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Research Article

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Synthesis and biological evaluation of some novel heterocyclic compounds as protein tyrosine phosphatase (PTP-1B) Inhibitor

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ABSTRACT

Diabetes is a metabolic disorder wherein blood glucose level is increased along with some other abnormal conditions like polyuria, polydipsia and polyphagia. As per WHO estimation, 380 million people will become diabetic by 2025. 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 ***4c, 4d, 4e, 4f*** *had shown promising anti-diabetic activity, while other compounds have shown lesser potency as anti-diabetic agent.*

Key words: Diabetes, Protein tyrosine phosphate, PTP1B

INTRODUCTION

Diabetes is often known as diabetes mellitus that represents a metabolic disorder in which the person has high blood glucose level, may be because of inadequate insulin production, or because the body cell are unable to 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 and PTP-1B acts as a negative regulator in insulin signaling pathway. Moreover, recent studies have shown that enhancement of insulin sensitivity in addition to the decrease in susceptibility to diet-induced obesity is may be a result of loss of protein tyrosine phosphatase-1B (PTP1B) activity [3]. Specific PTP1B inhibitors emerged as a new target for treatment of type-2 diabetes[4], obesity[5] and also for cancer [6],[8-10]. A class of PTP-1B inhibitor is synthesized by cyclization of three-components like aryl aldehydes, thiourea, and ethyl cyanoacetate in methanol using potassium hydroxide to form (6-Aryl substituted-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile) [7].

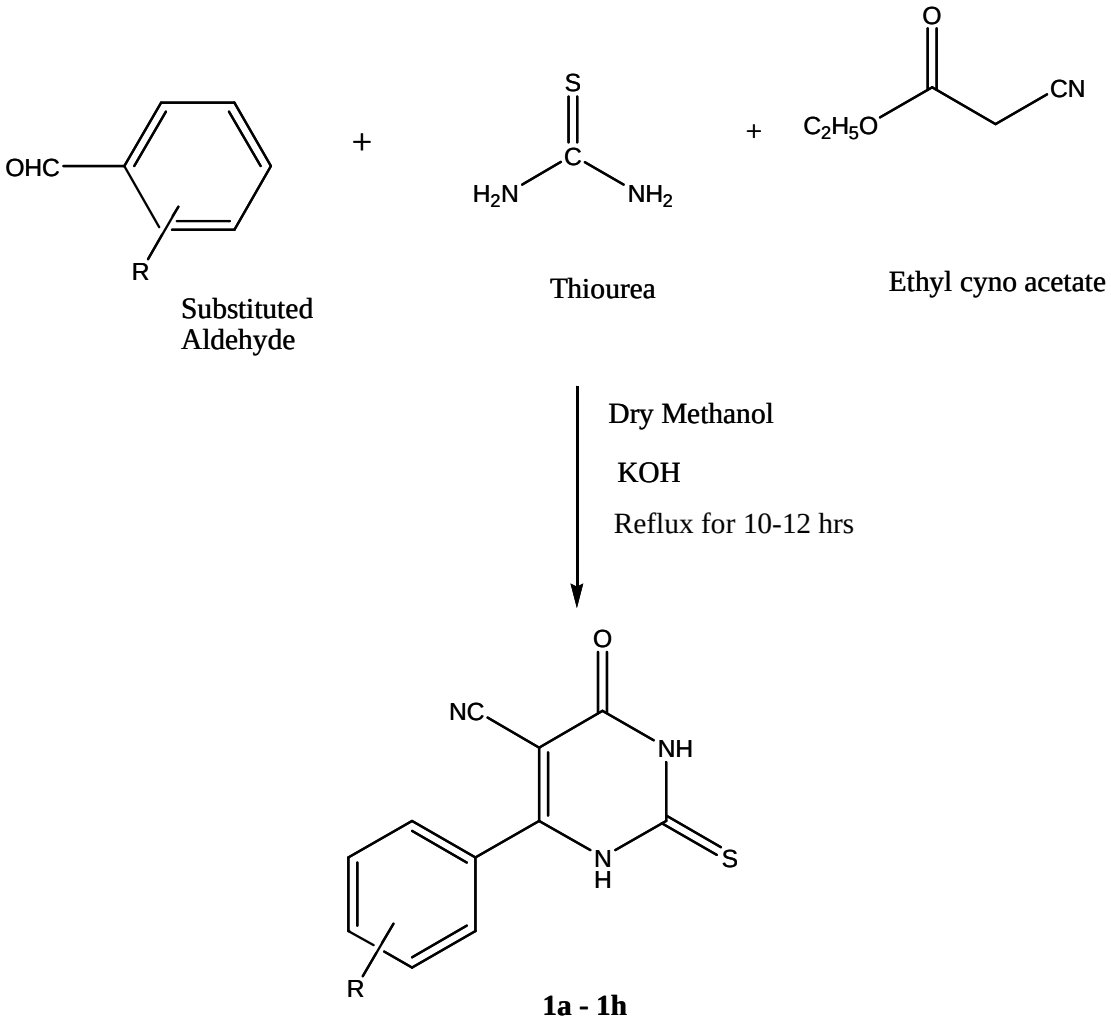
EXPERIMENTAL SECTION

1. Chemical Studies
2. Biological Study

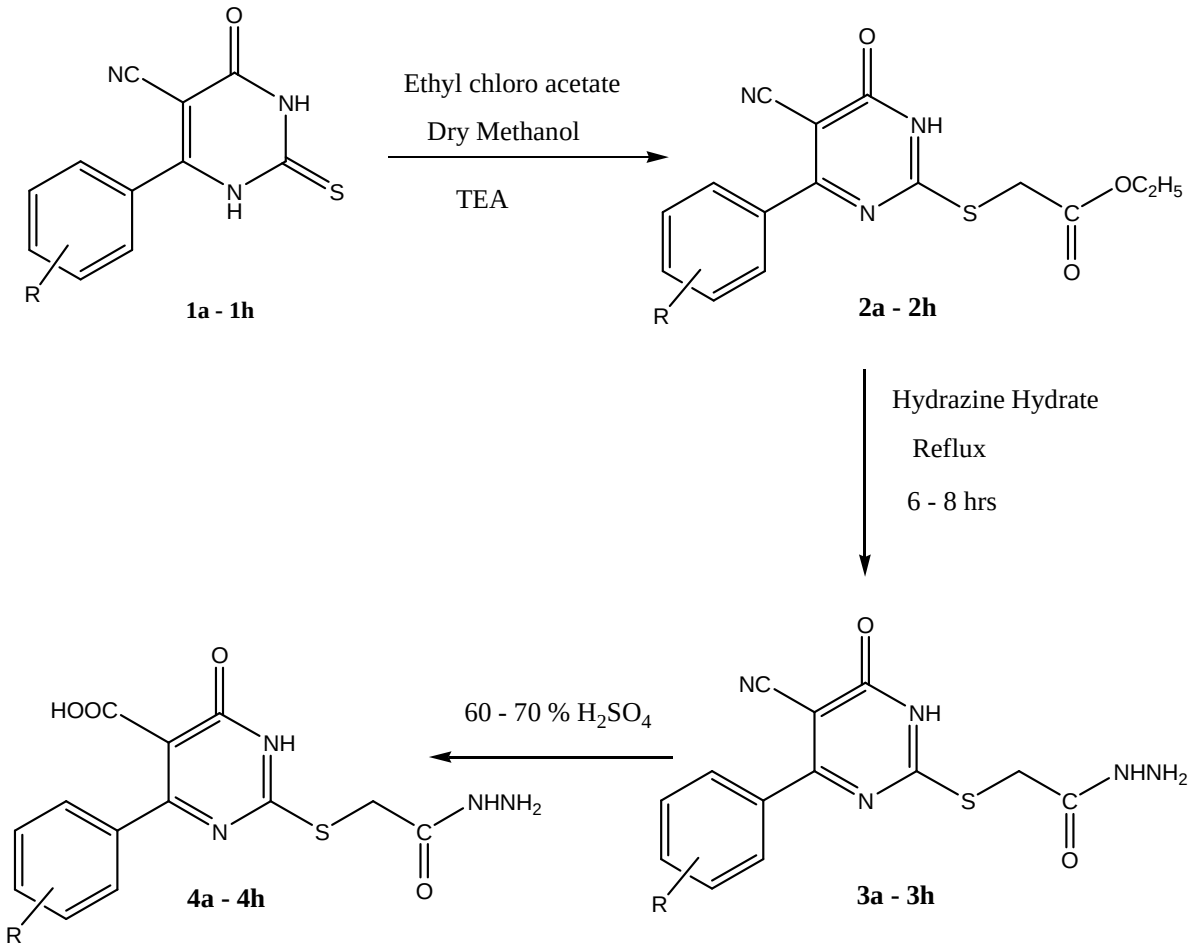
1. Chemical studies

All synthesized compounds were characterized by IR spectra (wave number in cm^{-1}) which was taken by using an ALPHA T BRUKER 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. All reagent used were of analytical grade, obtained from S.D fine chemicals, Spectrochem and Qualigens.

SCHEME:



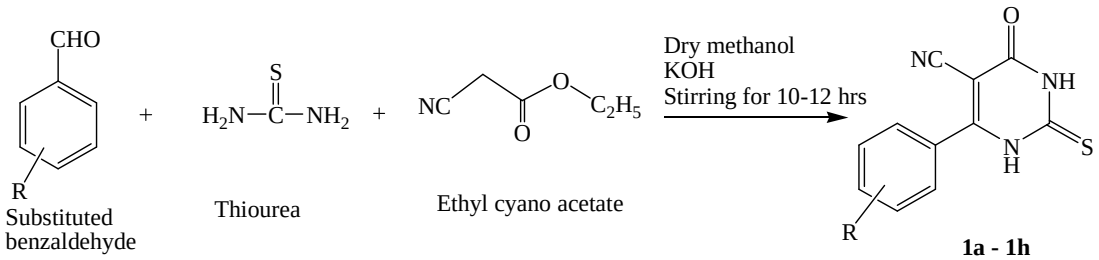
1a - 1h



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

Synthesis of 6-Aryl substituted-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydro pyrimidine-5- carbonitrile (1a – 1h)

The aryl aldehyde (0.033 mole), ethyl cyanoacetate (0.033 mole), thiourea (0.039 mole), potassium hydroxide (0.033 mole) and dry methanol (q.s.) was refluxed on a water bath for 10-12 hrs and reaction was monitored by TLC. After completion of reaction, reaction mixture was allowed to cool on ice-bath to obtain a solid which was then filtered. Collected solid was dissolved in hot water and neutralized with glacial acetic acid. Obtained precipitates were filtered out, washed with water and dried. Crude product was recrystallized from methanol and purity of compounds were confirmed by TLC and Melting Point.



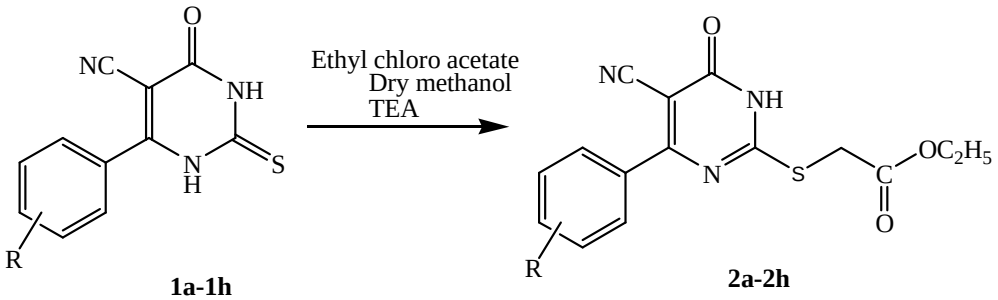
Sr. No	a	b	c	d	e	f	g	h
R	-3 NO_2	-4NO_2	-4 Cl	-3,4 OCH_3	-4 OCH_3	-4 CH_3	H	-4 F

Table: 1 Physicochemical properties of compound 1a – 1h

Sr. no.	Compound No	Mol. Formula	Mol.Wt. gm/mol	% Yield	R_f	IR (cm^{-1})
1	1a	$C_{11}H_6N_4O_3S$	274.51	73	0.46	-C=O (amide) ,1669 cm^{-1} -NO_2 ,1354 &1518 cm^{-1} -CH(aromatic), 3039 cm^{-1} -CN, 2210 cm^{-1} -C=S, 1118 cm^{-1}
2	1b	$C_{11}H_6N_4O_3S$	274.51	70	0.48	-C=O (amide) ,1645 cm^{-1} -NO_2 ,1350 &1536 cm^{-1} -CH(aromatic), 2997 cm^{-1} -CN, 2211 cm^{-1} -C=S, 1123 cm^{-1}
3	1c	$C_{11}H_6ClN_3OS$	263.45	72	0.52	-C=O (amide),1649 cm^{-1} C-Cl, 807 cm^{-1} -CH(aromatic), 3089 cm^{-1} -CN, 2235 cm^{-1} -C=S, 1118 cm^{-1}
4	1d	$C_{13}H_{10}N_3O_3S$	289.65	78	0.48	-C=O (amide) ,1680 cm^{-1} -C-O,1232 cm^{-1} -CH(aromatic), 3024 cm^{-1} -CN, 2218 cm^{-1} -C=S, 1093 cm^{-1}
5	1e	$C_{12}H_8N_3O_2S$	259.21	75	0.45	-C=O (amide) ,1644 cm^{-1} -C-O,1362 cm^{-1} -CH(aromatic), 2998 cm^{-1} -CN, 2217 cm^{-1} -C=S, 1139 cm^{-1}
6	1f	$C_{12}H_8N_3OS$	243.67	74	0.5	-C=O (amide) ,1679 cm^{-1} -CH(aromatic), 3012 cm^{-1} -CN, 2223 cm^{-1} -C=S, 1120 cm^{-1}
7	1g	$C_{11}H_7N_3OS$	229.43	74	0.54	C=O (amide),1668 cm^{-1} -CH(aromatic), 2998 cm^{-1} -CN, 2235 cm^{-1} -C=S, 1115 cm^{-1}
8	1h	$C_{11}H_6FN_3OS$	247.25	68	0.42	-C=O (amide) ,1649 cm^{-1} -CH(aromatic), 3089 cm^{-1} -CN, 2235 cm^{-1} -C=S, 1118 cm^{-1}

Synthesis of 4-Aryl substituted-ethyl-2-(5-cyano-1,6-dihydro-6-oxo-pyrimidine-2-ylthio) acetate (2a – 2h)

Synthesis of 4-Aryl substituted-ethyl-2-(5-cyano-1,6-dihydro-6-oxopyrimidine-2-ylthio)acetate**(2a-2h)** by compound **(1a-1h)** (0.0072mole) was dissolved in methanol and cooling condition was maintained at 0-5°C with continuous stirring then after 10-15 min, TEA (0.0086 mole) was added slowly, preferably drop by drop with the help of dropping funnel followed by drop wise addition of ethyl chloroacetate (0.0072 mole) over a period of 30 min. Further, cooling condition was maintained for additional 3 hours and then put the reaction at room temperature for further 3hr with stirring. The completion of reaction was confirmed by TLC and Recrystallized from methanol.



1a-1h **2a-2h**

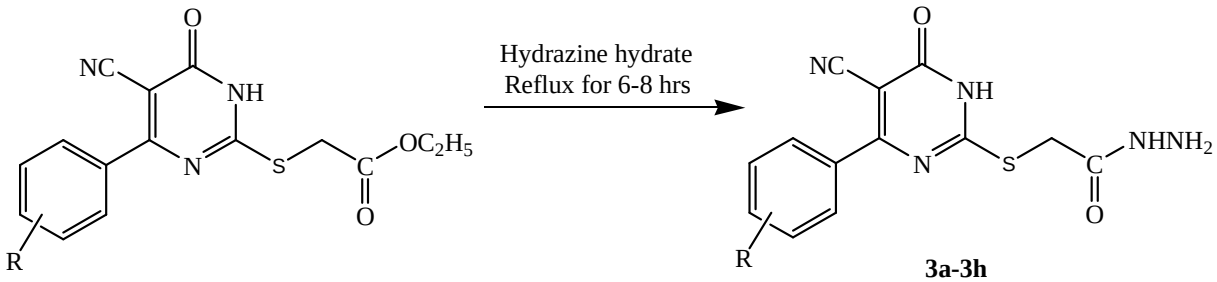
Sr. No	a	b	c	d	e	f	g	h
R	-3 NO_2	-4NO_2	-4 Cl	-3,4 OCH_3	-4 OCH_3	-4 CH_3	H	-4 F

Table: 2 Physicochemical properties of compound 2a – 2h

Sr no.	Compound No	Mol. Formula	Mol.Wt. gm/mol	% Yield	R_f	IR (cm^{-1})
1	2a	$C_{15}H_{12}N_4O_5S$	360.52	62.7	0.65	-C=O(ester) , 1739 cm^{-1} -C=O(amide) , 1663 cm^{-1} -CN, 2224 cm^{-1} -CH(aromatic) , 2992 cm^{-1}
2	2b	$C_{15}H_{12}N_4O_5S$	360.52	67.8	0.6	-C=O(ester) , 1732 cm^{-1} -C=O(amide) , 1663 cm^{-1} -CN, 2213 cm^{-1} -CH(aromatic) , 2981 cm^{-1}
3	2c	$C_{15}H_{12}ClN_3O_3S$	349.30	65	0.5	-C=O(ester) , 1740 cm^{-1} -C=O(amide), 1661 cm^{-1} -CN, 2219 cm^{-1} -CH(aromatic) , 2985 cm^{-1}
4	2d	$C_{17}H_{17}N_3O_5S$	375.45	70	0.48	-C=O(ester) , 1742 cm^{-1} -C=O(amide) , 1634 cm^{-1} -CN, 2209cm^{-1} -CH(aromatic) , 3007 cm^{-1}
5	2e	$C_{16}H_{15}N_3O_4S$	345.67	72	0.46	-C=O(ester) , 1743 cm^{-1} -C=O(amide) , 1653 cm^{-1} -CN, 2220cm^{-1} -CH(aromatic) , 2981 cm^{-1}
6	2f	$C_{16}H_{15}N_3O_3S$	329.21	67.3	0.5	-C=O(ester) , 1732 cm^{-1} -C=O(amide) , 1698 cm^{-1} -CN, 2210 cm^{-1} -CH(aromatic) , 2980 cm^{-1}
7	2g	$C_{15}H_{13}N_3O_3S$	315.37	65	0.42	-C=O(ester) , 1744 cm^{-1} -C=O(amide) , 1648 cm^{-1} -CN, 2207 cm^{-1} -CH(aromatic) , 2982 cm^{-1}
8	2h	$C_{15}H_{12}FN_3O_3S$	333.34	58	0.38	-C=O(ester) , 1735 cm^{-1} -C=O(amide) , 1652 cm^{-1} -CN, 2221 cm^{-1} -CH(aromatic) , 2998 cm^{-1}

Synthesis of 4-Aryl substituted 2-(5-cyano-1,6–dihydro-6-oxopyrimidine)-2-ylthio hydrazide (3a – 3h)

The compound **(2a-2h)** (0.0055 mole) was dissolved in methanol and refluxed for 10-15 min and then hydrazine hydrate (99%) (0.0094 mole) was added and then refluxed for 7-8 hrs. The completion of reaction was confirmed by TLC. After completion of reaction, methanol was evaporated to obtain solid which was recrystallized from methanol.



2a-2h **3a-3h**

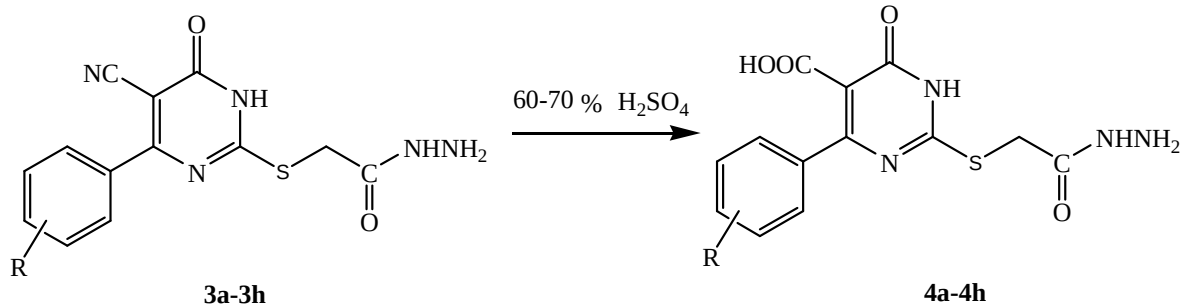
Sr. No	a	b	c	d	e	f	g	h
R	-3 NO_2	-4NO_2	-4 Cl	-3,4 OCH_3	-4 OCH_3	-4 CH_3	H	-4 F

Table: 3 Physicochemical properties of compound 3a – 3h

Sr no.	Compound No	Mol. Formula	Mol. Wt. gm/mol	% Yield	R_f	IR (cm^{-1})
1	3a	$C_{13}H_{10}N_6O_4S$	346.20	53	0.65	-NH_2, 3281,3288 cm^{-1} -C=O, 1680 cm^{-1} -CN , 2202 cm^{-1} -CH(aromatic),2916 cm^{-1}
2	3b	$C_{13}H_{10}N_6O_4S$	346.20	52.5	0.65	-NH_2, 3287,3295 cm^{-1} -C=O, 1682 cm^{-1} -CN , 2213 cm^{-1} -CH(aromatic),2922 cm^{-1}
3	3c	$C_{13}H_{10}ClN_5O_2S$	335.11	50	0.7	-NH_2, 3279,3287 cm^{-1} -C=O, 1684 cm^{-1} -CN , 2204 cm^{-1} -CH(aromatic),3110 cm^{-1} -C=C, 1592 cm^{-1}
4	3d	$C_{15}H_{15}N_5O_4S$	361.4	54.3	0.6	-NH_2, 3285,3393 cm^{-1} -C=O, 1678 cm^{-1} -CN , 2202 cm^{-1} -CH(aromatic),2917 cm^{-1} -C=C, 1601 cm^{-1}
5	3e	$C_{14}H_{13}N_5O_3S$	331.52	55	0.62	-NH_2, 3234,3242 cm^{-1} -C=O, 1667 cm^{-1} -CN , 2213 cm^{-1} -CH(aromatic),2996 cm^{-1}
6	3f	$C_{14}H_{13}N_5O_2S$	315.75	52.4	0.63	-NH_2, 3310,3316 cm^{-1} -C=O, 1665 cm^{-1} -CN , 2209 cm^{-1} -CH(aromatic),2982 cm^{-1}
7	3g	$C_{13}H_{11}N_5O_2S$	301.28	56	0.57	-NH_2, 3289,3282 cm^{-1} -C=O, 1677s cm^{-1} -CN , 2200 cm^{-1} -CH(aromatic),3111 cm^{-1}
8	3h	$C_{13}H_{10}FN_5O_2S$	319.32	62	0.48	-NH_2, 3282,3275 cm^{-1} -C=O, 1668s cm^{-1} -CN , 2210 cm^{-1} -CH(aromatic),3050 cm^{-1}

Synthesis of 4-Aryl substituted-2-(5-carboxylic acid-1,6-dihydro-6-oxo-pyrimidine)-2-ylthio hydrazide (4a – 4h)

The synthesized compound **(3a-3h)** was dissolved in methanol and then conc. H_2SO_4 (70-80%) was added drop wise and refluxed for 2-3 hrs. The completion of reaction was confirmed by TLC. After completion of reaction, basify the reaction mixture with NaOH solution and precipitated obtained was filtered to obtain solid product which was recrystallized from methanol.



3a-3h **4a-4h**

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

Table: 4 Physicochemical properties of compound 4a – 4h

Sr no.	Compound No	Mol. Formula	Mol. Wt. g/mol	% Yield	R_f	Melting Point (°C)
1	4a	$C_{13}H_{11}N_5O_6S$	365	59.2	0.7	300-304
2	4b	$C_{13}H_{11}N_5O_6S$	365	50	0.71	302-306
3	4c	$C_{13}H_{11}ClN_4O_4S$	354	52.4	0.7	284-286
4	4d	$C_{15}H_{16}N_4O_6S$	380	50	0.6	280-285
5	4e	$C_{14}H_{14}N_4O_5S$	350	54	0.62	296-298
6	4f	$C_{14}H_{14}N_4O_4S$	334	53	0.64	290-292
7	4g	$C_{13}H_{12}N_4O_4S$	320	53.3	0.61	280-284
8	4h	$C_{13}H_{11}FN_4O_4S$	338	51	058	288-292

2. Biological study

The synthesized compounds were evaluated for anti-diabetic activity using Calbiochem® PTP1B colorimetric assay kit and The PTP-1B inhibitor assay suramin is taken as a control [11-12].

Table: 5 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

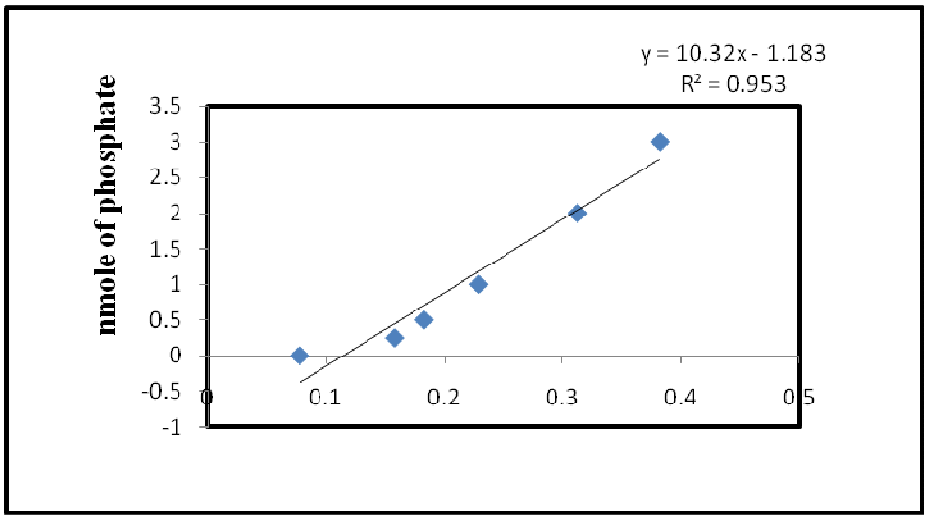


Fig.1 Phosphate Standard Curve

Y (n mole of phosphate) =10.32(Abs. X) –1.185
R^2= 0.961

RESULTS AND DISCUSSION

Table: 6 Spectral data of final compound 4a – 4h

Sr No.	Compound No.	IR Stretching (cm^{-1})	^1H NMR δ (ppm)	MASS (m/z)
1	**4a**	-C=O (amide),1641 -C=O (acid), 1739 -OH, 3474 -CH (Aromatic), 2981 -NO_2, 1568 & 1352	2.2-2.5 (d, 2H, NH_2), 3.26 (s, 2H, CH_2), 8.16 (s, 1H, NH), 8.02-8.12 (t, H, NH), 7.2-7.4 (d, 3H, Ar-H), 7.48 (s, 1H Ar-H), 10.24(s, 1H, COOH).	366 (M^+)
2	**4b**	-C=O (amide), 1653 -C=O (acid), 1730 -OH, 3637 -CH (Aromatic), 2984 -NO_2 , 1364,1554	2.0 (d, 2H, NH_2), 3.76 (s, 2H, CH_2), 8.0 (s, 1H, NH), 8.2 (t, 1H, NH), 7.54-7.89 (d, 4H, Ar-H), 11.6 (s, 1H, COOH).	366 (M^+)
3	**4c**	-C=O (amide), 1648 -C=O (acid), 1734 -OH, 3530 -CH (Aromatic),2992	2.2 (d, 2H, NH_2), 3.70 (s, 2H, CH_2), 8.1 (s, 1H, NH), 8.0 (t, 1H, NH), 7.22-7.35 (d, 4H, Ar-H), 10.9 (s, 1H, COOH).	354 (M^+) 356 (M+2)
4	**4d**	-C=O (amide), 1642 -C=O (acid), 1739 -OH, 3437 -CH (Aromatic), 2922 C-O,1255	2.4 (d, 2H, NH_2), 3.6 (s, 2H, CH_2),6.79(d,1H,Ar-H), 6.61(t,2H,Ar-H), 3.03 (s,3H,-OCH_3),3.73(s,3H,-OCH_3), 6.70(s,1H,Ar-H),11.0(s,1H,-COOH),8.0 (s,1H,-NH)	381 (M^+)
5	**4e**	-C=O (amide), 1643 -C=O (acid), 1731 -OH, 3457 -CH (Aromatic), 2947 C-O,1245	2.1 (d, 2H, NH_2), 3.7 (s, 2H, CH_2), 3.03 (s, 3H,-OCH_3), 8.0 (s, 1H, NH), 8.2 (t, 1H, NH), 7.54-7.89 (d, 4H, Ar-H), 11.9 (s, 1H, COOH).	351 (M^+)
6	**4f**	-C=O (amide), 1653 -C=O (acid), 1725 -OH, 3637 -CH (Aromatic), 2953	2.1 (d, 2H, NH_2), 3.7 (s, 2H, CH_2), 7.01-7.18(d,4H,Ar-H), 2.35(s,3H,-CH_3),10.8 (s,1H,-OH), 8.0(s,1H,-NH)	335 (M^+)
7	**4g**	-C=O (amide), 1673 -C=O (acid), 1734 -OH, 3500 -CH (Aromatic), 3010	2.5 (d, 2H, NH_2), 3.6 (s, 2H, CH_2), 7.28-7.37(d,2H,Ar-H), 7.11-7.22 (t,3H,Ar-H), 10.7 (s,1H,-COOH), 8.0(s,1H,-NH);	321 (M^{+1})
8	**4h**	C=O (amide) 1653, -C=O (acid) 1730, -OH, 3637 -CH (Aromatic) 2984	2.6 (d, 2H, NH_2), 3.75 (s, 2H, CH_2), 8.10 (s, 1H, NH), 8.25 (t, 1H, NH), 7.55-7.82 (d, 4H, Ar-H), 11.7 (s, 1H, COOH)	338.05 (M^{+1}) 340 (M+2)

Table: 7 % Inhibition as compared to Time zero and Suramin of compound 4a-4h

Compound no	Absorbance 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
4a	0.102	0.5012	35.72	43.2	125
4b	0.099	0.50	38.05	46.02	125
4c	**0.089**	**0.3771**	**50.89**	**61.17**	125
4d	**0.075**	**0.3680**	**71.48**	**86.42**	125
4e	**0.073**	**0.2535**	**73.85**	**88.80**	125
4f	**0.071**	**0.2238**	**74.84**	**89.90**	125
4g	0.130	0.7935	23.59	28.37	125
4h	0.091	0.4278	55.89	51.29	125
Time Zero	0.066	0.1872	100	120.25	-----
4a	0.100	0.5093	36.76	44.2	250
4b	0.125	0.7461	25.09	30.17	250
4c	**0.083**	**0.3483**	**53.75**	**64.63**	250
4d	**0.072**	**0.2451**	**80.16**	**97.21**	250
4e	**0.069**	**0.2132**	**84.21**	**101**	250
4f	**0.073**	**0.2535**	**73.85**	**87.82**	250
4g	0.112	0.5378	43.35	40.19	250
4h	0.087	0.3532	52.75	58.63	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 **4d,4e,4f** 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 **4c,4h** which is **chloro** and **fluoro** 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.

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