Journal of Enzyme Inhibition and Medicinal Chemistry, 2011; 26(6): 819–826

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2011.557022

informa
healthcare

RESEARCH ARTICLE

Synthesis and *in vivo* diuretic activity of some novel pyrimidine derivatives

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Abstract

A series of 1,6-dihydropyrimidine-2-amine derivatives and 1,6-dihydropyrimidine-2-thiol derivatives were synthesized by the reaction of substituted 1,3-diphenylprop-2-en-1-one (chalcones) with guanidine hydrochloride and thiourea, respectively. All the synthesized compounds were in good agreement with elemental and spectral data. The synthesized compounds were screened *in vivo* for diuretic activity. The four compounds **2d**, **2e**, **3d and 3e**, which showed moderate to good diuretic activity, were evaluated for their toxicity studies and none of the compounds showed any toxicity of the liver as compared with control. However, compounds **3e** and **3d** showed diuretic properties more than that of standard (acetazolamide) and were long acting. Overall, compound **3e**, 6-(2,6-dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol, was found to be the most promising candidate of the series.

Keywords: Pyrimidines, diuretic activity, chalcones, toxicity studies

Introduction

Hypertension is a very common chronic condition associated with increased mortality and multiple morbidities[1]. Blood pressure (BP) is directly associated with risks of several types of cardiovascular diseases, and the association of BP with disease risk are continuous with large proportion of most populations having non-optimal BP values[2]. Recently, in World Health Organization–International Society of Hypertension guidelines for the management of hypertension have also described the importance of diuretics as one of the most valuable class of drugs[3]. Diuretics reduce both systolic and diastolic BPs in the great majority of hypertensive patients. They are as effective as most other antihypertensive drugs and also enhance the antihypertensive efficacy of multidrug regimens and can be useful in achieving BP control. Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 6) guidelines, which were issued in 1997, and JNC 7 guidelines, which were issued in 2003, both recommend diuretics as first-line drugs in the treatment of uncomplicated hypertension[4].

From the literature, it is clear that diuretic plays a significant role in the management of hypertension; therefore, there is an urgent clinical need for the development of selective diuretics (high ceiling [loop]/potassium sparing/osmotic) devoid of many of the unpleasant side effects *viz* hypokalemia, hyperuricemia, hypercholestremia, etc. associated with current diuretic regimens. Because of the lack of oral activity and toxicities associated with current diuretic regimen, research efforts were focused on the development of orally effective agents. In medicinal chemistry, pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, uracil and cytosine, which are essential building blocks of nucleic acids, DNA and RNA, is one possible reason for their activity. They possess broad range of pharmacological activities such as anti-cancer[5], anti-viral[6], anti-HIV[7], anti-hypertensive[8], anti-convulsant[9], anti-tubercular[10], diuretic[11], anti-bacterial[12], anti-fungal[13] and anti-epileptics[14] properties, and many classes of chemotherapeutic agents containing pyrimidine nucleus are in clinical use[15]. However, pyrimidines are still least explored compounds for diuretic profile although a few promising diuretic drugs possess this ring[16,17]. Triamterene (2,4,7-triamino-6-phenylpteridine)

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(Received 25 August 2010; revised 12 January 2011; accepted 14 January 2011)

is a mild, clinically effective potassium sparing diuretic either used alone or as an adjunct to thiazide and loop diuretics. It is orally active but has very poor water solubility and may be contraindicated in people with kidney problems, diabetes and in elderly patients.

Diuretic agents such as furosemide and triamterene have been reported for their hepatotoxicities[18,19]. Therefore, the need of toxicity studies is significant.

Among the pyrimidines, aminopyrimidines and thiopyrimidines are broadly found in bioorganic and medicinal chemistry with applications in drug discovery and developments. They are reported to possess broad spectrum of biological activities as well[20,21]. In pursuit of this goal, it was proposed to carry out the synthesis and biological screening of aforesaid heterocycles with appropriate substitution, with improved efficacy and decreased toxicity.

Materials and methods

All the chemicals used were laboratory grade and procured from E.Merck (Germany) and S.D Fine Chemicals (India). Diagnostic kits for the estimation of biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were purchased from local supplier manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Melting points were determined by open tube capillary method and are uncorrected. Thin-layer chromatography plates (silica gel G 60 F_{254}, 0.2 mm) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reaction. Two different solvent systems toluene:ethyl acetate:formic acid (5:4:1, v/v/v) and benzene:acetone (9:1, v/v) were used for thin-layer chromatography. The spots were located under iodine vapors and ultraviolet light. Infrared (IR) spectra were obtained on a Perkin–Elmer 1720 Fourier transform infrared (FTIR) spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 400 MHz, spectrometer using Tetra Methyl Silane (TMS) as an internal standard in DMSO-d_6/$CDCl_3$. The FAB mass spectra were recorded on a JEOLSX 102/DA-6000 Mass Spectrometer. All the biochemical estimations were carried out using standard kits in semi auto-analyzer Screen Master 3000.

Methods

Chemistry

Synthesis of substituted 1,3-diphenylprop-2-en-1-ones (1a-f) An equimolar mixture of 2-acetyl pyridine and respective aryl aldehyde was stirred in ethanol (25 mL). To this mixture, an aqueous solution of NaOH (40%, 10 mL) was added at once and the reaction mixture was stirred for 40 min at room temperature. The mixture was kept overnight at room temperature, poured onto crushed ice and then acidified with dilute HCl. The solid separated was filtered and washed carefully with water until neutral. The resulting chalcone was purified by recrystallization with ethanol.

Synthesis of 1,6-dihydropyrimidine-2-amine derivatives (2a-f) A mixture of chalcone (1 mole), guanidine hydrochloride (1.5 mole) and sodium hydride (3.0 mole) in Di Methyl Formamide (DMF) (100 mL) was refluxed for 6–8 hours. After completion of reaction, mixture was poured into ice cold water, a solid so separated was filtered and purified by column chromatography to afford pure compounds.

Synthesis of 1,6-dihydropyrimidine-2-thiol derivatives (3a-f) A mixture of chalcone (0.01 mole), thiourea (0.012 mole) and sodium methoxide (0.025 mole) in ethanol (30 mL) was refluxed for 3–6 hours. The reaction mixture was concentrated and cooled. The solid separated out was filtered and recrystallized from DMF or water.

3-Phenyl-1-(pyridin-2-yl)prop-2-en-1-one (1a) Yield: 76%; mp: 160–162°C; Rf: 0.9556; FTIR (KBr) υ_{max} cm^{-1}: 1726 (C=O), 1648 (CH=CH), 1576 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.84 (2H, d, Ar H-3,5), 8.52 (1H, d, Ar H-6′), 8.41 (1H, m, Ar H-4), 8.01 (2H, d, Ar H-2,6), 7.94 (1H, m, Ar H-4′), 7.86 (1H, d, Hβ), 7.76 (2H, d, Ar H-3′,5′),7.23 (1H, d, Hα),. Anal. Calcd. for $C_{14}H_{11}NO$: C, 71.98; H, 5.64; N, 22.38. Found: C, 71.73; H, 5.61; N, 22.30.

3-(4-Methoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1b) Yield: 80%; mp: 128–130°C; Rf: 0.8837; FTIR (KBr) υ_{max} cm^{-1}: 1734 (C=O), 1658 (CH=CH), 1582 (C=N), 1172 (–OCH_3); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.10 (1H, d, Ar H-6′), 8.65 (1H, m, Ar H-4′), 8.44–8.52 (2H, m, Ar H-3, 5), 8.10 (2H, d, Ar H-2,6), 8.04 (2H, d, Ar H-3′,5′), 7.95 (1H, d, Hβ), 6.94 (1H, d, Hα), 3.84 (3H, s, OCH_3). Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.28; H, 5.46; N, 5.83.

3-(4-Fluorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1c) Yield: 84%; mp: 143–145°C; Rf: 0.9302; FTIR (KBr) υ_{max} cm^{-1}:1727 (C=O), 1654 (CH=CH), 1533 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.75 (1H, d, Ar H-6′), 8.65 (2H, d, Ar H-3, 6), 8.24(1H, d, Hβ), 8.15 (1H, s, Ar H-2), 7.82 (1H, m, Ar H-4′), 7.61 (1H, m, Ar H-5), 7.36 (2H, d, Ar H-3′,5′), 7.25 (1H, d, Hα),. Anal. Calcd. for $C_{14}H_{10}FNO$: C, 74.00; H, 4.44; N, 6.16. Found: C, 73.86; H, 4.41; N, 6.14.

3-(2-Chlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1d) Yield: 81%; mp: 167-169°C; Rf: 0.8837; FTIR (KBr) υ_{max} cm^{-1}: 1726 (C=O), 1642 (CH=CH), 1536 (C=N), 853 (C-Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.76 (1H, d, Ar H-6′), 7.92 (2H, d, Ar H-3,5), 7.68 (1H, d, Hβ), 7.66 (1H, m, Ar H-4′),7.52 (2H, d, Ar H-3′,5′),7.42 (1H, d, Hα), 7.02 (2H, d, Ar H-4,6). Anal. Calcd. for $C_{14}H_{10}ClNO$: C, 69.00; H, 4.14; N, 5.75. Found: C, 68.89; H, 4.12; N, 5.73.

3-(2,6-Dichlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1e) Yield: 68%; mp: 152–154°C; Rf: 0.9048; FTIR (KBr)

υ_{max} cm^{-1}: 1723 (C=O), 1648 (CH=CH), 1536 (C=N), 852 (C–Cl); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.78 (1H, d, Ar H-6′), 8.34 (1H, d, Hβ), 7.98 (1H, m, Ar H-4′), 7.84 (2H, d, Ar H-3,5), 7.56 (1H, d, Ar H-4), 7.46 (2H, d, Ar H-3′,5′),7.36 (1H, d, Hα). Anal. Calcd. for $C_{14}H_9Cl_2NO$: C, 60.46; H, 3.26; N, 5.04. Found: C, 60.33; H, 3.24; N, 5.02.

3-(3,4-Dimethoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (1f) Yield: 74%; mp: 136–138°C; Rf: 0.7627; FTIR (KBr) υ_{max} cm^{-1}: 1734 (C=O), 1647 (CH=CH), 1412 (C=N), 1173 (–OCH_3); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.14 (1H, d, Ar H-6′), 8.37 (1H, m, Ar H-4′), 8.11 (2H, d, Ar H-3′,5′), 7.86 (1H, d, Hβ), 7.64 (1H, s, Ar H-2), 7.32 (2H, d, Ar H-5, 6), 7.11 (1H, d, Hα), 3.87–3.95 (6H, s, 2 × –OCH_3). Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.28; H, 5.56; N, 5.18.

6-Phenyl-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (2a) Yield: 63%; mp: 210–212°C; Rf: 0.8750; FTIR (KBr) υ_{max} cm^{-1}: 3410 (NH_2), 3201 (NH), 3096 (Ar–H), 1536 (C=N); ^{1}H NMR (DMSO-d_6, in δ ppm): 8.8 (1H, d, Ar H-6′ ring-B), 8.1 (5H, m, Ar-H ring-A), 7.8 (1H, d, NH pyrimidine proton), 7.1–7.9 (3H, m, Ar H-3′,4′,5′ ring-B), 5.93 (1H, d,H-6 pyrimidine proton), 5.81 (1H, d, H-5 pyrimidine proton), 4.6 (2H, s, Ar-NH_2). FABMS *m/z*: 250 (M^+). Anal. Calcd. for $C_{15}H_{14}N_4$: C, 71.98; H, 5.64; N, 22.38. Found: C, 71.84; H, 5.61; N, 22.37.

6-(4-Methoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (2b) Yield: 72%; mp: 216–218°C; Rf: 0.5908; FTIR (KBr) υ_{max} cm^{-1}: 3412 (NH_2), 3201 (NH), 3096 (Ar–H), 1584 (C=N), 1174 (OCH_3); ^{1}H NMR (DMSO-d_6, in δ ppm): 9.12 (1H, d, Ar H-6′ ring-B), 8.09–8.68 (3H, m, Ar H-3′,4′,5′ ring-B), 7.81 (1H, d, NH pyrimidine proton), 6.6 (4H, m, Ar–H ring-A), 5.92 (1H, d, H-6 pyrimidine proton), 5.81 (1H, d, H-5 pyrimidine proton), 4.6 (2H, s, Ar–NH_2), 3.8 (3H, s, OCH_3). FABMS *m/z*: 278 (M^{-2}). Anal. Calcd. for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99. Found: C, 68.33; H, 5.72; N, 19.97.

6-(4-Fluorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (2c) Yield: 78%; mp: 214–216°C; Rf: 0.8551; FTIR (KBr) υ_{max} cm^{-1}: 3412 (NH_2), 3201 (NH), 3096 (ArH), 1584 (C=N), 1174 (OCH_3); ^{1}H NMR (DMSO-d_6, in δ ppm): 9.10 (1H, d, Ar H-6′ ring-B), 8.01–8.62 (3H, m, Ar H-3′,4′,5′ ring-B), 7.83(1H, d, NH pyrimidine proton), 6.7 (4H, m, Ar- H ring-A), 5.94 (1H, d, H-6 pyrimidine proton), 5.82 (1H, d, H-5 pyrimidine proton), 4.6 (2H, s, Ar–NH_2). FABMS *m/z*: 269 (M^{+1}). Anal. Calcd. for $C_{15}H_{13}FN_4$: C, 67.59; H, 6.03; N, 19.70. Found: C, 67.48; H, 6.01; N, 19.68.

6-(2-Chlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (2d) Yield: 68%; mp: 208–210°C; Rf: 0.8792; FTIR (KBr) υ_{max} cm^{-1}: 3411 (NH_2), 3215 (NH), 3089 (Ar–H), 1536 (C=N), 851 (C–Cl); ^{1}H NMR (DMSO-d_6, in δ ppm): 8.7 (1H, d, Ar H-6′ ring-B), 7.8 (1H, d, NH pyrimidine proton), 7.5–7.9 (3H, m, Ar H-3′,4′,5′ ring-B), 6.8 (4H, m, Ar- H ring-A), 5.92 (1H, d, H-6 pyrimidine proton), 5.81 (1H, d, H-5 pyrimidine proton), 4.5 (2H, s, Ar–NH_2). FABMS *m/z*: 285 (M^{+1}). Anal. Calcd. for $C_{15}H_{13}ClN_4$: C, 63.27; H, 4.60; N, 19.68. Found: C, 63.17; H, 4.57; N, 19.65.

6-(2,6-Dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine (2e) Yield: 76%; mp: 203–205°C; Rf: 0.7130; FTIR (KBr) υ_{max} cm^{-1}: 3410 (NH_2), 3216 (NH), 3085 (ArH), 1612 (C=N); ^{1}H NMR (DMSO-d_6, in δ ppm): 8.4 (1H, d, Ar H-6′ ring-B), 7.87 (1H, d, NH pyrimidine proton), 7.4–7.7 (3H, m, Ar H-3′,4′,5′ ring-B),7.4 (3H, m, Ar–H ring-A), 5.91 (1H, d, H-6 pyrimidine proton), 5.82 (1H, d, H-5 pyrimidine proton), 4.6 (2H, s, Ar–NH_2). FABMS *m/z*: 320 (M^{+1}). Anal. Calcd. for $C_{15}H_{12}Cl_2N_4$: C, 56.44; H, 3.79; N, 17.55. Found: C, 56.33; H, 3.77; N, 17.54.

6-(3,4-Dimethoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidin-2-amine(2f) Yield: 65%; mp: 236–238°C; Rf: 0.8336; FTIR (KBr) υ_{max} cm^{-1}: 3412 (NH_2), 3201 (NH), 3096 (Ar–H), 1412 (C=N), 1170 (OCH_3); ^{1}H NMR (DMSO-d_6, in δ ppm): 8.7 (1H, d, Ar H-6′ ring-B), 7.84 (1H, d, NH pyrimidine proton), 7.1–7.9 (3H, m, Ar H-3′,4′,5′ ring-B), 6.4 (3H, m, Ar–H ring-A), 5.92 (1H, d, H-6 pyrimidine proton), 5.81(1H, d, H-5 pyrimidine proton), 4.5 (2H, s, Ar–NH_2), 3.8–3.93 (6H, s, 2 × OCH_3). FABMS *m/z*: 308 (M^{-2}). Anal. Calcd. for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 65.67; H, 5.81; N, 18.03.

6-Phenyl-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3a) Yield: 81%; mp: 165–167°C; Rf: 0.9455; FTIR (KBr) υ_{max} cm^{-1}: 3201 (NH), 3120 (C–H str, Aromatic), 2591 (SH), 1640 (C=N), 1593 (Aromatic C=C str), 1520 (C–N str); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.72 (1H, s, S–H), 7.74 (1H, d, NH pyrimidine proton), 6.7–8.1 (11H, m, aromatic protons). FABMS *m/z*: 267 (M^+). Anal. Calcd. for $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72. Found: C, 67.29; H, 4.86; N, 15.70.

6-(4-Methoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3b) Yield: 67%; mp: 152–154°C; Rf: 0.7223; FTIR (KBr) υ_{max} cm^{-1}: 3119 (C–H str, aromatic), 2585 (SH), 1651 (C=N), 1582 (aromatic C=C str), 1516 (C–N str); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.68 (1H, s, S–H), 7.79 (1H, d, NH pyrimidine proton), 6.81–8.32 (10H, m, aromatic protons), 3.72 (3H, s,–OCH_3). FABMS *m/z*: 295 (M^{-2}). Anal. Calcd. for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.57; H, 5.06; N, 14.11.

6-(4-Fluorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3c) Yield: 73%; mp: 186–188°C; Rf: 0.8778; FTIR (KBr) υ_{max} cm^{-1}: 3082 (C–H str, aromatic), 2598 (S–H), 1635 (C=N), 1580 (aromatic C=C str), 1524 (C–N str); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.13 (1H, s, S–H), 7.72 (1H, d, NH pyrimidine proton), 6.52–8.18 (10H, m, aromatic protons). FABMS *m/z*: 286 (M^{+1}). Anal. Calcd. for $C_{15}H_{12}FN_3S$: C, 63.14; H, 4.24; N, 14.73. Found: C, 63.04; H, 4.21; N, 14.70.

6-(2-Chlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3d) Yield: 79%; mp: 173–175°C; Rf: 0.9303; FTIR (KBr) υ_{max} cm^{-1}: 3122 (C–H str, aromatic), 2540 (S–H), 1618 (C=N), 1598 (aromatic C=C str), 1526 (C–N str), 751 (C–Cl); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.99 (1H, s, S–H), 7.76 (1H, d, NH pyrimidine proton), 6.6–8.8 (10H, m, aromatic protons). FABMS *m/z*: 302 (M^{+1}). Anal. Calcd. for $C_{15}H_{12}ClN_3S$: C, 59.70; H, 4.01; N,13.92. Found: C, 59.61; H, 4.00; N, 13.89.

6-(2,6-Dichlorophenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3e) Yield: 64%; mp: 158–160°C; Rf: 0.8675; FTIR (KBr) υ_{max} cm^{-1}: 3125 (C–H str, aromatic), 2540 (S–H), 1614 (C=N), 1592 (aromatic C=C str), 1522 (C–N str), 753 (C–Cl); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.71 (1H, s, S–H), 7.70 (1H, d, NH pyrimidine proton), 6.24–8.64 (9H, m, aromatic protons). FABMS *m/z*: 337 (M^{+1}). Anal. Calcd. for $C_{15}H_{11}Cl_2N_3S$: C, 53.58; H, 3.30; N, 12.50. Found: C, 53.46; H, 3.28; N, 12.47.

6-(3,4-Dimethoxyphenyl)-4-(pyridin-2-yl)-1,6-dihydropyrimidine-2-thiol (3f) Yield: 61%; mp: 176–178°C; Rf: 0.9048; FTIR (KBr) υ_{max} cm^{-1}: 3086 (C–H str, aromatic), 2543 (S–H), 1618 (C=N), 1585 (aromatic C=C str), 1520 (C–N str); ^{1}H NMR ($CDCl_3$, in δ ppm): 9.56 (1H, s, S–H), 7.76 (1H, d, NH pyrimidine proton), 6.15–8.23 (9H, m, aromatic protons), 3.8–3.93 (6H, s, $2\times OCH_3$). FABMS *m/z*: 325 (M^{-2}). Anal. Calcd. for $C_{17}H_{17}N_3O_2S$: C, 62.36; H, 5.23; N, 12.83. Found: C, 62.28; H, 5.21; N, 12.79.

Biological activity

Diuretic activity was measured on healthy adult albino rats weighing 180–200 g according to an adaptation of the method of Lipschitz et al.[22] Each group was comprised of six animals ($n=6$). They were housed in standard environmental conditions (temperature: 25–30°C). The rats are fed with standard diet (Altromin® pellets) and water ad libitum. Food and water are withdrawn 15 hours prior to the experiment. Diuretic activity was measured by collecting total excreted urine of rat kept in metabolic cages designed to separate the urine and faeces. The cages together with the funnel and measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine with minimum loss. Each animal is placed in a metabolic cage provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves are placed in the funnel to retain feaces and to allow the urine to pass. Rats were placed in metabolic cages individually as soon as the treatments started. The urine sample was collected for a total period of 5 h (urine collected initially of 20 min was discarded). All the doses were administered with the aid of an oral dosing needle. The test compounds were administered orally at a dose of 45 mg/kg body weight in 5 mL of (0.5% carboxy methyl cellulose + 0.9% NaCl solution). Control group received 5 mL of 0.9% NaCl solution per kilogram body weight. The test compounds are compared with two standard diuretics, urea (1 g/kg body weight in 5 mL of 0.5% carboxy methyl cellulose + 0.9% NaCl solution) and acetazolamide[23–26] (45 mg/kg body weight in 5 mL of 0.5% carboxy methyl cellulose + 0.9% NaCl solution)[27]. The excreted urine was collected, measured and studied for cumulative urine output, diuretic action, diuretic activity, Lipschitz value and electrolyte excretion (Na^+, K^+ and Cl^-). Sodium and potassium are estimated by using lab model Mediflame photometer. Chloride was estimated by titrating the urine by Volhards method.

Assessment of liver function The compounds that have exhibited excellent diuretic profile have been selected for the study. The serum collected from the groups of albino rats was used for estimation of biochemical parameters to determine the functional state of the liver. SGOT and SGPT were estimated by a ultraviolet kinetic method based on the reference method of International federation of Clinical Chemistry[28]. Alkaline phosphatase was estimated by using King method[29]. Total protein, albumin and globulin were also measured according to the reported methods[30,31].

Statistical analysis

Results of biochemical estimation were reported as mean ± SEM. The determination of significant inter-group difference was analyzed separately and one-way analysis of variance was carried out[32]. Dunnett's test was used for individual comparisons[33].

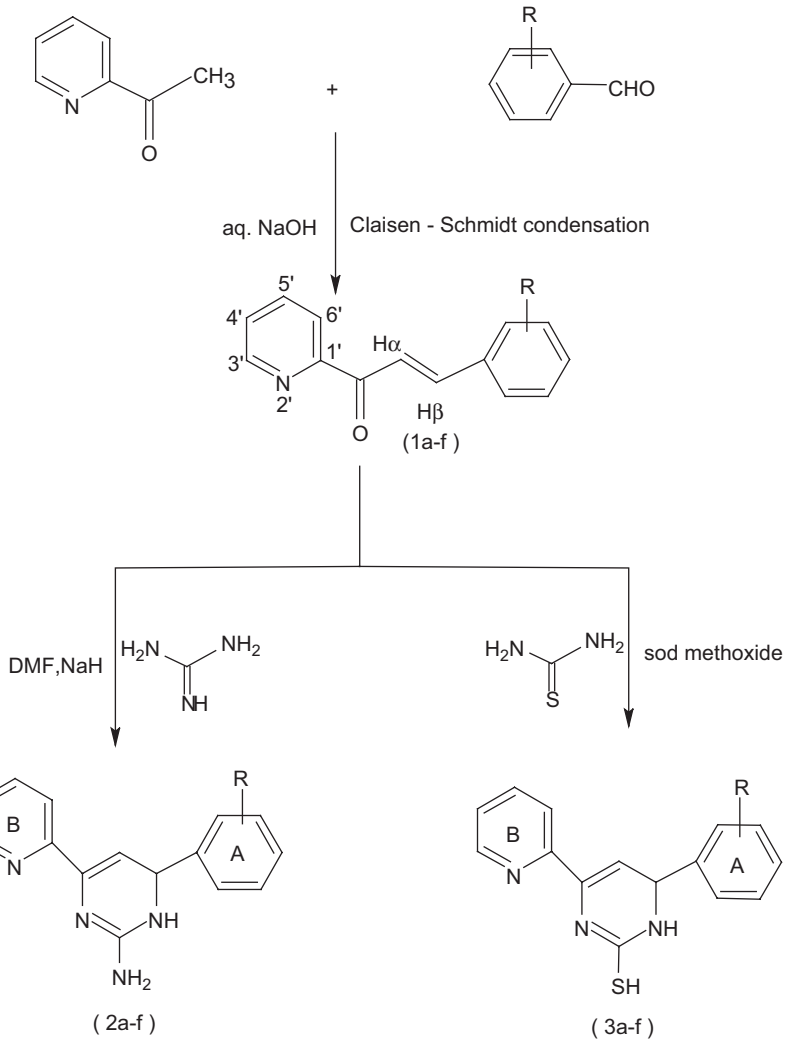
Result and discussion

Chemistry

The synthesis of chalcones from substituted benzaldehyde and 2-acetyl pyridine were carried out according to the Claisen–Schmidt condensation[34]. The 1,6-dihydropyrimidine-2-amine (**2a-f**) and 1,6-dihydropyrimidine-2-thiol (**3a-f**) derivatives were synthesized from chalcones (**1a-f**) using guanidine hydrochloride and thiourea, respectively. The synthetic sequences leading to the formation of targeted compounds are depicted in Scheme 1.

Diuretic activity

The *in vivo* diuretic activity of the synthesized compounds is summarized in (Table 1) and (Table 3). Cumulative urine excreted during 0–5 hours for each group (6 *albino* rats) is a measure of urinary excretion. After 5 hours of screening, the compounds **3e**, **3d**, **2e**, **2d** and **3c** showed good cumulative urine output. The urinary output of compound **3e** was highly significant 15.09 ± 0.540 ($P<0.01$), i.e., increased by 300% with respect to control. Urinary excretion of **3e** was 301.80% and that of **3d**, **2e**, **2d** and **3c** lies in between urea (138.09%) and standard acetazolamide (184.77%). Diuretic action was measured as the ratio (%) of the total volume of urine excreted during the 5 hours following the administration of the test drug at a dose of 45 mg/kg to the volume of urine excreted by the



R= Phenyl; 4-Methoxyphenyl; 4-Flourophenyl; 2-Chlrophenyl; 2,6-Dichlorophenyl; 3,4-Dimethoxyphenyl

Scheme 1. Protocol of synthesis.

Table 1. Diuretic activity of amino and thio pyrimidine derivatives.

Treatment	Dose (mg/Kg body weight)	Total urinary output (mL)	Normal saline intake (mL)	Urinary excretion (%)	Diuresis		
					Diuretic action	Diuretic activity	Lipschitz value
2a	45	4.31 ± 0.545	5.03 ± 0.401	85.68	0.7	0.43	0.57
2b	45	4.58 ± 0.419	4.66 ± 0.166	98.28	0.8	0.50	0.60
2c	45	5.83 ± 0.926	4.93 ± 0.633	118.25	1.02	0.63	0.77
2d	45	8.66 ± 0.310*	5.22 ± 0.247	165.90	1.4	0.87	1.14
2e	45	9.08 ± 0.864**	5.03 ± 0.256	180.51	1.5	0.93	1.20
2f	45	4.35 ± 0.545	4.96 ± 0.588	87.70	0.7	0.43	0.57
3a	45	4.43 ± 0.568	4.85 ± 0.387	91.34	0.7	0.43	0.58
3b	45	5.16 ± 0.459	5.01 ± 0.329	102.99	0.8	0.50	0.68
3c	45	8.03 ± 0.359*	5.04 ± 0.468	159.32	1.3	0.81	1.06
3d	45	11.56 ± 0.844**	4.94 ± 0.276	234.00	2.03	1.25	1.53
3e	45	15.09 ± 0.540**	5.00 ± 0.687	301.80	2.62	1.63	2.001
3f	45	6.28 ± 0.612	4.91 ± 0.416	127.90	1.1	0.68	0.83
CONT	—	5.65 ± 0.353	4.92 ± 0.387	114.83	1.0	0.62	—
UREA	1000	7.54 ± 0.436	5.46 ± 0.298	138.09	1.2	0.75	1.0
ACET	45	8.98 ± 0.523**	4.86 ± 0.533	184.77	1.6	1.0	1.19

Each value represents the mean ± SEM ($n = 6$).
*$P < 0.05$, **$P < 0.01$ (Dunnett's multiple comparison test).
CONT = control, ACET = acetazolamide.

saline control. The diuretic action of **3e** and **3d** was 2.62 and 2.03, respectively, in comparison with 1.2 and 1.6 for urea and acetazolamide, respectively. This activity was evaluated in terms of daily diuresis and was measured as the ratio of the diuretic action of the treated groups to the diuretic action of the standard (acetazolamide) group. As far as diuretic activity is concerned, compounds **3e** and **3d** was found to be 1.63 and 1.25, respectively, while **2e**, **2d** and **3c** were calculated as 0.93, 0.87 and 0.81, respectively (Table 1).

The Lipschitz value (the ratio *T/U*, in which *T* is the response of the test compound, and *U*, that of urea treatment, indices of 1.0 and higher are regarded as a positive effect in terms of diuretic activity) shows that **3e** is two times potent than urea as far as urinary output is concerned, and compounds **3d**, **2e**, **2d** and **3c** were nearly equal to urea (>1.0, means positive effects) (Table 1).

Compounds (**2a-f**) and (**3a-f**) were also tested for saluretic and kaliuretic effects in *albino* rat model. After 0–5 hours, compounds **3e**, **2e** and **3d** showed a significant increase in sodium excretion ($P<0.01$), i.e. 3.68 ± 0.284, 2.99 ± 0.150 and 2.83 ± 0.429, respectively, which was either almost similar or more than standards, i.e. urea (2.74 ± 0.154) and acetazolamide (3.36 ± 0.294). The Na^+ excretion of **2d** was also significant as compared with urea ($P<0.05$), i.e. 2.56 ± 0.278 and 2.74 ± 0.154, respectively (Table 2).

Compounds **3e** and **2e** were also found to have significant kaliuretic property ($P<0.01$), i.e. 1.72 ± 0.156 and 1.480 ± 0.405, respectively, similar to acetazolamide (1.523 ± 0.118) and with regards to Na^+/K^+ ratio, it was observed that **3e** is a stronger kaliuretic. Table 2 showed that compounds **3a**, **2b** and **3f** were potassium sparing (3.6, 3.4 and 3.3, respectively), while in rest of the compounds Na^+/K^+ ratio lies between 1.8 and 2.1, i.e. more kaliuretic than potassium sparing.

Chloride was estimated by titrating the urine by Volhards method. Chloride excretion was also increased to similar extent of sodium.

The extent of hepatic damage was assessed by the level of various biochemical parameters in circulation. Table 3 shows the liver function tests with reference to selected compounds. The estimation revealed that there was no significant increase in SGOT and SGPT alkaline phosphatase, and there is a decrease in protein level in serum as compared with the control level (Table 3). It was clearly indicated that none of the compound showed any toxicity of the liver as compared with control.

To summarize, an attempt to obtain an efficacious and non-toxic diuretic, we have designed and synthesized a series of pyrimidine derivatives. The structure–activity relationship (SAR) of pyrimidine diuretics may be rationalized by assuming that the newly synthesized compounds may possess an important site that involves

Table 2. Effects of amino and thio pyrimidine derivatives on electrolyte excretion.

Treatment	Dose (mg/kg body weight)	Electrolyte concentration (m Eq/L)				
		Na^+	K^+	Cl^-	Na^+/K^+	Na^+/Cl^-
2a	45	0.37 ± 0.278	0.18 ± 0.468	0.20 ± 0.128	2.05	1.85
2b	45	1.82 ± 0.150	0.53 ± 0.574	0.99 ± 0.133	3.4	1.83
2c	45	1.12 ± 0.089	0.62 ± 0.104	0.58 ± 0.187	1.80	1.93
2d	45	2.56 ± 0.278	1.34 ± 0.465	1.32 ± 0.614	1.91	1.93
2e	45	2.99 ± 0.150*	1.48 ± 0.405*	1.42 ± 0.895	2.02	2.10
2f	45	1.23 ± 0.149	0.63 ± 0.117	0.75 ± 0.140	1.95	1.64
3a	45	1.96 ± 0.404	0.53 ± 0.131	0.99 ± 0.686	3.6	1.97
3b	45	0.87 ± 0.253	0.48 ± 0.255	0.49 ± 0.151	1.81	1.77
3c	45	1.01 ± 0.117	0.58 ± 0.121	0.52 ± 0.946	1.74	1.94
3d	45	2.83 ± 0.429*	1.42 ± 0.428*	1.41 ± 0.382	1.99	2.0
3e	45	3.68 ± 0.284**	1.72 ± 0.156**	1.78 ± 0.454	2.13	2.15
3f	45	1.22 ± 0.255	0.36 ± 0.307	0.43 ± 0.224	3.38	2.83
CONT	—	0.90 ± 0.194	0.38 ± 0.428	0.44 ± 0.174	2.36	2.04
UREA	1000	2.74 ± 0.154	0.91 ± 0.426	0.93 ± 0.564	3.01	2.94
ACET	45	3.36 ± 0.294**	1.523 ± 0.118**	1.62 ± 0.407	2.206	2.07

*$P<0.05$, ** $P<0.01$ (Dunnett's multiple comparison test).
CONT = control, ACET = acetazolamide.

Table 3. Enzyme estimation of selected compounds.

Compound	Alkaline phosphatase ± SEM	SGOT ± SEM	SGPT ± SEM	Albumin ± SEM	Globulin ± SEM	Total protein ± SEM
Control	20.06 ± 0.48	31.33 ± 0.26	27.37 ± 0.48	3.88 ± 0.16	2.34 ± 0.88	7.10 ± 0.25
2d	20.66 ± 0.56	31.87 ± 0.21	27.57 ± 0.34	3.93 ± 0.68	2.86 ± 0.33	6.95 ± 0.53
2e	20.78 ± 0.38	33.77 ± 0.68	27.19 ± 0.90	3.46 ± 0.39	2.67 ± 0.49	6.53 ± 0.42
3d	19.46 ± 0.45	32.66 ± 0.24	26.18 ± 0.58	4.36 ± 0.24	2.13 ± 0.94	6.02 ± 0.28
3e	20.48 ± 0.06	31.98 ± 0.73	26.89 ± 0.97	3.76 ± 0.48	2.43 ± 0.68	6.43 ± 0.36

Values are given in mean ± SEM ($n=6$).

a basic centre of the drug, which may be N-1 or N-3 of pyrimidine nucleus or both. Groups that decrease the basic strength of pyrimidine nucleus reduce the diuretic activity. The other site involves the substituted phenyl ring at position 6, which may be hydrophobic in nature. This assumption is further supported in the structure of triamterene and its derivatives[35]. To conclude, the SAR based on the observed results indicated that the type of aryl group substitution attached to the position 6 of pyrimidine nucleus plays a significant role for diuretic activity. It has been noticed that the substitution of the phenyl group at the position 6 of pyrimidine heterocycle with a chlorine atom seems more favorable for an active diuretic agent than the case of using a methoxy residue. Additional research on the mechanisms of these compounds and modification is underway.

The preliminary *in vivo* diuretic studies suggested the following SAR,

1. Compound **3e** prototype of series-1 possess strong aquaretic and saluretic activity when given orally in a single dose. However, compound **2e** with NH_2 group showed comparable diuretic action with acetazolamide, whereas compound **3e** with–SH group showed improved activity, as compared with **2e** and acetazolamide.
2. Substitution at position 6 of pyrimidine ring system with electron-withdrawing group (Cl, F or Br) increases significant urinary excretion.
3. Compounds with electron-releasing group such as–CH_3 or–OCH_3 substitution at any position of pyrimidine ring reduces diuresis.

In short, the diuretic activity may be increased by incorporating strong electron-withdrawing and large size substituent on the phenyl ring. The toxicity studies also indicated that the selected compounds are safe for administration. The results obtained from the *in vivo* diuretic studies demonstrate the potential of searching for diuretic agents among the amino and thiopyrimidine derivatives.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.However, authors are thankful to Vice Chancellor, Jamia Hamdard for providing the necessary facilities.

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