SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW HYDRAZONE DERIVATIVES BEARING PYRIMIDINE RING AS ANALGESIC AND ANTI-INFLAMMATORY AGENTS

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Abstract: New hydrazone derivatives were synthesized from 4-chloro-2-(methylthio)pyrimidine-5-carbohydrazide. IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral data and elemental analysis characterized synthesized compounds. All compounds were investigated for analgesic and anti-inflammatory activity with hot plate test and biochemical assay, respectively. The results of anti-inflammatory activity showed that compound **2t** had maximal lipoxygenase (LOX) inhibition (66.30%) whereas inhibitions of **2a, 2c, 2i, 2k, 2l, 2m, 2p, 2s,** and **2u** were observed between approximately 45-15%. Our results also indicated that 15 compounds of 21 had an analgesic effect in hot plate test. Analgesic effect of **2l, 2s, 2t** began earlier than others while the effect of **2i** began at the latest time. According to the activity results, compound **2t** have both significant analgesic and anti-inflammatory effects.

Keywords: Hydrazones, analgesic activity, anti-inflammatory activity

Hydrazones, an important class of compounds (comprising –CO-NH-N=CH- pharmacophore group) in the structure (1), show numerous biological and pharmaceutical properties such as antibacterial (2), antifungal (3), antimalarial (4), analgesic (5), antioxidant (6), antituberculosis (7), anticancer (8), antidepressant (9) effects. Ansari et al. showed that hydrazone conjugates of metronidazole had better antiamoebic activity than reference drug metronidazole (10). Cordeiro et al. indicated antifungal activity of the isoniazid-derived hydrazones against *Coccidioides posadasii* (11). Rawat et al. also demonstrated the antimycobacterial activity of dipyrromethane hydrazone derivatives (12). In addition to these, Popiolek et al. proved antimicrobial activities of nitrofurazone derivatives. Hydrazone compounds showed more effective against *Staphylococcus spp.* than nitrofurantoin and cefuroxime reference drugs (13). Gadhawala et al. reported the antibacterial activity of phenyl and 2,4-dinitrophenyl hydrazones against *S.aureus* and *E.coli* (14).

International Association for the Study of Pain defined pain as an unpleasant sensory and emotional experience. Unfortunately, pain often lasts quite a long time, even after the precipitating insults have been resolved. Therefore, the management of pathological pain is a major clinical challenge (15). The prostaglandins and leukotrienes are naturally occurring fatty acid derivatives produced through arachidonic acid which cause inflammation, pain, fever, nausea, asthmatic and allergic reactions due to excessive production of prostaglandins and leukotrienes by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. The non-steroidal anti-inflammatory drugs (NSAIDs), another commonly used analgesic agent and as COX inhibitors are the leading prescription medicine worldwide for the cure of inflammation but their long-term use is restricted due to side effects (gastrointestinal, bronchoconstriction and hepatotoxic). Besides they increase production of LOX-derived eicosanoids such as leukotrienes (LTs), which limit analgesic and anti-

inflammatory effect of NSAIDs due to their bronchoconstriction, ulcerative, and inflammatory effects. Therefore COX and LOX inhibitor drugs are considered to enhance anti-inflammatory potency. The discovery of dual COX and LOX enzyme inhibitors are a big challenge for medicinal chemists (16, 17).

Kaplancikli et al. showed that some hydrazone derivatives containing cyclohexyl and substituted aromatic ring had anti-inflammatory activity (18). Kumar and Chauhan also reported the anti-inflammatory effect of new hydrazone derivatives bearing substituted aromatic ring such as pyridine, pyrazole, indole (19). In another study, Rodrigues et al. indicated good cytotoxic activity of their hydrazone derivatives when compared to the reference drug doxorubicin (20). In addition to this, Venkateshwarlu et al. found the analgesic activity of isatin hydrazone compounds with hot plate test (21).

Pyrimidine derivatives play vital roles in *N*-heterocyclic chemistry. These compounds have naturally occurring substances such as nucleotides, enzymes and vitamins. Therefore researchers studied with pyrimidine derivatives in different pharmaceutical and agrochemical areas (22, 23). Bonacorso et al. indicated a similar analgesic effect with ketoprofen in arthritic mouse pain model. Karoui et al. reported good analgesic and anti-inflammatory activity of pyrimidine derivatives when compared acetylsalicylate. In another study, Karoui showed anti-inflammatory activity of pyrimidine compounds by COX enzyme inhibition (24-26).

In this study, a new series of hydrazone derivatives bearing pyrimidine ring were synthesized from 4-chloro-2-(methylthio)pyrimidine-5-carbohydrazide. The potential analgesic and anti-inflammatory effects of synthesized compounds were investigated by hot plate test and LOX inhibition test. These compounds may lead to the development of new analgesic and anti-inflammatory drugs in future studies.

EXPERIMENTAL

Chemistry

All chemicals reagents and solvents were procured from Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany). The purity of the compounds was checked by thin layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F254) coated aluminum sheets (Merck) by using petroleum ether: ethyl acetate (30 : 70) as the solvent system. Visualization on TLC was done by ultra-violet (UV) light ($\lambda = 254$ nm). The purities of the synthesized compounds were checked by reversed phase HPLC (Chromasil C_{18} 4.6×150 mm column, 1311A Quat pomp, PDA detector) using acetonitrile and water (gradient flow) as the eluent. Melting points were determined by Kleinfield SMP-II (UK) basic model. IR spectra were recorded with Bruker Ultrashield TM (UK). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 300/75 MHz (UK) in DMSO-d_6 using tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) were expressed in parts per million relatives to TMS and the following abbreviations were used to describe the peak patterns when appropriate: s, (singlet); d, (doublet); t, (triplet); q, (quartet); m, (multiplet). MS spectra were recorded on an Ab-Sciex3200 QTRAP LCMS/MS (NY, USA). Elemental analysis (C, H, N, and S) was performed on a CHNS-Thermo Scientific Flash 2000 (Waltham, MA USA).

General procedure for the synthesis of the hydrazide derivatives

4-Chloro-2-(methylthio)pyrimidine-5-carbohydrazide [1]

Ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (3 mmol) was solved in methanol (5 mL). Then, hydrazine monohydrate (10 mmol) was added and the mixture stirred at room temperature for 4 h. The reaction was finalized by thin layer chromatography control and left 1 h in a refrigerator. The precipitate was filtered, washed with water and purified with methanol (27).

4-Chloro-2-(methylthio)pyrimidine-5-carbohydrazide [1]

White solid, yield 80%, m.p. 98-100. IR $v_{max.}$(cm^{-1}): 3361, 3309, 3277, 3000, 2984, 2903, 2872, 1678, 1602, 1562, 1473, 1413, 1357, 1172, 1080, 678. ^{1}H-NMR (300 MHz, DMSO-d_6) δ(ppm): 2.51 (s, 3H,-SCH$_3$), 4.70 (s, 2H, -NH$_2$), 8.49 (s, 1H, -NH-), 9.01 (s, 1H, pyrimidine -CH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ(ppm): 14.15-14.54 (-S-CH$_3$), 116.22 (C-3), 157.96 (C-2), 159.53 (C-4), 166.03 (C-5), 175.68 (C-1). For $C_6H_7ClN_4OS$ (M.W.: 218.66 g/mol) calcd: C, 32.96; H, 3.23; N, 25.62; S, 14.66%; found: C, 33.47; H, 3.21; N, 25.88; S, 14.81%; QTRAP (ES, MS m/z): 219.0 (M$^+$ + 1), 172.8, 156.98, 141.0, 112.8, 72.0, 40.0.

General procedure for the synthesis of the hydrazone derivatives [2a-w]

4-Chloro-2-(methylthio)pyrimidine-5-carbohydrazide (2.2 mmol) was heated with ethanol (10 mL) and a few drops of glacial acetic acid. Substituted

aldehyde (2.2 mmol) was added on the mixture and refluxed for 4 h. The reaction was finalized by thin layer chromatography control. The precipitate was filtered, dried and purified with methanol (28).

4-Chloro-N'-(4-methoxybenzylidene)-2-(methyl-thio)pyrimidine-5-carbohydrazide [2a]

White solid, yield 90%, m.p. 136-137. IR $v_{max.}(cm^{-1})$: 3231, 3070, 2987, 2887, 1681, 1601, 1567, 1512, 1485, 1438, 1419, 1098, 1025, 828, 673. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.52 (s, 3H, -O$_3$), 3.81 (s, 3H, H-8), 7.04 (d, 2H, J = 7.6 Hz, aromatic protons, H-5, H-6), 7.69 (d, 2H, J = 7.5 Hz, aromatic protons, H-4, H-7), 8.37 (s, 1H, H-3), 8.69 (s, 1H, H-1), 11.23 (s, 1H, H-2). For C$_{14}$H$_{13}$ClN$_4$O$_2$S (M.W.: 336.80 g/mol) calcd: C, 49.93; H, 3.89; N, 16.64; S, 9.52%; found: C, 49.87; H, 3.82; N, 16.61; S, 9.50%.

4-Chloro-N'-(2-hydroxybenzylidene)-2-(methyl-thio)pyrimidine-5-carbohydrazide [2b]

Yellow solid, yield 88%, m.p. 179-180. IR $v_{max.}(cm^{-1})$: 3217, 3100, 2986, 1678, 1619, 1556, 1478, 1360, 1132, 746, 602. ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.52 (s, 3H, -SCH$_3$), 6.91-7.44 (m, 4H, aromatic protons), 8.71, 8.68 (2s, 2H, pyrimidine CH and N=CH), 11.43 (s, 1H, Ar-OH), 11.65 (s, 1H, -CONH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta(ppm)$: 14.38 (-S-CH$_3$), 116.52, 118.22, 119.38 (C-3, C-7, C-9, C-11, C-12), 131,43 (C-10), 143.60 (C-6), 157.67-158.65 (C-8), 159.56-160.08 (C-2), 161.44 (C-4), 165.15 (C-5), 172.30 (C-1). For C$_{13}$H$_{11}$ClN$_4$O$_2$S (M.W.: 322.77 g/mol) calcd: C, 48.37; H, 3.44; N, 17.36; S, 9.93%; found: C, 48.24; H, 3.40; N, 17.32; S, 9.89%.

4-Chloro-2-(methylthio)-N'-((5-nitrothiophen-2-yl)methylene)pyrimidine-5-carbohydrazide [2c]

Yellow solid, yield 93%, m.p. 224-226. IR $v_{max.}(cm^{-1})$: 3232, 3087, 2979, 1693, 1577, 1552, 1528, 1487, 1424, 1366, 1303, 1033, 639. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.51 (s, 3H, -SCH$_3$), 7.47 (d, 1H, J = 4.2 Hz, H-4), 8.11 (d, 1H, J = 4.2 Hz, H-5), 8.65, 8.69 (2s, 2H, H-1 and H-3), 11.60 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta(ppm)$: 14.26 (S-CH$_3$), 121.52 (C-3), 129.30-130.30 (C-8 and C-9), 146.55 (C-6), 150.70 (C-7), 156.65 (C-2 and C-10), 158.65 (C-4), 165.29 (C-5), 172.30 (C-1). For C$_{11}$H$_8$ClN$_5$O$_3$S$_2$ (M.W.: 357.80 g/mol) calcd: C, 36.93; H, 2.25; N, 19.57; S, 17.92%; found: C, 36.96; H, 2.23; N, 19.54; S, 17.92%.

4-Chloro-2-(methylthio)-N'-(4-(trifluoromethyl)-benzylidene)pyrimidine-5-carbohydrazide [2d]

White solid, yield 88%, m.p. 179-180. IR $v_{max.}(cm^{-1})$: 3353, 3212, 3050, 2996, 2908, 2785, 1686, 1555, 1474, 1407, 1377, 1061, 837, 794, 663. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.51 (s, 3H, -SCH$_3$), 7.83 (d, 2H, J = 8.1 Hz, H-4 and H-7), 7.92 (d, 2H, J = 8.1 Hz, H-5 and H-6), 8.51 (s, 1H, H-3), 8.70 (s, 1H, H-1), 11.48 (s, 1H, H-2). For C$_{14}$H$_{10}$ClF$_3$N$_4$OS (M.W.: 374.77 g/mol) calcd: C, 44.87; H, 2.69; N, 14.95; S, 8.56%; found: C, 44.79; H, 2.60; N, 14.92; S, 8.58%.

4-Chloro-N'-(3,4-dichlorobenzylidene)-2-(methyl-thio)pyrimidine-5-carbohydrazide [2e]

White solid, yield 89%, m.p. 209-210. IR $v_{max.}(cm^{-1})$: 3231, 3151, 3048, 2962, 2905, 2817, 1681, 1610, 1558, 1475, 1409, 1404, 1380, 1364, 1011, 814, 675. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.51 (s, 3H, -SCH$_3$), 7.68-7.90 (m, 3H, aromatic protons), 8.39 (s, 1H, N=CH), 8.68 (s, 1H, pyrimidine -CH), 11.46 (s, 1H, CONH). For C$_{13}$H$_9$Cl$_3$N$_4$OS (M.W.: 375.66 g/mol) calcd: C, 41.56; H, 2.41; N, 14.91; S, 8.54%; found: C, 41.52; H, 2.40; N, 14.90; S, 8.50%.

4-Chloro-N'-(2,6-dichlorobenzylidene)-2-(methyl-thio)pyrimidine-5-carbohydrazide [2f]

White solid, yield 89%, m.p. 230-231. IR $v_{max.}(cm^{-1})$: 3241, 3140, 3063, 2979, 2923, 1673, 1606, 1561, 1484, 1438, 1422, 1366, 1067, 914. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.51 (s, 3H,-SCH$_3$), 7.30-7.75 (m, 3H, aromatic protons), 8.39 (s, 1H, N=CH), 8.68 (s, 1H, pyrimidine -CH), 11.45 (s, 1H, CONH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta(ppm)$: 14.14 (-S-CH$_3$), 129.73 (C-3), 130.64 (C-9 and C-11), 131.65 (C-8 and C-10), 134.49 (C-7 and C-12), 145.60 (C-6), 157.22 (C-2), 159.14 (C-4), 165.79 (C-5), 171.01 (C-1). For C$_{13}$H$_9$Cl$_3$N$_4$OS (M.W.: 375.66 g/mol) calcd: C, 41.56; H, 2.41; N, 14.91; S, 8.54%; found: C, 41.53; H, 2.38; N, 14.90; S, 8.55%. QTRAP (ES, MS m/z): 377.0(M$^+$ + 2), 359.0, 316.8, 112.8, 72.1.

4-Chloro-N'-(3,5-dichloro-2-hydroxybenzylidene)-2-(methylthio)pyrimidine-5-carbohydrazide [2g]

Yellow solid, yield 88%, m.p. 244-246. IR $v_{max.}(cm^{-1})$: 3550, 3233, 3075, 2981, 1682, 1611, 1561, 1480, 1450, 1434, 1390, 1366, 1377, 1083, 614. ^{1}H-NMR (300 MHz, DMSO-d_6) $\delta(ppm)$: 2.51 (s, 3H, -SCH$_3$), 7.50 (s, 1H, H-3), 7.61 (s, 1H, H-2), 8.29 (s, 1H, N=C<u>H</u>), 8.67 (s, 1H, H-1), 11.62 (s, 1H, CONH). ^{13}C-NMR (75 MHz, DMSO-d_6) $\delta(ppm)$: 14.10 (-S-CH$_3$), 121.8 (C-7), 123.4 (C-3), 126.9, 127.2 (C-9, C-11), 128.9 (C-12), 132.4 (C-10), 146.38 (C-6), 160.02 (C-2 and C-8), 162.47 (C-4),

166.05 (C-5), 171.0 (C-1). For $C_{13}H_9Cl_3N_4O_2S$ (M.W.: 391.66 g/mol) calcd: C, 39.87; H, 2.32; N, 14.30; S, 8.19%; found: C, 39.65; H, 2.17; N, 14.18; S, 8.05%.

4-Chloro-N'-(4-hydroxybenzylidene)-2-(methylthio)pyrimidine-5-carbohydrazide [2h]

Brown solid, yield 85%, m.p. 237-238. IR $\nu_{max.}$(cm^{-1}): 3212, 3052, 2930, 2883, 1681, 1610, 1571, 1492, 1440, 1414, 1413, 1362, 1080, 793. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 2.51 (s, 3H, -SCH$_3$), 6.84 (d, 2H, J = 8.4 Hz, H-4 and H-7), 7.57 (d, 2H J = 8.2 Hz, H-5 and H-6), 8.29 (s, 1H, H-3), 8.67 (s, 1H, H-1), 11.62 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.98 (-SCH$_3$), 115.72 (C-9 and C-11), 121.5 (C-3), 125.01 (C-7), 128.93 (C-8 and C-12), 147.8 (C-6), 160.9 (C-2 and C-10), 162.3 (C-4), 165.20 (C-5), 171.8 (C-1). For $C_{13}H_{11}ClN_4O_2S$ (M.W.: 322.77 g/mol) calcd: C, 48.37; H, 3.44; N, 17.36; S, 9.93%; found: C, 48.34; H, 3.43; N, 17.38; S, 9.91%.

4-Chloro-2-(methylthio)-N'-(quinolin-3-ylmethylene)pyrimidine-5-carbohydrazide [2i]

White solid, yield 82%, m.p. 241-242. IR $\nu_{max.}$(cm^{-1}): 3362, 3215, 3032, 2929, 2868, 1691, 1603, 1554, 1474, 1407, 1404, 1362, 1006, 675. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.32 (t, 3H, CH$_3$CH$_2$OH), 2.51 (s, 3H, -SCH$_3$), 4.35 (q, 2H, CH$_3$CH$_2$OH), 7.64-7.84 (m, 4H, H-3, H-4, H-5 and H-6), 8.54-8.70 (m, 3H, H-1, H-2 and N=CH), 9.31 (s, 1H, pyrimidine -CH), 11.49 (s, 1H, CONH). For $C_{16}H_{12}ClN_5OS.C_2H_5OH$ (M.W.: 403.89 g/mol) calcd: C, 53.53; H, 4.49; N, 17.34; S, 7.94%; found: C, 53.01; H, 4.21; N, 17.04; S, 7.35%.

4-Chloro-2-(methylthio)-N'-(4-(pyrrolidin-1-yl)benzylidene)pyrimidine-5-carbohydrazide [2j]

Brown solid, yield 88%, m.p. 232. IR $\nu_{max.}$(cm^{-1}): 3187, 3100, 2960, 2847, 1666, 1594, 1562, 1525, 1432, 1064, 638. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.96 (s, 4H, H-9 and H-10), 2.50 (s, 3H, -SCH$_3$), 3.28 (s, 4H, H-8 and H-11), 6.59 (d, 2H, J = 8.1 Hz, H-5 and H-6), 7.55 (d, J = 8.0 Hz, 2H, H-4 and H-7), 8.21 (s, 1H, H-3), 8.65 (s, 1H, H-1), 11.08 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 14.07 (-SCH$_3$), 25.49 (C-14 and C-15), 47.69 (C-13 and C-16), 112.04 (C-9 and C-11), 121.09 (C-7 and C-3), 129.23 (C-8 and C-12), 149.44-149.66 (C-6 and C-10), 156.59 (C-2), 158.82 (C-4), 166.21 (C-5), 175.98 (C-1). For $C_{17}H_{18}ClN_5OS$ (M.W.: 375.88 g/mol) calcd: C, 54.32; H, 4.83; N, 18.63; S, 8.53%; found: C, 54.12; H, 4.23; N, 18.00; S, 8.825%.

4-Chloro-2-(methylthio)-N'-((2-oxoindolin-3-yl)methylene)pyrimidine-5-carbohydrazide [2k]

Yellow solid, yield 88%, m.p. 268-269. IR $\nu_{max.}$(cm^{-1}): 3195, 3141, 3067, 2997, 2991, 2810, 1705, 1673, 1539, 1460, 1021, 635. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.33 (t, 3H, CH$_3$CH$_2$OH) 2.51 (s, 3H, -SCH$_3$), 4.35 (q, 2H, CH$_3$CH$_2$OH), 7.69-8.80 (m, 5H, aromatic protons), 10.88 (s, 1H, indol NH), 11.96 (s, 1H, CONH). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.74-13.92 (-SCH$_3$ and CH$_3$CH$_2$OH), 61.90 (CH$_3$CH$_2$OH), 111.16 (C-13), 115.16 (C-9), 122.07 (C-11), 123.07 (C-12), 123.47 (C-3), 132.09 (C-10), 138.28 (C-8), 143.76 (C-6), 157.40 (C-2), 159.10 (C-4), 164.49 (C-5), 166.00 (C-7), 176.27 (C-1). For $C_{14}H_{10}ClN_5O_2S.C_2H_5OH$ (M.W.: 393.85 g/mol) calcd: C, 48.79; H, 4.09; N, 17.78; S, 8.14%; found: C, 47.90; H, 4.00; N, 17.12; S, 7.99%.

4-Chloro-N'-((2-chloroquinolin-3-yl)methylene)-2-(methylthio)pyrimidine-5-carbohydrazide [2l]

Yellow solid, yield 94%, m.p. 277-279. IR $\nu_{max.}$(cm^{-1}): 3219, 3142, 3043, 2980, 2926, 2864, 1678, 1608, 1573, 1545, 1456, 1406, 1367, 1168, 678. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.34 (t, 3H, CH$_3$CH$_2$OH), 2.52 (s, 3H, -SCH$_3$), 4.30 (q, 2H, CH$_3$CH$_2$OH), 7.13-7.24 (m, 2H, H-6 and H-7), 7.44-7.47 (d, 1H, H-8), 7.79 (s, 1H, H-4), 8.44-8.47 (d, 1H, H-5), 8.57-8.65 (2s, 2H, H-3 and H-1) and 11.61 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 14.10-14.64 (CH$_3$CH$_2$OH and SCH$_3$), 61.47 (CH$_3$CH$_2$OH), 112.37 (C-3), 120.90–124.88 (C-10, C-9, C-8, C-8a, C-12 and C-11), 131.02 (C-7), 137.50 (C-12a), 145.42 (C-6), 156.65 (C-2 and C-13), 158.86 (C-4), 165.21 (C-5), 176.20 (C-1). For $C_{16}H_{11}Cl_2N_5OS.C_2H_5OH$ (M.W.: 438.33 g/mol) calcd: C, 49.32; H, 3.91; N, 15.98; S, 7.32%, found: C, 49.11; H, 3.20; N, 15.23; S, 7.15%.

4-Chloro-N'-(indolin-3-ylmethylene)-2-(methylthio)pyrimidine-5-carbohydrazide [2m]

Yellow solid, yield 87%, m.p. 216-218. IR $\nu_{max.}$(cm^{-1}): 3225, 3097, 2978, 2892, 1674, 1608, 1492, 1454, 1404, 1367, 1064, 666. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 2.60 (s, 3H, -SCH$_3$), 7.68-7.73 (t, 1H, H-7), 7.85-7.90 (t, 1H, H-8), 7.99 (d, J = 8.4 Hz, 2H, H-4 and H-6), 8.19 (d, J = 8.4 Hz, 1H, H-9), 8.72 (s, 1H, H-3), 8.87 (s, 1H, H-1) and 11.28 (s, 2H, H-2 and H-5). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 14.02 (-SCH$_3$), 111.61-111.86 (C-7 and C-11), 120.39, 122.09, 122.67, 124.36 (C-3, C-9, C-10, C-8, C-7a), 130.49 (C-12), 136.99 (C-11a), 144.88 (C-6), 156.03 (C-2), 158.29 (C-4), 165.72 (C-5), 175.48 (C-1). For $C_{15}H_{12}ClN_5OS$ (M.W.:

345.81 g/mol) calcd: C, 52.10; H, 3.50; N, 20.25; S, 9.27%, found: C, 51.23; H, 4.38; N, 20.76; S, 9.00%.

4-Chloro-N'-((6-(4-(methylsulfonyl)phenyl)pyridin-2-yl)methylene)-2-(methylthio)pyrimidine-5-carbohydrazide [2n]

Yellow solid, yield 91%, m.p. 194-196. IR $v_{max.}$(cm^{-1}): 3228, 3048, 2971, 2924, 2866, 1674, 1493, 1453, 1404, 1382, 1087, 687. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1,3 (t, 3H, CH$_3$CH$_2$OH), 2,51 (s, 3H, -SCH$_3$), 3.29 (s, 3H, H-9), 4.3 (q, 2H, CH$_3$CH$_2$OH), 7.97-8.14 (m, 5H, H-7, H-6, H-4 and H-5), 8.38 (d, 2H, J = 8.7, H-8), 8.51 (s, 1H, H-3), 8.70 (s, 1H, H-1), 11.57 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.66-13.99 (-SCH$_3$ and CH$_3$CH$_2$OH), 43.66 (C-18), 61.23 (CH$_3$CH$_2$OH), 119.75, 121.58 (C-3, C-8 and C-10), 127.42, 127.49 (C-13, C-17, C-15, C-14 and C-16), 138.33 (C-9), 141.09, 142.69 (C-6 and C-12), 153.34 (C-7), 154.13 (C-11), 159.20 (C-2), 160.90 (C-4), 164.80 (C-5), 172.68 (C-1). For C$_{19}$H$_{16}$ClN$_5$O$_3$S$_2$.1/4C$_2$H$_5$OH (M.W.: 473.47 g/mol) calcd: C, 49.47; H, 3.72; N, 14.79; S, 13.54%; found: C, 49.00; H, 3.21; N, 15.995 S, 13.73%.

4-Chloro-N'-(2-chlorobenzylidene)-2-(methylthio)pyrimidine-5-carbohydrazide [2o]

White solid, yield 92%, m.p. 144-145. IR $v_{max.}$(cm^{-1}): 3221, 3154, 3057, 2978, 2923, 2866, 1673, 1594, 1551, 1467, 1463, 1444, 1401, 1366, 1075, 749. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.30 (t, 3H, CH$_3$CH$_2$OH), 2.51 (s, 3H, SCH$_3$), 4.32 (q, 2H, CH$_3$CH$_2$OH), 7.42-7.55 (m, 3H, other aromatic protons), 7.98 (t, 1H, aromatic proton), 8.70 and 8.73 (2s, 2H, pyrimidine -CH and N=CH), 11.56 (s, 1H,-CONH). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 14.12-14.40 (-SCH$_3$ and CH$_3$CH$_2$OH), 62.19 (CH$_3$CH$_2$OH), 126.18 (C-11 and C-3), 126.63 (C-12), 130.41 (C-9), 131.58 (C-10), 134.45 (C-8), 137.01 (C-7), 143.94 (C-6), 156.11 (C-2), 159.48 (C-4), 165.32 (C-5), 176.80 (C-1). For C$_{13}$H$_{10}$Cl$_2$N$_4$OS.1/4C$_2$H$_5$OH (M.W.: 352.73 g/mol) calcd: C, 45.97; H, 3.29; N, 15.88; S, 9.09%; found: C, 46.20; H, 3.15; N, 16.55; S, 9.77%. QTRAP (ES, MS m/z): 342.8(M$^+$+ 1), 186.8, 144.8, 112.8, 72.0.

4-Chloro-2-(methylthio)-N'-(4-(piperidin-1-yl)benzylidene)pyrimidine-5-carbohydrazide [2p]

Yellow solid, yield 89%, m.p. 161-162. IR $v_{max.}$(cm^{-1}): 3243, 3038, 2923, 1685, 1603, 1560, 1487, 1400, 791, 671. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1,34 (t, 3H, CH$_3$CH$_2$OH), 1.58 (s, 6H, H-9, H-10 and H-11), 2.51 (s, 3H,-SCH$_3$), 3.27 (t,

4H, H-8 and H-12), 4.30 (q, 2H, CH$_3$CH$_2$OH), 6.97 (d, J = 8.7 Hz, 2H, H-4 and H-7), 7.56 (d, J = 7.8 Hz, 2H, H-5 and H-6), 8.26 (s, 1H, H-3), 8.66 (s, 1H, H-1), 11.14 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.56-13.98 (CH$_3$CH$_2$OH and -SCH$_3$), 61.06 (CH$_3$CH$_2$OH), 23.89 (C-15), 24.91 (C-14 and C-16), 48.30 (C-13 and C-17), 114.50 (C-3, C-9, C-11), 122.97 (C-7), 128.47 (C-8 and C-12), 147.51 (C-6), 152.36 (C-2 and C-10), 159.68 (C-4), 164.45 (C-5), 176.28 (C-1). For C$_{18}$H$_{20}$ClN$_5$OS.1/2C$_2$H$_5$OH (M.W.: 412.94 g/mol) calcd: C, 55.26; H, 5.61; N, 16.96; S, 7.77%; found: C, 55.03; H, 5.27; N, 16.53; S, 7.23%.

4-Chloro-2-(methylthio)-N'-(pyridin-4-ylmethylene)pyrimidine-5-carbohydrazide [2r]

Yellow solid, yield 81%, m.p. 238-240. IR $v_{max.}$(cm^{-1}): 3195, 3148, 3050, 2975, 2922, 1679, 1571, 1518, 1367, 1160, 839, 657. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 2,51 (s, 3H,-SCH$_3$), 6.84 (d, J = 8.1 Hz, 2H, H-4 and H-7), 7.12 (d, J = 7.2 Hz, 2H, H-5 and H-6), 8.28 (s, 1H, H-3), 8.67 (s, 1H, H-1), 11.18 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.97 (-SCH$_3$), 115.46 (C-3), 121.87 (C-8 and C-11), 147.91 (C-6 and C-7), 149.00 (C-9 and C-10), 158.90 (C-2), 162.30 (C-4), 165.70 (C-5), 172.80 (C-1). For C$_{12}$H$_{10}$ClN$_5$OS (M.W.: 307.76 g/mol) calcd: C, 46.83; H, 3.28; N, 22.76; S, 10.42%; found: C, 46.27; H, 3.05; N, 22.42; S, 10.31%.

4-Chloro-2-(methylthio)-N'-(thiophen-2-ylmethylene)pyrimidine-5-carbohydrazide [2s]

Yellow solid, yield 86%, m.p. 220-221. IR $v_{max.}$(cm^{-1}): 3212, 3002, 2932, 1675, 1596, 1572, 1365, 1162, 790, 725, 637. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 2.51 (s, 3H, -SCH$_3$), 7.13-7.68 (m, 3H, H-4, H-5 and H-6), 8.64-8.66 (2s, 2H, H-1 and H-3), 11.27 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.98 (-SCH$_3$), 118.60 (C-3), 127.88 (C-9 and C-10), 130.78 (C-8), 138.39 (C-7), 143.80 (C-6), 156.27 (C-2), 158.39 (C-4), 165.30 (C-5), 174.69 (C-1). For C$_{11}$H$_9$ClN$_4$OS$_2$ (M.W.: 312.80 g/mol) calcd: C, 42.24; H, 2.90; N, 17.91; S, 20.50%; found: C, 42.21; H, 2.79; N, 17.86; S, 20.04%.

4-Chloro-2-(methylthio)-N'-(pyridin-3-ylmethylene)pyrimidine-5-carbohydrazide [2t]

White solid, yield 80%, m.p. 165-167. IR $v_{max.}$(cm^{-1}): 3177, 3050, 2932, 1691, 1608, 1557, 1508, 1474, 1492, 1422, 1365, 1064, 647. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.31 (t, 3H, CH$_3$CH$_2$OH), 2.51 (s, 3H, -SCH$_3$), 4.33

(CH$_3$CH$_2$OH), 7.48-7.53 (t, 1H, H-6), 8.11 (s, 1H, H-3), 8.46-8.85 (m, 4H, H-1, H-4, H-5 and H-7), 11.27 (s, 1H, H-2). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.62-13.96 (-SCH$_3$ and CH$_3$CH$_2$OH), 61.19 (CH$_3$CH$_2$OH), 124.03 (C-3 and C-10), 130.07 (C-7), 130.43 (C-11), 144.94 (C-6), 148.56 (C-8), 150.63 (C-9), 156.60 (C-2), 158.43 (C-4), 165.58 (C-5) and 175.81 (C-1). For C$_{12}$H$_{10}$ClN$_5$OS.1/4C$_2$H$_5$OH (M.W.: 319.28 g/mol) calcd: C, 47.02; H, 3.63; N, 21.94; S, 10.04%; found: C, 46.02; H, 2.95; N, 21.98; S, 10.80%.

N'-((5-bromothiophen-2-yl)methylene)-4-chloro-2-(methylthio)pyrimidine-5-carbohydrazide [2u]

White solid, yield 80%, m.p. 218-219. IR $\nu_{max.}$(cm^{-1}): 3248, 3071, 2984, 2923, 1693, 1606, 1593, 1567, 1482, 1397, 1166, 794, 631. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 2,51 (s, 3H, -SCH$_3$), 7.27 (d, J = 3.9 Hz, 2H, thiophen protons), 8.56-8.67 (s, 2H, pyrimidine -CH and N=CH), 11.31 (s, 1H, CONH). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 14.03 (-SCH$_3$), 115.60 (C-10 and C-3), 131.71-131.85 (C-8 and C-9), 141.30 (C-6 and C-7), 159.42 (C-2), 165.30 (C-4 and C-5), 172.74 (C-1). For C$_{11}$H$_8$BrClN$_4$OS$_2$ (M.W.: 391.69 g/mol) calcd: C, 33.73; H, 2.06; N, 14.30; S, 16.37%; found: C, 33.19; H, 2.91; N, 15.03; S, 16.49%.

4-Chloro-2-(methylthio)-N'-((5-nitrofuran-2-yl)methylene)pyrimidine-5-carbohydrazide [2w]

White solid, yield 80%, m.p. 238-239. IR $\nu_{max.}$(cm^{-1}): 3236, 3139, 2980, 1697, 1554, 1493, 1452, 1339, 1093, 631. ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ(ppm): 1.30 (t, 3H, CH$_3$CH$_2$OH), 2.51 (s, 3H,-SCH$_3$), 4.30 (q, 2H, CH$_3$CH$_2$OH) 7.18 (d, J = 3.9 Hz, 1H, H-4), 7.79 (d, J = 3.9 Hz, 1H, H-5), 8.4 (s, 1H, H-3), 8.72 (s, 1H, H-1), 11.65 (s, 1H, CONH). ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ(ppm): 13.61-13.95 (-SCH$_3$ and CH$_3$CH$_2$OH), 61.31 (CH$_3$CH$_2$OH), 114.75-115.00 (C-3, C-8 and C-9), 143.50 (C-6), 151.68-151.84 (C-7 and C-10), 156.51 (C-2), 158.57 (C-4), 165.32 (C-5), 173.20 (C-1). For C$_{11}$H$_8$ClN$_5$O$_4$S.1/4C$_2$H$_5$OH (M.W.: 353.25 g/mol) calcd: C, 39.10; H, 2.71; N, 19.83; S, 9.08%; found: C, 38.45; H, 2.20; N, 20.21; S, 9.42%.

Animals

Male Balb/C mice (25–32 g) were used in this study (n = 10 per group). Animals were kept under standard laboratory conditions (room temperature: 25.0 ± 2.0°C, relative humidity: 55–65% and 12 h light/dark cycle) throughout the experiment. The animals were fed ad libitum standard mouse cubes and water. The animals were adapted to the labora-tory environment for a period of a week prior to per-forming the experiments. All experiments were per-formed according to Ethical Principles and Guidelines for Scientific Experiments and were approved by the Ethics Committee of Marmara University (2012.Mar).

Compounds and treatments

All compounds were homogeneously suspend-ed within 17% tween 80/saline. All compounds were intraperitoneally (i.p.) injected to mice at a dose of 50 mg/kg and 0.1 mL/10 g volume. A pilot study was conducted to determine the dosing inter-val for analgesic effect, which does not cause any changes in locomotor activity. Due to the ethical issues, the median dose was picked for scanning the biological activity of all compounds. Control group received an equivalent volume of 17% tween 80/saline solution with other groups.
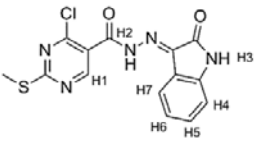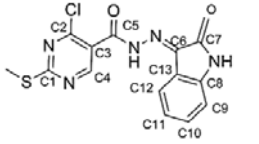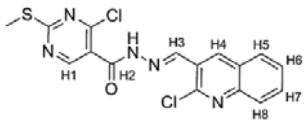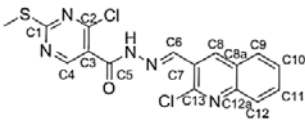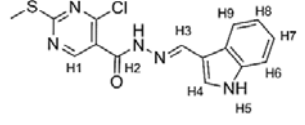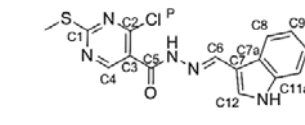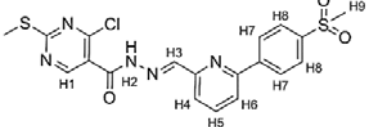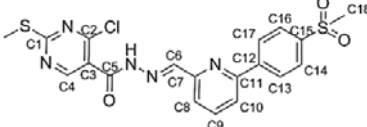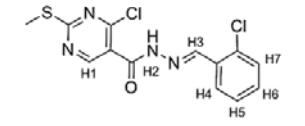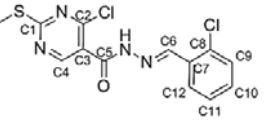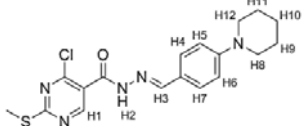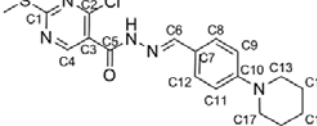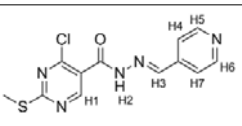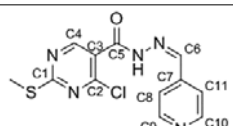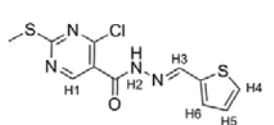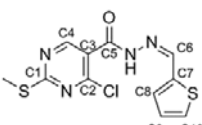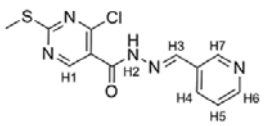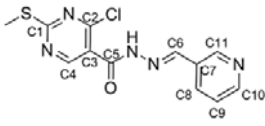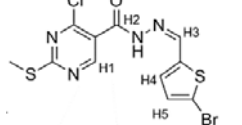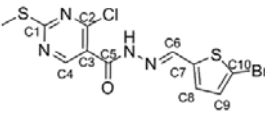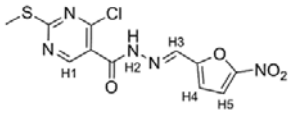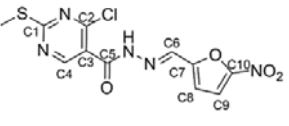
Antinociceptive activity test

Hot plate test was performed for evaluating the potential analgesic effect of various substances. Pain reflexes in response to a thermal stimulus were per-formed by hot plate test. The method was employed for the purpose of preferential assessment of possi-ble centrally mediated analgesic effects (29). Mice were divided into groups of 10 mice each. Mice were placed on a hot plate kept at a temperature of 52 ± 1°C. A cut off period of the 60 sec was main-tained to avoid paw tissue damage. The response in the form of forepaw licking, withdrawal symptom of the paws or jumping was recorded at 10, 20, 40, 60, 90, 120, 150 (if necessary) and 180 (if necessary) min following treatment. Baseline value as an indi-cator of basal algesia threshold recorded just before the treatments. Mice that did not respond to a ther-mal stimulus within 60 s of baseline evaluation were excluded from the experiment.

Statistical analysis

All results are presented as a mean ± standard error of the mean (SEM). Since pain threshold is a personalized value, the response of each group was evaluated by using basal values of the group. Statistical evaluation of the data was performed with Graph Pad Prism version 6.0 for Mac (GraphPad Software, San Diego, CA, USA). Repeated measures of one way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparison with baseline threshold (*: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$) and two way ANOVA followed by Sidak's post hoc test for comparison to control group (+: $p < 0.05$. ++: $p < 0.01$. +++: $p < 0.001$).

Compounds	Numbers for ¹H-NMR data	Numbers for ¹³C-NMR data
1		
2a		
2b		
2c		
2d		
2e		
2f		
2g		
2h		
2i		
2j		

Compounds	Numbers for ¹H-NMR data	Numbers for ¹³C-NMR data
2k		
2l		
2m		
2n		
2o		
2p		
2r		
2s		
2t		
2u		
2w		

Supporting information

LOX inhibitory activity

LOX inhibitory activity was measured by modifying the spectrophotometric method developed by Baylac and Racine (30). LOX (1.13.11.12, type I-B, Soybean), linoleic acid were purchased from Sigma (St. Louis, MO, USA). All chemicals used were of analytical grade. Potassium phosphate buffer (1.98 mL; 100 mM; pH 9.0), 40 µL of test compound solution and 20 µL of lipoxygenase solution were mixed and incubated for 10 min at 25°C. Test compounds and the standard (Nordihydroguaiaretic acid (NDGA)) were dissolved in DMSO. The reaction was then initiated by the addition of 10 µL linoleic acid solution, the change of absorbance at 234 nm was followed for 10 min (31).

All the kinetic experiments were performed in a quartz cuvette. Each test compound and control was run in triplicate at each concentration. The percent inhibition of enzymes activities (I%) was calculated by using the equation:

$$I\% = 100 - (OD_{sample}/OD_{control}) \times 100$$

All data are presented as the mean ± standard deviation (SD).

RESULT AND DISCUSSION

Synthesis

The synthetic route to the target compounds is outlined in Scheme 1. The structures of the compounds (**1, 2a-w**) were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral data, and elemental analysis. IR spectra of hydrazide-hydrazones (**2a-w**) revealed NH absorption bands around 3141-3362 cm^{-1} and C=O absorption bands in the 1666-1697 cm^{-1} region. IR spectra of all compounds (**1, 2a-w**) were described aromatic rings C-H stretching (3000-3100 cm^{-1}), aromatic rings C=C stretching (1452-1608 cm^{-1}) bands. The NH protons of hydrazide-hydrazones groups resonated as a singlet peak at 11.08-11.96 ppm. The aromatic protons appeared a multiplet at 6.59-8.87 ppm. Other protons were observed at expected regions. In their ^{13}C-NMR spectra, the signal due to the hydrazide-hydrazone carbon appeared at 141.09-149.44 ppm. The signal due to the carbonyl carbon was observed at 164.45-166.21 ppm. Other aromatic and aliphatic carbons were observed at expected regions. The analyses by mass spectrometry for compounds showed the presence of molecular ion peaks [M + H]$^+$. The elemental analysis of compounds was in agreement with the proposed structures of the compounds.

Antinociceptive Activity

In this study, the potential analgesic effects of 21 compounds were evaluated by hot plate test. 15 of the 21 substances had an analgesic effect on mice while 6 of the substances had no effect on pain threshold in hot plate test.

Injection of **2a**, **2b**, **2c**, **2d**, **2e**, and **2k** did not alter reaction time to the thermal stimulus of mice in hot plate tests. It was shown that **2f** injected mice has higher latencies time than the control group at 60 min after injection (p < 0.05). In **2g** group, time latencies to thermal stimulus were significantly

i: MeOH, NH$_2$NH$_2$.H$_2$O
ii: EtOH, g.AcOH, substituted aldehyde
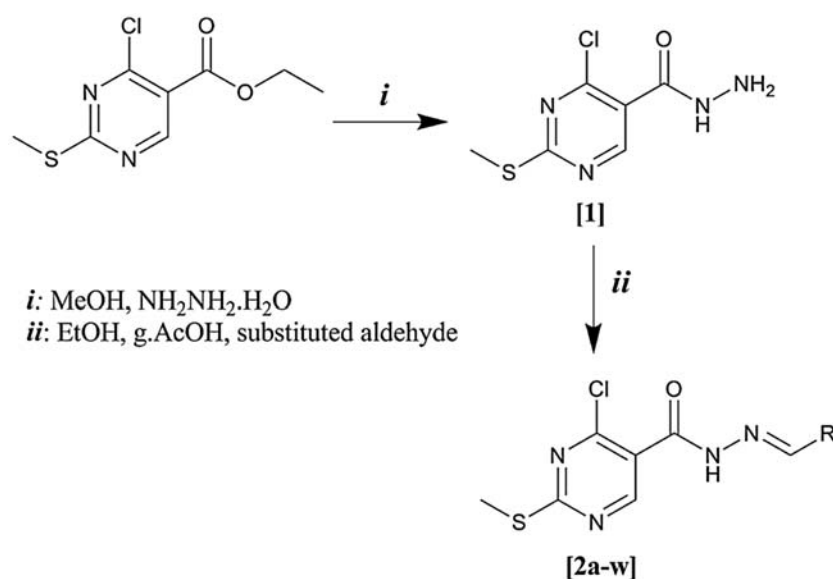
Scheme 1. The synthesis of the target compounds (**1, 2a-w**)

higher than baseline value of the same group at the min 120 (p < 0.01) and 180 (p < 0.001) while there was no significant difference compared with control group at any time. In **2h** injected mice, latency to thermal stimuli markedly increased at min 120 (p < 0.01), 150 (p < 0.05) and 180 (p < 0.05) compared with baseline and min 90 (p < 0.05), 120 (p < 0.001), 150 (p < 0.01) and 180 (p < 0.001) compared with control group. It has been found that **2i** elevated pain threshold from 40 min to 180 min (p < 0.05, p < 0.05, p < 0.001, p < 0.01, p < 0.001, p < 0.001, respectively) compared with baseline and it also increased latency time from min 40 to 180 (p < 0.01, p < 0.001, p < 0.001, p < 0.001, p < 0.001 and p < 0.001, respectively) compared with control group. In **2j** group, mice responded to thermal stimulus significantly later than its own baseline at min 120 (p < 0.05) and control group at min 120 (p < 0.05) and 150 (p < 0.01). It has also shown that **2l** injected mice had increased latency times at min 120 (p < 0.01) compared with its baseline value whereas it increased the threshold at min 20 (p < 0.01), 40 (p < 0.05), 60 (p < 0.001), 90 (p < 0.05), 120 (p < 0.01) and 150 (p < 0.05) compared with control group. **2m** increased reaction latency to thermal stimulus at min 60 (p < 0.05), 90 (p < 0.05), 150 (p < 0.05) and 180 (p < 0.001) in comparison with baseline latency. Latency times were also increased by **2m** at min 40 (p < 0.01), 60 (p < 0.05), min 90 (p < 0.001), 120 (p < 0.05), 150 (p < 0.05) and 180 (p < 0.001) compared to control group. The time latencies in the **2n** group was significantly higher than baseline value from min 90 to min 180 (p < 0.05, p < 0.05, p < 0.01, p < 0.05, respectively) while they were higher than latencies of control group at min 90, 120 and 150 (p < 0.05 for each). It was also indicated that **2o** injection increased latency times at min 90 (p < 0.05), 120 (p < 0.001) and 150 (p < 0.001) compared to baseline and min 90 (p < 0.01), 120 (p < 0.001) and 150 (p < 0.001) compared with control group. In **2p** group, latency times were markedly increased at min 60 (p < 0.05), 120 (p < 0.05), 150 (p < 0.001) and 180 (p < 0.01) compared with its baseline and from min 40 to min 150 compared with control group (p < 0.05, p < 0.001, p < 0.05, p < 0.01, p < 0.001, p < 0.001, respectively). It has found that **2r** elevated pain threshold from min 60 to 180 (p < 0.05, p < 0.05, p < 0.05, p < 0.001 and p < 0.001, respectively) compared with its baseline and from min 40 to 180 (p < 0.05, p < 0.001, p < 0.001, p < 0.001, p < 0.001 and p < 0.001, respectively) compared to control group. In **2s** group, response to painful stimulus was significantly delayed from min 40 to 150 (p < 0.05, p < 0.001, p < 0.001, p < 0.001,

p < 0.01) in comparison with its baseline and from min 10 to 150 (p < 0.05 for min 10 and p < 0.001 for others) compared with control animals. **2t** injected mice had increased latency times at min 60 (p < 0.05), min 120 (p < 0.001) and min 150 (p < 0.05) compared to its baseline latencies and from min 20 to 180 compared to control group (p < 0.05, p < 0.01, p < 0.001, p < 0.001, p < 0.001 and p < 0.001, respectively). It has demonstrated that **2u** elevated pain threshold at min 120 (p < 0.01), min 150 (p < 0.01) and min 180 (p < 0.05) compared with baseline latency and min 90 (p < 0.05), 120 (p < 0.001), 150 (p < 0.001) and 180 (p < 0.01) compared with control group. In **2w** group, time latencies were significantly higher than its baseline at min 150 (p < 0.05) and 180 (p < 0.001) while the latency was higher than control group at min 60 (p < 0.05), 90 (p < 0.05), 120 (p < 0.01), 150 (p < 0.05) and 180 (p < 0.001) (Table 1).

The anti-inflammatory activity of hydrazone compounds were measured by LOX activity. As a result of anti-inflammatory activity, the maximum inhibition was detected 66.30% for **2t.** Inhibitions of **2a**, **2c**, **2i**, **2k**, **2l**, **2m**, **2p**, **2s** and **2u** were observed between 45.11-15.12% (Table 2).

In summary, the analgesic effect of various numbers of newly synthesized substances was evaluated with hot plate test in this study. Our study showed that certain substances had rapid analgesic effect potential such as **2l** having 2-chloroquinoline, **2s** having thiophene and **2t** having pyridine ring whereas the analgesic effect of some substances such as **2i** having quinoline nucleus begun in a later time in hot plate test.

Heterocyclic ring imparts an important function in medicinal chemistry and serves as a key template for the new drug development studies. There is an extensive spectrum of biological activities shown by many compounds containing five or six-membered heterocyclic rings in their structure. Bioisosteric replacements play an important role in polarity, duration, and degree of activity. Substituted phenyl ring reduced analgesic activity while aromatic ring containing heteroatom provided good activity. Increased activity can be explained by electron density and strong binding interactions with the active side of the target enzyme. Furthermore, physicochemical parameters are important for drug candidates to explain their absorption, distribution, metabolism, excretion, toxicity and pharmacodynamic process (32). Physicochemical parameters such as Clog P and tPSA (topological polar surface area) describe drug behaviors in our body. Hydrogen bonding descriptors and polar surface

Table 1: Paw withdrawal latencies of mice at baseline and post-injection (10, 20, 40, 60, 90, 120, 150 and 180 minutes (min) after) in hot plate test. Data are expressed as mean ± SEM. Statistical analysis was performed with repeated measures of analysis of variance followed by Tukey's post hoc test for comparison with baseline threshold (*: $p < 0.05$. **: $p < 0.01$. ***: $p < 0.001$) and two way ANOVA followed by Sidak post hoc test for comparison to control group (+: $p < 0.05$. ++: $p < 0.01$. +++: $p < 0.001$). The data which has statistical significance in any comparison were expressed as bold in the table.

Groups	Dose (mg/kg)	Baseline threshold (sec)	Post Injection pain threshold (sec)							
			10 min	20 min	40 min	60 min	90 min	120 min	150 min	180 min
Control	17% Tween 80	21.2 ± 2.6	19.1 ± 2.4	17.0 ± 1.8	18.2 ± 3.5	18.3 ± 3.3	18.3 ± 3.0	18.1 ± 2.4	18.8 ± 4.0	20.5 ± 1.2
2a	50	8.7 ± 0.8	8.9 ± 1.7	9.6 ± 1.7	14.3 ± 5.2	18.4 ± 7.0	12.4 ± 5.4	21.3 ± 6.7	20.2 ± 6.7	-
2b	50	**7.6 ± 0.6 +**	16.7 ± 5.6	7.3 ± 6.9	21.0 ± 7.5	11.6 ± 1.3	17.3 ± 5.6	11.0 ± 1.6	9.6 ± 1.9	-
2c	50	**7.5 ± 0.5 +**	14.0 ± 5.3	6.2 ± 1.3	9.9 ± 1.5	13.8 ± 5.4	14.7 ± 5.1	8.9 ± 1.5	9.0 ± 1.2	14.6 ± 5.3
2d	50	8.0 ± 0.5	11.9 ± 1.5	13.2 ± 5.3	14.4 ± 5.3	14.1 ± 5.3	18.9 ± 7.0	18.4 ± 4.7	23.0 ± 6.3	24.8 ± 7.3
2e	50	9.7 ± 0.7	10.9 ± 5.6	15.3 ± 5.3	13.6 ± 5.3	18.4 ± 7.0	18.6 ± 7.0	27.5 ± 7.1	20.4 ± 6.7	21.8 ± 6.5
2f	50	24.2 ± 1.1	21.1 ± 1.9	22.4 ± 2.2	25.0 ± 2.3	**27.6 ± 3.6+**	25.8 ± 2.4	22.7 ± 2.4	23.5 ± 2.2	-
2g	50	22.1 ± 1.0	18.3 ± 2.1	15.9 ± 1.3	23.3 ± 2.0	21.0 ± 2.2	24.2 ± 4.2	22.3 ± 1.6	**38.4 ± 5.6**++**	**40.8 ± 5.8***+++**
2h	50	23.4 ± 1.8	27.0 ± 4.0	26.6 ± 1.9	27.1 ± 5.0	29.3 ± 4.0	**32.0 ± 4.7+**	**41.8 ± 6.3**+++**	**37.7 ± 4.5*+**	**39.6 ± 5.9*++**
2i	50	24.9 ± 1.7	25.3 ± 2.8	32.8 ± 5.9	**42.4 ± 6.4*++**	**42.0 ± 6.4*+++**	**50.9 ± 6.0***+++**	**45.0 ± 6.4*+++**	**51.6 ± 4.7***+++**	**54.4 ± 4.4***+++**
2j	50	24.9 ± 2.3	18.6 ± 1.8	22.5 ± 4.8	25.5 ± 5.8	26.0 ± 5.7	33.6 ± 6.8	**45.9 ± 6.2**+**	**42.0 ± 6.8++**	-
2k	50	22.6 ± 1.9	22.2 ± 2.9	23.6 ± 5.0	24.5 ± 5.1	25.3 ± 4.1	23.5 ± 3.4	28.5 ± 4.5	-	-
2l	50	25.6 ± 1.8	26.9 ± 2.6	**31.9 ± 4.1++**	**31.1 ± 3.2+**	**40.3 ± 5.5**+++**	**31.5 ± 2.1+**	**33.5 ± 2.3+**	**35.3 ± 5.2+**	30.1 ± 5
2m	50	26.9 ± 2.6	28.1 ± 4.6	31.3 ± 4.7	**38.0 ± 5.5++**	**42.1 ± 3.9*+**	**41.8 ± 6*+++**	**36.7 ± 4.9+**	**40.3 ± 6.7*+**	**48.8 ± 5.1***+++**
2n	50	22.8 ± 0.9	28.5 ± 4.5	25.3 ± 2.6	27.3 ± 2.4	31.1 ± 4.8	**32.4 ± 4.3*+**	**34.0 ± 4.8*+**	**35.0 ± 5.8**+**	**32.8 ± 4.7***
2o	50	22.2 ± 2.3	27.7 ± 4.6	23.5 ± 3.0	26.0 ± 3.3	29.5 ± 2.9	**37.8 ± 5.5*+++**	**43.3 ± 5.6***+++**	**40.7 ± 6.2**+++**	-
2p	50	23.7 ± 1.5	23.2 ± 2.7	28.8 ± 2.2	**33.2 ± 4.7+**	**38.8 ± 4.6*+++**	**32.4 ± 4.0+**	**37.5 ± 3.8*++**	**44.8 ± 5.8***+++**	**40.4 ± 5.9**+++**
2r	50	24.9 ± 1.5	27.7 ± 4.6	27.3 ± 2.4	**30.4 ± 3.9+**	**34.9 ± 3.0*+++**	**35.6 ± 4.4*+++**	**35.4 ± 3.9*+++**	**42.5 ± 4.4***+++**	**39.1 ± 3.2**+++**
2s	50	28.4 ± 2.1	**32.8 ± 1.0+**	**42.6 ± 4.2*+++**	**52.3 ± 2.6***+++**	**52.5 ± 4.1***+++**	**52.1 ± 5.3***+++**	**48.1 ± 6.0**+++**	**54.9 ± 4.5***+++**	-
2t	50	29.1 ± 1.7	28.3 ± 1.3	**31.4 ± 5.1+**	**36.0 ± 4.6++**	**41.4 ± 4.6*+++**	**37.0 ± 5.2++**	**47.5 ± 4.7*** +++**	**41.9 ± 5.9*+++**	**42.1 ± 5.9+++**
2u	50	25.8 ± 2.0	24.4 ± 2.0	25.1 ± 3.1	28.7 ± 4.5	27.0 ± 4.8	**35.8 ± 7.0+**	**45.9 ± 6.8*+++**	**47.2 ± 6.3**+++**	**43.0 ± 5.6*++**
2w	50	21.4 ± 2.1	244 ± 2.0	22.0 ± 2.2	24.0 ± 2.4	**33.5 ± 6.4+**	**32.5 ± 5.3+**	**36.1 ± 6.7++**	**36.8 ± 5.3+**	**47.0 ± 5.0***+++**

Table 2. LOX inhibitory activity results of the tested compounds.

Comp.	LOX inhibition % (Mean ± SD)	Comp.	LOX inhibition % (Mean ± SD)
2a	45.11 ± 2.60	2l	35.11 ± 2.40
2b	N A	2m	22.16 ± 1.25
2c	15.12 ± 1.18	2n	NA
2d	NA	2o	NA
2e	NA	2p	34.26 ± 1.74
2f	NA	2r	NA
2h	NA	2s	40.18 ± 2.08
2i	25.32 ± 1.80	2t	66.30 ± 2.10
2j	NA	2u	36 ± 1.91
2k	27 ± 1.30	2w	NA
Nordihydroguaiaretic acid (NDGA) (20 µg/mL):	92.61 ± 1.73		

*NA: inactive

area (PSA) are used explaining biological permeation (33). Compounds **2i** having quinoline, **2l** having 2-chloroquinoline, **2s** having thiophene and **2t** having pyridine ring provided lipophilicity and tPSA, so these compounds could be increased cell permeability.

CONCLUSION

In the present paper, we reported the synthesis of some new hydrazone derivatives from corresponding substituted aldehyde and 4-chloro-2-(methylthio)pyrimidine-5-carbohydrazide. All compounds were evaluated for their analgesic and anti-inflammatory activity with a hot plate and LOX inhibition tests. Our study showed that certain substances such as **2l, 2s,** and **2t** had rapid analgesic effect whereas the effect of compound **2g** latency was found significantly later in hot plate test. As results of the anti-inflammatory activity, the maximum LOX inhibition was detected for **2t** (66.30%). Our results demonstrate that compound **2t** containing pyridine ring has both significant analgesic and anti-inflammatory effects.

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