Full Paper

Microwave-Assisted Synthesis, Hypolipidemic and Hypoglycemic Activity of Some Novel 2-(4-(2-Amino-6-(4-substituted phenyl)-pyrimidin-4-yl)-phenoxy)-2-methyl Propanoic Acid Derivatives

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A novel series of aminopyrimidines containing the phenoxy isobutyric acid group as a pharmacophore was synthesized using conventional and microwave assisted methods of synthesis. The compounds were synthesized in good yields (70–89%) by the microwave-assisted one-pot protocol in much shorter reaction times. The synthesized compounds were evaluated for their hypolipidemic and hypoglycemic activity by high-fat diet-induced hyperlipidemia and hyperglycemia in male Sprague-Dawley rats. The present investigation showed significant antihyperlipidemic and antihyperglycemic activity for all compounds of the series when compared with the standard drug. Structure-activity relationship (SAR) for the series were developed by comparing total lipid profile data of synthesized compounds with fenofibrate as standard drug.

Keywords: Hypolipidemic / Microwave assisted synthesis / Phenoxy isobutyric acid / Pyrimidine

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Introduction

The major cause of death in the western world is vascular disease, of which the most prevalent form is atherosclerotic heart disease. Many causative factors of this disease are recognized (e.g. smoking, stress, diet). Atherosclerotic diseases can be treated through medication or surgery. Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease, it is a term used to describe elevated plasma levels of the lipids that are usually in the form of lipoproteins [1].

Hyperlipidemia mainly increases the level of total cholesterol (TC), triglycerides (TG), very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) along with decrease in high-density lipoprotein (HDL) [2].

Fibrates (Fig. 1) affect lipid metabolism as agonists of enzyme peroxisome proliferator activated receptor alpha (PPAR-α) by lowering TG, LDL and by increasing HDL cholesterol level.

It was observed that phenoxy isobutyric acid pharmacophores have been frequently used in the synthesis of hypolipidemic agents [3–8]. Phenoxy isobutyric acid moiety was frequently coupled with heterocyclic nucleus such as benzoxazole [3], pyrimidine [9], thiazole [10], trifluoromethane sulfonamide [11], thiadiazole [12] and morpholine [13] to have more potent antihyperlipidemic agent.

In view of this, we have attempted the synthesis of aminopyrimidine containing phenoxy isobutyric acid pharmacophore (Fig. 2) with improved antihyperlipidemic activity.

We report herein the synthesis of 2-(4-(2-amino-6-(4-substituted phenyl)-pyrimidin-4-yl)-phenoxy)-2-methyl propanoic acid under conventional heating and microwave-assisted solvent-free synthesis. The conventional procedures involved a solvent and heating condition which was not efficient, giving lower yields and requiring longer period of time for completion of reaction (24 h). But microwave synthesis could proceed within 5–7 min with higher yield.

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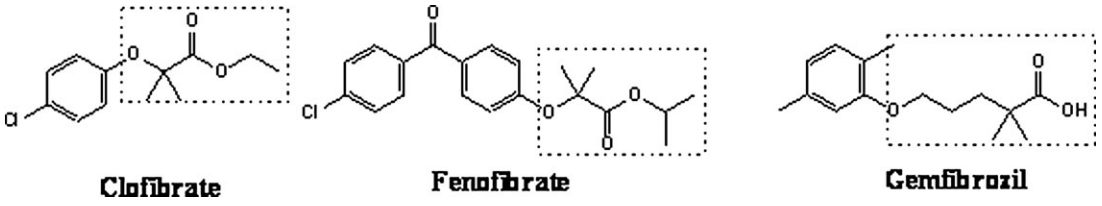


Figure 1. Structures of some known fibrates.

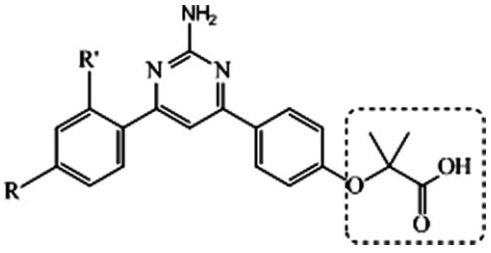


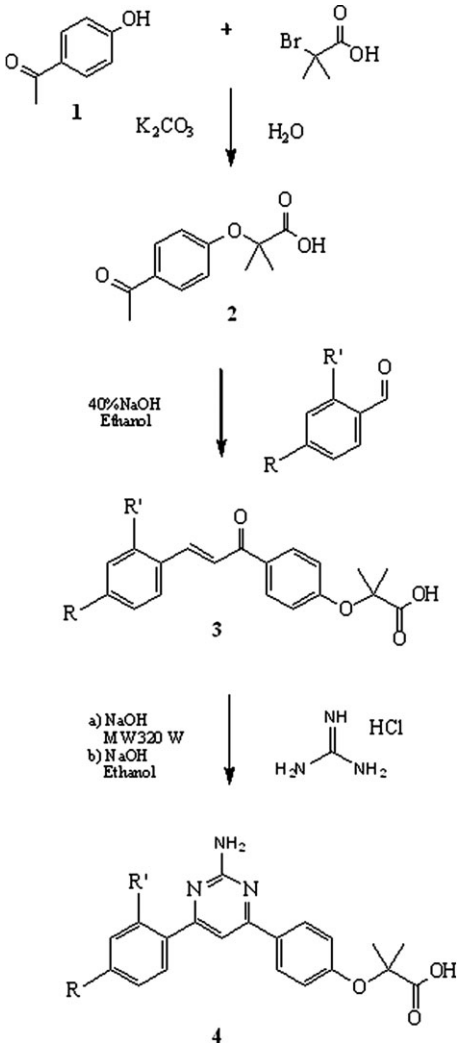
Figure 2. General structure of the target compound.

Results and discussion

Chemistry

The final compounds 2-(4-(2-amino-6-(4-substituted phenyl) pyrimidin-4-yl)-phenoxy)-2-methyl propanoic acid (**4a–4f**) were synthesized from the commercially available 1-(4-hydroxyphenyl) ethanone (**1,** Scheme 1). 1-(4-Hydroxyphenyl) ethanone (**1**) was subjected to nucleophilic substitution reaction with 2-bromo-2-methyl propanoic acid in alkaline condition to give 2-(4-acetyl phenoxy)-2-methyl propanoic acid (**2,** Scheme 1, Table 1). 2-(4-Acetyl-phenoxy)-2-methyl propanoic acid **(2)** was subjected to Claisen-Schmidt reaction with substituted benzaldehyde in alkaline condition to give the corresponding substituted chalcones (**3a–f,** Scheme 1, Table 1).

2-(4-(2-Amino-6-(4-substituted phenyl) pyrimidin-4-yl) phenoxy)-2-methyl propanoic acid compounds (**4a–f,** Scheme 1, Table 2) were prepared by cyclocondensation of substituted chalcones **(3a–f)** with guanidine HCl salt in alkaline conditions. These derivatives were synthesized by conventional as well as microwave-assisted methods. The comparison between the two methods is given in Table 3.



Scheme 1. General synthetic route for the synthesis of the compounds.

Pharmacological activity

The hypolipidemic and hypoglycemic activities of the synthesized compounds were studied in the high-fat diet-induced hyperlipidemic rats for 30 days by oral administration of the drug and compounds and the lipid profile was determined using Autoanalyser [14]. The results were compared with Group II (positive control). The obtained results revealed that feeding rats with high-fat diet for 30 days significantly elevated the serum level of total cholesterol, triglycerides, VLDL, LDL and blood sugar when compared with normal control rats. Moreover, induction of hyperlipidemia significantly decreased serum HDL level compared

Table 1. Experimental data of synthesized compounds **2–3f**.

Compound	Melting point (°C)	Molecular formula	Molecular weight
2	102	$C_{12}H_{14}O_4$	222.18
3a	170	$C_{19}H_{17}ClO_4$	345.32
3b	200	$C_{19}H_{16}Cl_2O_4$	379.21
3c	180	$C_{19}H_{18}O_5$	326.29
3d	185	$C_{19}H_{18}O_5$	326.29
3e	158	$C_{19}H_{17}FO_4$	328.34
3f	175	$C_{19}H_{18}O_4$	310.32

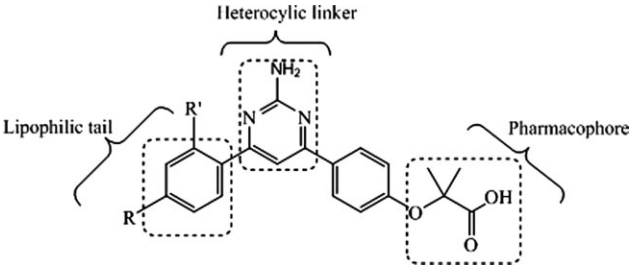


Figure 3. Structure highlighting important pharmacophores of the target compound.

to that of normal control rats. The obtained data revealed that the tested compounds produced variable effects on the serum levels of TC, TG, VLDL, LDL and HDL as compared with Group II (positive control).

The hyperlipidemia is a complex condition based upon alteration in concentration of individual components, of which TC, TG, VLDL, LDL and HDL are most important.

The pathological investigation revealed that compounds **4a** and **4e** are potential compounds for hyperlipidemia and cause significant HDL and LDL level modulation.

In case of hypoglycemic activity, high-fat diet fed for 30 days significantly increased sugar level in rats and compounds have shown significant decrease in blood sugar level after 7 days treatment. Significant glucose lowering activity has been shown by compounds **4d**, **4e** and **4a**.

Structure-activity relationship (Fig. 3)

1. All fibrates are analogues of phenoxy isobutyric acid which is very important for hypolipidemic activity. So, we have used phenoxy isobutyric acid as a pharmacophore in the present series.
2. Literature reveals that use of spacer in between phenoxy isobutyric acid and lipophilic tail increases the activity. So, we have used aminopyrimidine heterocycle as spacer which shows promising activity.
3. Substitution on aminopyrimidine with 4-substituted phenyl ring (lipophilic tail) influences hypolipidemic activity.
   a. 4-Substituted phenyl with electron withdrawing group showing highest activity. Activity decreases in following order (**4e > 4a > 4f**) F > Cl > H.
   b. Substitution with electron donating group (-OH) on phenyl ring decreases the hypolipidemic activity (**4c, 4d**).

Table 2. Experimental data of synthesized compounds 2-(4-(2-amino-6-(4-substituted phenyl) pyrimidin-4-yl)-phenoxy)-2-methyl propanoic acid (**4a–4f**).

Compound	R	R′	Molecular formula	Molecular weight	Melting point (°C)
4a	-Cl	-H	$C_{20}H_{18}ClN_3O_3$	383	280
4b	-Cl	-Cl	$C_{20}H_{17}Cl_2N_3O_3$	418	220
4c	-OH	-H	$C_{20}H_{19}N_3O_4$	365	234
4d	-H	-OH	$C_{20}H_{19}N_3O_4$	365	240
4e	-F	-H	$C_{20}H_{18}FN_3O_3$	367	260
4f	-H	-H	$C_{20}H_{19}N_3O_3$	349	295

Table 3. Comparative data of synthesized compounds (**4a–4f**) using conventional and microwave assisted synthetic methods.

Compound	Time required		% Yield	
	Conventional method	Microwave-assisted method	Conventional method	Microwave-assisted method
4a	24 Hrs	7 min	58	80
4b	24 Hrs	7 min	67	75
4c	24 Hrs	5 min	49	70
4d	24 Hrs	5 min	60	83
4e	24 Hrs	5 min	55	86
4f	24 Hrs	7 min	65	89

Conclusion

Novel 2-(4-(2-amino-6-(4-substituted phenyl) pyrimidin-4-yl) phenoxy)-2-methyl propanoic acid derivatives were synthesized by two method, conventional (solvent phase) and microwave-assisted (solid phase), and their hypolipidemic and hypoglycemic activities were screened in the high-fat diet-induced hyperlipidemic rats for 30 days. The present investigation showed significant antihyperlipidemic and antihyperglycemic activity to all compounds of the series when compared with standard drug. Among the synthesized compounds **4e** was found to be the most active hypolipidemic agent affording significant effects on the serum levels of TC, TG, LDL and HDL as compared with Group II (positive control) and seems to be a good candidate for developing hypolipidemic agent.

On the other hand compounds from the series also showed capacity to lower the plasma glucose level associated with hyperlipidemia.

Hence the present series could be developed as a novel class of antihyperlipidemic and antihyperglycemic agents. However, further structural modification is planned to increase the antihyperlipidemic and antihyperglycemic activities.

Experimental

General

The compounds were synthesized by conventional (solvent phase) and microwave-assisted (solid phase) methods using Catalyst Systems Cata Scientific Microwave. Melting points of the synthesized compounds were determined on scientific melting point apparatus in open capillaries and were uncorrected. ^{1}H 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 time of flight mass spectrometer.

The total lipid profile and blood sugar level was determined by Autoanalyser using cholesterol, triglycerides, HDL and glucose assay kit (Agappe Diagnostics).

Chemistry

*Synthesis of 2-(4-acetyl phenoxy)-2-methyl propanoic acid **2***

In 500 ml of RBF provided with reflux condenser were placed 4-hydroxy acetophenone (0.01 mol), 2-bromo-2-methyl propanoic acid (0.01 mol) in 25 mL of distilled ethanol and K_2CO_3 (0.03 mol). Then the resultant solution was refluxed in a water bath. After 1 h, pH of the solution was dropped to 7, and further 1 g of K_2CO_3 was added. Refluxing was continued for 12 h. The hot solution was acidified with conc. HCl. The product was extracted with diethyl ether. Ethereal layer was washed with 50 mL of saturated solution of sodium bicarbonate. The aqueous layer was acidified with dil. HCl. White colored solid was filtered off, washed with water, dried and recrystallized by hot water to get the desired compounds **2**.

*General procedure for preparation of compounds **3a–3f***

Into a 250-mL conical flask were placed 2-(4-acetyl-2-substituted phenoxy)-2-methyl propanoic acid (0.01 mol), substituted benzaldehyde (0.01 mol) in 40 mL of ethanol and 15 mL of 40% sodium hydroxide solution. Then the reaction mixture was kept aside for 48 h. On the next day crushed ice was added in reaction mixture and it was acidified by dil. HCl. The crude product obtained was filtered and recrystallized by ethanol to get the desired product **(3a–f).**

*General procedure for preparation of compounds **4a–4f***

Method A: Solvent phase synthesis (conventional method)

The reaction mixture of 2-(4-(3-(4-substituted phenyl)-3-oxoprop-1-enyl)-phenoxy)-2-methyl propanoic acid (0.01 mol), guanidine hydrochloride (0.03 mol), sodium hydroxide (0.02 mol) and 30 mL of ethanol was refluxed for 12 h in a water bath. The reaction was monitored on TLC and heating was continued till single spot. The reaction mixture was poured in 50 mL of 10% cold hydrochloric acid solution, the precipitate was filtered washed with water until free from acid, dried and recrystallized from methanol to get desired product **(4a–f).**

Method B: Solid phase synthesis (microwave method)

Mixture of chalcone (0.01 mol), guanidine HCl (0.01 mol) and NaOH pellets (0.5 mol) was finely powdered by pestle-mortar. This reaction mixture was irradiated at 320 W for 5–7 min in oven. The reaction was monitored on TLC and heating was continued till single spot. Distilled water was added to remove excess alkali, filtered, dried. The derivatives were recrystallized from ethanol to get desired product **(4a–f).**

*2-(4-(2-Amino-6-(4-chlorophenyl) pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4a**)*

IR (KBr): 3558, 3514, 3034, 2983, 1726, 1658, 1596, 1806, 1394, 1281, 1048, 1105, 944, 584 cm^{-1};

^{1}H NMR (400 MHz, DMSO): δ = 11 (s, 1H), 8.56 (s, 2H), 7.06–7.96 (m, 9H), 1.5 (s, 6H); MS (TOF, 1.99 e4): m/z = 383;

$C_{20}H_{18}ClN_3O_3$ (383); requires (Found): C, 62.58 (62.48); H, 4.73 (4.6); N, 10.95 (10.84).

*2-(4-(2-Amino-6-(2,4-dichlorophenyl) pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4b**)*

IR (KBr): 3548, 3511, 3044, 2973, 1736, 1648, 1576, 1390, 1271, 1038, 1115, 954, 587 cm^{-1};

^{1}H NMR (400 MHz, DMSO): δ = 11.1 (s, 1H), 6.88 (s, 2H), 7.05–8.03 (m, 8H), 1.53 (s, 6H); MS (TOF, 1.99 e4): m/z = 417;

$C_{20}H_{17}Cl_2N_3O_3$ (418); requires (Found): C, 57.43 (57.41); H, 4.10 (4.08); N, 10.05 (10.00).

*2-(4-(2-Amino-6-(4-hydroxyphenyl) pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4c**)*

IR (KBr): 3663, 3544, 3488, 2815, 1716, 1683, 1685, 1636, 1413, 1278, 1253, 925 cm$^{-1;}$

^{1}H NMR (400 MHz, DMSO): δ = 10.9 (s, 1H), 6.98 (s, 2H), 6.07–8.08 (m, 9H), 5.35 (s, 1H), 1.58 (s, 6H);

MS (TOF, 1.99 e4): m/z = 365;

$C_{20}H_{19}N_3O_4$ (365); requires (Found): C, 65.73 (65.61); H, 5.24 (5.18); N, 11.50 (11.35).

Table 4. Data of total lipid profile (CH, TG, LDL, VLDL and HDL).

Compound	Parameter in mg/dL				
	CH	TG	VLDL	LDL	HDL
Normal control	72.17 ± 0.601	75.17 ± 0.601	15.03 ± 0.120	23.13 ± 1.179	34.5 ± 0.7638
Positive control	121.5 ± 1.335[a]	135 ± 1.592[a]	27 ± 0.3183[a]	75.5 ± 1.24[a]	19 ± 0.5774[a]
Fenofibrate	105.2 ± 1.939[a]	73.17 ± 0.945[a]	14.67 ± 0.261[a]	55.33 ± 2.90[a]	33.5 ± 0.7638[a]
4a	93.17 ± 1.537[a]	77.67 ± 1.647[a]	15.53 ± 0.329[a]	45.15 ± 0.70[a]	33.5 ± 0.7638[a]
4b	103.3 ± 2.186[a]	82.5 ± 0.7638[a]	16.5 ± 0.152[a]	55.25 ± 1.48[a]	33 ± 1.065[a]
4c	107.3 ± 1.687[a]	76.67 ± 0.882[a]	15.33 ± 0.176[a]	58.42 ± 2.33[a]	32.67 ± 0.8819[a]
4d	113 ± 1.054[d]	77.5 ± 1.784[a]	15.5 ± 0.356[a]	53.07 ± 1.98[a]	32.5 ± 0.7638[a]
4e	93.33 ± 1.783[a]	76.33 ± 1.856[a]	15.27 ± 0.371[a]	45.48 ± 2.56[a]	33.83 ± 0.9458[a]
4f	109.5 ± 1.478[a]	78.17 ± 2.358[a]	15.87 ± 0.548[a]	59.98 ± 2.28 [a]	32.83 ± 0.6009[a]

a) Test compound = 250 mg/kg
b) Reference standard, fenofibrate = 250 mg/kg
c) The results are expressed as mean ± SEM. The data is analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. (n = 6), [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$, [d] non significant.

*2-(4-(2-Amino-6-(2-hydroxyphenyl) pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4d**)*

IR (KBr): 3654, 3564, 3478, 2839, 1700, 1687, 1672, 1656, 1423, 1280, 1263, 923 cm^{-1};

^1H NMR (400 MHz, DMSO): δ = 11.2 (s, 1H), 6.99 (s, 2H), 6.9–8.18 (m, 9H), 5.4 (s, 1H), 1.54 (s, 6H); MS (TOF, 1.99 e4): *m/z* = 365;

$C_{20}H_{19}N_3O_4$ (365); requires (Found): C, 65.73 (65.66); H, 5.24 (5.20); N, 11.50 (11.45).

*2-(4-(2-Amino-6-(4-fluorophenyl) pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4e**)*

IR (KBr): 3570, 3512, 3034, 2981, 1716, 1681, 1596, 1634, 1450, 1299, 1230, 1088, 1108 cm^{-1};

^1H NMR (400 MHz, DMSO): δ = 11.1 (s, 1H), 6.96 (s, 2H), 7.06–8.15 (m, 9H), 1.52 (s, 6H); MS (TOF, 1.99 e4): *m/z* = 367;

$C_{20}H_{18}FN_3O_3$ (367); requires (Found): C, 65.39 (65.26); H, 4.94 (4.88); N, 11.44 (11.35).

*2-(4-(2-Amino-6-phenyl pyrimidin-4-yl) phenoxy)-2-methylpropanoic acid (**4f**)*

IR (KBr): 3575, 3458, 3022, 1907, 1719, 1658, 1612, 1398, 1332, 1243, 939 cm^{-1};

^1H NMR (400 MHz, DMSO): δ = 11.01 (s, 1H), 8.67 (s, 2H), 7.2–8.0 (m, 10H), 1.53 (s, 6H); MS (TOF, 1.99 e4): *m/z* = 349;

$C_{20}H_{19}N_3O_3$ (349); requires (Found): C, 68.75 (68.66); H, 5.48 (5.38); N, 12.03 (11.99).

Pharmacological activity

Male Sprague Dawley rats (120–150g) were divided into 9 groups of six animals and they were numbered individually. Normal diet was made available for 30 days to Group I. High-fat diet was made available for 23 days to Group II and vehicle was administered for last 7 days. High-fat diet was made available for 23 days to Group III–IX and test compounds **4a–f** (250 mg/kg) and standard drug (fenofibrate, 250 mg/kg) was administered for last 7 days respectively. On 31st day, 2 mL blood was withdrawn by retroorbital method and total lipid profile was determined by standared method (Table 4). Along with lipid profile blood sugar level was also determined (Table 5).

Table 5. Data of blood sugar level.

Compound	Blood sugar (mg/dL)
Normal control	57.83 ± 0.6009
Positive control	106.3 ± 0.6666 [a]
Fenofibrate	70.5 ± 0.7638 [a]
4a	70.17 ± 0.601 [a]
4b	72.67 ± 0.882 [a]
4c	71.67 ± 1.542 [a]
4d	66.67 ± 1.282 [a]
4e	68 ± 0.574 [a]
4f	71.17 ± 2.088 [a]

a) Test compound = 250 mg/kg
b) Reference standard, fenofibrate = 250 mg/kg
c) The results are expressed as mean ± SEM. The data is analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. (n = 6), [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$, [d] non significant.

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