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Graphical Abstract

Synthesis and antihyperglycemic evaluation of new 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids having pyrazolyl pharmacophores

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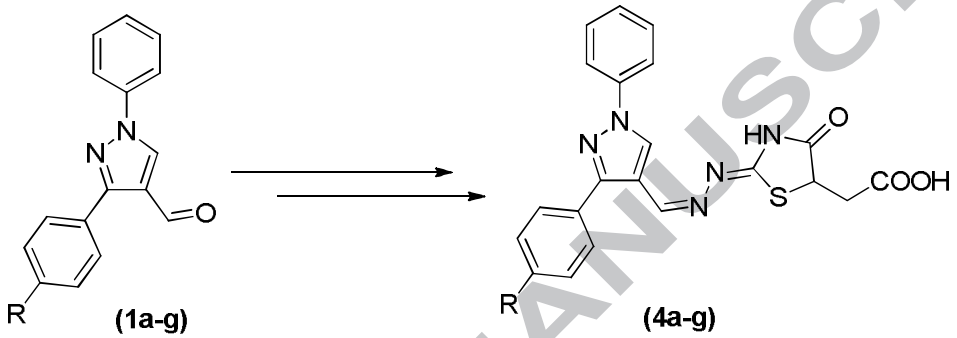

Synthesis and antihyperglycemic evaluation of new 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids having pyrazolyl pharmacophores

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Abstract

In the search of new antihyperglycemic agents and following rational approach of drug designing here new 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids (**4a–g)** with pyrazolyl pharmacophore have been synthesized *via* thia Michael addition reaction of 1-((3-(4-substituted phenyl)-1-phenyl-*1H*-pyrazol-4-yl)methylene)thiosemicarbazides (**3a-g**) with malic anhydride. The required precursors, (**3a-g**) were obtained by condensing known 3-(4-substitutedphenyl)-1-phenyl-*1H*-pyrazole-4-carbaldehydes (**1a-g**) with thiosemicarbazide in ethanol. The newly synthesized compounds (**4a–g)** have been evaluated for the antihyperglycemic activity in sucrose loaded rat model and among these compounds **4d, 4f** and **4g** have displayed significant antihyperglycemic activity.

Keywords: Type 2 Diabetes mellitus, Oral Sucrose Tolerance Test**,** antihyperglycemic activity, 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids, pyrazole

Type 2 diabetes mellitus (T2DM) is most prevalent and serious disease in the world.[1] T2DM is also responsible for morbidity, mortality and overall health problems in the developed and fast developing countries.[2] Continuous quest is found to be directed in search of effective and safer antihyperglycemic/antidiabetic agents for treating T2DM. It has been observed that several thiazolidinediones (TZDs) are established as clinical agents for treating and controlling type 2 DM. The thiazolidinediones containing medicaments presently used are pioglitazone,[3] and rosiglitazone.[4] Although these synthetic drugs have shown significant therapeutic potential but each one of them is accompanying with one or other kind of side effects.[5]

Literature survey reveals that more attention has been found to synthesize 2,4-TZDs bearing five/six membered biodynamic heterocyclic moieties for obtaining synergetic antihyperglycemic activity. The acidic functionality of the TZD ring is considered essential for its binding to PPARγ.[6] Thiazolidine-2,4-dione having carboxylic ester appendage at N-3 has also been reported to have antihyperglycemic activity.[7] Recently the saroglitazar has been launched as antihyperglycemic agent for type 2 DM having noncyclic ethoxy phenyl propanoic acid as one of the functionality in its molecular frame work.[8]

The study of pyrazoles has become of much interest. A systematic investigation of this class of compounds revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry and displayed anti-inflammatory, antibacterial, antifungal, antiviral, antitubercular, antioxidant and antiandrogenic activities.[9-15] It has been also reported that some of the pyrazole derivatives are emerging as potential antihyperglycemic agents.[16] According to literature survey, there are no reports on the designing of the molecules having 2,4-thiazolidinediones-5-carboxylic acid and pyrazole ring system in one molecular framework.

Keeping in view the multifarious applications of pyrazoles, carboxy thiazolidinediones and in continuation of our earlier interest in designing and synthesizing new analogues of antidiabetic agents, 2,4-thiazolidinediones[17] here new 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids has been synthesized from readily available reactants with hope to obtain the titled, 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids with enhanced antihyperglycemic activity with minimal side effects.

The multistep synthetic route (**Scheme 1**) for the desired/titled compounds has been developed starting from 4-formyl pyrazoles. Formyl pyrazoles, 3-(4-substitutedphenyl)-1-phenyl-*1H*-pyrazole-4-carbaldehydes (**1a-g**) were synthesized using reported procedure[18] i.e. carrying Vilsmeier Haack reaction of phenyl/aryl hydrazones with $POCl_3$ and DMF. Phenyl hydrazones were obtained by condensing substituted acetophenones and phenyl hydrazine in ethanol. Thus obtained pyrazole aldehydes (**1a-g**) were then condensed with thiosemicarbazide (**2**) in ethanol in presence of desiccant, molecular sieves under reflux for 1h and obtained 1-((3-(4-substituted phenyl)-1-phenyl-*1H*-pyrazol-4-yl)methylene)thiosemicarbazides (**3a-g**) with good to excellent yields (**Table 1**). The pyrazolyl thiosemicarbazones (**3a-g**) when allowed to react with maleic anhydride in refluxed toluene gave new 2-(4-(1-phenyl-3-p-substituted-

pyrazol-4-yl) methylene-2-hydrazolyl)-1,3-thiazolidin-5-yl-acetic acids (**4a-g**), by thia Michael addition reaction (**Scheme 1, Table 1**).

The structures of the synthesized compounds have been confirmed from IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. ^{1}H NMR spectrum of compound **4a** indicates the formation of the product as it shows a characteristic triplet at δ 4.42 ppm which corresponds to the methine proton of the 4-thiazolidinone ring. Methylene protons of the propionic acid group give signal as pair of doublet at δ 2.97-3.34 ppm. A broad singlet was appeared at 12.35 ppm for carboxylic proton. The presence of four characteristics carbon signals are observed at δ 36.80, 43.67, 171.73 and 175.40 ppm in ^{13}C NMR spectrum of **4a** due to the carbon of methylene (CH_2-COOH), methine (-CH-), carbonyl (-NH-C=O) and carbonyl of carboxylic acid (-COOH) groups, respectively which confirms the presence of 4-thiazolidinone ring with carboxylic acid in the product. The mass spectrum further provided support to the structure, assigned 2-(4-(1-phenyl-3--pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4a**) as displays $(M+H)^+$ ion peak at m/z 420 for the molecular formula $C_{21}H_{17}N_5O_3$.

Antihyperglycemic activity evaluation: The compounds (**4a-g**) were evaluated for their antihyperglycemic activity in male albino rats of Sprague Dawley strain.[19]

Procurement and Selection of Animals: Male albino rats of Sprague Dawley strain (8 to 10 weeks of age: body weight range 160 ± 20 g) were procured from National Laboratory Animal Centre (NLAC) of the Institute. All the experiments on animals were conducted in accordance to instructions of Institute Animal Ethics Committee.

Improvement on Oral Sucrose Tolerance Test (OSTT) in normal rats: The overnight fasted rats showing blood glucose level between 60 to 80 mg/dl measured by glucometer using glucostrips were finally selected, divided into groups. Each consisted of five animals each. Rats of experimental groups were respectively administered the suspensions of the test samples orally prepared in 1.0 % gum acacia (vehicle) at pre-selected dose levels i.e. 100 mg/kg. The standard antidiabetic drug i.e. Metformin was administered at 100 mg/kg dose levels. Animals of control group were given an equal amount of 1.0 % gum acacia and termed as sham treated control. An oral sucrose load of 10g/kg body weight was given to each animal exactly after 30 min post administration of the test sample/vehicle. Blood glucose of each rat was again determined from the tail vein at 30, 60, 90 and 120 min post administration of sucrose by glucostrips. Quantitative glucose tolerance of each animal was calculated by Area under curve (AUC) method (Prism

Software). The average fall in AUC of experimental group compared to control group was always termed as % improvement on oral sucrose tolerance test (OSTT) post sucrose load and also termed as % antihyperglycemic activity in normal rats. As compounds **4a-g**, are thiazolidinone derivatives. The mode of action thiazolidinediones is to increase the insulin sensitivity by acting on adipose, muscle, and liver to increase glucose utilization and decrease glucose production [21-23] as well as metformin also decreases hyperglycemia by suppressing glucose production by the liver (hepatic gluconeogenesis).[24] Therefore, for the study of antihyperglycemic activity of selected compounds, we used metformin as a positive control.

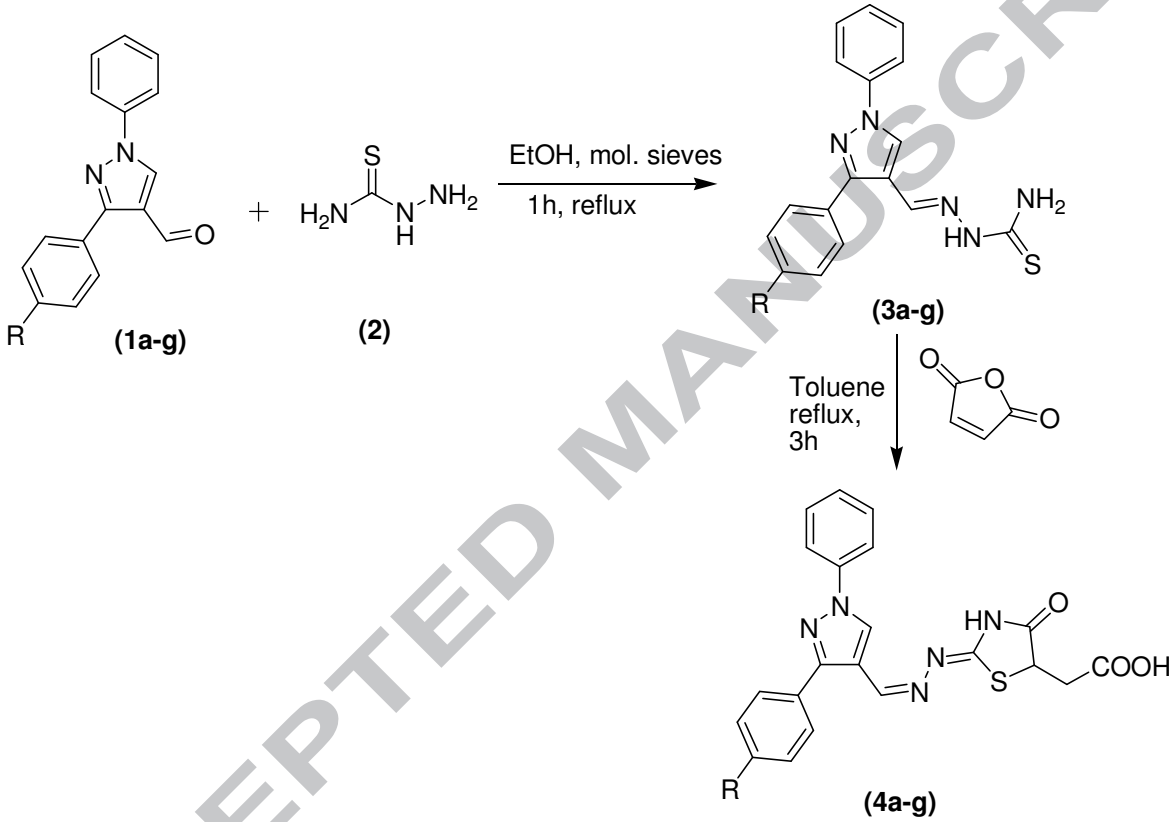
Statistical Analysis: Analysis of statistical significance of differences in measurements between samples was done by using Dunnet's test following ANOVA software. It is denoted by p values. Statistically Significance difference was set at following levels * represents $p< 0.05$, ** represents $p< 0.01$ and *** represents $p< 0.001$.

Effect of test samples in normal rats: **Table 2** and **Fig 1** Represents the average blood glucose profile of compound code **4a-4g** on the improvement of oral glucose tolerance post sucrose load in normal rats. Out of 7 compounds tested, compound **4d**, **4f** and **4g** showed significant reduction on OSTT to the tune of 7.64% ($p<0.05$), 7.57($p<0.05$) and 7.97% ($p<0.05$), While the standard antidiabetic drug, metformin showed improvement on OSTT by 14.1% ($p<0.01$) at 100 mg/kg in normal rats. Among these **4a-g** compounds **4d**, **4f** and **4g** have considerably reduce the blood glucose level compare to **4a** in normal rats. These compounds are having additional active pharmacophores like fluro, nitro and hydroxyl on one of the benzonoid ring that probably be enhancing acidic behavior through conjugation/mesomeric effect of the 2,4-TZD scaffold, which may be responsible for their significant activity.

In conclusion we have synthesized new 2-hydrazolyl-4-thiazolidinone-5-carboxylic acids with pyrazolyl moiety. The newly synthesized compounds **4a-g** has been evaluated for the antihyperglycemic activity by Oral Sucrose Tolerance Test (OSTT) in normal rat model. Among these compounds **4d**, **4f** and **4g** have displayed significant reduction on OSTT.

All the chemicals used were of laboratory grade. Melting points of all the synthesized compounds were determined in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 400 spectrometer operating at 300 MHz, 200 MHz and 75 MHz using DMSO-d_6 or $CDCl_3$ solvent and tetramethylsilane (TMS) as the internal

standard and chemical shift in δ ppm. Mass spectra were recorded on a Sciex, Model; API 3000 LCMS/MS Instrument. Elemental analyses were done on EA1108 (Carlo-Erba). The purity of each compound was checked by TLC using silica-gel, $60F_{254}$ aluminum sheets as adsorbent and visualization was accomplished by iodine/ultraviolet light. Metformin and STZ were purchased from Sigma Aldrich Co., USA. One touch glucometer and glucostrips was purchased from Roche Diagnostics India Ltd.



Scheme 1 Synthesis of 2-(4-(1-phenyl-3-p-substituted-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acids

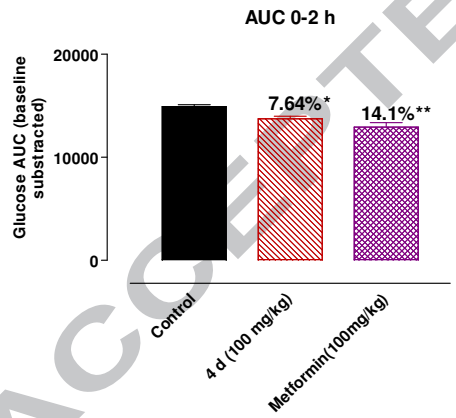
Table 1: Physical data of 1-((3-(4-substituted phenyl)-1-phenyl-*1H*-pyrazol-4-yl)methylene) thiosemicarbazides (**3a-g**) and 2-(4-(1-phenyl-3-p-substituted-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acids (**4a-g**).

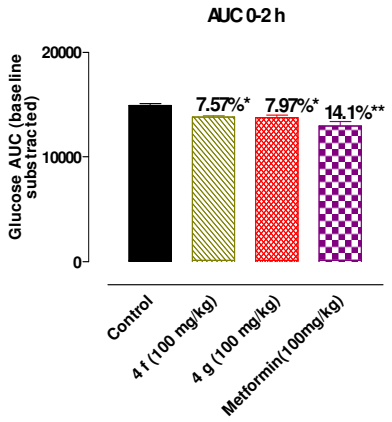
Entry	Compound	R	Yield (%)[b]	Melting Point (°C)
1	**3a**	-H	92	210-212
2	**3b**	$-CH_3$	86	201-202
3	**3c**	$-OCH_3$	91	162-163
4	**3d**	-F	94	218-220
5	**3e**	-Br	87	214-216
6	**3f**	$-NO_2$	91	310-312
7	**3g**	-OH	82	215-217
8	**4a**	-H	85	236-238
9	**4b**	$-CH_3$	96	230-232
10	**4c**	$-OCH_3$	80	242-243
11	**4d**	-F	82	249-250
12	**4e**	-Br	71	200-202
13	**4f**	$-NO_2$	78	260-262
14	**4g**	-OH	80	224-226

[b]Isolated Yield

Table 2: Effect of compound code 4a-4g and standard antidiabetic drug metformin on the improvement of Oral Sucrose Tolerance Test (OSTT) in normal rats.

Compound	Dose (mg/dL)	% improvement on OSTT	Significance
4a	100	Nil	-
4b	100	6.09	p<0.05
4c	100	6.97	p<0.05
4d	100	**7.64**	p<0.05
4e	100	5.69	p<0.05
4f	100	**7.57**	p<0.05
4g	100	**7.97**	p<0.05
Metformin	100	**14.1**	p<0.01





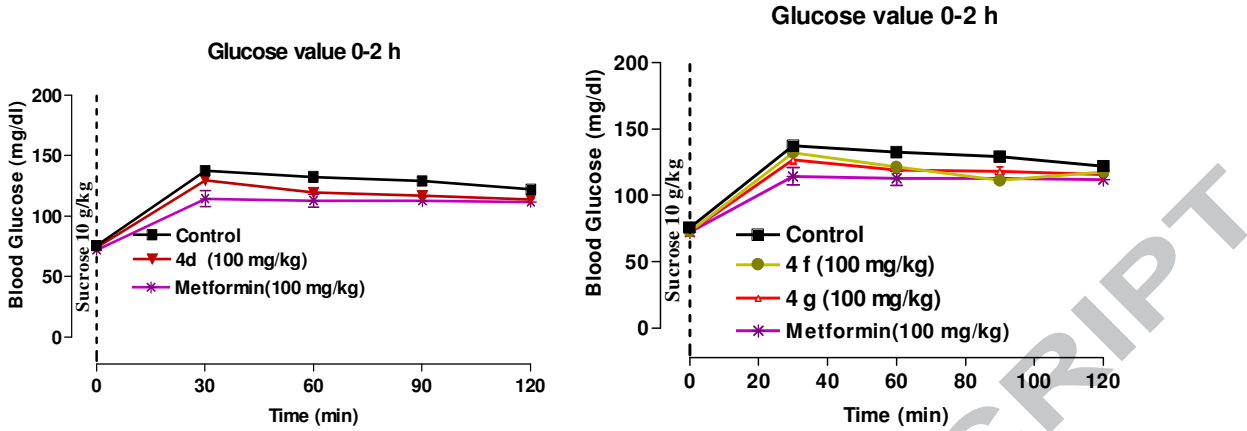


Fig:1 Effect of compounds code **4d**, **4f**, **4g** and standard antidiabetic drug metformin on the improvement of Oral Sucrose Tolerance Test (OSTT) in normal rats.

Acknowledgments

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20. *Synthesis of 1-((3-(4-phenyl)-1-phenyl-1H-pyrazol-4-yl) methylene) thiosemicarbazide* **(3a)** A mixture of 3-(4-phenyl)-1-phenyl-*1H*-pyrazole-4-carbaldehyde (**1a**) (10 mmol) and thiosemicarbazide (**2**) (10 mmol) was refluxed in ethanol in the presence of activated molecular sieves of size $4A^0$ (10 g). After 1h of reflux the reaction mixture was filtered to remove molecular sieves. Ethanol was removed under vacuum. The residual mass was poured on crushed ice. The solid obtained was filtered and washed with water. The crude solid was purified by crystallization. Similarly the other compounds, (**3b-g)** of the series were prepared. The melting points and the yields of the derivatives are recorded in **Table 1** 1-((3-(4-phenyl)-1-phenyl-*1H*-pyrazol-4-yl)methylene)thiosemicarbazide (3a). IR (KBr, ν cm^{-1}): 3410, 3100, 1597, 1590, 1334 and 1250. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 7.55 (s, 1H, -CH=N-), 8.01 (s, 1H, pyrazoline-H), 8.29-8.77 (m, 10H, Ar-H), 9.10 (s, 1H, NH) 9.36 (s, 1H, NH, tautomeric) and 11.73 (s, 1H, SH, enolic). MS (ESI^+, m/z): 322 (M^+).

Synthesis of 2-(4-(1-phenyl-3--pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid **(4a):** 1-((3-(4-substituted phenyl)-1-phenyl-1H-pyrazol-4-yl)methylene) thiosemicarbazide (**3a**) (3 mmol) and maleic anhydride (4 mmol) were refluxed in toluene. Thia Michael addition was monitored by thin layer chromatography. After 3h of reflux the reaction mixture was cooled. Excess toluene was removed on rota evaporator. The residual mass was poured on crushed ice and extracted with ethyl acetate. From extract organic medium was removed under reduced pressure. The residual solid obtained was dried and purified by crystallization. Similarly the other compounds, (**4b-g)** of the series were prepared. The melting points and the yields of the derivatives are recorded in **Table 1**

Spectral analysis: 2-(4-(1,3-dipheny--pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4a**): IR (KBr, ν cm^{-1}): 3433, 3271, 2924, 1643, 1720, 1597 and 1215. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.97-3.34 (dd, overlapped, 2H, methylene), 4.42 (t, 1H, methine), 7.23-8.02 (m, 10H, Ar-H), 8.42 (s, 1H), 8.94 (s, 1H), 11.31 (s, 1H, NH) and 12.35 (bs, 1H, -COOH). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 36.80, 43.67, 116.68, 118.85, 127.06, 128.49 (2C), 128.59 (2C), 128.84 (2C), 129.48

(2C), 129.59, 132.00, 138.91, 149.09, 151.64, 171.73 and 175.40. MS (ESI^+ , m/z): 420 (M^+). Elemental Analysis: Anal. Calcd for $C_{21}H_{17}N_5O_3S$: C, 60.13; H, 4.09; N, 16.70; S, 7.64 found C, 60.33; H, 4.64; N, 16.57 and S, 7.61.

2-(4-(1-phenyl-3-4-methylphenyl-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4b**): IR (KBr, ν cm^{-1}): 3446, 3266, 2937, 1635, 1726, 1599 and 1214. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 2.85-3.01 (dd, overlapped, 2H, methylene), 4.37 (t, 1H, methine), 7.30-8.00 (m, 9H, Ar-H), 8.41 (s, 1H), 8.93 (s, 1H), 12.05 (bs, 1H, NH) and 12.40 (bs, 1H, -COOH). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 20.93, 36.85, 43.70, 116.60, 118.84, 127.01, 128.47 (2C), 129.13 (2C), 129.60 (2C), 130.83, 138.01, 138.95, 149.19, 151.71, 162.73, 166.75, 171.77 and 175.44. MS (ESI^+, m/z):434 (M^+). Elemental Analysis: Anal. Calcd for $C_{22}H_{19}N_5O_3S$: C, 60.96; H, 4.42; N, 16.16; S, 7.40 found C, 61.00; H, 4.48; N, 16.20 and S, 7.35.

2-(4-(1-phenyl-3-4-methoxyphenyl-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4c**): IR (KBr, ν cm^{-1}): 3440, 3260, 2930, 1640, 1725, 1597 and 1218. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3), 2.73-3.09 (dd, overlapped, 2H, methylene), 4.39 (t, 1H, methine), 7.05-7.07 (d, 2H), 7.14-7.99 (m, 7H, Ar-H), 8.42 (s, 1H), 8.92 (s, 1H), 12.30 (bs, 1H, NH) and 12.35 (bs, 1H, -COOH). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 36.88, 43.74, 55.20, 113.92, 114.29, 116.44, 118.77 (2C), 124.41 (2C), 126.95 (2C), 139.61, 130.00, 138.96, 149.34, 151.48, 159.58, 162.67, 171.83 and 175.46. MS (ESI^+, m/z): 450 (M^+). Elemental Analysis: Anal. Calcd for $C_{22}H_{19}N_5O_4S$: C, 58.79; H, 4.26; N, 15.58; S, 7.13 found C, 58.60; H, 4.30; N, 15.52 and S, 7.20.

2-(4-(1-phenyl-3-4-flurophenyl-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4d**): IR (KBr, ν cm^{-1}): 3438, 3258, 2935, 1649, 1729, 1592 and 1214. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.86-3.11 (dd, overlapped, 2H, methylene), 4.38 (t, 1H, methine), 7.23-8.20 (m, 10H, Ar-H), 8.94 (s, 1H), 11.31 (s, 1H, NH) and 12.31 (bs, 1H, -COOH). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 36.75, 43.70, 114.23, 115.19, 115.40, 117.17 (2C), 127.09 (2C), 128.58 (2C), 129.61, 130.80, 130.88, 138.85, 150.44, 162.84, 163.49, 171.80 and 175.41. MS (ESI^+, m/z):438 (M^+). Elemental Analysis: Anal. Calcd for $C_{21}H_{16}FN_5O_3S$: C, 57.66; H, 3.69; 4.34; N, 16.01; S, 7.33 found C, 57.60; H, 4.00; N, 16.08 and S, 7.29.

2-(4-(1-phenyl-3-4-nitrophenyl-pyrazol-4-yl) methylene-2-hydrazolyl) -1,3-thiazolidin-5-yl-acetic acid (**4f**): IR (KBr, ν cm^{-1}): 3432, 3260, 2939, 1641, 1730, 1593 and 1220. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.91-3.13 (dd, overlapped, 2H, methylene), 4.39 (t, 1H, methine), 7.36-8.32 (m, 10H, Ar-H), 8.46 (s, 1H), 11.35 (s, 1H, NH) and 12.33 (bs, 1H, -COOH). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 36.73, 43.77, 117.46, 117.98, 118.67, 118.92 (2C), 123.86 (2C), 127.39 (2C), 128.98, 129.67, 131.37, 134.33, 138.79, 148.86, 163.22, 171.75 and 175.40. MS (ESI^+, m/z):465 (M^+). Elemental Analysis: Anal. Calcd for $C_{21}H_{16}N_6O_5S$: C, 54.31; H, 3.47; N, 18.09; S, 6.90 found C, 54.60; H, 3.40; N, 18.04 and S, 6.89.

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