International Journal of PharmTech Research

CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.8, pp 136-143, 2015

Synthesis and Biological Evaluation of 4-Aryl substituted -2-(5-carboxylicacid-1, 6-dihydro)-2-thiophenylethylene-6-oxo-pyrimidine as Protein Tyrosine Phosphatase (PTP-1B) Inhibitors

Ashish Patel*[1], T.Y.Pasha[2], Arjun Modi[3]

[1]PhD scholar, School of Pharmacy, R.K University and Assistant Professor, Department of Pharmacy, Parul University,Gujarat-391760,India
[2]Principal, Parul Institute of Pharmacy and Research, Parul University, Gujarat-391760, India
[3]Department of Pharmacy, Parul University, Gujarat-391760, India

Abstract: Diabetes is a group of metabolic diseases which characterized by increased blood glucose level along with symptoms of polyuria, polydipsia and polyphagia. Tyrosine residues are selectively dephosphorylated by Protein tyrosine phosphatases (PTPs) and thus a wide variety of cellular processes are regulated by their action. Protein tyrosine phosphatase 1B (PTP1 B) has shown to be a negative regulator in the insulin signaling pathways. Recent gene knockout studies carried out on mice portrays PTP1B as an effective target for drug discovery process related to anti-diabetic and anti-obese agents. PTPs are also involved in several other disorders like cancer. The structure of compounds synthesized by the present method were confirmed by TLC, IR, NMR and Mass spectroscopy. The anti-diabetic activity of the synthesized compounds were tested against PTP1B enzyme by using Calbiochem® PTP1B colorimetric assay kit. Among all synthesized compounds **3c, 3d, 3e, 3f** had shown promising anti-diabetic activity, while other compounds had shown lesser potency as anti-diabetic agent.
Key words: Diabetes, Protein tyrosine phosphate, PTP1B.

Introduction

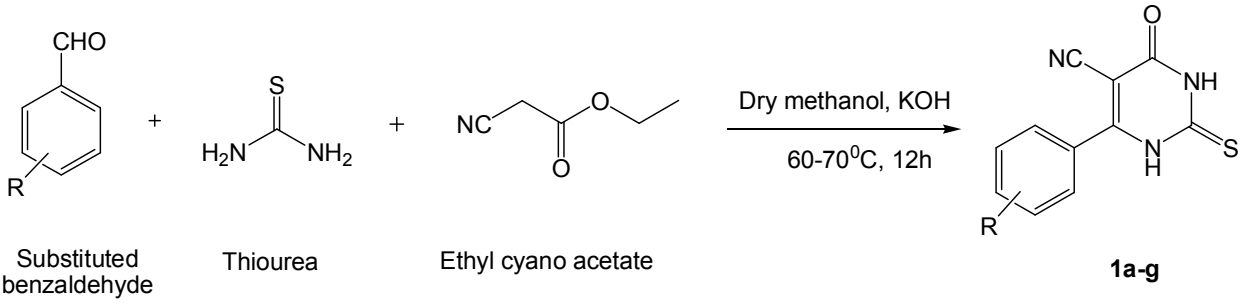
Diabetes is often referred as diabetes mellitus, which describes as a group of metabolic disorder in which the person has high blood glucose (blood sugar) either because insulin production is inadequate or because the body's cells do not respond properly to insulin or both[1]. Protein tyrosine phosphatases (PTPs) are responsible for selective dephosphorylation of tyrosine residues and regulate a wide variety of cellular processes[2]. Protein tyrosine phosphatase produces dephosphorylation of insulin receptor, PTP-1B is the negative regulator in insulin signaling pathway[11]. Recent studies have demonstrated that loss of protein tyrosine phosphatase-1B (PTP1B) activity resulted in enhancement of insulin sensitivity in addition to decrease in susceptibility to diet-induced obesity[3]. Specific PTP1B inhibitors emerged as a new target for treatment of type-2 diabetes[4], obesity[5] and also for cancer[6-9]. A class of PTP-1B inhibitor is synthesized by cyclization of three-components like arylaldehydes, thiourea, and ethyl cyanoacetate in dry methanol using potassium hydroxide to form (6-Aryl substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile)[10].

Experimental

1. Chemical Studies
2. Biological Study

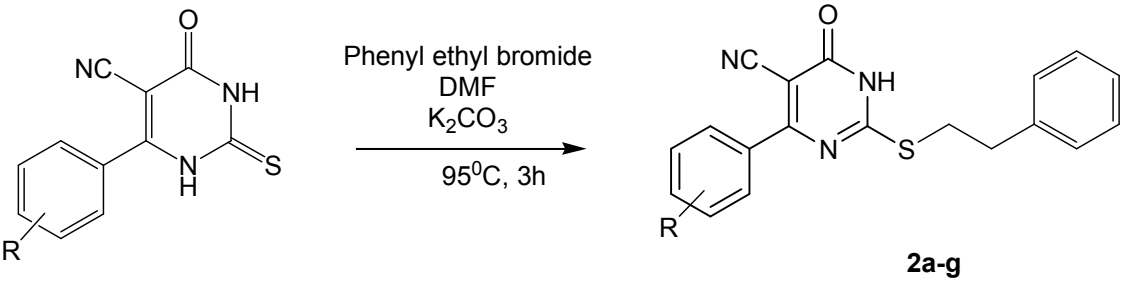
1. Chemical studies

Step 1. Synthesis of 6-Aryl substituted-4-oxo—2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1a-g)



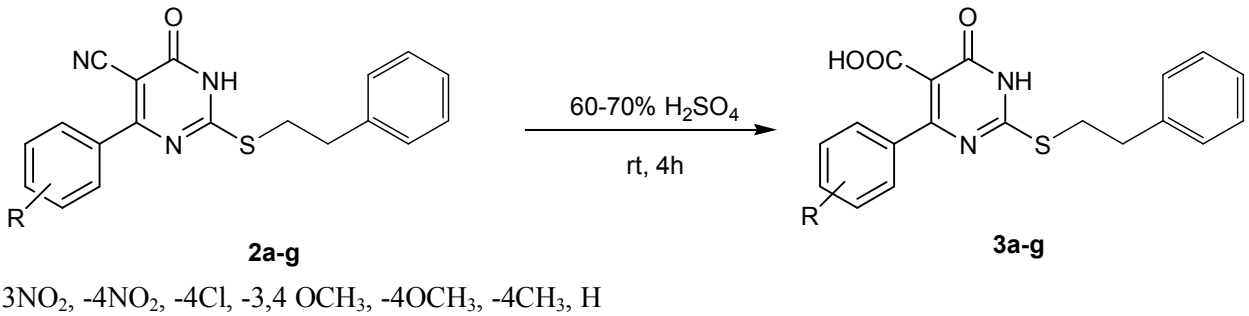
3NO_2, -4NO_2, -4Cl, -3,4 OCH_3, -4OCH_3, -4CH_3, H

Step 2. Synthesis of 4-Aryl substituted -2-(5-cyno-1,6-dihydro)2-thiophenylethylene-6-oxo-pyrimidine (2a-g)



R= -3NO_2, -4NO_2, -4Cl, -3,4 OCH_3, -4OCH_3, -4CH_3, H

Step 3. Synthesis of 4-Aryl substituted -2-(5-carboxylicacid-1,6-dihydro)-2-thiophenylethylene 6-oxo-pyrimidine (3a-g)



3NO_2, -4NO_2, -4Cl, -3,4 OCH_3, -4OCH_3, -4CH_3, H

Melting points were determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA T FT-IR spectrophotometer using press pellet technique. ^1H NMR spectra were recorded on 400MHz BRUKER AVANCE II NMR instrument in DMSO and $CDCl_3$ with TMS as internal standard for ^1H NMR and chemical shift values are mentioned in δ ppm. The progress of all reaction was monitored by TLC on 2 cm X 5 cm pre-coated silica gel 60 F254 (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV (254 nm) and/or exposure to iodine vapours. All reagents used were of analytical reagent grade, obtained

from S.D.fine, Spectrochem and Qualigens. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glasswares.

General procedure for synthesis of 6-Aryl substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile. (1a-g)

Mixture of substituted aryl aldehydes, ethyl cyanoacetate, thiourea, potassium hydroxide and dry methanol (q.s.) was refluxed on a water bath for 10-12 hrs. Reaction mixture was allowed to cool on ice-bath to obtain a solid and then filtered it. Collected solid was dissolved in hot water and neutralize with glacial acetic acid. The solid was filtered, washed with water and the pure material was dried and recrystallized from methanol.

6-(3-nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1a)

The Compound **1a** was obtained as white solids in 73% yield; mp 275-278˚C; IR(KBr): peak at C=O (1669 cm^{-1}), NO_2 (1354 & 1518 cm^{-1}), -CH aromatic (3039 cm^{-1}), CN (2210 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.05 (1H, s, CH), 8.01 (1H, d, Ar-H), 8.0 (1H, s, -NH), 7.51 (1H, d, Ar-H), 7.47 (2H, t, Ar-H), 4.1 (1H, d, -CH), 4.05 (1H, s, -CONH), 2.0 (1H, s, -NH); m/z 275.2 ([M^{+1}]), calcd for $[C_{11}H_6N_4O_3S]^+$ 274.26.

Synthesis of 6-(4-nitrophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1b)

The Compound **1b** was obtained as white solid in 70% yield; mp 276-278˚C; IR (KBr): peak at C=O (1645 cm^{-1}), NO_2 (13540 & 1536 cm^{-1}), -CH aromatic (2927 cm^{-1}), CN (2211 cm^{-1});^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.0 (1H, s, -NH), 7.3 (1H, d, Ar-H), 7.2 (1H, d, Ar-H), 7.2 (1H, d, Ar-H), 2.0 (2H, d, -NH); m/z 275.2 ([M^{+1}]), calcd for $[C_{11}H_6N_4O_3S]^+$ 274.26.

6-(4-chlorophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1c)

The Compound **1c** was obtained as white solid in 72% yield; mp 260-262˚C; IR (KBr): peak at C=O (1649 cm^{-1}), Cl (807 cm^{-1}), -CH aromatic (3089 cm^{-1}), CN (2235 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.0 (1H, s, -NH), 7.24 (1H, d, Ar-H), 7.22 (1H, d, Ar-H), 7.22 (1H, d, Ar-H), 2.0 (2H, d, -NH); m/z 264 ([M^{+1}]), calcd for $[C_{11}H_6ClN_4O_3S]^+$ 263.

6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile(1d)

The Compound **1d** was obtained as white solid in 78% yield; mp 270-272˚C; IR (KBr): peak at C=O (1680 cm^{-1}), -OCH_3 (1232 cm^{-1}), -CH aromatic (3024 cm^{-1}), CN (2218 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.0 (1H, s, -NH), 6.75 (1H, d, Ar-H), 6.70 (3H, q, -CH), 6.61 (1H, d, Ar-H), 3.03 (3H, s, -OCH_3), 3.73 (3H, s, -OCH_3), 2.0 (1H, d, -NH); m/z 290.53 ([M^{+1}]), calcd for $[C_{13}H_9N_3O_3S]^+$ 289.31.

6-(4-methoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1e)

The Compound **1e** was obtained as white solid in75% yield; mp 272-276˚C; IR (KBr): peak at C=O (1644 cm^{-1}), -OCH_3 (1262 cm^{-1}), -CH aromatic (2988 cm^{-1}), CN (2217 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.19 (1H, d, Ar-H), 6.72 (1H, d, Ar-H), 3.73 (3H, s, -OCH_3), 6.7 (1H, d, Ar-H), 7.19 (1H, d, Ar-H), 2.0 (1H, d, -NH), 8.0 (1H, s, -NH); m/z 260.17 ([M^{+1}]), calcd for $[C_{12}H_9N_3O_2S]^+$ 259.2.

6-(4-methylphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1f)

The Compound **1f** was obtained as white solid in 74%yield; mp 260-262˚C;IR (KBr): peak at C=O (1679 cm^{-1}), -CH_3 (1442 cm^{-1}), -CH aromatic (2939 cm^{-1}), CN (2223 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.18 (1H, d, Ar-H), 7.01 (1H, d, Ar-H), 2.35 (2H, t, -CH_3), 7.01 (1H, d, Ar-H), 7.18 (1H, d, Ar-H), 2.0 (1H, d, -NH), 8.0 (1H, s, -NH); m/z 244.3 ([M^{+1}]), calcd for $[C_{12}H_9N_3OS]^+$ 243.5.

6-(4-phenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile(1g)

The Compound **1g** was obtained as white solid in74%yield; mp 256-260˚C; IR (KBr): peak at C=O (1668 cm^{-1}), -CH aromatic (2924 cm^{-1}), CN (2235 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.0 (1H, s, -NH), 7.30 (1H, d, Ar-H), 7.30 (1H, d, Ar-H), 7.21 (2H, t, Ar-H), 7.21 (2H, t, Ar-H), 7.14 (2H, t, Ar-H), 2.0 (1H, d, -NH); m/z 230.14 ([M^{+1}]), calcd for $[C_{11}H_7N_3OS]^+$ 229.2.

General procedure for synthesis of 4-Aryl substituted-2-(5-cyno-1,6-dihydro)2-thiophenylethylene-6-oxo-pyrimidine. (2a-g)

The compounds **1a-f** was dissolved in DMF, cooling condition was maintained at 0-5˚C and then K_2CO_3 was added. After 10-15 min phenyl ethyl bromide was added drop wise. The cooling condition was maintained for 3hr and then put the reaction at room temperature for further 3hr stirring. After completion of reaction, reaction mixture was added in chilled water. Precipitate obtained was filtered and recrystallized from methanol.

2-(5-cyano-1,6-dihydro)-4-(3-nitrophenyl)-2-thiophenylethylene-6-oxo-pyrimidine. (2a)

Compound **2a** was obtained as white solid in 63.3% yield; mp 290-292˚C; IR (KBr) peak at C=O amide (1679 cm^{-1}), -NO_2 (1517 & 1352 cm^{-1}), -CH aromatic (2925 cm^{-1}), CN (2205 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.23 (1H, d, Ar-H), 8.07 (2H, s, Ar-H), 8.0 (1H, s, -NH), 7.69 (2H, s, Ar-H), 7.47 (4H, d, Ar-H), 7.21 (4H, d, Ar-H), 7.21 (4H, d, Ar-H), 7.12 (2H, s, Ar-H), 7.12 (2H, s, Ar-H), 7.08 (4H, d, Ar-H), 3.22 (2H, s, -CH), 3.20 (2H, s, -CH); m/z 379.2 ([M^{+1}]), calcd for $[C_{19}H_{14}N_4O_3S]^+$ 378.7.

2-(5-cyano-1,6-dihydro)-4-(4-nitrophenyl)-2-thiophenylethylene-6-oxo-pyrimidine. (RK 2b)

The Compound **2b** was obtained as white solid in 65% yield; mp 294-298˚C; IR (KBr): peak at C=O amide (1655 cm^{-1}), -NO_2 (1551 & 1351cm^{-1}), -CH aromatic (2929 cm^{-1}), CN (2205 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 8.14 (2H, s, Ar-H), 8.0 (1H, s, -NH), 7.56 (2H, s, Ar-H), 7.21 (4H, d, Ar-H), 7.12 (2H, s, Ar-H), 7.56 (2H, s, Ar-H), 3.20 (2H, s, -CH); m/z 379.1 ([M^{+1}]), calcd for $[C_{19}H_{14}N_4O_3S]^+$ 378.33.

4-chlorophenyl 2-(5-cyano-1,6-dihydro)-2-thiophenylethylene-6-oxo-pyrimidine (2c)

The Compound **2c** was obtained as white solid in 64.5% yield; mp 288-290˚C; IR (KBr): peak at C=O amide (1672 cm^{-1}), -Cl (774 cm^{-1}), -CH aromatic (3023cm^{-1}), CN (2217 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.24 (2H, s, Ar-H), 7.22 (2H, s, Ar-H), 8.0 (1H, s, -NH), 3.20 (2H, s, -CH), 7.12 (2H, s, Ar-H), 7.21 (4H, d, Ar-H), 7.08 (4H, s, Ar-H); m/z 368.12 ([M^{+1}]), calcd for $[C_{19}H_{14}ClN_3OS]^+$ 367.09.

2-(5-cyano-1,6-dihydro)-4-(3,4-dimethoxyphenyl)-2-thio phenyl ethylene 6-oxo-pyrimidine. (2d)

The Compound **2d** was obtained as white solid in 67%yield; mp 285-288˚C; IR (KBr) peak at C=O amide (1672 cm^{-1}), -OCH_3 (1252 cm^{-1}), -CH aromatic (3003cm^{-1}), CN (2212 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 6.75 (2H, s, Ar-H), 6.61 (2H, s, Ar-H), 3.73 (3H, s, -CH_3), 3.30 (3H, s, -CH_3), 6.70 (1H, s, Ar-H), 8.0 (1H, s, -NH), 3.20 (2H, s, -CH_2), 7.12 (2H, d, Ar-H), 7.21 (4H, s, Ar-H); m/z 394.0 ([M^{+1}]), calcd for $[C_{21}H_{19}N_3O_3S]^+$ 393.2.

2-(5-cyano-1,6-dihydro)-4-(4-methoxyphenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine. (2e)

The Compound **2e** was obtained as a white solid in 64.3% yield; mp 282-284˚C; IR (KBr): peak at C=O amide (1645 cm^{-1}), -OCH_3 (1255 cm^{-1}), -CH aromatic (2922cm^{-1}), CN (2203 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.19 (2H, d, Ar-H), 6.72 (2H, d, Ar-H), 3.73 (3H, s, -OCH_3); 8.0 (1H, s, -NH), 7.12 (2H, d, Ar-H), 7.21 (4H, q, Ar-H), 7.08 (4H, s, Ar-H); m/z 364.7 ([M]), calcd for $[C_{20}H_{17}N_3O_3S]^+$ 363.8.

2-(5-cyano-1,6-dihydro)-4-(4-methylphenyl)-2-thio phenyl ethylene -6-oxo-pyrimidine (2f)

The Compound **2f** was obtained as a white solid in 67% yield; mp 284-288˚C; IR (KBr): peak at C=O amide (1634 cm^{-1}), -CH_3 (1400 cm^{-1}), -CH aromatic (2944cm^{-1}), CN (2221 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.18 (2H, d, Ar-H), 7.01 (2H, d, Ar-H), 2.35 (4H, s, -CH_3), 8.0 (1H, s, -NH), 3.20 (3H, s, -CH_2), 7.12 (2H, s, Ar-H), 7.21 (3H, t, Ar-H); m/z 348.9 ([M^{+1}]), calcd for $[C_{20}H_{17}N_3OS]^+$ 348.0.

2-(5-cyano-1,6 dihydro)-4-(phenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine. (2g)

The Compound **2g** was obtained as white solid in 65.4% yield; mp 280-282˚C; IR (KBr): peak at C=O amide (1663 cm^{-1}), -CH_2 (1466 cm^{-1}), -CH aromatic (3060cm^{-1}), CN (2203 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 7.14-7.30 (2H, d, Ar-H), 8.0 (1H, s, -NH), 3.20 (2H, d, -CH_2), 7.21 (2H, d, Ar-H); m/z 334.1 ([M^{+1}]), calcd for $[C_{19}H_{15}N_3OS]^+$ 333.3.

General procedure for synthesis of 4-Aryl substituted-2-(5-carboxylicacid-1,6-dihydro)-2-thiophenyl ethylene-6-oxo-pyrimidine. (3a-g)

The compounds **2a-g** was dissolved in methanol and then conc. H_2SO_4 (70-80%) was added drop wise and refluxed for 3-4 hrs. after completion of reaction mixture was kept at room temperature and Basify the reaction mixture with NaOH solution. Precipitated obtained was filtered, dried and recrystaliized from methanol.

2-(5-carboxylic acid1,6-dihydro)-4-(3-nitrophenyl) 2-thio phenyl ethylene-6-oxo-pyrimidine. (3a)

The Compound **3a** was obtained as white solid in 52% yield; mp >300˚C; IR (KBr): peak at

-CH (aromatic 2959 cm^{-1}), -OH (3481 cm^{-1}), -NO_2(1523 & 1351 cm^{-1}), -C=O amide (1647 cm^{-1}), C=O acid (1710 cm^{-1}), -CH_2 (1469 cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 3.2-3.39 (4H, s, CH_2), 8.0 (1H, s, -NH), 10.9 (1H, s, -OH), 7.10-7.32 (3H, d, Ar-H), 7.35 (1H, s, Ar-H), 7.46-7.70 (5H, d, Ar-H); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 132.5-137.8 (-CH), 166.3 (-COOH), 167.0 (C=O), 160.7 (C-S), 26.6 (-CH_2), 127.8-138.7 (-CH_2); m/z 398.0 ([M^{+1}]), calcd for $[C_{19}H_{15}N_3O_5S]^+$ 397.1.

2-(5-carboxylic acid 1,6 dihydro)-4-(4-nitrophenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine. (3b)

The Compound **3b** was obtained as white solid in 54% yield; mp >300˚C; IR (KBr): peak at-CH aromatic (2959 cm^-), -OH(3481 cm^-), -NO_2(1531 & 1355 cm^-), -C=O(1652 cm^-); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 3.2-3.42 (4H, s, -CH_2), 8.1 (1H, s, -NH), 11.0 (1H, s, OH), 7.23-7.43 (4H, d, Ar-H), 7.50-7.82 (5H, d, Ar-H); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 178.1 (-COOH), 128.7 (-CH), 121.0 (-CH), 145.6 (C-4), 163 (C-S), 172.1 (C=O), 178.1 (-COOH), 26.5 (CH_2-S), 127.8 (-CH); m/z 398.0 ([M^{+1}]), calcd for $[C_{19}H_{15}N_3O_5S]^+$ 397.1.

2-(5-carboxylic acid 1,6-dihydro)-4-(4-chlorophenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine. (3c)

The Compound **3c** was obtained as white solid in 50.4% yield; mp 290-292˚C; IR (KBr): peak at –CH aromatic (3025cm^{-1}) ,-OH (3499 cm^{-1}), -Cl (876 cm^{-1}), -C=O amide (1647 cm^{-1}), -C=O acid (1739 cm^{-1}), -CH_2 (1455 cm^{-1}); ^{1}H-NMR (400 MHz, DMSO-*d6*): δ 3.11-3.38 (4H, t, -CH_2), 8.064 (1H, s, -NH), 10.26 (1H, s, -OH), 7.03-7.27 (4H, d, Ar-H), 7.29-7.53 (5H, d, Ar-H); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 129.2 (-CH), 131.5 (C-Cl),163.0 (C-S), 172.1 (C=O), 178.0 (-COOH), 26.5 (CH_2-S), 127.8-128.7 (-CH); m/z 386.9 ([M]), calcd for $[C_{19}H_{15}ClN_2O_3S]^+$ 386.

2-(5-carboxylic acid 1,6-dihydro)-4-(3,4-dimethoxyphenyl)-2-thiophenyl ethylene-6-oxo-pyrimidine.(3d)

The Compound **3d** was obtained as white solid in 51.3% yield; mp 290-292˚C; IR (KBr): peak at –CH aromatic (2930 cm^{-1}), -OH (3700 cm^{-1}), -OCH_3 (1251 cm^{-1}), -C=Oamide (1655 cm^{-1}), -C=O acid (1742cm^{-1}), -CH_2 (1472cm^{-1}); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 6.57-6.61 (2H, d, Ar-H), 3.73 (-3H, s, -OCH_3), 3.03 (3H, s, -OCH_3), 11.0 (1H, s, -COOH), 8.0 (1H, s, -NH), 3.20 (2H, s, -CH2), 7.12-7.21 (5H, d, Ar-H); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 178 (-COOH), 50.2-56.2 (-OCH_3), 172.1 (-C=O), 163.0 (C-S), 26.5 (CH_2-S), 127.8-128.7 (-CH); m/z 397.3 ([M^{+1}]), calcd for $[CH_{21}H_{20}N_2O_4S]^+$ 396.4.

2-(5-carboxylic acid 1,6-dihydro)-4-(4-methoxyphenyl)-2-thiophenyl ethylene-6-oxo-pyrimidine(3e)

The Compound **3e** was obtained as white solid in 53% yield; mp 286-288˚C; IR (KBr): peak at –CH aromatic (2958 cm^-), -OH (3595 cm^-), -OCH_3 (1257 cm^-), -C=O amide (1654 cm^-), C=O acid (1725 cm^-), -CH_2 (1466 cm^-); ^{1}H-NMR (400MHz, DMSO-*d6*): δ 11.0 (1H, s, -COOH), 8.0 (1H, s, -NH), 7.12-7.21 (5H, d, Ar-H), 3.20-3.22 (4H, t, -CH_2); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 178.0 (-COOH), 172.1 (C=O), 157.9 (C-O), 55.9 (-OCH_3), 163 (-C-S), 26.5-38.0 (-CH_2), 127.8-126.0 (-CH); m/z 383.2 ([M^{+1}]), calcd for $[CH_{20}H_{18}N_2O_4S]^+$ 382.1.

2-(5-carboxylic acid 1,6-dihydro)-4-(4-methylphenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine (3f)

The Compound **3f** was obtained as white solid in 54.4% yield; mp 288-292˚C; IR (KBr): peak at –CH aromatic (2958 cm^{-1}), -OH (3577 cm^{-1}), -CH_3 (1470 cm^{-1}), -C=O amide (1649 cm^{-1}), -C=O acid (1732cm^{-1}), -CH_2 (1470cm^{-1}); 1H-NMR (400MHz, DMSO-*d6*): δ 11.0 (1H, s, -COOH), 8.0 (1H, s, -NH), 7.0-7.01 (3H, d, Ar-H), 7.12-7.21 (5H, d, Ar-H), 3.20-3.22 (2H, d, -CH_2), 2.35 (3H, s, -CH_3); ^{13}C-NMR (100.6MHz, DMSO-*d6*):

δ 178 (-COOH), 24.3 (-CH_3), 127.7-129 (-CH), 172.1 (C=O), 163.0 (C-S), 26.5-38.0 (-CH_2), 127.8-128.7 (-CH); m/z 367.1 ([M^{+1}]), calcd for $[CH_{20}H_{18}N_2O_3S]^+$ 366.3.

2-(5-carboxylic acid 1,6-dihydro)-4-(phenyl)-2-thio phenyl ethylene-6-oxo-pyrimidine (3g)

The Compound **3g** was obtained as white solid in 53.2% yield; mp 284-286˚C; IR (KBr) peak at –CH aromatic (2954 cm^{-1}), -OH (3636 cm^{-1}), -C=O amide(1655 cm^{-1}), -C=O acid (1725 cm^{-1}), CH_2 (1455 cm^{-1}); 1H-NMR (400MHz, DMSO-*d6*): δ 11.0 (1H, s, -COOH), 8.0 (1H, s, -NH), 7.21-7.12 (5H, d, Ar-H), 3.20-3.22 (2H, s, -CH_2); ^{13}C-NMR (100.6MHz, DMSO-*d6*): δ 127.8-128.7 (-CH), 178.1 (-COOH), 26.5-38.0 (-CH_2), 127.8-128.7 (-CH); m/z 334 ([M^{+1}]), calcd for $[CH_{19}H_{16}N_2O_3S]^+$333.

2. Biological study

The synthesized compounds were evaluated for anti-diabetic activity using Calbiochem® PTP1B colorimetric assay kit and The PTP-1B inhibitor suramin is taken as a control[12].

Table 1: Inhibition of test samples as compared to Time zero and Suramin.

Concentration of Phosphate (nmole)	Absorbance (655 nm)
0.00	0.076
0.25	0.154
0.50	0.182
1.00	0.219
2.00	0.312
3.00	0.381

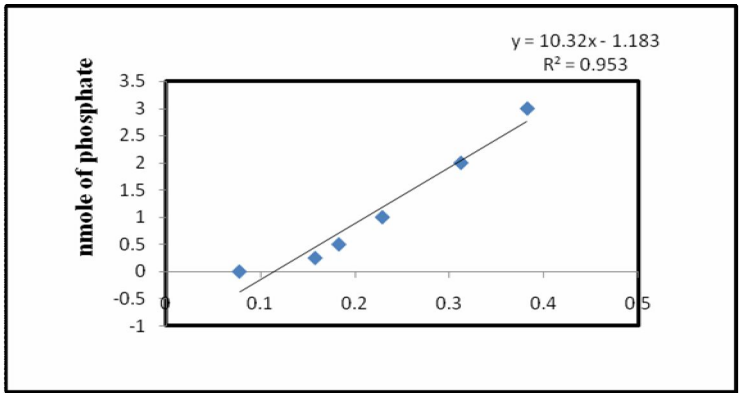
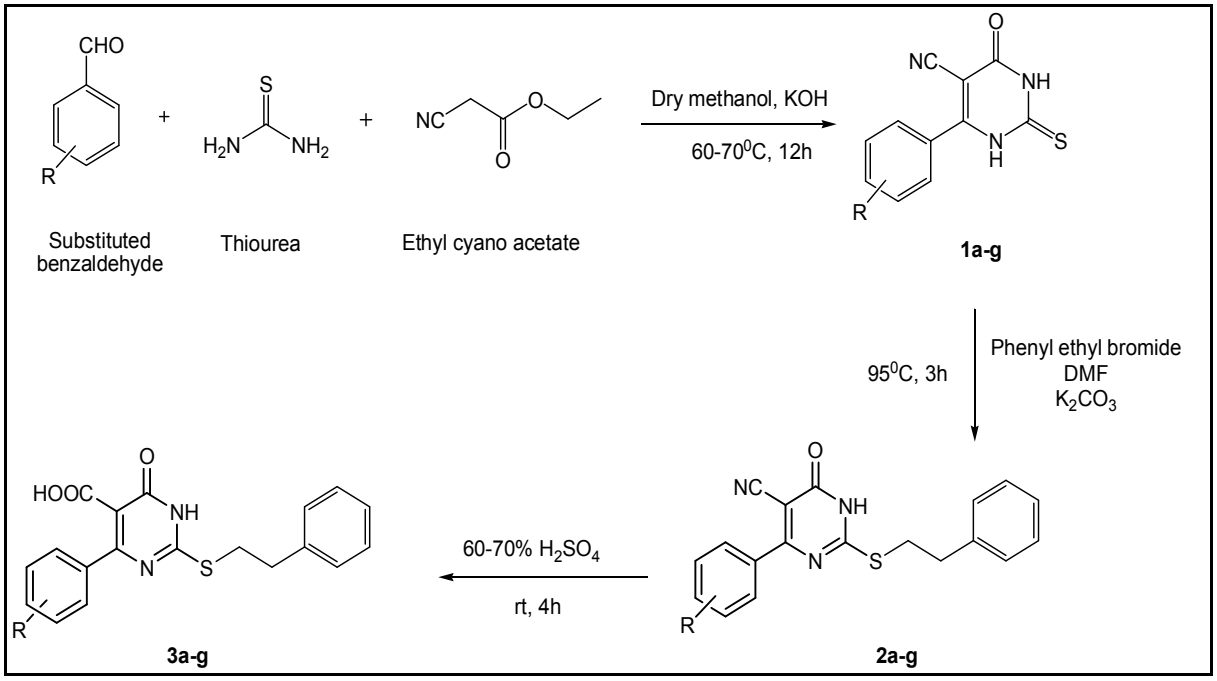


Fig.1 Phosphate Standard Curve Y (n mole of phosphate) =10.32(Abs. X) –1.185 R^2= 0.953

Results and Discussion

Series of 6-Aryl substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile was synthesized by refluxing the mixture of aryl aldehyde, ethyl cynoacetate, thiourea, potassium hydroxide and dry methanol (q.s). The reaction mixture was neutralized and thus obtained crude was recrystallized to obtain **1a-g** (Step-1). Compounds 4-Aryl substituted-2-(5-cyano-1,6-dihydro)-2-thiophenylethylene-6-oxo-pyrimidine **(2a-g)** were synthesized from **1a-g** by dissolving it in DMF at 0-5˚C in inert atmosphere and in presence of K_2CO_3 which was then added phenyl ethyl bromide after 10-15 minutes and further stirred for 3 hr at same condition. The resulting precipitate was recrystallized to obtain **2a-g** (Step-2).4-Aryl substituted-2-(5-carboxylicacid-1,6-dihydro)-2-thiophenylethylene-6-oxo-pyrimidine (**3a-g**) were synthesized by hydrolysis of cyano group in presence of strong acid to obtain **3a-g** (Step-3). All the synthesized compounds were confirmed by TLC, IR, Melting point, Mass and ^{1}H-NMR.

Scheme



Sr. No	a	b	c	d	e	f	g
R	-3 NO_2	-4NO_2	-4 Cl	-3,4 OCH_3	-4 OCH_3	-4 CH_3	H

Table 2: % Inhibition as compared to Time zero and Suramin of compound 3a-3g

Compound no	Absrbance at 655 nm	nmole of phosphate	% inhibition with compare to Time zero	% inhibition with compare to suramin	Concentration of compound (µM)
Suramin	0.070	0.2251	83.16	100	10
3a	0.105	0.5329	32.76	40.25	125
3b	0.094	0.4589	43.76	50.12	125
3c	**0.086**	**0.3478**	**54.19**	**64.24**	125
3d	**0.077**	**0.4085**	**69.41**	**84.32**	125
3e	**0.072**	**0.2465**	**74.55**	**89.90**	125
3f	**0.074**	**0.2568**	**71.74**	**87.80**	125
3g	0.127	0.7642	28.59	34.87	125
Time Zero	0.066	0.1872	100	120.25	-----
3a	0.102	0.5013	35.76	43.28	250
3b	0.122	0.7391	25.89	30.94	250
3c	**0.089**	**0.4051**	**46.21**	**55.57**	250
3d	**0.071**	**0.2231**	**74.16**	**88.91**	250
3e	**0.073**	**0.2532**	**82.26**	**99.23**	250
3f	**0.069**	**0.2135**	**84.85**	**101.43**	250
3g	0.098	0.4973	39.78	47.69	250

Conclusion

The yield of synthesized compounds ranged from 50% to 75% and their structures were established by spectral data (IR, NMR, and MS). Moreover, Result of inhibition of PTP-1B enzyme showed that substituted dihydropyrimidine derivative posses moderate to high anti-diabetic activity. Among all the compounds, those with OCH_3, CH_3 Substitution was found to be more active. After conducting the *in vitro* studies it was observed

that compounds **3d,3e,3f** having **-OCH_3, 3,4-OCH_3** and **CH_3** substitution on phenyl ring in the basic moiety shows good anti-diabetic activity and compound **3c** which is **chloro** substituted shows moderate anti diabetic activity. So in a nutshell, electron donating groups give more anti-diabetic activity owing to its ability to provide more hydrophobicity as compared to electron withdrawing groups.

References

1. Rayapureddi JP, Kattamuri C, Steinmetz BD, Frankfort BJ, Ostrin EJ, Mardon G, Hegde RS Eyes absent represents a class of protein tyrosine phosphatases. Nature (Pub Med)., 2003, 426: 295–298.
2. Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukuda T, Del VR, Tonks NK, and Moller NPH, A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. J. Faseb (Pub Med)., 2004, 18: 8–30.
3. Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA, Selengut JD, Parlikar BW, and Rebay I, The transcription factor Eyes absent is a protein tyrosine phosphatase. Nature (Pub Med)., 2003, 426: 299–302
4. Viktor VV, Andre YA, Daniel R, Herbert W, The therapeutic potential of phosphatase inhibitors. Current Opinion in Chem. Bio., 2009, 13: 272–283.
5. Theodore OJ, Jacques E and Michael RJ, Protein tyrosine phosphatase 1b inhibitors for diabetes. Nature (Pub Med)., 2002, 1:696-698.
6. Jia Z, Barford D, Flint AJ and Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. Science (pub med)., 1995, 268:1754–1758.
7. Yuanhua C, Zhou M, Chen-Ho T, Mingjuan J, Zhang F, Studies on two types of PTP1B inhibitors for the treatment of type 2 diabetes: Hologram QSAR for OBA and BBB analogues. Bio. & Med. Chem. Let., 2010, 20: 3329–3337
8. Harrie J M, Didier B, Michel A J, Cleyn D, Geuens L, Brône B, Marc M, Tricyclic 3,4-dihydropyrimidine-2-thione derivatives as potent TRPA1 antagonists. Bio. & Med. Chem. Let., 2012, 22:797–800
9. Ramesh B, Chetan MB, Novel dihydropyrimidine and its pyrazole derivatives. Eur. J. Med. Chem., 2011, 46:1882-1891.
10. Singh K, Arora D, Poremsky E, Lowery J, Moreland RS, N-1 Alkylated 3,4- dihydropyrimidine-2(1H)-ones: Convenient one-pot selective synthesis and evaluation of their calcium channel blocking activity. Eur. J. Med. Chem., 2009, 44:1997–2001
11. Zhang S and Zhang ZY, PTP1B as a drug target: Recent developments in PTP1B inhibitor discovery. Drug Disc. Today., 2007, 12:373-81.
12. Calbiochem PTP1B Assay Kit, Colorimetric, *User Protocol.* 2008, Catalogue No:539736.

*****