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ORIGINAL ARTICLE

Synthesis and pharmacological screening of 4, 6-substituted di-(phenyl) pyrimidin-2-amines

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KEYWORDS

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Abstract A new series of 4, 6-substituted di-(phenyl) pyrimidin-2-amine (**IIa–d**) were synthesized by reacting chalcone derivatives with guanidine hydrochloride in the presence of dimethylformamide. The synthesized compounds were characterized by spectral analysis and were screened for anti-inflammatory activity. Two compounds 4-(4-nitrophenyl)-6-phenylpyrimidine-2-amine **(IIa)** and 4-(4-methoxyphenyl)-6-phenylpyrimidine-2-amine **(IIb)** showed highly significant reduction in oedema volume

1. Introduction

Inflammation is a local reaction of the vascular tissue to injury resulting in the formation of protein-rich exudates. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumour (swelling), and functio laesa (loss of function). Inflammation is caused by various agents such as physical agents, chemical agents, immunological reactions, and infection by pathogenic organism (Wilson et al., 2003). Inflammation is of two types acute and chronic. The acute inflammation is the exudation of fluid and plasma proteins (oedema) and the emigration of leukocytes, especially neutrophils. Chronic inflammation is of prolonged duration in which active inflammation, tissue destruction, and attempts at repair are occurring simultaneously. Chronic inflammation includes the most common diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases (Collins et al., 2001). Several pyrimidine derivatives have wide varieties of usages and their nucleus is also present in vitamin B2 and folic acid.

Pyrimidines are an important class of heterocyclic compounds, which shows a wide range of pharmacological activities such as antimicrobial (Gossnitzer et al., 2002), anti-inflammatory (Hogale et al., 1986), anticancer (Mattew et al., 1984), antiviral (Ahluwalia et al., 1987), antitubercular (Jani et al., 1994) antihypertensive (Ishitsuka et al., 1982; Ninomyia et al., 1990) and anticonvulsant (Calis and Koksal, 2001) activities. Along with these derivatives various research papers showed that pyrimidine derivatives also have other diverse pharmacological activities such as H_1-antihistamines (Alagarsamy et al., 2007) and 4-phosphodiesterase inhibitors (Crespo et al., 1998).

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2. Experimental

2.1. Materials and reagents

Melting points were determined by the open capillary method and were uncorrected. IR spectra were recorded by the KBr pellet technique using JACO FT/IR 410-spectrophotometer. ^{1}HNMR spectra were recorded on Bruker model DPX 300 (300 MHz NMR) spectrometer in DMSO-d_6/$CDCl_3$ using tetramethylsilane as an internal standard. The purity of the synthesized compounds was analysed by thin-layer chromatography using silica gel as the stationary phase. Compounds were visualized by U.V. visualizing cabinet. Mass spectra were obtained using a Kratos-AEI MS-902S instrument. All chemicals used were analytical grade and purified before use in different reactions.

2.2. General procedure for synthesis of substituted chalcones (Ia-d)

The substituted chalcones were prepared by reacting an equimolar quantity of aromatic aldehyde (0.01) and acetophenone (0.01) in absolute ethanol; add 40% NaOH solution to the reaction mixture drop wise with stirring maintaining the temperature 0–2 °C. After completion of the reaction the solid was separated out and was poured into ice, the precipitate was filtered and recrystallized with ethanol. This leads to the formation of substituted chalcones i.e. α, β unsaturated ketones which were carried out by the clasien-schmidt condensation reaction.

2.2.1. 1-(4-Nitrophenyl)-3-phenylprop-2-en-1-one (**Ia**)

Yield: 67%; mp: 122–124 °C; IR (KBr cm^{-1}): 3067(Ar C-H stretching), 1660 (C=C stretching), 1608 (Ar. C=C stretching), 1218 (Ar. C–O), 655(Ar. C–H bend) 1462 (–NO_2 stretching); ^{1}H NMR (DMSO): δ 7.14–7.30 (m, 5H), 8.12–8.32 (m, 4H), 7.56–7.82 (s, 2H, olefinic proton). Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.0; H, 4.26; N, 5.42; O, 18.75. Found: C, 71.14, H, 4.38, N, 5.53, O, 18.95.

2.2.2. 3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (**Ib**)

Yield: 65.54%; mp: 80–82 °C; IR (KBr cm^{-1}): 3061(Ar C–H stretching), 1448(Ar C=C stretching), 1606 (C=C Stretching), 1215 (Ar–CO stretching), 748 (C–H vibrational).1157(–OCH_3 stretching); ^{1}HNMR (DMSO): δ 6.72–7.13 (m, 4H), 7.65–7.81 (m, 5H), 7.51–7.59 (s, 2H, olefinic proton), 3.73 (s, 3H, OCH_3). Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.56; H, 5.82; O, 13.36. Found: C, 80.65, H, 5.92 O, 13.43.

2.2.3. 3-(4-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**Ic**)

Yield: 78%; mp: 150–152 °C; IR (KBr cm^{-1}): 3067(Ar C–H stretching), 1663 (C=C stretching), 1608 (Ar. C=C stretching), 1218 (Ar. C–O), 655(Ar. C–H bend), 1462 (–NO_2 stretching), 1166 (-OCH_3 stretching); ^{1}H NMR (DMSO): δ 7.41-7.56 (s, 2H, olefinic proton), 6.81–7.13 (m, 4H), 8.07–8.31 (m, 4H), 3.63 (s, 3H, OCH_3). Anal. Calcd. for $C_{16}H_{13}NO_4$: C, 67.82; H, 4.60; N, 4.84; O, 22.54. Found: C, 67.84; H, 4.63; N, 4.94; O, 22.59;

2.2.4. 3-(4-Methoxyphenyl)-1-p-tolylprop-2-en-1-one (**Id**)

Yield: 63.2%; mp:174–176 °C; IR (KBr cm^{-1}): 3162(Ar C–H stretching), 1653 (C=C stretching), 1608 (Ar. C=C stretching), 1228 (Ar. C–O), 657 (Ar. C-H bend); ^{1}H NMR (DMSO): δ 2.35 (s, 3H, –CH_3), 3.71 (s, 3H, OCH_3), 6.89–7.21 (m, 4H, ArH), 7.45–7.69 (m, 4H, ArH), 7.82 (s, 2H, olefinic proton). Anal. Calcd. for $C_{17}H_{16}O_2$: C, 80.86; H, 6.27; O, 12.62. Found C, 80.93, H, 6.39, O, 12.68;

2.3. General procedure for Synthesis of 4, 6-substituted di(phenyl)pyrimidin-2-amine derivatives (IIa-d)

Substituted pyrimidines were prepared by refluxing an equimolar quantity of substituted chalcones (0.01 mol) with guanidine hydrochloride (0.01 mol) in the presence of dimethylformamide (DMF) at 50–60 °C for 6–7 h, the reaction mixture was cooled and poured into crushed ice, kept overnight for complete precipitation. The product was filtered and recrystallized with methanol and later was washed with petroleum ether (Scheme 1).

2.3.1. 4-(4-Nitrophenyl)-6-phenylpyrimidine-2-amine (**IIa**)

Yield: 52.6%; mp: 180–182 °C; I.R. (KBr, cm^{-1}) 3403 (C–H aromatic stretching), 3322 (free NH_2 stretching), 1627 (C=C aromatic stretching), 1515 (C=N stretching), 755, 690 (C-H aromatic bending). ^{1}H NMR (DMSO): δ 7.22–7.43 (m, 5H, ArH), 7.74 (s, 2H, ArH), 8,25 (s, 2H, ArH), 6.89 (s, 1H, pyrimidinyl proton), 4.21 (s, 2H, –NH_2). MS (m/z): (292.10, 246.10, 231.09, 170.07, 155.06, 79.02, 53.02, 26.01). Anal. Calcd for $C_{16}H_{12}N_4O_2$: C, 65.70; H, 4.10; N, 19.12; O, 10.88. Found: C, 65.75, H, 4.14; N, 19.17; O, 10.95.

2.3.2. 4-(4-Methoxyphenyl)-6-phenylpyrimidine-2-amine (**IIb**)

Yield: 62.6%; mp: 212–214 °C; IR(KBr, cm^{-1}) 3400 (C–H aromatic stretching), 3310 (free NH_2 stretching), 1657 (C=C aromatic stretching), 1589 (C=N stretching), 1015 (C–O–C stretching), 777, 689 (C–H aromatic bending). ^{1}H NMR (DMSO): δ 7.22–7.41 (s, 5H, ArH), 6.81–7.11 (m, 4H, ArH), 6.81 (s, 1H, pyridinyl proton), 3.71 (s, 3H, OCH_3), 3.81 (s, 3H, NH_2). MS(m/z): (277.12, 246.13, 231.09 170.07, 155.06, 94.04, 79.02, 53.02, 26.01)Anal. Calcd. for $C_{17}H_{15}N_3O$: C, 73.60; H, 5.35; N, 15.10; O, 5.72. Found: C, 73.63, H, 5.45, N, 15.15, O, 5.77.

2.3.3. 4-(4-Methoxyphenyl)-6-(4-nitrophenyl) pyrimidin-2-amine: (**IIc**)

Yield: 59.9%; mp: 190-192 °C; IR(KBr, cm^{-1}) 3450 (C-H aromatic stretching), 3328 (free NH_2 stretching), 1657 (C=C aromatic stretching), 1581 (C=N stretching), 1033 (C-O-C stretching), 721, 697(C-H aromatic bending). ^{1}H NMR(DMSO): δ 6.85-7.21 (m, 4H, ArH), 7.79-8.12 (m, 4H, ArH), 6.86 (s, 1H, pyridinyl proton), 3.69 (s, 3H, OCH_3), 4.12 (s, 2H, NH_2). Anal. Calcd. for $C_{17}H_{14}N_4O_3$: C, 63.30; H, 4.32; N, 17.32; O, 14.82. Found: C, 63.35, H, 4.38, N, 17.38; O, 14.89.

2.3.4. 4-(4-Methoxyphenyl)-6-p-tolylpyrimidin-2-amine (**IId**)

Yield: 55.5%; mp: 222-224 °C; IR (KBr, cm^{-1}) 3480 (C-H stretching aromatic), 3310 (free NH_2 stretching), 1656 (C=C aromatic stretching), 1510 (C=N stretching), 1033 (C–O–C stretching), 672, 629 (C–H aromatic bending); ^{1}H NMR(DMSO): δ 3.71 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3),

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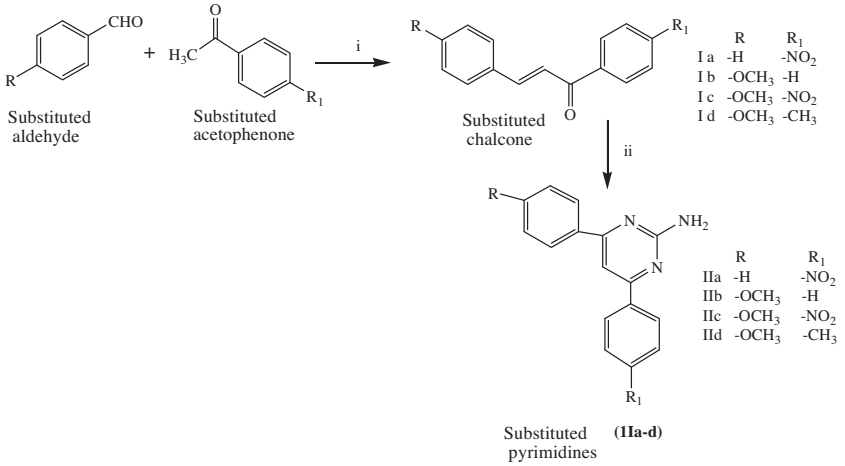

Scheme 1 Synthesis of 4, 6-substituted di-(phenyl) pyrimidin-2-amines derivatives.

3.91 (s, 2H, NH_2), 7.12–7.41 (m, 8H, ArH), 6.81 (s, 1H, pyridinyl proton). Anal. Calcd. for $C_{18}H_{17}N_3O$: C, 74.18; H, 5.82; N, 14.38; O, 5.42. Found: C, 74.20, H, 5.88, N, 14.42, O, 5.49.

Reagents and conditions: (i) Absolute ethanol, 40%NaOH, stirring 3-4 hrs at 0-2 °C *(ii)* Guanidine hydrochloride, DMF, reflux for 6-7 hrs at 50-60 °C.

3. Results and discussion

3.1. Chemistry

Firstly aromatic aldehyde was treated with acetophenone and sodium hydroxide to obtain substituted chalcone. Then the title compounds (IIa-d) were successfully obtained by substituted chalcone and guanidine hydrochloride. Compounds (IIa-d) were characterized by IR, ^{1}HNMR and elemental analysis. All results are in full agreement with the proposed structure. For example ^{1}H NMR spectrum showed the most important signal at δ 7.75 pspm for pyrimidinyl moiety and different other signals were observed at the expected chemical shift. IR spectra showed the absorption band in the region of 1510-1599 cm^{-1}assigned for aromatic C=N stretching and 3310–3328 cm^{-1}assigned for free $-NH_2$ which plays an important role in the formation of pyrimidine moiety.

3.2. Anti-inflammatory activity

All the synthesized compounds were screened for their antiinflammatory activity. Antiinflammatory activity is performed in albino rats using the carrageenan induced rat paw oedema method (Winter et al.,1962) at a dose of 10 mg/kg body weight. The test compounds were made into homogeneous suspension with distilled water and 1%CMC solution and were administered orally. The oedema volume was noted at the end of the 1st and 3rd hr of administration of carrageenan and the test compound was compared with known standard(Indomethacin). The anti-inflammatory activity of all the synthesized compounds (Ia-d) is presented in Table 1. All compounds were tested at a dose of 10 mg/kg p.o. and have shown considerable anti- inflammatory activity in Fig. 1. From Fig. 1, we could find these compounds showed significant anti-inflammatory activities. Compounds with the electron withdrawing group at the 4th position such as **IIa** increased the activity and compounds with electron donating groups such as **IId** decreased the activity.

Table 1 Anti-inflammatory activity data (paw volume) of synthesized compounds

COMPOUND CODE	MEAN(oedema volume) ± SEM	
	1 h	3 h
Std	0.26 ± 0.02	0.50 ± 0.026
IIa	0.20 ± 0.025	0.22 ± 0.028***[a]
IIb	0.35 ± 0.040	0.25 ± 0.035***
IIc	0.28 ± 0.030	0.36 ± 0.020*
IId	0.43 ± 0.045*	0.45 ± .044

All the synthesized compounds were screened for their antiinflammatory activity by the carrageenan induced rat paw oedema method at a dose of 10 mg/kg body weight and mean of oedema volume is given in table. It was observed that compounds **IIa** and **IIb** showed a highly significant reduction in oedema after 3hr.

[a] All the values are expressed as Mean ± SEM of six animals in each group. ***($p \leqslant 0.001$) indicates the level of statistical significance.

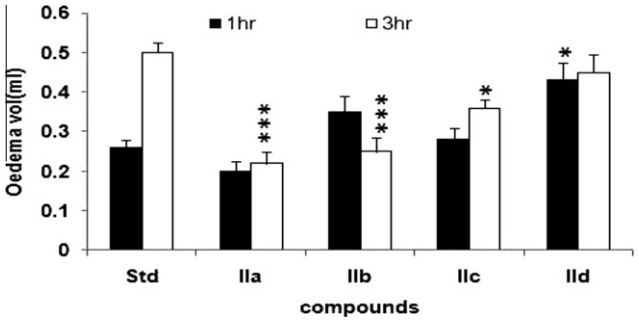

Figure 1 Anti-inflammatory activity of synthesized compounds.

3.3. Statistical analysis

Anti-inflammatory activity of synthesized compounds was measured by rat paw edema method using plethysmometer.

All the values are expressed as Mean ± SEM and $^{***}p < 0.001$ indicates the level of significance compared with standard. Statistical analysis was performed by one way-ANOVA and post hoc analysis was done by tukey's test.

4. Conclusions

All the newly synthesized 4, 6-substituted di-(phenyl) pyrimidin-2-amine derivatives were tested *in-vivo* in order to evaluate their anti-inflammatory activity by the carrageenan induced rat paw oedema method. Compound having the electron withdrawing group increased the activity. It was observed that compounds **IIa** and **IIb** showed a highly significant reduction in oedema after 3hr whereas compounds **IIc** and **IId** showed a less significant reduction in oedema volume. All the tested compounds were compared with standard drug Indomethacin.

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