2H, ArH_c), 5.04 (*s*, 2H, CH_{2j}) and 4.26 (*s*, 2H, CH_{2k}).

**84****85**

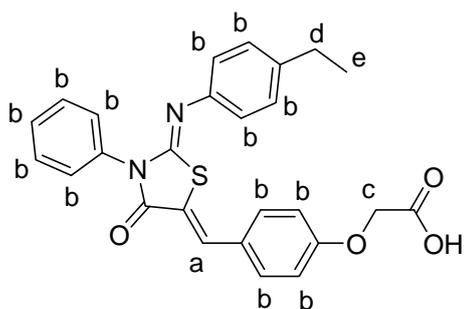
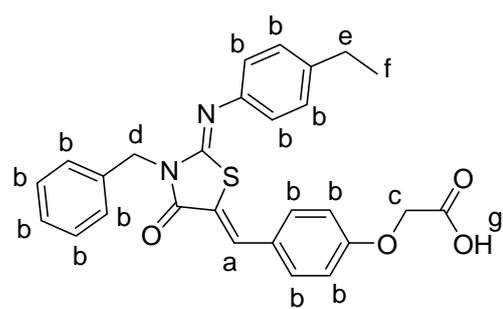
2-{4-[(2-(benzylimino)-4-oxo-3-(pyridin-3-ylmethyl)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**85**) was synthesized by hydrolysis of compound (**C38**) showed IR peaks at 3185 cm^{-1} (OH str), 1705 cm^{-1} (C=O str), 1638 cm^{-1} (C=N) and 1594 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at m/z 459.73 (M^+).

2-{4-[(4-oxo-3-phenyl-2-(o-tolylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**86**) was synthesized by hydrolysis of compound (**C39**). Compound (**86**) displayed the IR peaks at 3127 cm^{-1} (OH str), 1719 cm^{-1} (C=O str), 1644 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**86**) appeared at δ 7.67 (*s*, 1H, CH_a), 6.78-7.52 (*m*, 13H, ArH_b), 4.59 (*s*, 2H, CH_{2c}) and 2.22 (*s*, 3H, CH_{3d}).

**86****87**

2-[4-[(3-benzyl-4-oxo-2-(o-tolylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**87**) was synthesized by hydrolysis of compound (**C40**) showed IR peaks 3200 cm^{-1} (OH str), 1717 cm^{-1} (C=O str), 1643 cm^{-1} (C=N) and 1601 cm^{-1} (C=C) while its Mass spectrum showed molecular ion peak at $m/z\ 459.89$ (M^+).

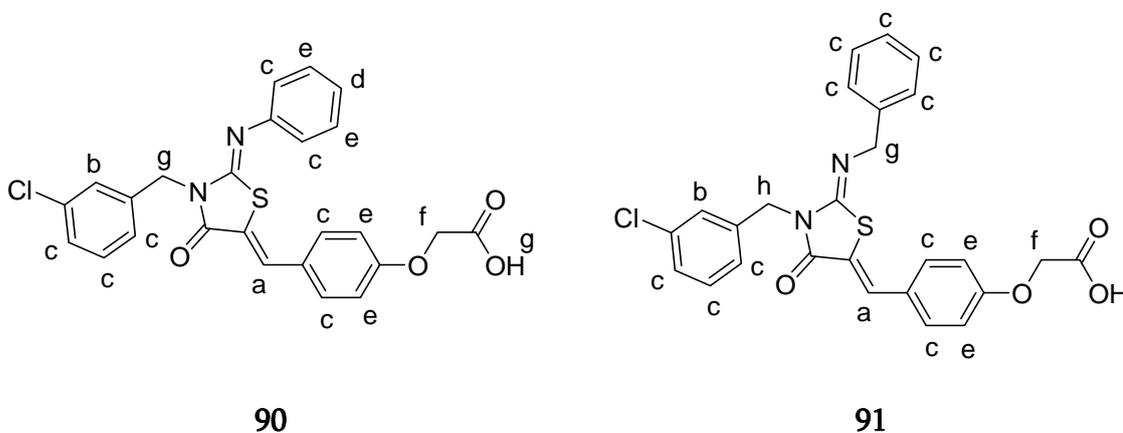
2-[4-[(2-((4-ethylphenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**88**) was synthesized by hydrolysis of compound (**C41**). Compound (**88**) displayed the IR peaks at 3128 cm^{-1} (OH str), 1715 cm^{-1} (C=O str), 1634 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**88**) appeared at $\delta\ 7.79$ (*s*, 1H, CH_a), 6.78-7.50 (*m*, 13H, ArH_b), 4.58 (*s*, 2H, CH_{2c}), 2.63-2.69 (*m*, 2H, CH_{2d}) and 1.16-1.24 (*m*, 3H, CH_{3e}).

**88****89**

2-[4-[(3-benzyl-2-((4-ethylphenyl)imino)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**89**) was obtained by hydrolysis of compound (**C42**) showed IR peaks 3140 cm^{-1} (OH str), 1715 cm^{-1} (C=O str), 1643 cm^{-1} (C=N) and 1595 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at $\delta\ 12.83$ (*bs*, 1H, OH_g), 7.77 (*s*, 1H, CH_a),

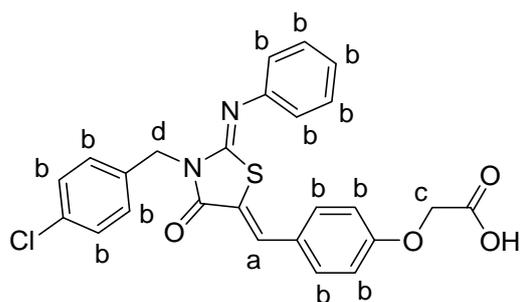
6.45-7.63 (*m*, 13H, ArH_b), 4.68 (*s*, 2H, CH_{2c}), 4.31 (*s*, 2H, CH_{2d}), 2.50-2.56 (*m*, 2H, CH_e) and 1.12-1.18 (*m*, 4H, CH_{2f}).

2-{4-[(3-(3-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**90**) was synthesized by hydrolysis of compound (**C47**). Compound (**90**) displayed the IR peaks at 3126 cm⁻¹ (OH str), 1708 cm⁻¹ (C=O str), 1638 cm⁻¹ (C=N) and 1595 cm⁻¹ (C=C). ¹H-NMR signals for the compound (**90**) appeared at δ 7.64 (*s*, 1H, CH_a), 7.48 (*s*, 1H, ArH_b), 7.27-7.40 (*m*, 7H, ArH_c), 7.13-7.17 (*t*, 1H, ArH_d), 6.88-6.96 (*m*, 3H, ArH_e), 5.09 (*s*, 2H, CH_{2f}) and 4.44 (*s*, 2H, CH_{2g}).

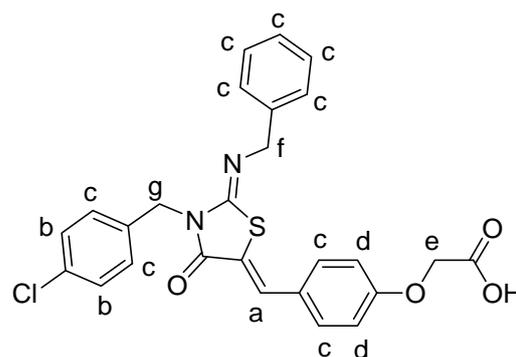


2-{4-[(2-(benzylimino)-3-(3-chlorobenzyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**91**) was obtained by hydrolysis of compound (**C48**) showed IR peaks 3121 cm⁻¹ (OH str), 1733 cm⁻¹ (C=O str), 1695 cm⁻¹ (C=N) and 1647 cm⁻¹ (C=C) while its ¹H-NMR spectrum displayed peaks at δ 7.70 (*s*, 1H, CH_a), 7.55 (*s*, 1H, ArH_b), 7.18-7.41 (*m*, 10H, ArH_c), 7.00-7.03 (*d*, 1H, ArH_d), 6.42-6.45 (*m*, 1H, ArH_e), 5.02 (*s*, 2H, CH_{2f}), 4.69 (*s*, 2H, CH_{2g}) and 4.28 (*s*, 2H, CH_{2h}).

2-{4-[(3-(4-chlorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**92**) was synthesized by hydrolysis of compound (**C49**). Compound (**92**) displayed the IR peaks at 3408 cm⁻¹ (OH str), 1709 cm⁻¹ (C=O str), 1641 cm⁻¹ (C=N) and 1595 cm⁻¹ (C=C). ¹H-NMR signals for the compound (**92**) appeared at δ 6.86-7.69 (*m*, 14H, CH_a&ArH_b), 5.03(*s*, 2H, CH_{2d}) and 4.46 (*s*, 2H, CH_{2d}).



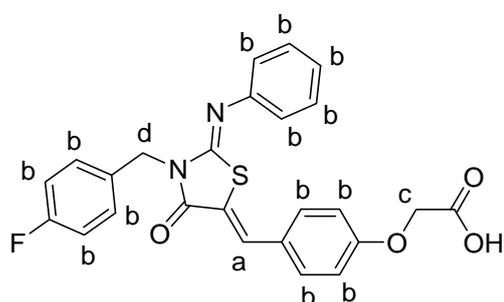
92



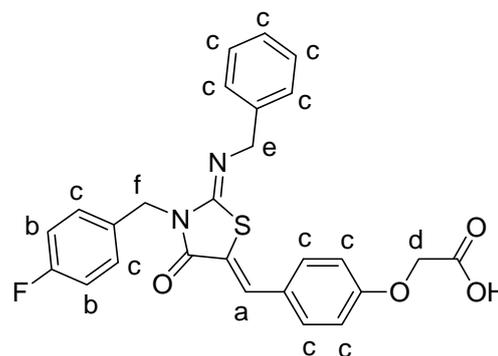
93

2-[4-[(2-(benzylimino)-3-(4-chlorobenzyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**93**) was obtained by hydrolysis of compound (**C50**) showed IR peaks 3122 cm^{-1} (OH str), 1694 cm^{-1} (C=O str), 1645 cm^{-1} (C=N) and 1595 cm^{-1} (C=C) while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.69 (*s*, 1H, CH_a), 7.53-7.55 (*d*, 2H, ArH_b), 7.23-7.36 (*m*, 9H, ArH_c), 7.01-7.03 (*d*, 2H, ArH_d), 5.03 (*s*, 2H, CH_{2e}), 4.67 (*s*, 2H, CH_{2f}) and 4.60 (*s*, 2H, CH_{2g}).

2-[4-[(3-(4-fluorobenzyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**94**) was synthesized by hydrolysis of compound (**C51**). Compound (**94**) displayed the IR peaks at 3418 cm^{-1} (OH str), 1700 cm^{-1} (C=O str), 1649 cm^{-1} (C=N) and 1595 cm^{-1} (C=C). $^1\text{H-NMR}$ signals for the compound (**94**) appeared at δ 6.91-7.67 (*m*, 14H, CH_a & ArH_b), 5.03 (*s*, 2H, CH_{2d}) and 4.40 (*s*, 2H, CH_{2d}).



94

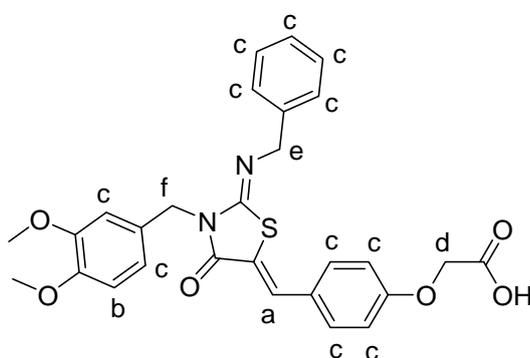


95

2-[4-[(2-(benzylimino)-3-(4-fluorobenzyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy]acetic acid (**95**) was obtained by hydrolysis of compound (**C52**) showed IR peaks 3112 cm^{-1} (OH str), 1700 cm^{-1} (C=O str), 1649 cm^{-1} (C=N) and 1595 cm^{-1} (C=C)

while its $^1\text{H-NMR}$ spectrum displayed peaks at δ 7.69 (*s*, 1H, CH_a), 7.54-7.56 (*d*, 2H, ArH_b), 7.02-7.43 (*m*, 10H, ArH_c), 5.00 (*s*, 2H, CH_{2d}), 4.69 (*s*, 2H, CH_{2e}) and 4.61 (*s*, 2H, CH_{2f}).

2-{4-[(2-(benzylimino)-3-(3,4-dimethoxybenzyl)-4-oxothiazolidin-5-ylidene)methyl]phenoxy}acetic acid (**96**) was synthesized by hydrolysis of compound (**C53**). Compound (**96**) displayed the IR peaks at 3118 cm^{-1} (OH str), 1697 cm^{-1} (C=O str), 1624 cm^{-1} (C=N) and 1586 cm^{-1} (C=C). Mass spectrum showed molecular ion peak at m/z 418.26 (M^+).

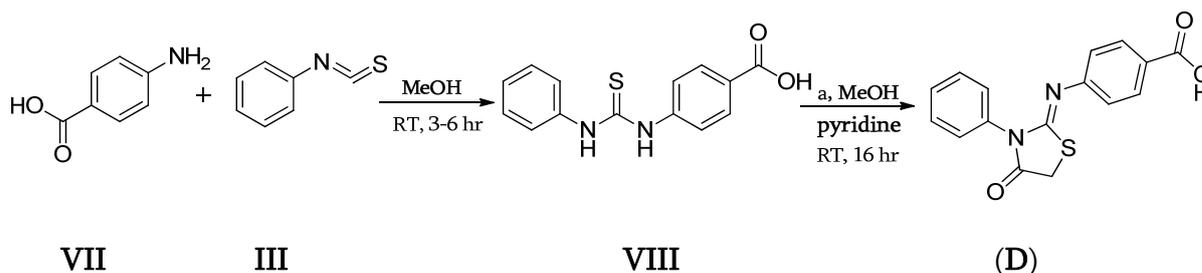


96

4.2.3. Synthesis of substituted 4-[[5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (97-114).

4.2.3.1. Synthesis of 4-[[4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (D).

Synthesis of 4-[[4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (D) was accomplished by **Scheme VII**. A methanolic solution of 4-aminobenzoic acid (**VII**) was reacted with phenyl isothiocyanate (**III**) to afford 4-(3-phenylthioureido)benzoic acid (**VIII**) which was directly cyclised to 4-[[4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (D) with ethyl chloroacetate in presence of pyridine. It showed IR peak of 1716 cm^{-1} (C=O str), 1686 cm^{-1} (C=O str), 1631 cm^{-1} (C=N str), 1592 cm^{-1} (C=C str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 7.94-7.96 (*d*, 2H, ArH), 7.37-7.55 (*m*, 5H, ArH), 6.92-6.95 (*d*, 2H, ArH) and 4.08 (*s*, 2H, CH_2).

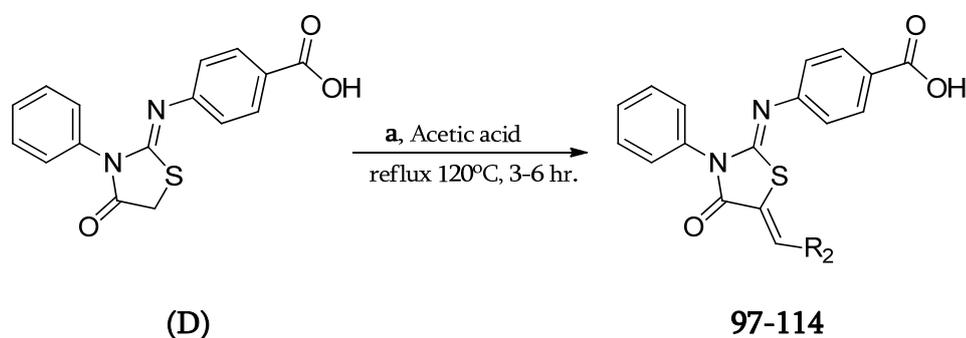


a = Ethylchloro acetate

Scheme VII

4.2.3.2. Synthesis of derivatives of 4-[[5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (97-114).

The common intermediate 4-[[4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (D) was synthesized as per the above discussed method (Scheme-VII). Various commercially available substituted aldehydes were procured (Table-6). The selection of these aldehydes was based on literature reports that suggested optimal PTP1B enzyme inhibitory activity associated with these groups. The 4-(4-oxo-2-phenylimino-thiazolidin-3-yl)-benzoic (D) were reacted with different substituted aldehydes according to above discussed procedure in section 4.2.1.2 to yield the desired derivatives of 4-(4-oxo-3-phenyl-thiazolidin-2-ylideneamino)-benzoic acid (97-114) (Scheme-VIII). These derivatives of 4-(4-oxo-3-phenyl-thiazolidin-2-ylideneamino)-benzoic acid were purified by repeated washing with cold acetic acid followed by water.



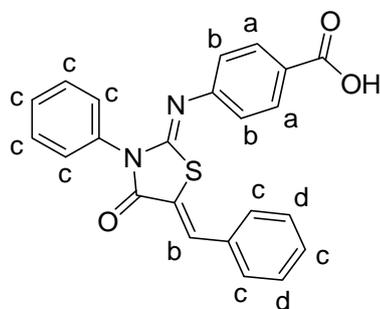
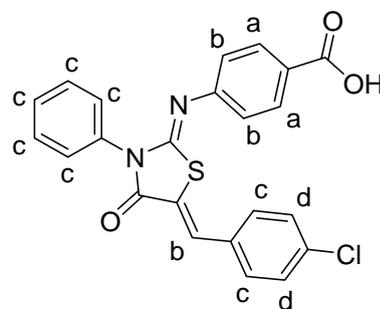
a = Substituted aldehyde, β -alanine.

Scheme VIII

Table-6: List of substituted aldehydes

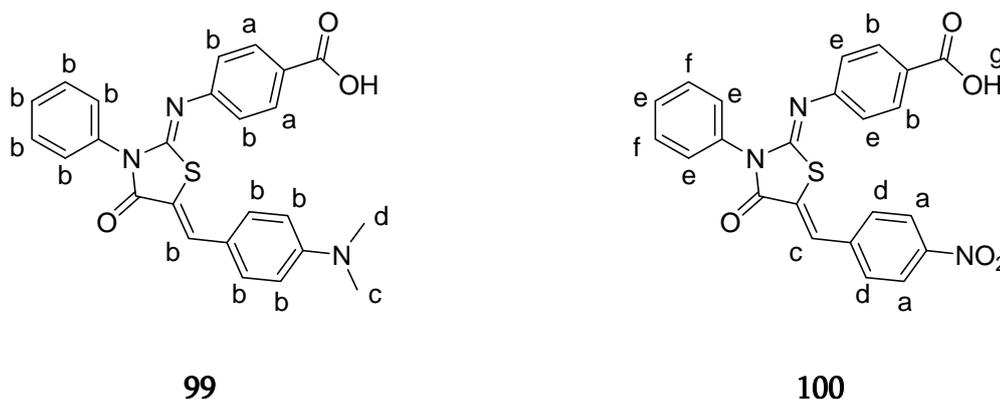
S. No.	R ₂	S. No.	R ₂	S. No.	R ₂
1		7		13	
2		8		14	
3		9		15	
4		10		16	
5		11		17	
6		12		18	

The IR spectrum of 4-[[5-Benzylidene-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**97**) showed the peaks at 3125 cm⁻¹ (OH str), 1718 & 1687 cm⁻¹ (C=O str), 1640 cm⁻¹ (C=N str) and 1596 cm⁻¹ (C=C, str). In ¹H-NMR spectrum signals were observed at δ 7.93-7.95 (*d*, 2H, ArH_a), 7.75 (*s*, 1H, CH_{2b}), 7.34-7.56 (*m*, 10H, ArH_c) and 6.94-6.96 (*d*, 2H, ArH_d).

**97****98**

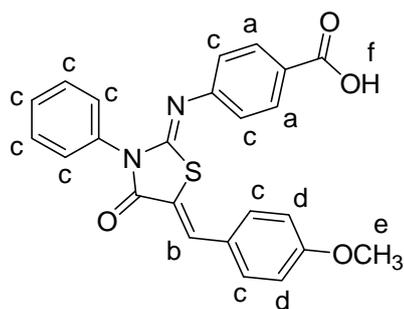
4-[[5-(4-Chlorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**98**) gave peaks at 3122 cm^{-1} (OH str), 1715 & 1681 cm^{-1} (C=O str), 1632 cm^{-1} (C=N str) and 1586 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 12.69 (*bs*, 1H, OH_e), 7.96-7.98 (*d*, 2H, ArH_a), 7.81 (*s*, 1H, CH_{2b}), 7.45-7.58 (*m*, 8H, ArH_c) and 7.02-7.04 (*d*, 2H, ArH_d).

4-[[5-(4-(Dimethylamino)benzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino] benzoic acid (**99**) showed the peaks at 3386 cm^{-1} (OH str), 1710 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1591 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 7.91-7.93 (*d*, 2H, ArH_a), 6.92-7.68 (*m*, 12H, ArH_b) 3.06 (*s*, 3H, NCH_{3c}) and 3.03 (*s*, 3H, NCH_{3d}).

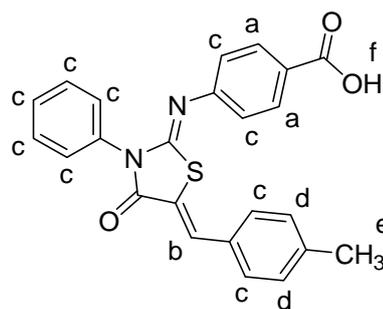


4-[[5-(4-Nitrobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**100**) gave peaks at 3123 cm^{-1} (OH str), 1717 & 1683 cm^{-1} (C=O str), 1630 cm^{-1} (C=N str) and 1585 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 12.64 (*bs*, 1H, OH_g) 8.27-8.29 (*d*, 2H, ArH_a), 7.98-8.00 (*d*, 2H, ArH_b), 7.90 (*s*, 1H, CH_c), 7.75-7.77 (*d*, 2H, ArH_d), 7.48-7.59 (*m*, 5H, ArH_e) and 7.02-7.04 (*d*, 2H, ArH_f).

4-[[5-(4-Methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**101**) showed the peaks at 3126 cm^{-1} (OH str), 1715 & 1689 cm^{-1} (C=O str), 1639 cm^{-1} (C=N str) and 1595 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 12.67 (*bs*, 1H, OH_f), 7.97-7.99 (*d*, 2H, ArH_a), 7.77 (*s*, 1H, CH_b), 7.41-7.57 (*m*, 9H, ArH_c) 7.01-7.03 (*d*, 2H, ArH_d) and 3.81 (*s*, 3H, OCH_{3e}).



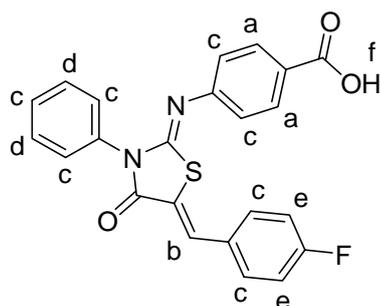
101



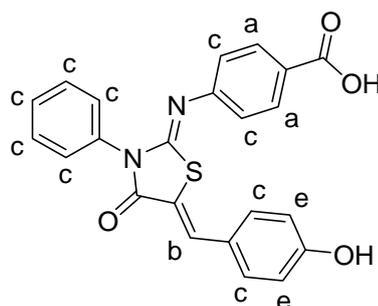
102

4-[[5-(4-Methylbenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**102**) gave peaks at 3122 cm^{-1} (OH str), 1710 & 1680 cm^{-1} (C=O str), 1630 cm^{-1} (C=N str) and 1583 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed at δ 12.71 (*bs*, 1H, OH), 7.97-7.99 (*d*, 2H, ArH_a), 7.78 (*s*, 1H, CH_b), 7.26-7.58 (*m*, 9H, ArH_c), 7.01-7.04 (*d*, 2H, ArH_d) and 2.36 (*s*, 3H, CH_{3e}).

4-[[5-(4-Fluorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**103**) showed the peaks at 3124 cm^{-1} (OH str), 1718 & 1687 cm^{-1} (C=O str), 1646 cm^{-1} (C=N str) and 1596 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 12.78 (*bs*, 1H, OH), 7.96-7.98 (*d*, 2H, ArH_a), 7.82 (*s*, 1H, CH_b), 7.46-7.61 (*m*, 7H, ArH_c), 7.23-7.28 (*m*, 2H, ArH_d) and 7.02-7.04 (*d*, 2H, ArH_e).



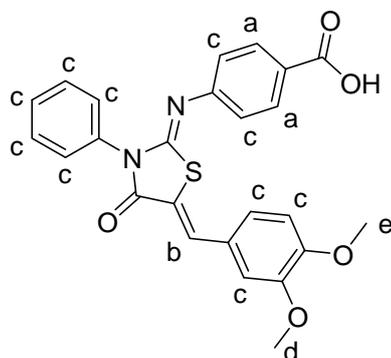
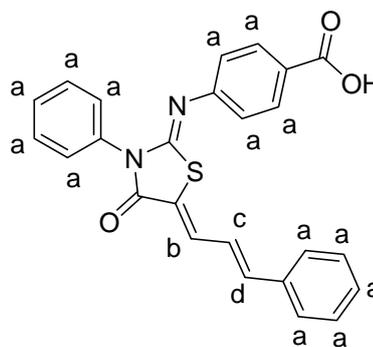
103



104

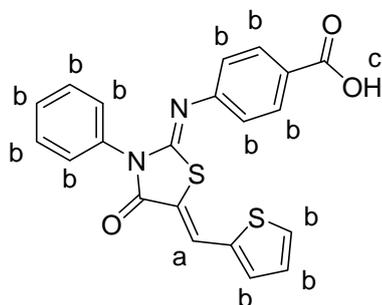
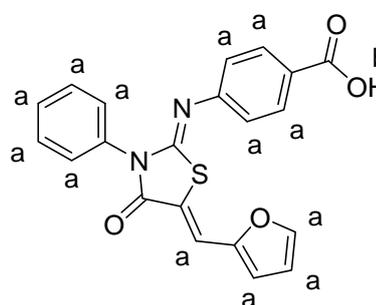
4-[[5-(4-Hydroxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**104**) gave peaks at 3391 cm^{-1} (OH str), 1705 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1592 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 7.96-7.98 (*d*, 2H, ArH_a), 7.72 (*s*, 1H, CH_b), 7.36-7.57 (*m*, 9H, ArH_c) and 6.87-6.89 (*d*, 2H, ArH_d).

4-[[5-(3,4-Dimethoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**105**) showed the peaks at 3130 cm^{-1} (OH str), 1715 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1593 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 7.96-7.99 (*d*, 2H, ArH_a), 7.78 (*s*, 1H, CH_b), 7.37-6.92 (*m*, 10H, ArH_c), 3.84 (*s*, 3H, OCH_{3d}) and 3.81 (*s*, 3H, OCH_{3e}).

**105****106**

4-[[4-Oxo-3-phenyl-5-(3-phenylallylidene)thiazolidin-2-ylidene]amino]benzoic acid (**106**) gave peaks at 3125 cm^{-1} (OH str), 1708 & 1684 cm^{-1} (C=O str), 1632 cm^{-1} (C=N str) and 1583 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 6.90-8.72 (*m*, 17H, $\text{ArH}_a\text{CH}_{b,c,d}$).

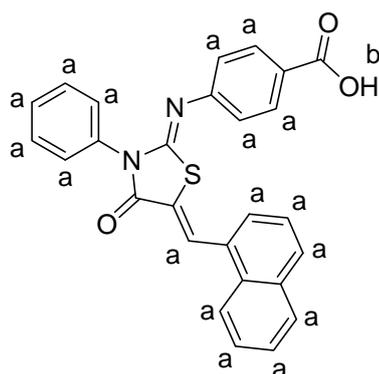
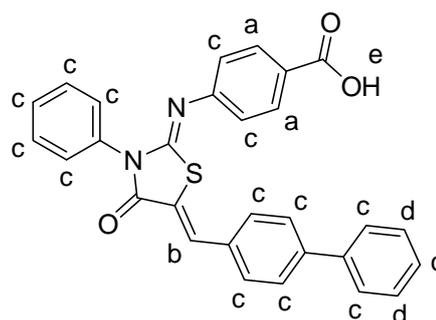
4-[[5-(Thiophen-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**107**) showed the peaks at 3091 cm^{-1} (OH str), 1705 cm^{-1} (C=O str), 1640 cm^{-1} (C=N str) and 1594 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 12.39 (*bs*, 1H, OH_c), 8.07 (*s*, 1H, CH_b) and 6.93-7.99 (*m*, 12H, ArH_b).

**107****108**

4-[[5-(Furan-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**108**) gave peaks at 3391 cm^{-1} (OH str), 1715 & 1687 cm^{-1} (C=O str), 1637 cm^{-1} (C=N

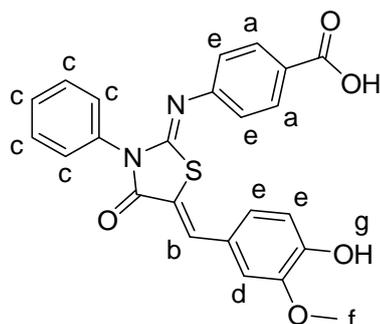
str) and 1593 cm^{-1} (C=C, str) in its IR spectrum. Mass spectrum showed molecular ion peak at m/z 391 (M^+).

4-[[5-(Naphthalen-1-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**109**) showed the peaks at 3128 cm^{-1} (OH str), 1716 & 1686 cm^{-1} (C=O str), 1639 cm^{-1} (C=N str) and 1593 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 12.71 (*bs*, 1H, OH_b) and 6.93-8.47 (*m*, 17H, ArH_a).

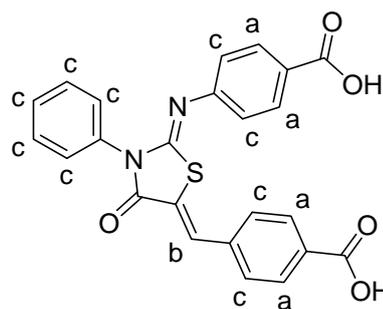
**109****110**

4-[[5-((1,1'-Biphenyl)-4-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**110**) gave peaks at 3391 cm^{-1} (OH str), 1711 & 1688 cm^{-1} (C=O str), 1633 cm^{-1} (C=N str) and 1589 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 12.60 (*bs*, 1H, OH_e), 6.97-8.99 (*d*, 2H, ArH_a), 7.85 (*s*, 1H, CH_b), 7.35-7.77 (*m*, 14H, ArH_c), and 7.04-7.06 (*d*, 2H, ArH_d). Mass spectrum showed molecular ion peak at m/z 476.9 (M^+).

4-[[5-(4-Hydroxy-3-methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**111**) showed the peaks at 3380 cm^{-1} (OH str), 1706 cm^{-1} (C=O str), 1635 cm^{-1} (C=N str) and 1590 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 7.96-7.98 (*d*, 2H, ArH_a), 7.74 (*s*, 1H, CH_b), 7.45-7.57 (*m*, 5H, ArH_c), 7.09 (*s*, 1H, ArH_d), 6.89-7.03 (*m*, 4H, ArH_e), 3.81 (*s*, 3H, OCH_3) and 1.93 (*s*, 1H, OH_g)



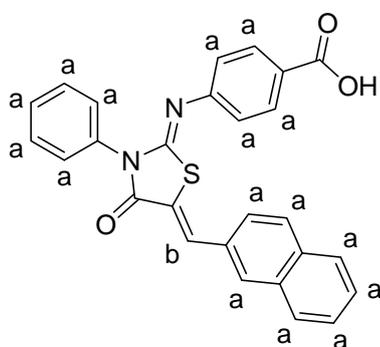
111



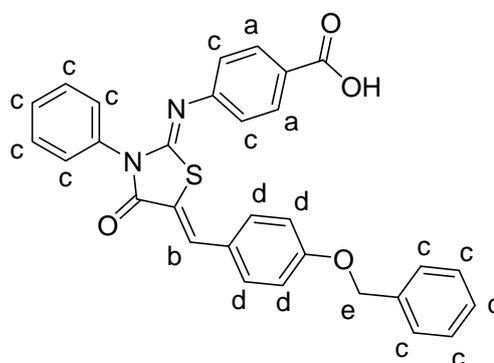
112

4-[[5-(4-Carboxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**112**) gave peaks at 3117 cm^{-1} (OH str), 1718 and 1688 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1595 cm^{-1} (C=C, str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 7.99-8.05 (*m*, 4H, ArH_a), 7.84 (*s*, 1H, CH_b) and 6.92-7.64 (*m*, 10H, ArH_c).

4-[[5-(Naphthalen-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**113**) showed the peaks at 3125 cm^{-1} (OH str), 1710 & 1682 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1588 cm^{-1} (C=C, str). In $^1\text{H-NMR}$ spectrum signals were observed at δ 6.82-8.19 (*m*, 17H, ArH_a and CH_b).



113



114

4-[[5-(4-(Benzyloxy)benzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene]amino]benzoic acid (**114**) gave peaks at 3113 cm^{-1} (OH str), 1710 & 1685 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) 1595 cm^{-1} (C=C, str) and 1258 (C-O-C str) in its IR spectrum. $^1\text{H-NMR}$ spectrum showed δ 7.93-7.95 (*d*, 2H, ArH_a), 7.70 (*s*, 1H, CH_b), 7.26-7.56 (*m*, 12H, ArH_c), 6.87-7.08 (*m*, 4H, ArH_d) and 5.07 (*s*, 2H, CH_e).

4.2.4. Synthesis of substituted 2-{4-[(5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (115-132).

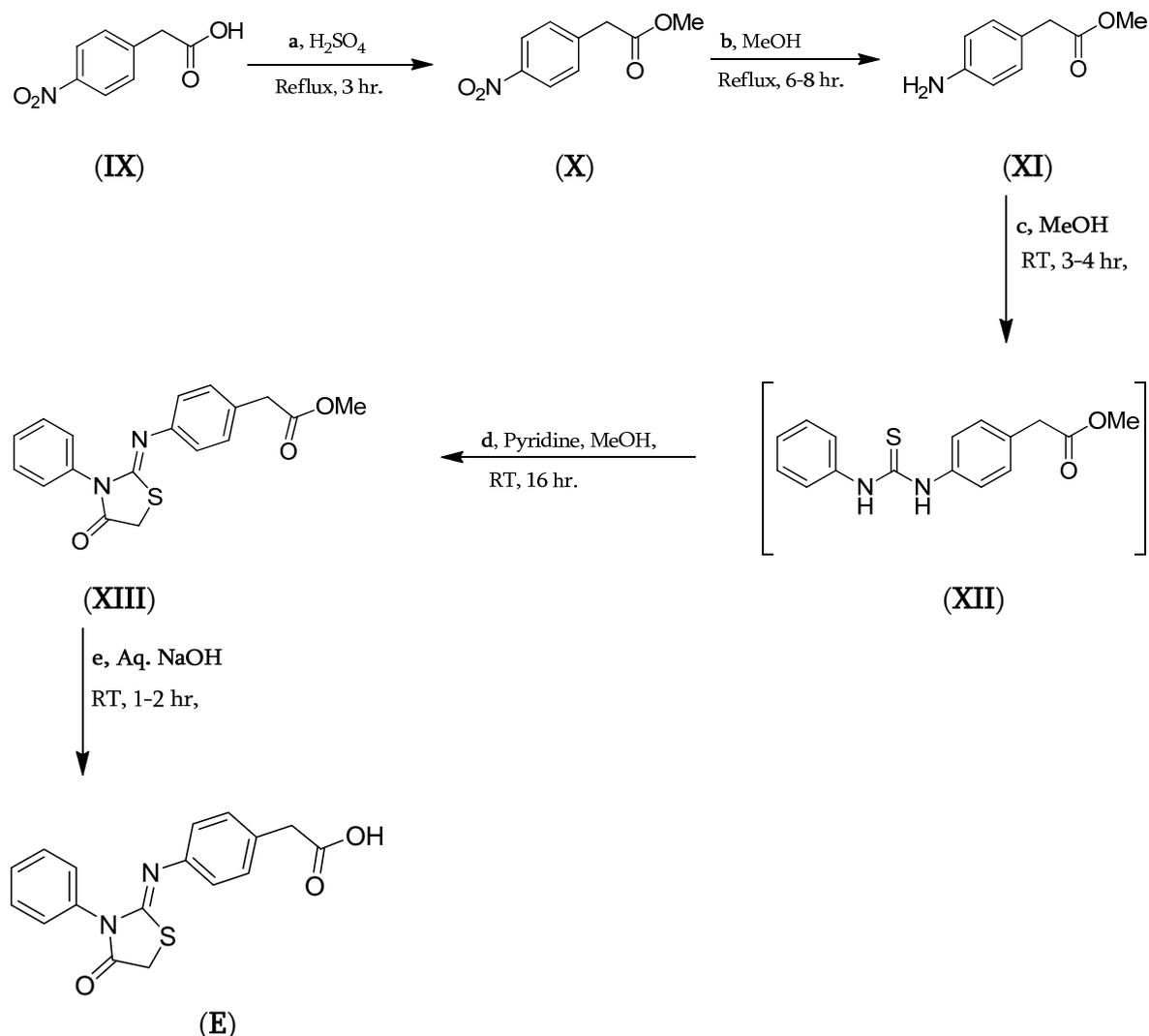
4.2.4.1. Synthesis of 2-{4-[(4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (E).

Commercially available 4-Nitrophenylacetic acid (**IX**) was used for the preparation of (4-Nitro-phenyl)-acetic acid methyl ester (**X**) by the reported procedures of Fischer esterification reaction. (4-Nitro-phenyl)-acetic acid methyl ester (**X**) was reduced in presence of iron powder (Fe) and NaCl solution in methanol to produce the amino derivative (**XI**) which shows characteristic doublet peak of NH₂ str. at 3450 & 3339 cm⁻¹. This amino derivative (**XI**) was stirred with phenyl isothiocyanate to afford methyl 2-[4-(3-phenylthioureido)phenyl]acetate (**XII**). The methyl 2-[4-(3-phenylthioureido)phenyl]acetate *in situ* cyclised to the methyl 2-{4-[(4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetate (**XIII**) by reacting (**XII**) with ethyl chloroacetate in presence of pyridine. The intermediate (**XIII**) obtained was hydrolysed in alkaline media to afford the desired 2-{4-[(4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**E**) (**Scheme IX**). It showed a broad peak of -OH at 3325 cm⁻¹ and C=O str at 1728 cm⁻¹ and ¹H-NMR spectrum showed δ 7.46-7.49 (*t*, 2H, ArH), 7.34-7.41 (*m*, 3H, ArH), 7.16-7.19 (*d*, 2H, ArH), 6.77-6.79 (*d*, 2H, ArH), 4.07 (*s*, 2H, -S-CH₂) and 3.48 (*s*, 2H, CH₂).

4.2.4.2. Synthesis of derivatives of 2-{4-[(5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (115-132).

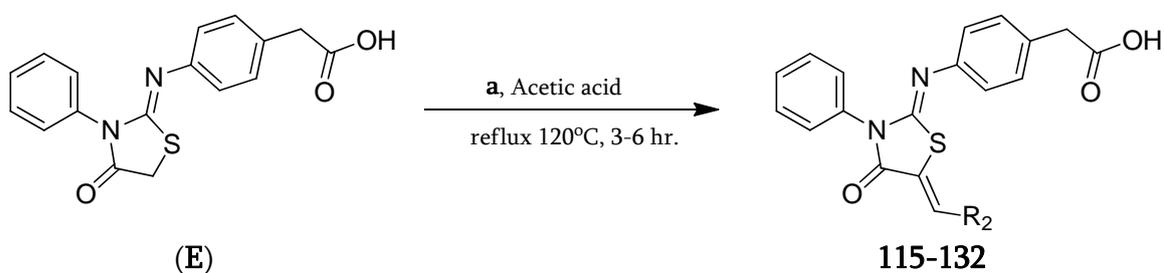
The common intermediate 2-{4-[(4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**E**) synthesized as per the above discussed method (**Scheme-IX**) was reacted with various commercially available substituted aldehydes to yield the desired derivatives of 2-{4-[(5-ylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (115-132) (**Scheme-X**). The selection of these aldehydes was based on literature reports that suggested optimal PTP1B enzyme inhibitory activity associated with these groups. These derivatives of 2-{4-[(5-ylidene-

4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid were purified by repeated washing with cold acetic acid followed by water.



a = MeOH, **b** = Fe, NaCl, **c** = Phenyl isothiocyanate. **d** = Ethylchloroacetate, **e** = MeOH

Scheme-IX

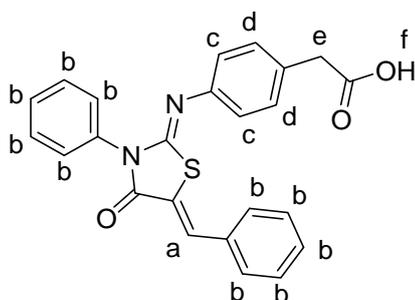
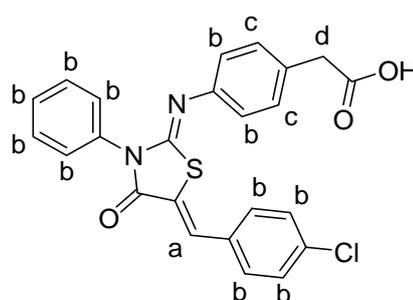


a = Substituted aldehyde, β -alanine

Scheme-X

2-{4-[(5-Benzylidene-4-oxo-3-phenylthiazolidin-2-ylidene)amino] phenyl}acetic acid (**115**) displayed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1719 cm^{-1} (C=O str), 1632 cm^{-1} (C=N str) and 1603 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 12.17 (*bs*, 1H, OH), 7.72 (*s*, 1H, CH_a), 7.34-7.50 (*m*, 10H, ArH_b), 7.21-7.23 (*d*, 2H, ArH_c), 6.83-6.85 (*d*, 2H, ArH_d) and 3.51 (*s*, 2H, CH_{2e}).

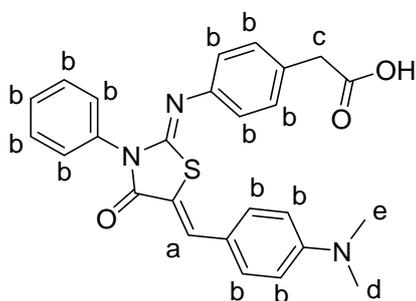
2-{4-[5-(4-Chlorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**116**) showed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1718 cm^{-1} (C=O str), 1635 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C, str). It offered signals at δ 7.75 (*s*, 1H, CH_a), 7.27-7.57 (*m*, 11H, ArH_b), 6.89-6.95 (*d*, 2H, ArH_c) and 3.65 (*s*, 2H, CH_{2d}) in its $^1\text{H-NMR}$ spectrum.

**115****116**

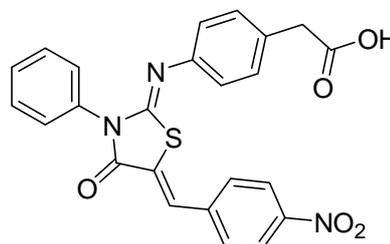
2-(4-{5-[4-(Dimethylamino)phenyl]methylidene}-4-oxo-3-phenyl-1,3-thiazolidin-2-ylidene]amino}phenyl)acetic acid (**117**) displayed the characteristic IR peaks at 3123 cm^{-1} (OH str), 1711 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1589 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 7.79 (*s*, 1H, CH_a), 6.59-7.70 (*m*, 13H, ArH_b), 3.64 (*s*, 2H, CH_{2c}), 3.10 (*s*, 3H, CH_{3d}) and 3.04 (*s*, 3H, CH_{3e}). Mass spectrum showed molecular ion peak at m/z 458.2 (M⁺).

2-{4-[(5-(4-Nitrobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**118**) showed the characteristic IR peaks at 3112 cm^{-1} (OH str), 1717 cm^{-1} (C=O str), 1644 cm^{-1} (C=N str) and 1609 cm^{-1} (C=C, str). It offered signals at δ 8.25-8.27 (*d*, 2H, ArH_a), 7.83 (*s*, 1H, CH_b), 7.18-7.63 (*m*, 9H, ArH_c), 6.93-6.96 (*t*, 2H, ArH_d) and

3.67 (*s*, 2H, CH_{2e}) in its 1H -NMR spectrum. Mass spectrum showed molecular ion peak at m/z 458.2 ($M+1$).



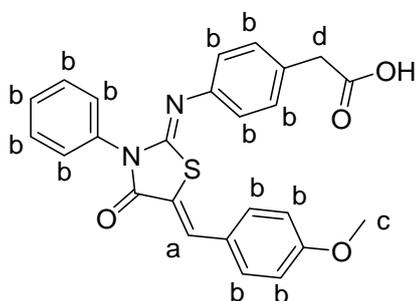
117



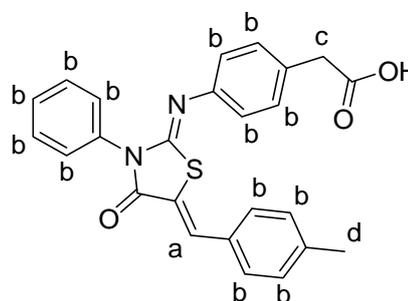
118

2-{4-[(5-(4-Methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**119**) displayed the characteristic IR peaks at 3126 cm^{-1} (OH str), 1713 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1594 cm^{-1} (C=C, str). Its 1H -NMR spectrum displayed signals at δ 7.66 (*s*, 1H, CH_a), 6.82-7.47 (*m*, 13H, ArH_b), 3.76 (*s*, 3H, CH_{3c}) and 3.57 (*s*, 2H, CH_{2d}).

2-{4-[(5-(4-Methylbenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**120**) showed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1717 cm^{-1} (C=O str), 1637 cm^{-1} (C=N str) and 1598 cm^{-1} (C=C, str). It offered signals at δ 7.66 (*s*, 1H, CH_a), 6.82-7.47 (*m*, 13H, ArH_b), 3.67 (*s*, 2H, CH_{2c}) and 2.37 (*s*, 3H, CH_{3d}) in its 1H -NMR spectrum.



119

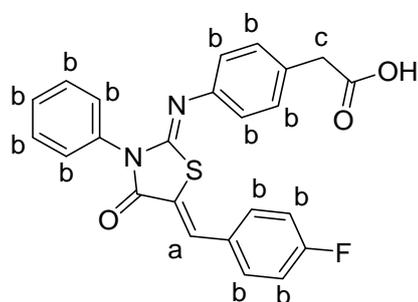


120

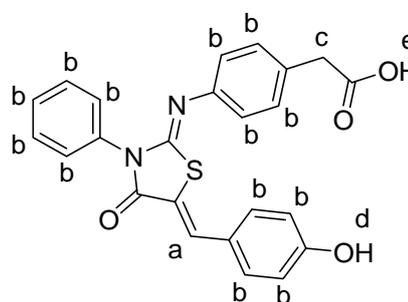
2-{4-[(5-(4-Fluorobenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**121**) displayed the characteristic IR peaks at 3125 cm^{-1} (OH str),

1714 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1595 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 7.79 (*s*, 1H, CH_a), 6.93-7.56 (*m*, 13H, ArH_b) and 3.70 (*s*, 2H, CH_2c).

2-{4-[(5-(4-Hydroxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**122**) showed the characteristic IR peaks at 3369 cm^{-1} (OH str), 1713 cm^{-1} (C=O str), 1634 cm^{-1} (C=N str) and 1588 cm^{-1} (C=C, str).It offered signals at δ 10.09 (*bs*, 1H, OH_e), 7.62(*s*, 1H, CH_a), 6.79-7.48 (*m*, 13H, ArH_b), 3.57 (*s*, 2H, CH_2c) and 2.49 (*s*, 1H, OH_d) in its $^1\text{H-NMR}$ spectrum.



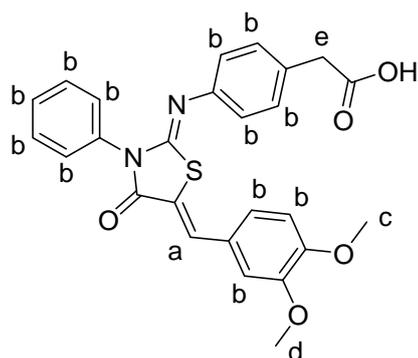
121



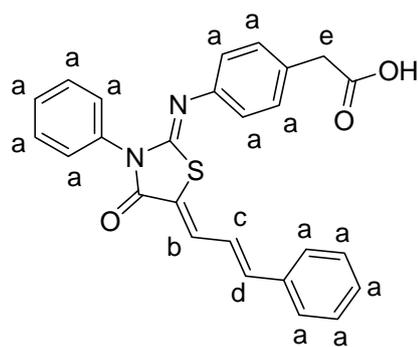
122

2-{4-[(5-(3,4-dimethoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**123**) displayed the characteristic IR peaks at 3127 cm^{-1} (OH str), 1712 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1592 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 7.69 (*s*, 1H, CH_a), 6.82-7.46 (*m*, 13H, ArH_b), 3.83 (*s*, 3H, OCH_3c), 3.79 (*s*, 3H, CH_3d) and 3.62 (*s*, 2H, CH_2e).

2-{4-[(4-oxo-3-phenyl-5-(3-phenylallylidene)thiazolidin-2-ylidene)amino]phenyl}acetic acid (**124**) showed the characteristic IR peaks at 3128 cm^{-1} (OH str), 1712 cm^{-1} (C=O str), 1634 cm^{-1} (C=N str) and 1607 cm^{-1} (C=C, str).It offered signals at δ 7.70(*s*, 1H, CH_b), 6.68-7.49 (*m*, 16H, ArH_a & $\text{CH}_{c,d}$)and 3.57 (*s*, 2H, CH_2e) in its $^1\text{H-NMR}$ spectrum.



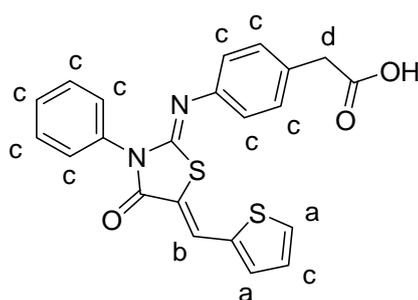
123



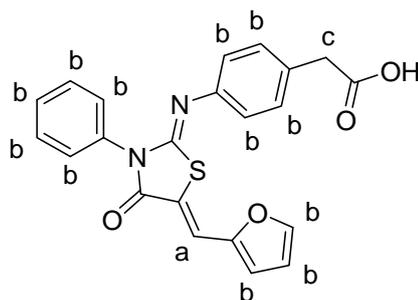
124

2-[4-[(5-(thiophen-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl]acetic acid (**125**) displayed the characteristic IR peaks at 3109 cm^{-1} (OH str), 1712 cm^{-1} (C=O str), 1633 cm^{-1} (C=N str) and 1595 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 7.97-8.00 (*d*, 2H, ArH_a), 7.72 (*s*, 1H, CH_b), 6.90-7.54 (*m*, 10H, ArH_c) and 3.64 (*s*, 2H, CH_2d).

2-[4-[(5-(furan-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl]acetic acid (**126**) showed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1712 cm^{-1} (C=O str), 1637 cm^{-1} (C=N str) and 1615 cm^{-1} (C=C, str). It offered signals at δ 7.68 (*s*, 1H, CH_a), 6.51-7.50 (*m*, 12H, ArH_b) and 3.57 (*s*, 2H, CH_2c) in its $^1\text{H-NMR}$ spectrum.



125

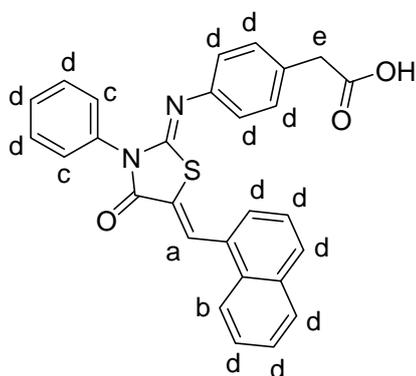
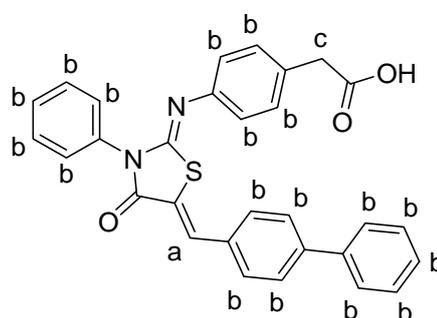


126

2-[4-[(5-(naphthalen-1-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl]acetic acid (**127**) displayed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1709 cm^{-1} (C=O str), 1638 cm^{-1} (C=N str) and 1592 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum

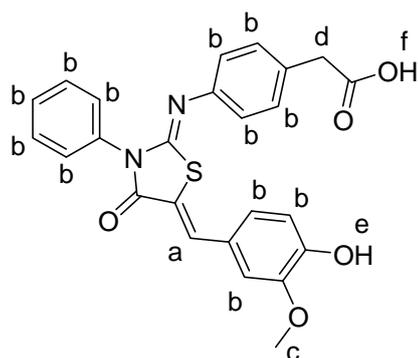
displayed signals at δ 8.55 (*s*, 1H, CH_a), 8.13-8.15 (*d*, 1H, ArH_b), 7.85-7.87 (*d*, 2H, ArH_c), 6.90-7.64 (*d*, 13H, ArH_d) and 3.70 (*s*, 2H, CH_{2e}).

2-{4-[(5-((1,1'-biphenyl)-4-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**128**) showed the characteristic IR peaks at 3125 cm^{-1} (OH str), 1701 cm^{-1} (C=O str), 1639 cm^{-1} (C=N str) and 1596 cm^{-1} (C=C, str). It offered signals at δ 7.83 (*s*, 1H, CH_a), 6.91-7.71 (*m*, 18H, ArH_b) and 3.66 (*s*, 2H, CH_{2c}) in its $^1\text{H-NMR}$ spectrum. Mass spectrum showed molecular ion peak at m/z 491.3 (M^+).

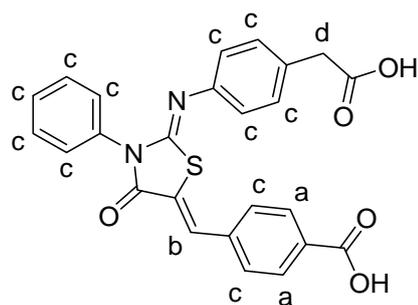
**127****128**

2-{4-[(5-(4-hydroxy-3-methoxybenzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**129**) displayed the characteristic IR peaks at 3380 cm^{-1} (OH str), 1711 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1589 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 9.50 (*bs*, 1H, OH_f), 7.70 (*s*, 1H, CH_a), 6.89-7.56 (*m*, 12H, ArH_b), 3.84 (*s*, 3H, OCH_{3c}), 3.64 (*s*, 2H, CH_{2d}) and 3.57 (*s*, 1H, OH_e).

4-[[2-((4-(carboxymethyl)phenyl)imino)-4-oxo-3-phenylthiazolidin-5-ylidene]methyl]benzoic acid (**130**) showed the characteristic IR peaks at 3126 cm^{-1} (OH str), 1717 cm^{-1} (C=O str), 1639 cm^{-1} (C=N str) and 1606 cm^{-1} (C=C, str). It offered signals at δ 7.98-8.00 (*d*, 2H, ArH_a), 7.73 (*s*, 1H, CH_b), 6.82-7.50 (*m*, 11H, ArH_c) and 3.58 (*s*, 2H, CH_{2d}) in its $^1\text{H-NMR}$ spectrum.



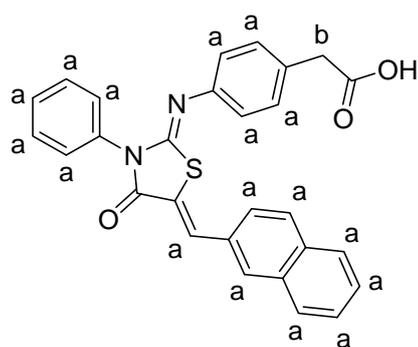
129



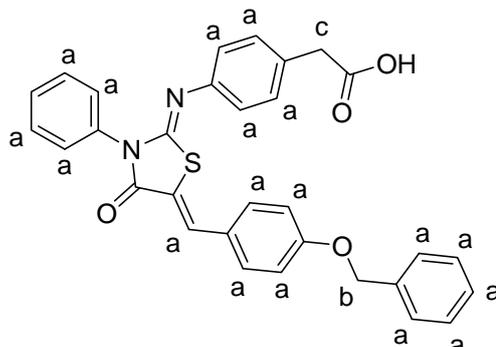
130

2-{4-[(5-(naphthalen-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**131**) displayed the characteristic IR peaks at 3124 cm^{-1} (OH str), 1713 cm^{-1} (C=O str), 1636 cm^{-1} (C=N str) and 1591 cm^{-1} (C=C, str). Its $^1\text{H-NMR}$ spectrum displayed signals at δ 6.84-7.93 (*m*, 17H, ArH_a) and 3.58 (*s*, 2H, CH_{2b}).

2-{4-[(5-(4-(benzyloxy)benzylidene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino]phenyl}acetic acid (**132**) showed the characteristic IR peaks at 3113 cm^{-1} (OH str), 1701 cm^{-1} (C=O str), 1634 cm^{-1} (C=N str), 1596 cm^{-1} (C=C, str) and 1259 cm^{-1} (C-O-C str). It offered signals at δ 6.77-7.66 (*m*, 19H, ArH_a), 5.04 (*s*, 2H, CH_{2b}) and 3.44 (*s*, 2H, CH_{2c}) in its $^1\text{H-NMR}$ spectrum.



131



132

4.3. BIOLOGICAL STUDIES

The *in vitro* studies were carried out at National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai, P. R. China and at pharmacology division of this department. All the compounds were evaluated for their *in vitro* inhibitory potency towards the PTP1B enzymes. The test compounds were grouped in three different Sets I, II & III (Table-7) for evaluation of their *in vitro* PTP1B inhibitory activity.

Table-7: Set wise distribution of compounds

Set No.	Compound No.	Total No. of Compounds
I	1, 3, 5, 7, 9, 11, 13, 15, 17, 18, 19, 20, 22, 24, 25, 26, 27, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 68, 69, 71, 73, 75, 77, 79, 81, 82, 84, 86, 88, 90, 92 and 94	52
II	2, 4, 6, 8, 10, 12, 14, 16, 21, 23, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 70, 72, 74, 76, 78, 80, 83, 85, 87 89, 91, 93, 95 and 96	44
III	97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131 and 132	36

4.3.1 Enzyme-based assay of PTP1B

A colorimetric assay to measure inhibition against PTP1B was performed in 96-well plates¹³⁴. The test compounds were solubilised in DMSO and serially diluted in concentrations for the inhibitory test. The assays were carried out in a final volume of 100 μ L containing 50 mmol/L MOPS, pH 6.5, 2 mmol/L pNPP, 30 nmol/L GST-PTP1B and 2% DMSO, and the catalysis of pNPP was continuously monitored on a Spectra Max 340 microplate reader at 405 nm for 3 min at 30 °C. The IC₅₀ value was calculated from the nonlinear curve fitting of the percent inhibition [**inhibition (%)**] vs the inhibitor concentration [**I**] using the following equation.

$$\text{Percent inhibition} = \frac{100}{\{1+(IC_{50}/[I])^k\}}$$

Where, **k** is the Hill coefficient.

The results for the first set of compounds (**Set-I**) is given in the **Table-8**. The initial concentration, 20 µg/mL was selected for determination of percent inhibitory activity. Compounds which have shown the inhibitory activity more than 50% were subjected for evaluation for the determination of IC₅₀.

Table-8: *In vitro* activity of Set-I compounds against human PTP1B

S. No.	Compd Code	Percent Inhibition	IC ₅₀	S. No.	Compd Code	Percent Inhibition	IC ₅₀
1	1	33.57±4.05		27	46	28.46±9.77	
2	3	17.08±3.58		28	49	92.93±6.55	3.08±0.41
3	5	97.09±2.03	0.21±0.03	29	51	97.31±3.31	2.96±0.47
4	7	26.74±4.87		30	53	80.58±7.02	4.21±0.41
5	9	32.28±14.84		31	55	80.64±3.94	6.77±0.84
6	11	28.01±6.13		32	57	76.03±2.05	5.6±0.65
7	13	48.78±12.09		33	59	17.08±3.58	
8	15	67.51±6.1	19.68±3.6	34	61	83.38±4.11	6.64±1.37
9	17	5.91±2.61		35	63	90.27±4.27	2.28±0.49
10	18	61.87±9.44	5.45±0.88	36	65	64.91±3.11	16.21±3.0
11	19	83.62±2.46	4.21±0.52	37	67	90.03±4.13	5.61±0.42
12	20	8.81±5.63		38	68	72.41±4.04	9.75±0.79
13	22	30.32±7.61		39	69	33.41±10.68	
14	24	45.11±3.34		40	71	77.06±3.81	14.33±1.3
15	25	6.28±8.82		41	73	74.09±7.55	17.38±2.0
16	26	2.65±2.23		42	75	73.55±7.03	9.51±1.46
17	27	49.12±12.03		43	77	41.03±7.33	
18	28	63.76±2.17	7.11±1.3	44	79	93.38±4.33	2.67±0.44
19	30	60.72±5.88	9.55±2.19	45	81	92.62±5.02	5.07±1.06
20	32	28.88±3.96		46	82	99.05±6.21	5.09±0.91
21	34	35.76±4.07		47	84	48.08±7.23	
22	36	43.39±9.08		48	86	48.11±2.95	
23	38	32.06±8.75		49	88	74.72±4.88	9.72±0.98
24	40	59.72±2.27	13.73±2.15	50	90	73.16±5.81	1.45±0.13
25	42	47.46±6.85		51	92	82.69±10.41	2.67±0.91
26	44	31.95±8.93		52	94	82.11±0.03	6.14±0.51

In first set of compounds total 56 compounds were evaluated at fixed concentration of 20 µg/mL for the PTP1B inhibitory activity. Out of 56 compounds 31 compounds show more than 50% inhibitory activity and were further evaluated in order to obtain IC₅₀ values. Compound **5**, **63**, **79**, **90** and **92** were found to be most potent inhibitors with IC₅₀ of 0.21, 2.28, 2.67, 1.45 and 2.67 µM respectively. To

determine the selectivity over other homologous PTPs, compound **5**, **63**, **79**, **90** and **92** were evaluated for the selectivity which is discussed in Section 4.3.2.

To gain more insight, second set of compounds (**Set-II**) were synthesized using benzyl isothiocyanate (**4.2.1.1**) and evaluated for their PTP1B inhibitory activity at a concentration of 10 $\mu\text{g/mL}$. (**Table-9**)

Table-9: *In vitro* activity of Set-II compounds against human PTP1B

S. No.	Compd Code	Percent Inhibition	IC ₅₀	S. No.	Compd Code	Percent Inhibition	IC ₅₀
1	2	39.92±7.33		23	52	8.3±7.03	
2	4	18.58±3.58		24	54	56.92±2.49	
3	6	40.71±6.25		25	56	74.33±10.7	7.58±1.55
4	8	72.43±8.9	8.81±1.43	26	58	33.99±4.07	
5	10	51.78±12.03		27	60	89.52±10.01	2.38±0.39
6	12	65.93±3.33	8.76±1.61	28	62	74.7±1.05	
7	14	50.2±2.33		29	64	81.42±0.7	
8	16	90.12±4.13		30	66	85.38±4.45	
9	21	-		31	70	70.75±2.22	
10	23	76.28±2.00		32	72	74.7±7.55	
11	29	73.52±5.43		33	74	64.43±3.11	
12	31	47.83±6.85		34	76	72.73±4.04	
13	33	73.91±2.03		35	78	-	
14	35	57.71±2.27		36	80	71.94±5.75	
15	37	40.32±7.23		37	83	76.28±2.05	
16	39	49.41±1.23		38	85	70.75±6.1	
17	41	57.71±2.27		39	87	39.53±2.5	
18	43	65.22±3.33		40	89	43.48±9.25	
19	45	55.73±12.03		41	91	58.1±10.1	
20	47	64.03±4.04		42	93	62.85±5.51	
21	48	67.19±4.89		43	95	54.94±2.09	
22	50	47.73±0.98		44	96	64.03±6.11	

Compound **16** and **60** were found to be the most active compounds with percent inhibition of 90.12 and 89.52 (IC₅₀=2.38±0.39) respectively.

The third set of compounds was designed based on the modification of polar group which is required for the inhibitory activity against PTP1B. The first 18 compounds in **Set-III** were designed without spacer in between the aromatic ring and polar functional

group. A further 18 compounds were designed with one carbon spacer between the aromatic ring and the polar functional group (Table-10).

Table-10: *In vitro* activity of Set-III compounds against human PTP1B

S. No.	Compd Code	Percent Inhibition	IC ₅₀	S. No.	Compd Code	Percent Inhibition	IC ₅₀
1	97	10.46±5.03	-	19	115	37.41±1.56	-
2	98	58.30±2.01	3.70±0.41	20	116	54.08±4.56	8.87±1.61
3	99	-	-	21	117	-	-
4	100	85.34±6.12	4.75±1.06	22	118	12.41±2.69	-
5	101	83.26±2.22	4.50±0.52	23	119	56.16±2.75	7.26±.98
6	102	56.11±5.11	6.11±8.82	24	120	24.91±5.15	-
7	103	93.66±0.97	1.94±0.09	25	121	58.24±9.09	6.75±0.51
8	104	20.86±0.4	-	26	122	-	-
9	105	-	-	27	123	-	-
10	106	31.26±10.56	-	28	124	14.50±5.66	-
11	107	-	-	29	125	39.49±4.58	-
12	108	-	-	30	126	-	-
13	109	-	-	31	127	-	-
14	110	29.18±3.49	-	32	128	16.58±2.89	-
15	111	-	-	33	129	-	-
16	112	37.50±2.87	-	34	130	99.00±1.03	3.35±0.84
17	113	60.38±4.54	1.79±0.13	35	131	62.41±5.15	3.50±1.09
18	114	99.90±1.01	1.43±0.7	36	132	12.41±14.02	-

Compound **98**, **103**, **113**, **114**, **130** and **131** were found to be the most potent inhibitors with IC₅₀ of 3.70, 1.94, 1.79, 1.43, 3.35 and 3.50 μM respectively.

4.3.2 Selectivity study against PTPs

PTPase family members, such as homology Src domain 2 (SH2)-containing tyrosine phosphatase-1(SHP1), T-Cell protein tyrosine phosphatase (TCPTP), leukocyte antigen-related phosphatase (LAR), CDC25B were prepared for the selectivity assay of compounds¹³⁵. Assays for these PTPases were performed at the optimal pH for each individual enzyme activity. These enzymes and inhibitors were preincubated for 3 min at 4 °C, and the assays were initiated by adding substrates.

OMFP was used as the substrate in the assays performed for CDC25B, SHP1 and LAR, as well as pNPP as the substrate for TC-PTP. The assays were carried out and the IC₅₀ values were calculated in the same manner as for PTP1B.

4.3.2.1 Selectivity study against CDC25B

M-phase inducer phosphatase 2 is an enzyme encoded by the CDC25B gene in humans¹³⁶. CDC25B is a member of the CDC25 family of phosphatases. CDC25B activates the cyclin dependent kinase CDC2 by removing two phosphate groups that is required for entry into mitosis. CDC25B shuttles between the nucleus and the cytoplasm due to nuclear localization and nuclear export signals. The protein is nuclear in the M and G1 phases of the cell cycle and moves to the cytoplasm during S and G2. CDC25B has oncogenic properties, although its role in tumor formation has not been determined.

OMF is the product of dephosphorylation of fluorescent substrate OMFP by CDC25B. OMF is has 485nm excitation wavelength at on excitation emits wavelength of 535 nm, this fluorescence signal is detected and observed activity is calculated.

Selected compounds **5**, **60**, **63**, **79**, **90** and **92**) from Set-I and II were evaluated for their selectivity over CDC25B (**Table-11**).

Table-11: *In vitro* activity of compounds against CDC25B

S No.	Compd. Code	Percent Inhibition @ 20 µg/mL	IC ₅₀
1	5	98.10±0.11	0.13±0.01
2	60	3.03±8.41	-
3	63	97.51±0.41	0.93±0.17
4	79	62.04±6.01	2.34±0.43
5	90	98.39±0.32	2.57±0.58
6	92	96.46±0.41	0.89±0.07

Compound **60**, **79** and **90** showed some degree of selectivity for PTP1B. Compound **5**, **63** and **92** showed more selectivity towards CDC25B than PTP1B.

4.3.2.2 Selectivity study against Leukocyte Antigen-Related phosphatase (LAR)

Leukocyte common antigen-related protein (leukocyte antigen-related protein, LAR) is a receptor-type tyrosine phosphatase (Protein Tyrosine Phosphatase, PTP LAR knockout mice shows defects in insulin and glucose levels as well as in liver glycogen which is reduced to glucose. Thus, selectivity and high activity of PTP-LAR inhibitors have significant value in the treatment of diabetes¹³⁷.

Selected compounds **5**, **60**, **63**, **79**, **90** and **92** from Set-I and II were evaluated for their selectivity over LAR (**Table:-12**). All compounds show good selectivity towards PTP1B.

Table-12: *In vitro* activity of compounds against LAR

S. No.	Compd. Code	Percent Inhibition @ 20 μ g/mL
1	5	6.86 \pm 1.17
2	60	15.15 \pm 1.55
3	63	14.76 \pm 1.01
4	79	2.32 \pm 6.25
5	90	9.64 \pm 4.96
6	92	13.61 \pm 1.84

4.3.2.3 Selectivity study against Src domain 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1)

SHP-1, a Src homology 2 (SH2) domain containing intracellular PTPase, is predominantly expressed in multiple hematopoietic lineages and to a lesser degree in epithelial cells. This enzyme has been described to interact with numerous activated growth factor, cytokine, and antigen receptors. It is also implicated in the negative regulation of various immunoreceptor transduction pathways by dephosphorylating and inactivating these receptors or their cognate substrates. The essential role of SHP-1 as a negative regulator of hematopoietic cell signal transduction is consistent with the multiple defects in hematopoietic cells observed in moth eaten mice characterized by mutations in the SHP-1 gene and loss of SHP-1 activity. SHP-1 is a negative regulator

of the insulin signaling, and upon activation, *sst2* negatively modulates insulin signaling by accelerating and amplifying the regulatory functions of SHP-1¹³⁸. Among somatostatin receptors, *sst2* has been found to play a critical role in the negative control of cell growth and to act as a tumor suppressor gene for pancreatic cancer¹³⁹.

Selected compounds **5**, **60**, **63**, **79**, **90** and **92** from Set-I and II were evaluated for their selectivity over SHP-1 (**Table:-13**). All these compounds show good selectivity towards PTP1B.

Table-13: *In vitro* activity of compounds against SHP-1

S No.	Compd. Code	Percent Inhibition @ 20 µg/mL	IC ₅₀
1	5	99.71±0.14	2.15±0.09
2	60	7.13±5.33	-
3	63	39.54±2.54	
4	79	9.44±2.91	
5	90	98.04±0.61	8.41±0.46
6	92	93.66±0.97	9.79±1.04

4.3.2.4 Selectivity study against T-Cell protein tyrosine phosphatase (TCPTP).

Tyrosine-protein phosphatase non-receptor type 2 is an enzyme that in humans is encoded by the PTPN2 gene. The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. Members of the PTP family share a highly conserved catalytic motif, which is essential for the catalytic activity. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. Epidermal growth factor receptor and the adaptor protein Shc were reported to be substrates of this PTP, which suggested the role in growth factor mediated cell signaling. Three alternatively spliced variants of this gene, which encode isoforms differing at their extreme C-termini, have been described. The different C-termini are thought to determine the substrate specificity, as well as the cellular localization of the isoforms¹⁴⁰.

Selected compounds **5**, **60**, **63**, **79**, **90** and **92** from Set-I and II were evaluated for their selectivity over TCPTP (Table:-14). All compounds have shown good selectivity towards PTP1B.

Table-14: *In vitro* activity of compounds against TCPTP

S No.	Compd. Code	Percent Inhibition @ 20 µg/mL	IC ₅₀
1	5	99.09±0.07	0.76±0.14
2	60	5.56±2.58	-
3	63	63.15±1.21	12.71±1.51
4	79	59.38±0.88	14.31±2.01
5	90	100.5±0.39	3.76±0.69
6	92	99.27±0.94	4.42±0.41

Compound **5**, **63**, **79**, **90** and **92** show moderate selectivity towards PTP1B while compound **60** displayed highest selectivity over TCPTP.

4.3.3 The nature of PTP1B inhibitors (Kinetic studies)

Compound **5**, **60** and **90** were screened to determine the type of inhibitor.

The K_m values were calculate by Michaelis - Menten equation

$$V = V_{max} * [S] / (K_m + [S])$$

V: Initial reaction rate

[S]: Substrate concentration

V_{max}: Maximum reaction rate

K_{cat} is calculated by following formula

$$V_{max} = k_{cat} * [E]$$

V_{max}: Maximum reaction rate

[E]: Enzyme concentration

4.3.3.1 Compound 5 PTP1B inhibitory effects.

Compound 5 (0.5 μM) and PTP1B (14 nM) incubated for 0-16 min. and inhibition were measured at selected different time intervals. The inhibitory efficiency of compound 5 for PTP1B inhibition varies with time change, indicating that it belongs to the fast binding inhibitor agent (**Figure-10**).

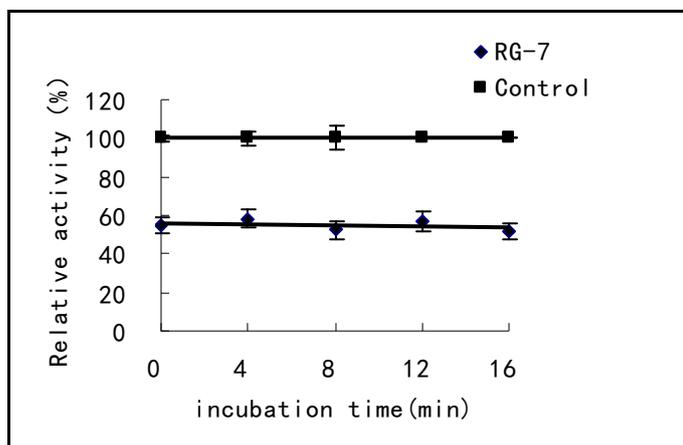


Figure-10: Enzyme kinetic properties of compound 5

The $K_m = 1.68$ mM, $V_{max} = 65.48$ mOD/ min were calculated by equation 1 and $K_{cat} = 136.41$ s^{-1} , $K_{cat} / K_m = 81.40$ $\text{mM}^{-1}.\text{s}^{-1}$ were calculated by equation 2 at 14 nM PTP1B concentration at pH 6.5.

Compound 5 ($IC_{50} 0.47 \pm 0.07\mu\text{M}$) was tested with different concentrations (0-0.8 μM). The enzyme kinetic properties of the PTP1B showed that with increasing concentration, the K_m values of PTP1B increased accordingly, while the V_{max} remained unchanged in case of compound 5. Based on these findings, we initially proposed compound 5 of competitive inhibitor (**Figure-11**)

Table-15: V_{max} (mOD/Min) of compound 5

[I] μM	$K_m(\text{mM})$	V_{max} (mOD/Min)
0	1.68	65.48
0.2	2.61	66.96
0.4	3.31	69.26
0.6	4.45	70.49
0.8	5.79	70.83

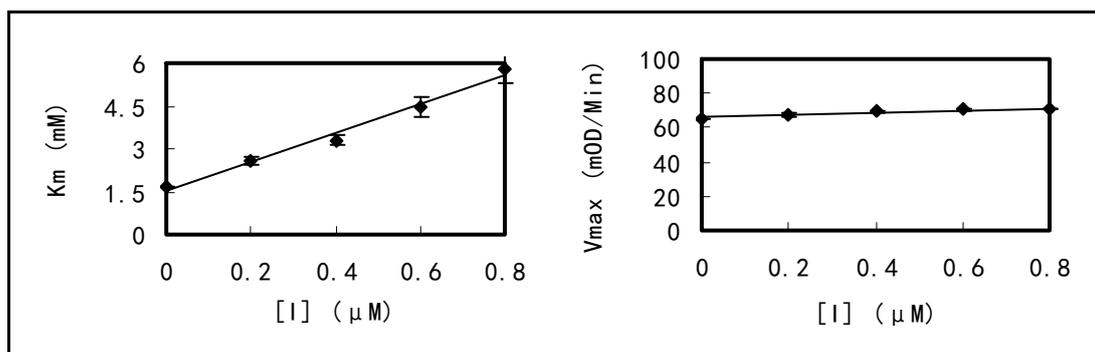


Figure-11: Graph of V_{max} vs I of compound **5**

The double reciprocal plot of reciprocal of the substrate concentration ($1/[S]$) to the corresponding reaction rate reciprocal ($1/[V]$) confirmed **5** as a competitive inhibitor of PTP1B (**Figure-12**).

$1/[S]$	$[I] (\mu M)$				
	0.000	0.200	0.400	0.600	0.800
1.600	0.040	0.050	0.059	0.076	0.090
0.800	0.031	0.036	0.039	0.046	0.057
0.400	0.026	0.030	0.032	0.035	0.039
0.200	0.024	0.026	0.026	0.028	0.032
0.100	0.021	0.022	0.022	0.023	0.025
0.050	0.018	0.019	0.019	0.019	0.020
0.025	0.015	0.016	0.015	0.016	0.016
0.013	0.014	0.013	0.013	0.014	0.014

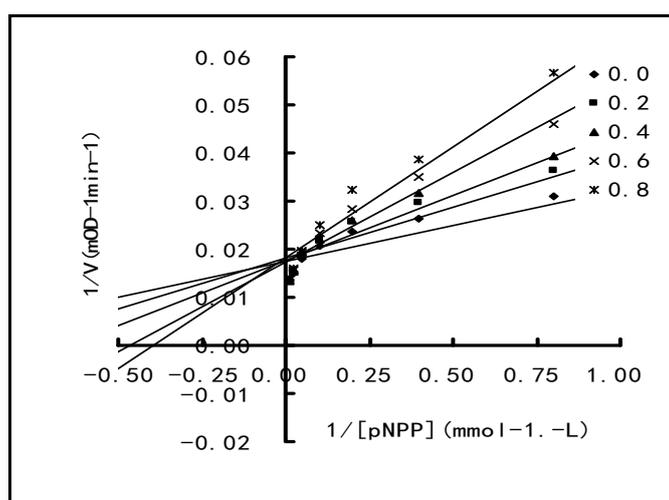


Figure-12: Double reciprocal plot of compound **5**

After determining compound **5** as a competitive inhibitor of PTP1B the plot of inhibitory concentration [I] to K_m of **5**, results shown in the **Figure-13** showed a linear correlation, fitting a straight line with the X-axis intersection of the absolute value of the compound K_i value; calculated $K_i = 0.31\mu\text{M}$.

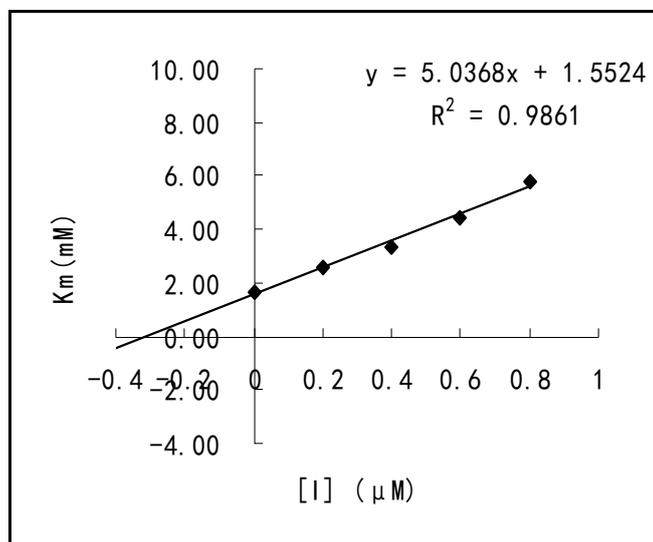


Figure-13: Plot of inhibitory concentration [I] to K_m of **5**

4.3.3.2 Compound **60** PTP1B inhibitory effects.

Compound **60** ($5\mu\text{M}$) and PTP1B (14 nM) were incubated for 0-16 min. and experimental results were measured at selected different time intervals. The inhibitory efficiency of compound **60** for PTP1B inhibition varies with time change, indicating that it belongs to the fast binding inhibitor type (**Figure-14**).

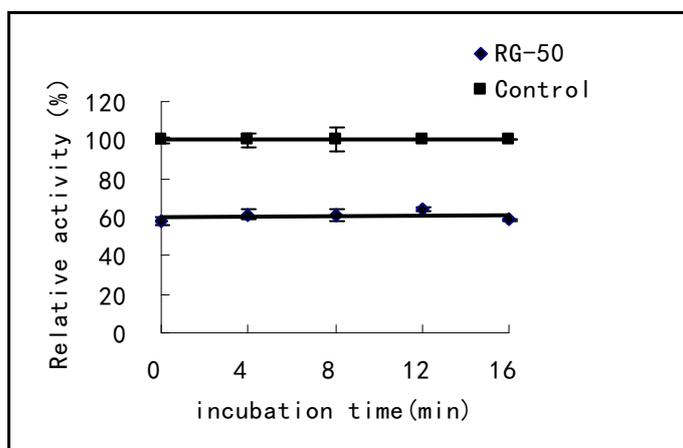


Figure-14: Enzyme kinetic properties of compound **60**

The $K_m = 2.00$ mM, $V_{max} = 75.70$ mOD/ min were calculated by equation 1 and $K_{cat} = 157.71$ s⁻¹, $K_{cat} / K_m = 78.83$ mM⁻¹.s⁻¹ were calculated by equation 2 at 14 nM PTP1B concentration at pH 6.5.

Compound **60** ($IC_{50} 3.02 \pm 0.07 \mu\text{M}$) was tested with different concentrations (0-12 μM). The enzyme kinetic properties of the PTP1B showed that with increasing concentration of compound **60**, the K_m values of PTP1B increased accordingly, while the V_{max} decreased. Based on these findings, we initially concluded compound **60** of mixed inhibitor type (**Figure-15**).

Table-16: V_{max} (mOD/Min) of compound **60**

[I] μM	K_m (mM)	V_{max} (mOD/Min)
0	2.00	75.70
3	2.86	74.48
6	3.60	72.46
9	5.49	70.06
12	6.07	65.17

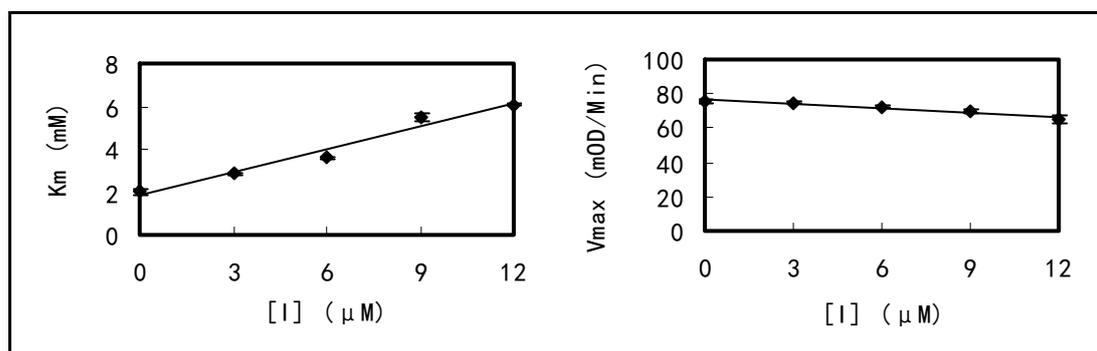


Figure-15: Graph of V_{max} vs I of compound **60**

The double reciprocal plot of reciprocal of the substrate concentration ($1 / [S]$) to the corresponding reaction rate reciprocal ($1 / [V]$) further confirmed **60** a mixed inhibitor of PTP1B (**Figure-14**).

4.3.3.3 Compound **90** PTP1B inhibitory effects.

Compound **90** (5 μM) and PTP1B (14 nM) were incubated for 0-16 min. and inhibition results were measured at selected different time intervals. The inhibitory efficiency of compound **90** for PTP1B inhibition varies with time change, indicating that it belongs to the category of fast binding inhibitors (**Figure-17**).

1/[S] \ 1/[V]	[I] (μM)				
	0	3	6	9	12
0.000	0.000	0.000	0.000	0.000	0.000
0.800	0.028	0.032	0.039	0.054	0.067
0.400	0.027	0.029	0.032	0.042	0.048
0.200	0.020	0.023	0.026	0.032	0.036
0.100	0.019	0.020	0.021	0.025	0.027
0.050	0.016	0.017	0.018	0.018	0.021
0.025	0.014	0.014	0.015	0.017	0.018
0.013	0.012	0.012	0.013	0.014	0.016

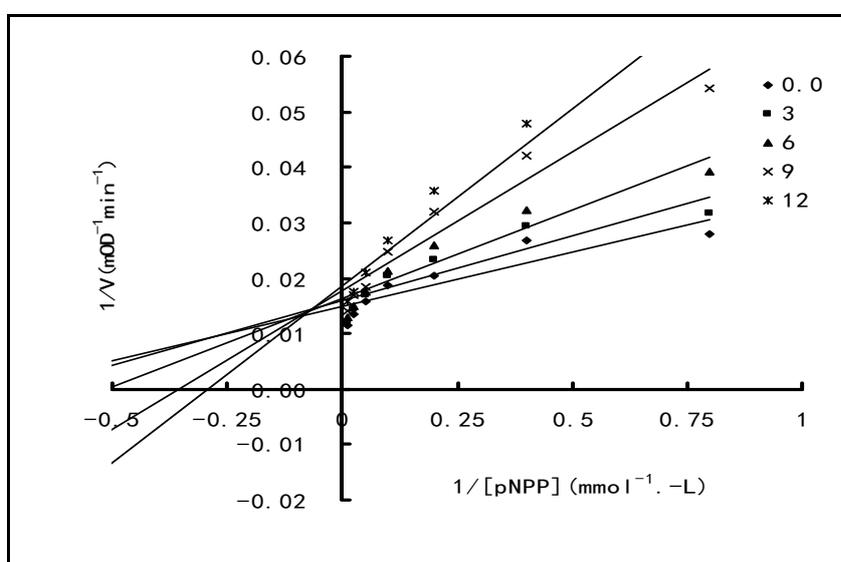


Figure-16: Double reciprocal plot of compound **60**

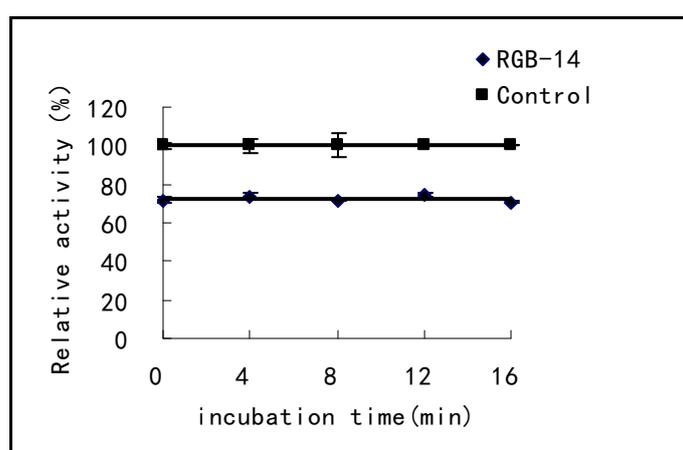


Figure-17: Enzyme kinetic properties of compound **90**

The $K_m = 2.21$ mM, $V_{max} = 70.80$ mOD/ min were calculated by equation 1 and $K_{cat} = 147.50$ s⁻¹, $K_{cat} / K_m = 66.79$ mM⁻¹/s were calculated by equation 2 at 14 nM PTP1B concentration at pH 6.5.

Compound **90** ($IC_{50} 4.86 \pm 0.80\mu\text{M}$) was tested with different concentrations (0-12 μM). The enzyme kinetic properties of PTP1B showed that with increasing compound **90** concentrations, the K_m values of PTP1B increased accordingly, while the V_{max} remained unchanged. Based on these findings, we initially thought compound **90** competitive inhibitor (**Figure-18**).

Table-17: V_{max} (mOD/Min) of compound **90**

[I] μM	$K_m(\text{mM})$	V_{max} (mOD/Min)
0	2.21	70.80
2	3.51	73.30
4	5.13	74.69
6	7.44	74.64
8	9.76	73.86

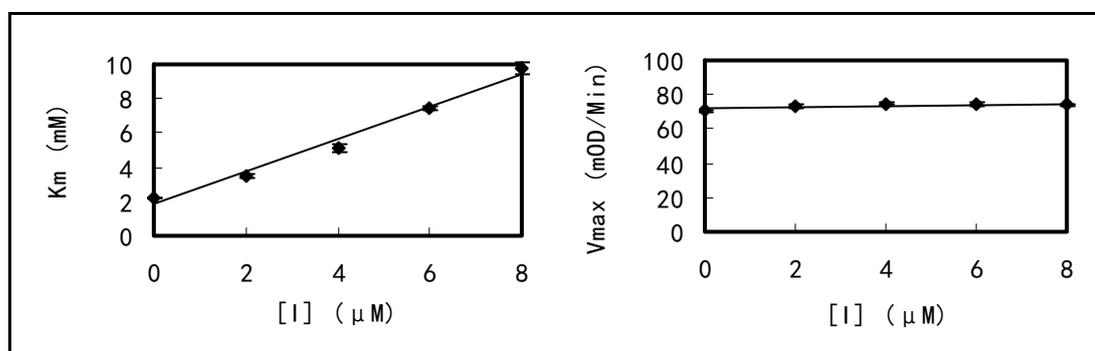


Figure-18: Graph of V_{max} vs I of compound **90**

The double reciprocal plot of reciprocal of the substrate concentration ($1 / [S]$) to the corresponding reaction rate reciprocal ($1 / [V]$) further confirmed **90** as a competitive mixed inhibitor of PTP1B (**Figure-19**).

After determining compound **90** as a competitive inhibitor of PTP1B the graph of inhibitory concentration $[I]$ to K_m of **90**, shows a linear correlation (**Figure-20**), fitting a straight line with the x-axis intersection of the absolute value of the compound K_i value calculated $K_i = 1.89\mu\text{M}$.

1/[S]	1/[V]	[I] (μM)				
		0	2	4	6	8
1.600	0.039	0.049	0.062	0.088	0.122	
0.800	0.031	0.038	0.044	0.053	0.064	
0.400	0.027	0.031	0.035	0.041	0.048	
0.200	0.025	0.026	0.028	0.034	0.042	
0.100	0.020	0.022	0.025	0.029	0.030	
0.050	0.018	0.018	0.019	0.020	0.022	
0.025	0.014	0.015	0.015	0.016	0.017	
0.013	0.012	0.012	0.013	0.014	0.014	

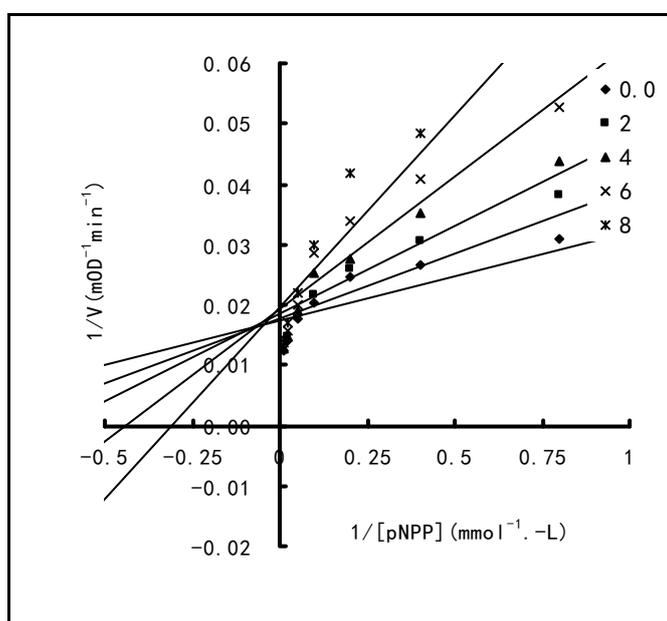


Figure-19: Double reciprocal plot of compound 90

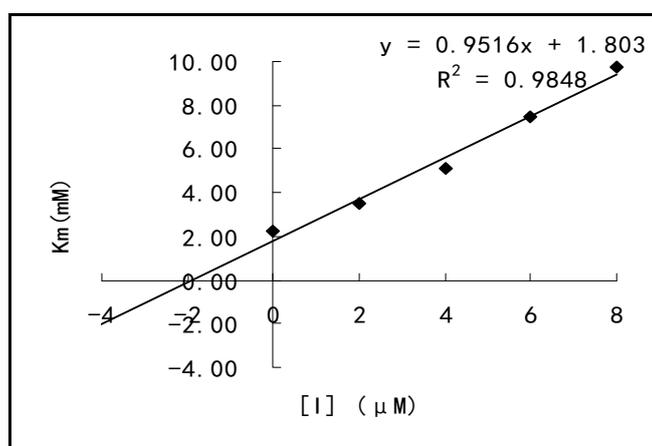


Figure-20: Plot of inhibitory concentration [I] to K_m of 90