Design, Synthesis and Docking Studies on 6-chloro-N^2, N^4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine derivatives as PTP1B inhibitors

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Abstract

A set of sixteen derivatives of 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine were synthesised and assessed for their ability to inhibit the PTP1B enzyme in a research environment. The molecular docking studies were conducted using the PTP1B enzyme receptor (PDB ID 1XBO). The synthesised compounds, specifically molecules 2a to 2i (9 compounds) and 3a to 3i (9 compounds), demonstrated PTP1B inhibition with activity ranging from 24.11% to 62.88% at a concentration of 10 µM. The docking studies indicated that the presence of hydrogen bond interactions with ASP181, ARG221, GLY220, and ASP48 is crucial for the binding affinity and inhibitory activity of PTP1B.

Keywords: Protein Tyrosine Phosphatase 1B (PTP1B), 1,3,5-Triazine, Docking

Introduction

Protein tyrosine phosphatases (PTPases) are a subset of protein phosphatases, a larger group that also encompasses protein serine/threonine phosphatases (PSTPs). The protein tyrosine phosphatases (PTPases) are frequently associated with cellular malfunction and serve as significant contributors to various diseases, including diabetes, obesity, inflammation, cancer, and neurodegeneration.[1-3] Type-2 diabetes and obesity are known to be associated with impaired insulin receptor signalling, which can potentially be restored by inhibiting specific protein tyrosine phosphatases (PTPases). This inhibition leads to sustained phosphorylation of the insulin receptor kinase. Protein tyrosine phosphatase 1B (PTP1B) is a pivotal component in cellular signalling pathways and is implicated in various human diseases, notably diabetes and obesity. [4-5] This protein is an intracellular phosphatase that does not bind to receptors. It is found in a wide range of human tissues. The compound exerts its inhibitory effects on the insulin receptor (IR) by inducing dephosphorylation. The PTP1B knockout mice exhibit enhanced glucose tolerance and improved insulin sensitivity due to enhanced insulin action in skeletal and liver muscle, while adipose tissue insulin action remains unaffected.[3] Furthermore,

the disruption of the PTP1B gene in mice surprisingly confers protection against obesity when subjected to a high-fat diet. Therefore, it is believed that potent, orally active, and selective inhibitors of PTP1B have the potential to be effective pharmacological agents for treating obesity and non-insulin dependent diabetes mellitus (NIDDM).[6-9]

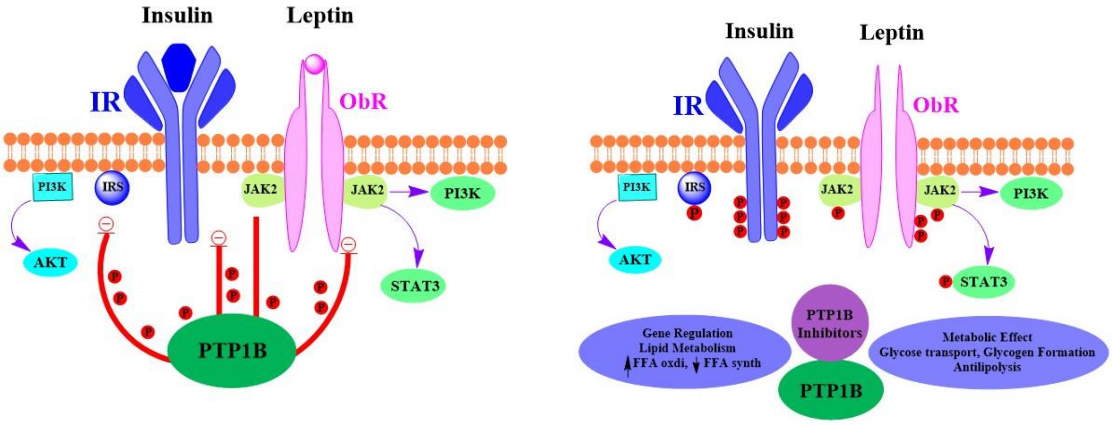

Fig. 1: Mechanism of action and dephophorylation of of PTP1B

In recent years, various classes of PTP1B inhibitors have been documented, such as 2-(oxalylarylamino)-benzoic acids, biphenyl derivatives, aminothiazoles, bidentate ligands, and acetamide derivatives.[7] Considering the aforementioned information and employing the substructural analysis methodology, a set of sixteen modified 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine compounds was devised and assessed for their PTP1B inhibitory activity in vitro. The overall procedure for the production of 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine derivatives is presented in fig.2 (reaction scheme).

The reaction between cyanuric chloride and thiazol-2-amine involves a substitution process. Specifically, the chloride group in cyanuric chloride is substituted by the amino group in thiazole-2-amine. This reaction takes place in the presence of acetone as a solvent at a temperature of 60°C. The resulting products are identified as 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine[8-9]. These products were characterised using various analytical techniques including IR spectroscopy, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, and mass spectrometry. The nucleophilic substitutions of para substituted 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine with various substituted phenol and amine derivatives in acetone at a temperature of 0°C (using an ice bath) over a period of 2 hours resulted in the formation of the desired compounds (2a to 2i and 3a to 3i). The synthesised

compounds underwent in vitro evaluation to assess their inhibitory activity against PTP1B at a concentration of 10 μM. This evaluation was conducted following the established principles of the conventional Malachite green assay method[11], with suramin used as a reference standard. The findings from the in vitro inhibition assays are presented in Table 1, revealing significant variations in the inhibitory effects of these analogues as a result of the diverse substituents present in the thiazole ring. Among these compounds, the analogues 3h, 3i, 2h, and 2i exhibited notable in vitro inhibitory activity. Specifically, these compounds, which possess a 4-O-CH_3 and 4-fluorine group, demonstrated significant inhibitory effects (% activity of 3h = 24.11%, 3i = 24.33%, 2h = 27.0%, and 2i = 25.55% at a concentration of 10 μM). The substitution of the chlorine group in 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine with 4-methoxyphenol led to a minor decrease in PTP1B inhibitory activity, with a percentage inhibition of 24.0% at a concentration of 100 μM. The compound 2a, with the ortho-directed Cl group, exhibited nearly equivalent inhibition (% activity = 60.00% at 10 μM) compared to the meta and para-directed groups. The introduction of 4-methoxyphenol (3h) and 4-fluorophenyl (3i) resulted in superior inhibition compared to other amine and phenyl derivatives. However, the substitution of 2-methoxyaniline, 3-methoxyaniline, and 4-methoxyaniline led to a decrease in activity when compared to 3h and 3i. On the other hand, compounds containing both O-CH_3 and fluorine groups exhibited an enhanced inhibitory effect compared to other substituted phenyl and aniline derivatives. A meticulous analysis unveiled that the presence of the O-CH_3 and fluorine group at the para position of the phenyl ring exhibited a favourable effect on the inhibitory activity. In order to validate this discovery, we conducted the synthesis of eight analogues through nucleophilic substitution. Specifically, we replaced the chlorine atom of 6-chloro-N2, N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine with various aniline derivatives such as methoxyaniline, chloroaniline, and floroaniline. Additionally, we substituted the chlorine atom with phenol derivatives including chlorophenol, florophenol, and methoxyphenol. Given the observed inhibitory activity exhibited by these analogues.

Experimental protocols

Chemistry and Synthesis Scheme:

The synthesis of all derivatives of 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine was carried out following the established procedure. 1, In a reaction vessel, introduce 0.01 mole of 6-chloro-N2, N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine along with 0.01 mole

of various phenyl and aniline derivatives (2a to 2i and 3a to 3i). The reaction is commonly conducted in a solvent, specifically acetone, at a temperature of 0°C (in an ice bath) for a duration of 2 hours. In this temporal period, the nucleophilic ylide undergoes an attack on the electrophilic chlorine atom located on the triazine ring. As a result, the chlorine atom is replaced by the phenyl and aniline derivatives, respectively.

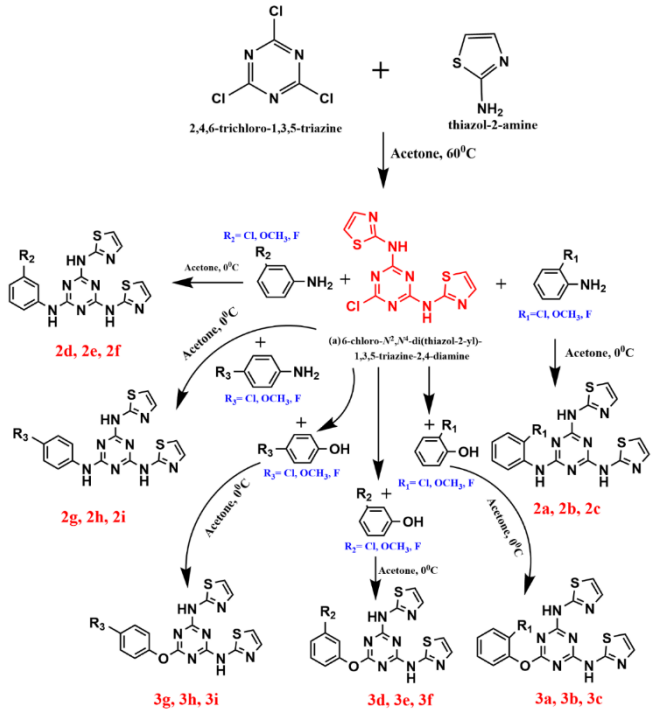

Fig 2; Synthesis scheme for the triazine derivatives

Synthesis of N^2-(2-chlorophenyl)-N^4, N^6-di(thiazol-2-yl)-1, 3, 5-triazine-2, 4, 6-triamine (2a)

White solid (2.85 g, 71%), M.P. 180-183 ºC. **IR ν_{max} (KBr)/ cm-^{1}**: 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 402.1 [M+2] 403.1 **Elemental Analysis (CHN)** for C_{15} H_{11} Cl N_8 S_2: [C, 44.72; H, 2.75; N, 27.81]. Found: [C, 44.82, H, 2.84, N, 27.75]. %.

Synthesis of N2-(2-methoxyphenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2b):

White solid (2.65 g, 66.5%), M.P. 170-172 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3329.42, 3289.30 cm-1 (multiple band 2x-NH str.), 3022.23 (C-H str. Aromatic), 2901.35 (Ali. C-H str. Asym.), 2761.21 (Ali. C-H str. Sym.), 1581.37 (C=N ring str.), 1464.98, 1412.21(C=C ring str.) 1332.28 (C-N str.) 1251.67 & 1159.25 (C-O str. Of C-O-C group) 869.05 (s-triazine), 686.64 (C-S Str.); **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.58 (s, 2H, m, -CH2), 3.31 (s, 3H, - OCH3), 3.49 (d, 1H, -CH2-S), 3.81 (-CH2-S), 5.58 (s, H, N-CH-S), 6.92-7.50 (m, 3H, H4, H5, H6, -OCH3-Ar-H), 8.21 (s, H, N-H), 8.34 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 39.52, 55.21, 122.81, 134.42, 152.82, 147.67 (C-C, OCH3-Ar-C-CH2) 149.64 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 398.1 [M+2] 399.2

Elemental Analysis (CHN) for $C_{16}H_{14}N_8OS_2$: [C, 48.23; H, 3.54; N, 28.12] Found: [C, 48.33; H, 3.62; N, 28.20]. %.

Synthesis of N2-(2-fluorophenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2c):

White solid (2.75 g, 71.23%), M.P. 185-187 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3252 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1392.3 (NH bending), 1425 (C-H bending, CH3), 1440.11 (C=C ring str.), 829 (s-triazine), 734.8 (Ar.C-F str), 592.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.62 (s, 2H, m, -CH2), 3.27 (d, 1H, -CH2-S), 3.89 (-CH2-S), 6.18 (s, H, N-CH-S), 6.62-7.89 (m, 3H,H4, H5, H6, Ar-H, F-Ar-H)), 8.19 (s, H, N-H), 8.32 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 38.13, 44.18, 65.79, 125.50, 128.90, 137.83 (F-C, NH-Ar-C-CH2) 167.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 387.1 [M+2] 389.1

Elemental Analysis (CHN) for $C_{15}H_{11}FN_8S_2$ [C, 46.62; H, 2.87; N, 29.00] Found: [C, 46.68; H, 2.91; N, 29.12]. %.

Synthesis of N2-(3-chlorophenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2d):

White solid (2.76 g, 68.65%), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 402.1 [M+2] 403.1 **Elemental Analysis (CHN)** for $C_{15}H_{11}ClN_8S_2$: [C, 44.72; H, 2.75; N, 27.81]. Found: [C, 44.82, H, 2.84, N, 27.75]. %.

Synthesis of N2-(3-methoxyphenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2e)

White solid (2.73 g, 68.58%), M.P. 190-192 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3329.42, 3289.30 cm-1 (multiple band 2x-NH str.), 3022.23 (C-H str. Aromatic), 2901.35 (Ali. C-H str. Asym.), 2761.21 (Ali. C-H str. Sym.), 1581.37 (C=N ring str.), 1464.98, 1412.21(C=C ring str.) 1332.28 (C-N str.) 1251.67 & 1159.25 (C-O str. Of C-O-C group) 869.05 (s-triazine), 686.64 (C-S Str.); **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.58 (s, 2H, m, -CH2), 3.31 (s, 3H, - OCH3), 3.49 (d, 1H, -CH2-S), 3.81 (-CH2-S), 5.58 (s, H, N-CH-S), 6.92-7.50 (m, 3H, H4, H5, H6, -OCH3-Ar-H), 8.21 (s, H, N-H), 8.34 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 39.52, 55.21, 122.81, 134.42, 152.82, 147.67 (C-C, OCH3-Ar-C-CH2) 149.64 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 398.1 [M+2] 399.2

Elemental Analysis (CHN) for $C_{16}H_{14}N_8OS_2$: [C, 48.23; H, 3.54; N, 28.12] Found: [C, 48.33; H, 3.62; N, 28.20]. %.

Synthesis of N2-(3-fluorophenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2f):

White solid (2.67g, 69.16%), M.P. 220-223 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3252 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1392.3 (NH bending), 1425 (C-H bending, CH3), 1440.11 (C=C ring str.), 829 (s-triazine), 734.8 (Ar.C-F str), 592.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.62 (s, 2H, m, -CH2), 3.27 (d, 1H, -CH2-S), 3.89 (-CH2-S), 6.18 (s, H, N-CH-S), 6.62-7.89 (m, 3H,H4, H5, H6, Ar-H, F-Ar-H)), 8.19 (s, H, N-H), 8.32 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 38.13, 44.18, 65.79, 125.50, 128.90, 137.83 (F-C, NH-Ar-C-CH2) 167.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 387.1 [M+2] 389.1

Elemental Analysis (CHN) for $C_{15}H_{11}FN_8S_2$ [C, 46.62; H, 2.87; N, 29.00] Found: [C, 46.68; H, 2.91; N, 29.12]. %.

Synthesis of N2-(4-chlorophenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2g)

White solid (2.69 g, 66.91 %), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C**

NMR (100 MHz, DMSOd^6, δ, ppm): 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 402.1 [M+2] 403.1 **Elemental Analysis (CHN)** for $C_{15}H_{11}ClN_8S_2$: [C, 44.72; H, 2.75; N, 27.81]. Found: [C, 44.82, H, 2.84, N, 27.75]. %.

Synthesis of N2-(4-methoxyphenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2h):

White solid (2.78 g, 69.83 %), M.P. 190-192 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3329.42, 3289.30 cm-1 (multiple band 2x-NH str.), 3022.23 (C-H str. Aromatic), 2901.35 (Ali. C-H str. Asym.), 2761.21 (Ali. C-H str. Sym.), 1581.37 (C=N ring str.), 1464.98, 1412.21(C=C ring str.) 1332.28 (C-N str.) 1251.67 & 1159.25 (C-O str. Of C-O-C group) 869.05 (s-triazine), 686.64 (C-S Str.); **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.58 (s, 2H, m, -CH2), 3.31 (s, 3H, - OCH3), 3.49 (d, 1H, -CH2-S), 3.81 (-CH2-S), 5.58 (s, H, N-CH-S), 6.92-7.50 (m, 3H, H4, H5, H6, -OCH3-Ar-H), 8.21 (s, H, N-H), 8.34 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 39.52, 55.21, 122.81, 134.42, 152.82, 147.67 (C-C, OCH3-Ar-C-CH2) 149.64 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 398.1 [M+2] 399.2

Elemental Analysis (CHN) for $C_{16}H_{14}N_8OS_2$: [C, 48.23; H, 3.54; N, 28.12] Found: [C, 48.33; H, 3.62; N, 28.20]. %.

Synthesis of N2-(4-fluorophenyl)-N4,N6-di(thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine (2i):

White solid (2.89 g, 74.86 %), M.P. 220-223 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3252 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1392.3 (NH bending), 1425 (C-H bending, CH3), 1440.11 (C=C ring str.), 829 (s-triazine), 734.8 (Ar.C-F str), 592.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.62 (s, 2H, m, -CH2), 3.27 (d, 1H, -CH2-S), 3.89 (-CH2-S), 6.18 (s, H, N-CH-S), 6.62-7.89 (m, 3H,H4, H5, H6, Ar-H, F-Ar-H)), 8.19 (s, H, N-H), 8.32 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 38.13, 44.18, 65.79, 125.50, 128.90, 137.83 (F-C, NH-Ar-C-CH2) 167.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 387.1 [M+2] 389.1

Elemental Analysis (CHN) for $C_{15}H_{11}FN_8S_2$ [C, 46.62; H, 2.87; N, 29.00] Found: [C, 46.68; H, 2.91; N, 29.12]. %.

Synthesis of 6-(2-chlorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3a)

White solid (2.80 g, 69.64 %), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm-^{1}**: 3363 cm-1 (Phenolic compound str.), 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-

H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 403.01 [M+2] 405.0 **Elemental Analysis (CHN)** for $C_{15}H_{10}ClN_7OS_2$: [C, 44.72; H, 2.50; N, 24.28]. Found: [C, 44.78, H, 2.74, N, 24.67]. %.

Synthesis of 6-(2-methoxyphenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3b)

White solid (2.81 g, 70.59%), M.P. 116-118 ºC. **IR ν_{max} (KBr)/ cm-^{1}**: 3363 cm-1 (Phenolic compound str.), 3188 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H,), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (O-CH_3, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 399.06 [M+2] 400.1 **Elemental Analysis (CHN)** for $C_{16}H_{13}N_7O_2S_2$: [C, 48.11; H, 3.28; N, 24.55]. Found: [C, 44.0, H, 3.34, N, 24.83]. %.

Synthesis of 6-(2-fluorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3c)

White solid (2.83 g, 73.30%), M.P. 116-118 ºC. **IR ν_{max} (KBr)/ cm-^{1}**: 3318 cm-1 (Phenolic compound str.), 3274 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1529.3 (NH bending), 1385 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-F str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, F-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (F-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 387.02 [M+2] 388.03 **Elemental Analysis (CHN)** for $C_{15}H_{10}N_7FOS_2$: [C, 46.50; H, 2.60; N, 25.31]. Found: [C, 46.86, H, 2.73, N, 25.62]. %.

Synthesis of 6-(3-chlorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3d)

White solid (2.82 g, 70.14%), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm^{-1}**: 3363 cm-1 (Phenolic compound str.), 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 403.01 [M+2] 405.0 **Elemental Analysis (CHN)** for $C_{15}H_{10}ClN_7OS_2$: [C, 44.72; H, 2.50; N, 24.28]. Found: [C, 44.78, H, 2.74, N, 24.67]. %.

Synthesis of 6-(3-methoxyphenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3e)

White solid (2.80 g, 70.33%), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm^{-1}**: 3363 cm-1 (Phenolic compound str.), 3188 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H,), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (O-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 399.06 [M+2] 400.1 **Elemental Analysis (CHN)** for $C_{16}H_{13}N_7O_2S_2$: [C, 48.11; H, 3.28; N, 24.55]. Found: [C, 44.0, H, 3.34, N, 24.83]. %.

Synthesis of 6-(3-fluorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3f):

White solid (2.84 g, 73.56 %), M.P. 116-118 °C. **IR ν_{max} (KBr)/ cm^{-1}**: 3318 cm-1 (Phenolic compound str.), 3274 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1529.3 (NH bending), 1385 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-F str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, F-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (F-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 387.02 [M+2] 388.03 **Elemental Analysis (CHN)** for $C_{15}H_{10}N_7FOS_2$: [C, 46.50; H, 2.60; N, 25.31]. Found: [C, 46.86, H, 2.73, N, 25.62]. %.

Synthesis of 6-(4-chlorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3g):

White solid (2.81g, 69.89 %), M.P. 116-118 ^{o}C. 3363 cm-1 (Phenolic compound str.), 3258 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-Cl str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, Cl-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (Cl-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 403.01 [M+2] 405.0 **Elemental Analysis (CHN)** for $C_{15}H_{10}ClN_7OS_2$: [C, 44.72; H, 2.50; N, 24.28]. Found: [C, 44.78, H, 2.74, N, 24.67]. %.

Synthesis of 6-(4-methoxyphenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3h):

White solid (2.83 g, 71.09 %), M.P. 116-118 ^{o}C. **IR ν_{max} (KBr)/ cm-^{1}**: 3363 cm-1 (Phenolic compound str.), 3188 cm-1 (NH str.), 2870 (C-H, Ar-H str.), 1499.3 (NH bending), 1475 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H,), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (O-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum (MS (EI) m/z) :** [M^+] 399.06 [M+2] 400.1 **Elemental Analysis (CHN)** for $C_{16}H_{13}N_7O_2S_2$: [C, 48.11; H, 3.28; N, 24.55]. Found: [C, 44.0, H, 3.34, N, 24.83]. %.

Synthesis of 6-(4-fluorophenoxy)-N2,N4-di(thiazol-2-yl)-1,3,5-triazine-2,4-diamine (3i):

White solid (2.83 g, 73.30%), M.P. 116-118 ^{o}C. **IR ν_{max} (KBr)/ cm-^{1}**: 3318 cm-1 (Phenolic compound str.), 3274 cm-1 (NH str.), 2770 (C-H, Ar-H str.), 1529.3 (NH bending), 1385 (C-H bending, CH3), 1440.11 (C=C ring str.), 827 (s-triazine), 721.8 (Ar.C-F str), 693.4 (C-S Str.) ; **1H NMR (400 MHz, DMSO-d^6, δ, ppm);** 2.57 (s, 2H, m, -CH2), 3.34 (d, 1H, -CH2-S), 3.68 (-CH2-S), 5.58 (s, H, N-CH-S), 6.77-7.33 (m, $H,H3, H4, H5, H6, Ar-H, F-Ar-H)), 8.10 (s, H, N-H), 8.22 (s, H, C-NH); **13C NMR (100 MHz, DMSOd^6, δ, ppm):** 35.13, 42.28, 68.89, 124.50, 127.90, 136.83 (F-C, NH-Ar-C-CH2) 163.75 (-CH-NH, triazine) ; **Mass Spectrum**

(MS (EI) m/z) : [M^+] 387.02 [M+2] 388.03 **Elemental Analysis (CHN)** for $C_{15}H_{10}N_7FOS_2$: [C, 46.50; H, 2.60; N, 25.31]. Found: [C, 46.86, H, 2.73, N, 25.62]. %.

Molecular Docking Studies

To enhance understanding of the interaction between these compounds and the enzyme with a known X-ray structure, docking studies were conducted using the libdock module of BIOVIA Discovery Studio software version 2020 on a Windows-based workstation. The docking procedure for the newly developed set of eighteen inhibitors, including the standard drug suramin, was conducted using the Libdock modules within the Biovia Discovery Studio 2020 software. The libdock scores obtained from the molecular docking analysis between the PTP1B receptor (1XBO) and the 18 derivatives of 6-chloro-N2, N4-di(thiazol-2-yl)-1, 3, 5-triazine-2,4-diamine, along with the reference drug Suramin, were recorded. The crystal structure of PTP1B, in complex with a co-crystal of a isoxazole carboxyl acid , was obtained from the Protein Data Bank (PDB) with an X-ray resolution of 2.5 Å. This structure was utilised for the present docking investigations. The libdock score was measured for compounds 3h and 3i, resulting in values of 120.713 and 119.781, respectively. Additionally, the libdock score (measured at 153.025) of the reference drug suramin exhibited a comparatively greater value in comparison to the libdock scores of the triazine derivatives.

Based on the obtained data, it can be inferred that all eighteen compounds exhibit binding affinity with the active site of the protein, particularly compounds 3h and 3i. These compounds potentially demonstrate comparable or potentially equivalent binding affinity towards PTP1B when compared to other ligands and the standard drug (suramin). The amino acids that play a key role in the interactions between protein (1XBO) and ligand are ASP181, ALA217, CYS215, TYR46, ASP48, ARG24, SER216, GLY218, ARG221, and VAL48 (as depicted in Figure 3, 4).

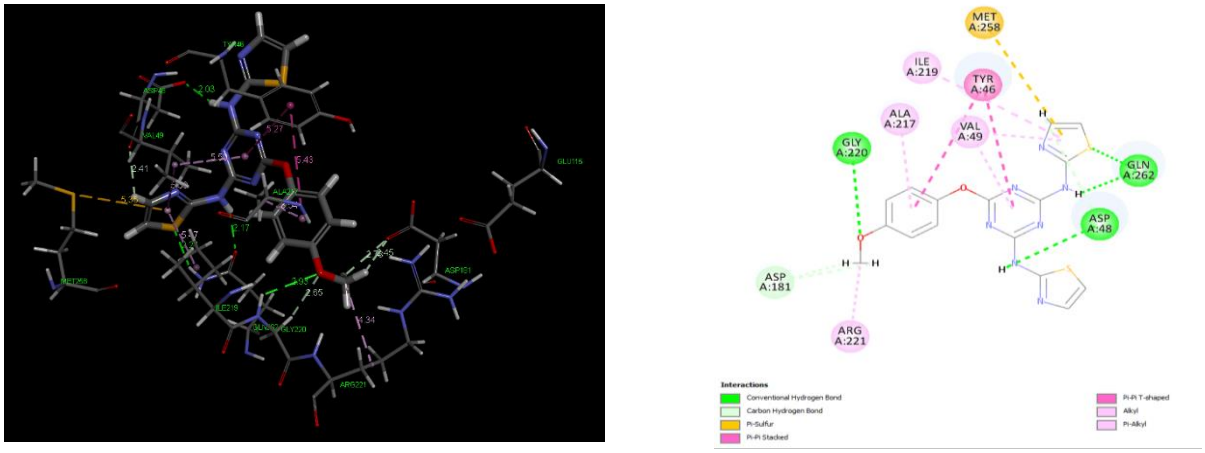

Fig. 3: Interaction between Protein (PDB ID: 1XBO) and Ligand 3h

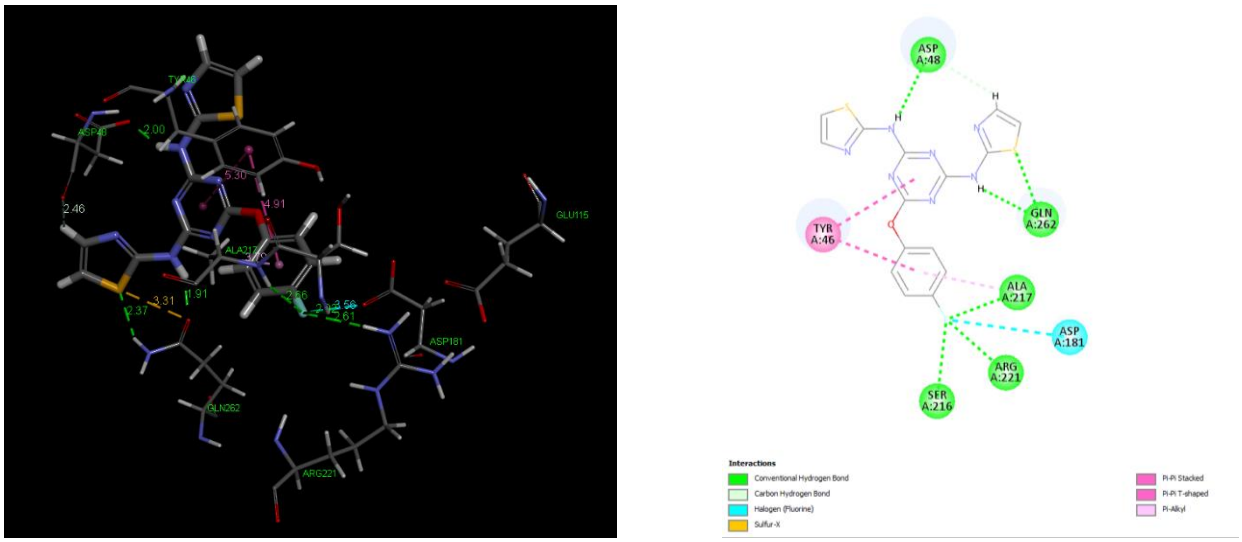

Fig. 4: Interaction between Protein (PDB ID: 1XBO) and Ligand 3i

Biological activities

In vitro PTP1B inhibition assays:

The PTP1B tyrosine phosphatase drug discovery kit (BML-AK 822, Enzo Life Sciences, USA) was utilised for conducting in vitro experimentation to evaluate the synthesised compounds (fig.5)' activity against PTP1B. Our state-of-the-art technology employs a colorimetric, non-radioactive technique to precisely measure the amount of phosphate released in the reaction solution. [10] The concentration of free phosphate was then measured using biomol red, a reagent used for detecting phosphate. A 96-well microtiter plate was employed for the assay, with 10mM dimethyl sulfoxide (DMSO) used as the control. The detection of liberated phosphate ions is predicated upon the fundamental principles underlying the conventional Malachite

green assay.[10] The measurement of optical density at a wavelength of 620 nm (OD620) was determined using a microplate reader.[10-11] This value was then converted into precise nanomoles of phosphate using the data obtained from our carefully constructed phosphate standard curve (Table1).

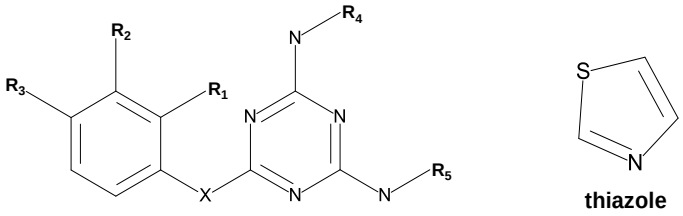

Fig.5: Triazine ring with their substitution position

Table: 1: Subset of triazine derivatives with its % of activity

Novel Triazine Derivatives								
SN	**Name**	**X**	**R1**	**R2**	**R3**	**R4**	**R5**	**% Activity at 10 µM**
1	Suramin (STD2)							15.556
2	**2a**	N	Cl	H	H	Thiazole	Thiazole	60.000
3	**2b**	N	O-CH3	H	H	Thiazole	Thiazole	62.889
4	**2c**	N	F	H	H	Thiazole	Thiazole	61.667
5	**2d**	N	H	Cl	H	Thiazole	Thiazole	37.667
6	**2e**	N	H	O-CH3	H	Thiazole	Thiazole	59.889
7	**2f**	N	H	F	H	Thiazole	Thiazole	58.778
8	**2g**	N	H	H	Cl	Thiazole	Thiazole	59.778
9	**2h**	N	H	H	O-CH3	Thiazole	Thiazole	27.000
10	**2i**	N	H	H	F	Thiazole	Thiazole	25.556
11	**3a**	O	Cl	H	H	Thiazole	Thiazole	58.556
12	**3b**	O	O-CH3	H	H	Thiazole	Thiazole	61.778
13	**3c**	O	F	H	H	Thiazole	Thiazole	53.889
14	**3d**	O	H	Cl	H	Thiazole	Thiazole	55.444
15	**3e**	O	H	O-CH3	H	Thiazole	Thiazole	61.889
16	**3f**	O	H	F	H	Thiazole	Thiazole	62.556
17	**3g**	O	H	H	Cl	Thiazole	Thiazole	65.333
18	**3h**	O	H	H	O-CH3	Thiazole	Thiazole	24.111
19	**3i**	O	H	H	F	Thiazole	Thiazole	24.333

Summary and Conclusion

A standardised procedure was employed to synthesise a range of compounds composed of different amine and phenol derivatives. The advancement of the reactions was observed by utilising thin-layer chromatography (TLC) with a solvent system comprising n-Hexane and Ethyl acetate in an 8:2 proportion. The synthesised compounds demonstrated a range of melting points between approximately 160 to 192 ^{0}C. Subsequently, the compounds were subjected to in vitro experimentation to evaluate their inhibitory activity against PTP1B (table 1). A non-radioactive colorimetric assay was employed to precisely measure the amount of phosphate released in the reaction solution compound 3h and 3i having the better inhibitory activity as compare to other compounds. The Libdock modules of Biovia Discovery Studio 2020 software were utilised to perform molecular docking screening on thirty-six recently developed inhibitors, which included the standard drug suramin. The amino acids that play a significant role in these interactions include ASP181, ALA217, CYS215, TYR46, ASP48, ARG24, SER216, GLY218, ARG221, and VAL48 (as illustrated in Figure 3 & 4).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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