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Synthesis and in-vivo hypolipidemic activity of some novel substituted phenyl isoxazol phenoxy acetic acid derivatives

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ABSTRACT

The present study was undertaken to evaluate in-vivo hypolipidemic activity of a novel series of 2-methyl-2-(substituted phenyl isoxazol)phenoxyacetic acid derivatives by triton induced hyperlipidemia in rats. The newly synthesized compounds **5a**, **5d** and **5g** showed significant decrease in the serum TCH, TG, LDL and VLDL along with an increase in serum HDL levels as compared to standard drug Fenofibrate. The treated groups also showed significant decrease in the atherogenic index and increase in % protective activity compared to control group.

Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream which includes cholesterol, cholesterol esters, phospholipids, and triglycerides. Lipids are transported in the blood as large 'lipoproteins'. Lipoproteins are divided into five classes, based on density: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Most triglyceride is transported in chylomicrons or VLDL, and most cholesterol is carried in LDL and HDL. Hyperlipidemia is a major, modifiable risk factor for atherosclerosis and cardiovascular disease, including coronary heart disease.[1,2]

Fibrates affect lipid metabolism as agonists of enzyme peroxisome proliferator activated receptor alpha (PPAR-α) by lowering TG, LDL and by increasing HDL cholesterol level.[3–5]

The phenoxy acetic acid pharmacophore has been frequently used in synthesis of potent hypolipidemic agent. Phenoxy acetic acid was frequently couple with many heterocyclic nucleus such as pyrimidine, isoxazol, thiazole, morpholine, oxadiazole, indole, benzisoxazol and piperidine[6,7] to have more potent hyperlipidemic activity.

It was observed from literature survey that isoxazol scaffold has never used previously for the development of antihyperlipidemic agent. In view of this, we have attempted the synthesis of isoxazol derivative containing phenoxy acetic acid pharmacophore to have potent hyperlipidemic activity (Fig. 1).

The target compounds 2-methyl-2-(4-(5-(substituted phenyl) isoxazol-3-yl)phenoxy)acetic acid and 2-methyl-2-(4-(3-(substituted phenyl)isoxazol-5-yl)phenoxy)acetic acids (**5a–j**) were synthesized using substituted acetophenones and 4-hydroxyl benzaldehyde or substituted benzaldehydes and 4-hydroxyl acetophenone starting material as outlined in Scheme 1. The substituted hydroxy chalcones (**3a–j**, Table 1) were synthesized using substituted benzaldehydes and 4-hydroxyl acetophenone or substituted acetophenones and 4-hydroxyl benzaldehyde in presence of aq alkali. The substituted isoxazol phenols (**4a–j**, Table 2) were synthesized by cyclization of substituted hydroxy chalcones with hydroxylamine HCl. In this reaction cyclisation takes place due to removal of water molecule to form substituted isoxazol phenols. The final derivatives were obtained by condensing chloro acetic acid with substituted isoxazol phenols (**5a–j**, Table 3). The proposed derivatives were verified by IR, ^{1}H NMR, ^{13}C NMR and LC–MS spectroscopy.

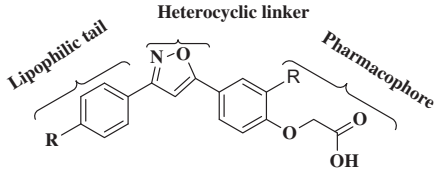

Figure 1. Designed scaffold.

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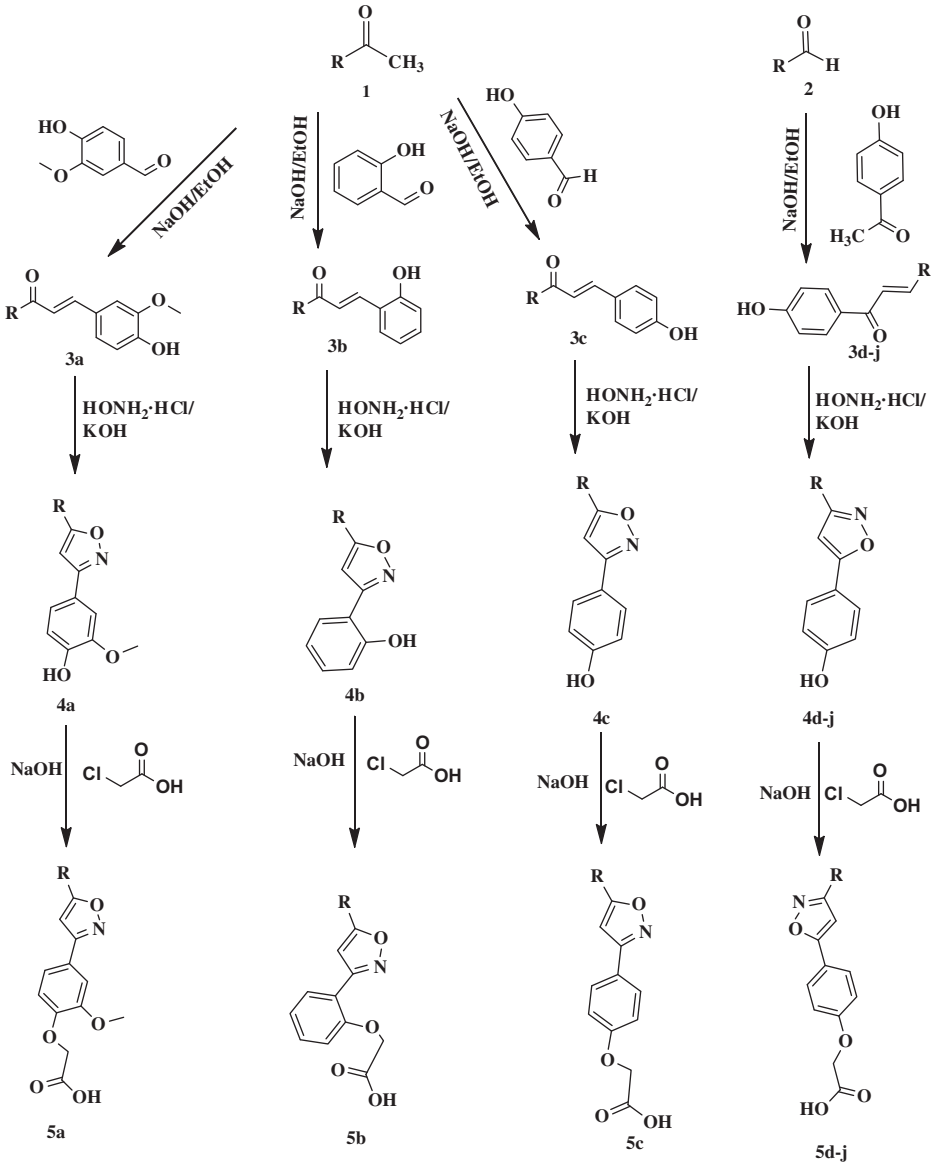


Scheme 1. Synthesis of designed compounds (**5a–j**).

Albino Wistar rats of either sex (200–250 g) were kept in a room with controlled temperature (25–26 °C), humidity (60–80%) and 12/12 h light/dark cycle under hygienic conditions. Animals were acclimatized for one week before starting the experiment with free access to the normal diet and water.

The hypolipidemic activity of the synthesized compounds was studied in the triton induced hyperlipidemic rats for 7 days by oral administration of the drug and compounds. Hyperlipidemia was developed by intra-peritoneal administration of triton WR-1339 (Sigma Aldrich, USA) at a dose of 400 mg/kg to all animals except the control. The compounds were suspended in Tween 80 and administered orally at a dose 250 mg/kg for 7 days. Animals of control and triton group without treatment with compounds were given vehicle only. Fenofibrate (250 mg/kg) was used as standard for the hypolipidemic activity.[8,9]

The serum was analysed for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL). The LDL and VLDL were calculated using Friedwald formula (Table 4). The atherogenic index (AI) was calculated as (TC-HDL/HDL, Table 4).[10] Data obtained in the test were compared against the control group using

Table 1
Characterization data for substituted hydroxy chalcone

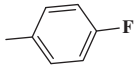
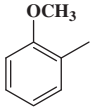
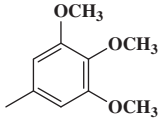
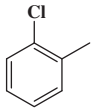
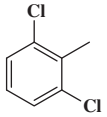
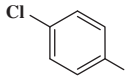
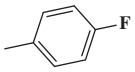
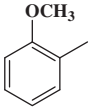
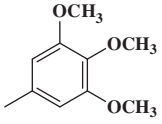
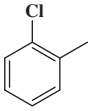
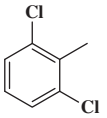
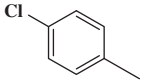
Compd	R	% Yield	Melting point	R_f value**
3a		68.12	130	0.54
3b		71.42	123	0.49
3c		69.20	127	0.62
3d		66.32	126	0.51
3e		65.90	125	0.48

Table 1 (*continued*)

Compd	R	% Yield	Melting point	R_f value**
3f		71.24	120	0.60
3g		67.49	128	0.52
3h		65.20	131	0.61
3i		66.10	130	0.53
3j		68.20	124	0.56

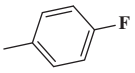
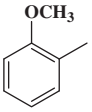
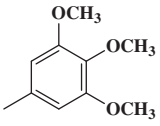
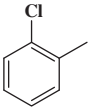
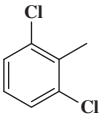
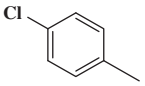
** Solvent system chosen for R_f value determination is chloroform/methanol (4:1).

Table 2
Characterization data for substituted isoxazol phenols

Compd	R	% Yield	Melting point*	R_f value**
4a		70.23	156	0.57
4b		62.61	158	0.60
4c		63.45	161	0.50
4d		69.50	169	0.59
4e		59.36	165	0.63
4f		63.15	160	0.60
4g		64.00	154	0.58
4h		60.85	153	0.61
4i		68.52	158	0.56
4j		62.34	146	0.60

* Melting points are uncorrected.
** Solvent system chosen for R_f value determination is ethyl acetate/*n*-hexane (1:1).

Table 3
Characterization data for of substituted phenyl isoxazol phenoxy acetic acid

Compd ID	R	Molecular formula	Molecular weight	% Yield	Melting point*	R_f value**
5a		$C_{18}H_{15}NO_5$	325.10	76.63	180	0.56
5b		$C_{17}H_{13}NO_4$	295.08	71.65	165	0.52
5c		$C_{17}H_{12}FNO_4$	313.08	70.36	145	0.52
5d		$C_{18}H_{15}NO_5$	325.10	65.12	150	0.45
5e		$C_{20}H_{19}NO_7$	385.12	65.78	163	0.51
5f		$C_{17}H_{12}ClNO_4$	329.08	67.20	168	0.48
5g		$C_{17}H_{11}Cl_2NO_4$	363.01	68.25	120	0.50
5h		$C_{15}H_{11}NO_5$	285.25	72.89	190	0.46
5i		$C_{17}H_{13}NO_4$	295.08	66.14	168	0.56
5j		$C_{17}H_{12}ClNO_4$	329.05	70.52	156	0.53

** Solvent system chosen for R_f value determination is ethyl acetate/*n*-hexane (1:1).
*Melting points are uncorrected.

the one-way analysis of variance method and followed by a post-hoc Dunnett test. For all statistical analysis, alpha was set to 0.05. Statistical analysis was performed using the SPSS 16 stat software. Results were presented as mean ± standard error mean (mean ± SEM).

In SAR, isoxazol was used as heterocyclic spacer in between phenoxy acetic acid pharmacophore and lipophilic tail. All the synthesized derivatives have ability to lower the elevated lipid levels. The substitution on phenoxy acetic acid ring has influence on the hypolipidemic activity. The substitution with OCH_3 (**5a**) on phenoxy acetic acid ring showed highest activity in comparison with unsubstituted derivatives. The substitution lipophilic tail also has influence on the hypolipidemic activity. The 2-OCH_3 showed highest activity on lipophilic tail (**5d**) followed by 2,6 dichloro derivative (**5g**).

In conclusion, a new series of substituted phenyl isoxazol phenoxy acetic acid was synthesized and evaluated for hypolipidemic activity. Most of these compounds showed better potency, amongst which **5a**, **5d** and **5g** were found to be the most active agents as compared to standard. The pharmacological screening of synthesized molecules indicates that isoxazol ring is important for hypolipidemic activity.

Table 4
Interpretation of pathological investigation (mg/dl) after 7 days and atherogenic index for target compounds

Groups	CH	TG	HDL	LDL	VLDL	Atherogenic index	% Protection
Normal	126 ± 1.57	116.5 ± 0.79	33 ± 1.42	58.6 ± 1.58	23.3 ± 0.58	2.53	—
Untreated	209 ± 1.45[#]	190 ± 1.98[#]	17 ± 1.70[#]	154 ± 1.82[#]	38 ± 1.80[#]	10.18	—
Std	116.5 ± 1.41[a]	119 ± 0.86[a]	44.5 ± 1.51[a]	48.2 ± 1.24[a]	23.8 ± 0.91[a]	1.67	83.6
5a	94 ± 1.22[a]	115 ± 1.01[a]	46 ± 1.60[a]	25 ± 1.11[a]	23 ± 1.01[a]	1.5	85.27
5b	91 ± 2.22[a]	123.5 ± 0.99[a]	42.5 ± 1.20[a]	23.3 ± 0.78[a]	25.2 ± 1.60[a]	1.91	81.24
5c	81 ± 2.10[a]	105.5 ± 1.62[a]	43.5 ± 1.70[a]	16.4 ± 1.01[a]	21.1 ± 1.51[a]	1.43	85.95
5d	77.5 ± 0.67[a]	117.5 ± 1.06[a]	44.5 ± 2.10[a]	10.5 ± 1.04[a]	23.5 ± 1.88[a]	1.64	83.89
5e	82.5 ± 2.18[a]	125 ± 0.91[a]	40.5 ± 1.10[a]	29 ± 0.81[a]	25.5 ± 1.84[a]	2.09	79.47
5f	148.5 ± 1.87[a]	110 ± 1.99[a]	40 ± 2.20[a]	86.5 ± 0.98[a]	22 ± 1.07[a]	1.75	82.81
5g	90 ± 1.91[a]	113.5 ± 1.10[a]	44.5 ± 1.80[a]	22.8 ± 0.88[a]	22.7 ± 1.22[a]	1.55	84.77
5h	87 ± 1.00[a]	113 ± 1.13[a]	42 ± 1.90[a]	22.4 ± 0.94[a]	22.6 ± 1.95[a]	1.69	83.4
5i	88 ± 1.29[a]	132 ± 0.81[a]	40.5 ± 1.69[a]	11.1 ± 1.52[a]	26.4 ± 1.00[a]	2.26	77.8
5j	83.5 ± 1.41[a]	131.5 ± 0.87[a]	41 ± 1.53[a]	16.2 ± 1.13[a]	26.3 ± 1.80[a]	2.21	78.29

Values are in mean ± SEM; number of animals in each group = 6.
[a] $p < 0.001$ versus untreated.
[#] $p < 0.001$ versus normal.

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14. *General:* Melting points were determined on scientific melting point apparatus in open capillaries and were uncorrected. FT-IR spectra were recorded on JASCO FT-IR 4000 using KBr powder. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AVANCE II 400 spectrometer (400 MHz) with TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on a Varian Inc., 410 Prostar Binary LC with 500 MS IT PDA detectors. All the reagents and solvents used were of analytical grade.
General procedure for the synthesis of substituted hydroxy chalcones:[11] In to 250 ml of conical flask placed 0.01 mol of substituted benzaldehyde and 0.01 mol of 4-hydroxyl acetophenone or substituted acetophenone and 4-hydroxyl benzaldehyde in 40 ml of ethanol containing 15 ml of 40% sodium hydroxide solution. The resulting solution stir for 2 h and reaction mixture was kept aside for 48 h. On next day crushed ice was added in reaction mixture and acidified it by dil.HCL. The crude product obtained was filtered and recrystallized by ethanol.
General procedure for synthesis of substituted isoxazol phenols:[12] In 250 ml of RBF, provide with reflux condenser placed substituted hydroxy chalcone (0.01 mol) and hydroxylamine HCl (0.01 mol) in a solution of KOH (0.02 mol) in 50 ml of methanol. The resultant solution was refluxed in water bath for 12 h. The hot solution was acidified with concd HCl. The solid which was obtained filtered off, washed with water, dried and recrystallized by ethanol.
General procedure for the synthesis substituted phenyl isoxazol phenoxy acetic acid:[13,14] In 500 ml of RBF provided with reflux condenser, placed substituted isoxazol phenols (0.01 mol), NaOH (0.02 mol) and mono chloro acetic acid (0.01 mol) in methanol. The resultant solution was refluxed in oil bath. After 1 h, pH of solution had dropped to 7 and further 1 g NaOH was added. Refluxing was continued for 2 h and 1 g NaOH added and again refluxes for 2 h. The hot solution was acidified with concd HCl and crystallization take place on cooling. The solid was filtered off, washed with water, dried and recrystallized by ethanol.
*2-(2-Methoxy-4-(5-phenylisoxazol-3-yl)phenoxy)acetic acid (**5a**):* IR (KBr): 3019, 3066, 2908, 1592, 744 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H, –OH), 8.1–7.5 (m, 5H, Ar-H), 6.9–7.3 (m, 3H, Ar-H), 6.7 (s, 1H, isoxazole), 4.7 (s, 2H, –CH_2), 3.8 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, DMSO): δ = 174.2, 170.1, 161.2, 151.3, 148.7, 129.9, 128.4, 127.6, 125.4, 123.2, 115.2, 98.1, 65.3, 55.9; MS: *m*/*z* = 326.1 [M+1].
*2-(2-(5-Phenylisoxazol-3-yl)phenoxy)acetic acid (**5b**):* IR (KBr): 3019, 3066, 2908, 1592, 744 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H, –OH), 7.8–7.4 (m, 5H, Ar-H), 7.0–7.3 (m, 4H, Ar-H), 6.6 (s, 1H, isoxazole), 4.7 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 173.7, 169.3, 160.7, 158.3, 132.7, 129.3, 128.4, 128.1, 127.5, 125.4, 116.7, 114.8, 98.4, 64.8; MS: *m*/*z* = 296.1 [M+1].
*2-(4-(3-(4-Fluorophenyl)isoxazol-5-yl)phenoxy)acetic acid (**5c**):* IR (KBr): 3089, 3016, 1893, 2568, 686, 590 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.8 (s, 1H, –OH), 8.2, 7.4 (m, 4H, Ar-H), 7.4–7.0 (m, 4H, Ar-H), 6.6 (s, 1H, isoxazole), 4.7 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 173.1, 169.2, 162.4, 158.7, 131.4, 131.2, 128.5, 127.3, 125.4, 123.7, 122.8, 118.9, 114.3, 98.4, 67.6; MS: *m*/*z* = 314.3 [M+1].
*2-(4-(3-(2-Methoxyphenyl)isoxazol-5-yl)phenoxy)acetic acid (**5d**):* IR (KBr): 3016, 2480, 1727, 1901, 574 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 11.1 (s, 1H, –OH), 7.6–7.4 (m, 4H, Ar-H), 7.2–7.0 (m 4H, Ar-H), 6.3 (s, 1H, isoxazole), 4.6 (s, 2H, –CH_2), 3.7 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, DMSO): δ = 170.2, 161.3, 160.1, 158.9, 132.4, 129.6, 128.5, 127.2, 123.2, 122.6, 117.4, 114.7, 110.2, 65.3, 53.2; MS: *m*/*z* = 326.2 [M+1].
*2-(4-(3-(3,4,5-Trimethoxyphenyl)isoxazol-5-yl)phenoxy)acetic acid (**5e**):* IR (KBr): 3016, 2480, 1727, 1901, 574 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 11.0 (s, 1H, –OH), 7.7–7.1 (m, 4H, Ar-H), 6.8 (m, 2H, Ar-H), 6.4 (s, 1H, isoxazole), 4.5 (s, 2H, –CH_2), 3.8-3.7 (s, 9H, –OCH_3); ^{13}C NMR (100 MHz, DMSO): δ = 174.4, 163.7, 161.2, 158.5, 153.4, 132.1, 129.7, 127.5, 124.4, 122.1, 115.3, 112.7, 99.9, 64.8, 58.2, 53.3; MS: *m*/*z* = 386.2 [M+1].
*2-(4-(3-(2-Chlorophenyl)isoxazol-5-yl)phenoxy)acetic acid (**5f**):* IR (KBr): 3016, 2480, 1727, 1901, 574 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H, –OH), 7.7–7.4 (m, 4H, Ar-H), 7.2–7.0 (m, 4H, Ar-H), 6.6 (s, 1H, isoxazole), 4.6 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 171.9, 161.2, 159.7, 155.2, 134.5, 131.7, 128.3, 126.4, 124.8, 121.5, 118.6, 115.1, 114.9, 64.5, 52.8; MS: *m*/*z* = 331.1 [M+1].
*2-(4-(3-(2,6-Dichlorophenyl)isoxazol-5-yl)phenoxy)acetic acid (**5g**):* IR (KBr): 3056, 2488, 1756, 1680, 1940, 650, 764 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H, –OH), 7.6–7.4 (m, 3H, Ar-H), 7.1–7.0 (m, 4H, Ar-H), 6.7 (s, 1H, isoxazole), 4.6 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 173.2, 170.4, 161.7, 156.4, 136.9, 132.2, 130.4, 129.7, 128.9, 119.3, 115.2, 114.7, 98.7, 66.3; MS: *m*/*z* = 366.1 [M+2].
*2-(4-(3-(Furan-2-yl)isoxazol-5-yl)phenoxy)acetic acid (**5h**):* IR (KBr): 3023, 3108, 1727, 1600, 640, 590 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H, –OH), 7.7, 7.6, 7.0 (m, 3H, furan ring), 7.2–7.1 (m, 4H, Ar-H), 6.6 (s, 1H, isoxazole), 4.6 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 170.1, 169.3, 160.7, 157.8, 154.9, 144.1, 128.5, 125.4, 116.3, 112.4, 108.8, 98.4, 67.6; MS: *m*/*z* = 286.2 [M+1].
*2-(4-(3-Phenylisoxazol-5-yl)phenoxy)acetic acid (**5i**):* IR (KBr): 3023, 3108, 1727, 1600, 640, 590 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 10.8 (s, 1H, –OH), 7.8–7.5 (m, 5H, Ar-H), 7.3–7.0 (m, 4H, Ar-H), 6.7 (s, 1H, isoxazole), 4.5 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 171.2, 164.5, 161.5, 145.4, 129.8, 128.5, 127.9, 126.3, 126.2, 115.2, 111.7, 109.9, 98.3, 67.4; MS: *m*/*z* = 296.1 [M+1].
*2-(4-(3-(4-Chlorophenyl)isoxazol-5-yl)phenoxy)acetic acid (**5j**):* IR (KBr): 3108, 2923, 1689, 2564, 2676, 1920, 763, 628 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ = 11.1 (s, 1H, –OH), 7.8–7.6 (m, 4H, Ar-H), 7.4–7.1 (m, 4H, Ar-H), 6.6 (s, 1H, isoxazole), 4.7 (s, 2H, –CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 174.2, 168.7, 161.5, 148.9, 136.6, 129.8, 128.3, 127.2, 126.3, 125.8, 117.9, 114.7, 115.1, 97.2, 66.9; MS: *m*/*z* = 331.7 [M+2].