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New Thiazoles Containing Pyrazolopyrimidine Moiety as Possible Analgesic Agents

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Abstract: Few new 2-[(1-phenyl-1H-pyrazolo [3,4-d] pyrimidin-4-yl) oxy]-N'-(4-aryl-1,3-thiazol-2-yl) acetohydrazide derivatives have been prepared and characterized by analytical and spectral analysis. The newly synthesized compounds have been screened for their analgesic activity. Some of the compounds have exhibited excellent analgesic activity.

Key words: Thiazoles, pyrazolopyrimidine, analgesic activity

Introduction

Pyrazolopyrimidine derivatives are reported to have antileishmanial (Garg *et al*., 1990), antihypertensive (El-Feky *et al*., 1996), antibacterial and antifungal (Ismail *et al*., 2003), anti-angiogenic activity (Devesa *et al*., 2004). 4-Hydroxy-1-phenyl pyrazolo[3,4-d]pyrimidine that is used as starting material in our studies is a precursor for a novel class of potent enterovirus inhibitors (Khafagy *et al*., 2004; Chern *et al*., 2004). Analgesic activity of 4-hydroxy-1-phenyl pyrazolo[3,4-d]pyrimidine derivative has been reported in the literature (Amin *et al*., 2003). We have reported the synthesis and biological activity of few thiazole derivatives derived from some biologically active building blocks. (Narayana *et al*., 2004; 2006a,b). Since pyrazolopyrimidine thiazoles are reported to possess antinflammatory and analgesic activities (Russo *et al*., 1999) we aimed at synthesizing some new thiazoles containing pyrazolopyrimidine moiety and investigate their analgesic activity.

Materials and Methods

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian (300 MHZ) spectrometer using TMS as internal standard and ^{13}C NMR spectra were recorded in $CDCl_3$ and in DMSO-d_6 on a Varian (75 MHZ) spectrometer. FABMS was recorded on a JEOL SX 102/DA-6000 Mass spectrometer using argon/xenon (6 kv, 10 mA) as the FAB gas.

Synthesis of 4-hydroxy-1-phenyl pyrazolo [3,4-d] pyrimidine (1)

This compound was prepared as per the procedure described by Druey and Schmidt (1958). Ethylethoxymethylenecyanoacetate in ethanol was treated slowly with phenylhydrazine

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followed by treatment with formamide gave the product as off white flaky crystals. m.p 286-287°C (Lit 286-288°C).

Synthesis of [(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetic acid methyl ester (2)

4-Hydroxy-1-phenyl pyrazolo[3,4-d]pyrimidine 1, 21.2 g (0.1 mole) in 180 mL acetone was stirred with 16.5 g (0.12 mole) of potassium carbonate at room temperature and 10.8 g (0.1 mole) of methylchloroacetate was added to it in drops. The reaction mixture was then refluxed for 8 h. Progress of the reaction was monitored by TLC. The acetone was distilled out and the residue was diluted with 250 mL water. The solid obtained was filtered, washed with water and then recrystallised in methanol to obtain the compound as white needles. Yield 6.35 g (95%), mp 148-152°C

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.78 (s, 3H, $-CH_3$), 4.73 (s, 2H, $-CH_2$), 7.34 (t, 1H, ArH), 7.48 (t, 2H, ArH), 7.94(s, 1H, ArH), 7.99 (d (J=6.08), 1H, ArH), 8.23 (s, 1H, ArH)

Synthesis of 2-[(1-Phenyl-1H-pyrazolo[3,4-d] pyrimidin-4-yl)oxy]acetohydrazide (3)

[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetic acid methyl ester 2, 15 g (0.052 mol) in 60 mL ethanol was refluxed with 13.5 mL of hydrazine hydrate for 1 h. The reaction mixture was then cooled to 0-5°C and filtered, washed with ethanol and dried. The product was obtained as white powder with a yield of 13.2 g (88%), mp 268-272°C.

^{1}H-NMR (400 MHZ, $CDCl_3$): δ 4.87 (s, 3H,$-CH_2$), 7.39 (t, 1H,ArH), 7.53 (t,2H,ArH), 8.06 ($dd_{(J=7.9)}$, 2H, Ar-H), 8.30 (s,1H, ArH), 8.21 (s, 1H, ArH), 8.35 (s, 1H, ArH), 10.75 (s, H, NH).

Synthesis of 1-{[(1-phenyl-1H-pyrazolo [3,4-d] pyrimidin-4-yl) oxy] acetyl} thiosemi carbazide (4)

2-[(1-Phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetohydrazide 3, 9.26 g (0.0325 mol) was refluxed with potassium thiocyanate, 5.6 g (0.057 mol) in 75 mL water and 6.7 mL conc. HCl for 3 h. The reaction mixture was then cooled to room temperature and stirred overnight. The precipitated product was then filtered and washed with water and recrystallised from ethanol. The product was isolated as white powder with an yield of 8.6 g (77.3%), mp 236 (dec).

IR (KBr, v in cm^{-1}): 3406 and 3302 ($-NHNH_2$), 2928 and 2856 (-CH), 1667(-C = O), 1533 (-C = S), 1072 (-C = N).
FABMS: m/z 344 (I = 55%, M^+), 253 (I = 100%, $M-H_2NCSNH_2$), 212 (I = 25%, $C_{11}H_8N_4O$).

Synthesis of 2-[(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl) oxy]-N'-(4-aryl-1,3-thiazol-2-yl)acetohydrazide (5a-h)

1-{[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetyl}thiosemicarbazide 4 (0.0011 mol) was refluxed with aromatic acylbromides (0.0012 mol) in 5 mL ethanol was refluxed for 8 h. The reaction mixture was then cooled to room temperature and stirred for 2 h. The product was filtered and recrystallised from ethanol/DMF mixture. All the compounds are obtained in a yield of 60-80%. The spectral data are given below.

Spectral Data

2-[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]-N'-(4-(2-hydroxy-5-benzamide)-1,3-thiazol-2-yl)acetohydrazide (5a)

IR(KBr, v in cm^{-1}): 3288.4 and 3182.3 (-NH), 1691.5 and 1649.0 (-C = O).

^{1}H-NMR (400MHz, DMSO-d_6): δ 4.85 (s, 2H,$-CH_2$), 6.91 (d (J=6.92), 1H, ArH), 7.12 (s, 1H,

ArH), 7.42 (t, 1H, ArH), 7.58 (t, 2H, ArH), 8.34 (dd (J=6.86), 1H, Ar-H), 7.96 (bs, 1H,-OH), 8.037 (d (J=5.95), 2H, NH), 8.30 (s, 1H, ArH), 8.40 (s, 1H, ArH), 8.512 (s, 1H, ArH), 10.75 (s, 1H, NH).

^{13}C-NMR (75MHz, DMSO-d_6): δ 47.82, 102.86, 107.95, 115.85, 118.76, 123.08, 126.63, 126.75, 128.59, 130.59, 132.84, 137.40, 139.31, 150.70, 152.56, 153.15, 157.58, 161.68, 168.31, 173.01, 173.09, FABMS: m/z 503 (I = 40%, M^+), 486 (I = 10%,M-NH_2).

2-[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]-N'-(4-phenyl-1, 3-thiazol-2-yl)acetohydrazide (5f).

IR(KBr, v in cm^{-1}): 3288.2 and 3182.0 (-NH),1690.0 and 1652.0 (-C = O).

^{1}H-NMR (400MHz, DMSO-d_6): δ 4.84 (s, 2H,-CH_2), 7.28 (s, 2H, ArH), 7.40 (m, 4H, ArH), 7.58 (t, 2H, ArH), 7.82 (t, 2H, ArH), 8.34 (d (J=6.08), 2H, Ar-H), 8.40 (s, 1H, ArH), 8.52 (s,1H, ArH), 10.75 (s, 1H, NH).

^{13}C-NMR (75MHz, DMSO-d_6): δ 46.4, 103.37, 106.58, 121.71, 125.53, 127.21, 127.49, 128.48, 129.22, 134.29, 136.04, 137.94, 150.11, 151.18, 151.78,156.12, 166.94, 171.74, FABMS: m/z 444 (I = 100%, M+1), 443(I = 20%,M^+).

2-[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]-N'-(4-(4-chlorophenyl)-1, 3-thiazol-2-yl)acetohydrazide (5g)

^{1}H-NMR (300MHz, DMSO-d_6): δ 4.99 (s, 2H,-CH_2), 6.84 (s, 1H, ArH), 7.45-7.60 (m, 8H, ArH), 7.71 (d (J=7.1), 2H, Ar-H), 8.34 (s, 1H, ArH), 8.42 (s, 1H, ArH).

FABMS: m/z 478 (I = 100%, M^+).

2-[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]-N'-(4-(4-methoxyphenyl)-1, 3-thiazol-2-yl)acetohydrazide (5h.).

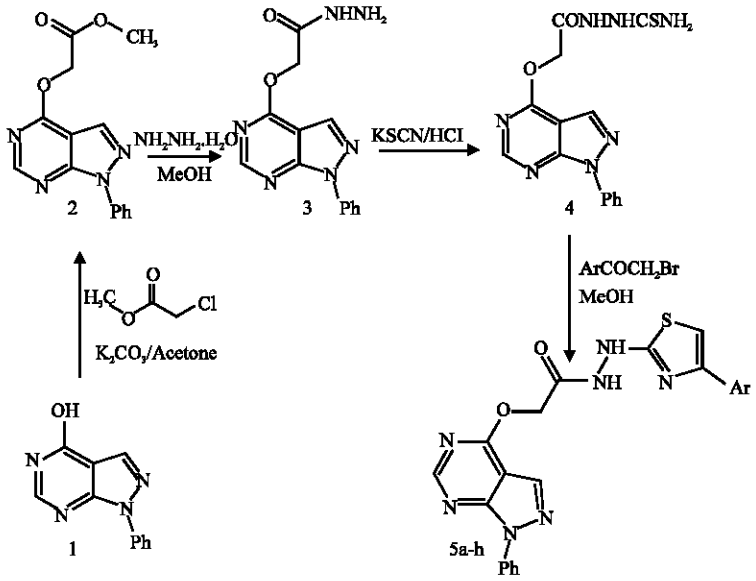
^{1}H-NMR (300MHz, DMSO-d_6): δ 3.86 (s, 3H,-OCH_3), 5.10 (s, 2H,-CH_2), 6.71(s,1H,ArH), 6.98 (d (J=8. 7),2H,Ar-H), 7.54 (m,5H,ArH), 7.65 (dd (J=8.13), 2H, ArH), 8.44 (s,1H,ArH), 8.48 (s,1H,ArH).

FABMS: m/z 474 (I = 100%, M+1), 473 (I = 25%,M^+).

Results and Discussion

[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetic acid methylester 2 was prepared by reacting 4-hydroxy-1-phenyl pyrazolo[3,4-d]pyrimidine 1 with methyl-2-chloroacetate in presence of an alkali metal carbonate. [(1-Phenyl-1H-pyrazolo[3,4-d] pyrimidin-4-yl)oxy]acetic acid methylester was then converted to 2-[(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetohydrazide 3 by treating with hydrazine hydrate. 2-[(1-Phenyl-1H-pyrazolo [3,4-d] pyrimidin-4-yl) oxy] acetohydrazide was then converted to 1-{[(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetyl}thiosemicarba-zide 4 by treating with KSCN and Conc. HCl. The 1-{[(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]acetyl} thiosemicarbazide 4 was then refluxed with different aromatic acyl bromides in ethanol yielded 2-[(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]-N'-(4-aryl-1,3-thiazol-2-yl) acetohydrazide derivatives 5a-h, Scheme-1. All the compounds were isolated in 64-94% yield after recrystallising from methanol-DMF mixture. Few selected compounds were characterized by IR, ^{1}H-NMR and mass spectral analysis. Characterization data are given in Table 1 and spectral data are given in the experimental section.

All the newly synthesized compounds were evaluated for their analgesic activity by acetic acid induced writhing test. Swiss albino mice (25-30 g) of either sex were used for the experiment. They were housed in clean polypropylene cages and kept under room temperature (24±2°C), relative



Scheme 1

Table 1: Characterization data of the compounds 5a-h

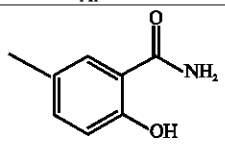
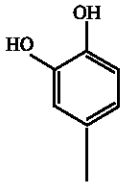
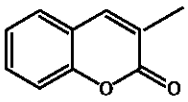
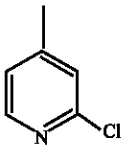
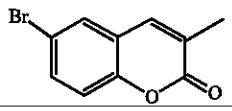
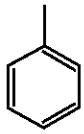
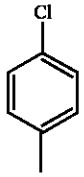
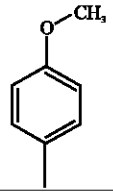
Compound No.**	Ar-	Yield * (%)	m.p (°C)	Molecular formula	Nature of crystals***
5a		93	240-242	$C_{23}H_{18}N_8O_4S$	Cream crystals
5b		94	224-226	$C_{22}H_{17}N_7O_4S$	Tan crystals
5c		90	>250	$C_{25}H_{17}N_7O_4S$	Light yellow micro crystals
5d		64	226-229	$C_{21}H_{15}ClN_8O_2S$	Dark yellow crystals
5e		80	>250	$C_{25}H_{16}BrN_7O_4S$	Yellow crystals

Table 1: Continued

Compound No.**	Ar-	Yield * (%)	m.p (°C)	Molecular formula	Nature of crystals***
5f		65	>250	$C_{22}H_{17}BrN_7O_2S$	Cream crystals
5g		91	246-250	$C_{22}H_{16}ClN_7O_2S$	Light yellow crystals
5h		85	>250	$C_{23}H_{19}N_7O_3S$	Cream microcrystals

* All the yields are on the isolated basis, ** All the compounds gave satisfactory results for elemental analysis, ***Recrystallisation solvent, methanol-DMF mixture

Table 2: Analgesic activity (Acetic acid induced writhing test) of compounds (5a-h)

Groups	Drugs	Dose (mg kg^{-1})	Average time taken for onset writhing (S)	No. of writhes for 15 min	Protection (%)
1	2% gum acacia	10 mL kg^{-1}	246	16	-
2	Diclofenac sodium	2.5	692	6	62.5
3	5a	50	105	8	50.0
4	5b	50	394	8	50.0
5	5c	50	422	14	12.5
6	5d	50	425	14	12.5
7	5e	50	331	13	18.75
8	5f	50	524	14	12.5
9	5g	50	689	6	62.5
10	5h	50	385	9	43.75

N = 2 in each group

humidity 60-70% in a 12 h light-dark cycle. The animals were fed a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental procedures. The experiment was conducted after obtaining approval from institutional animal ethics committee.

The mice were divided in to 10 groups of two each and received the drugs in 2% gum acacia orally by gavage feeding as shown in Table 2. Group-1 received 2% gum acacia. Group-2 received diclofenac sodium at a dose of 2.5 mg kg^{-1}. 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th group administered the test compounds 5a-h, respectively at a dose of 50 mg kg^{-1} suspended in 10 mL kg^{-1} of 2% gum acacia orally by gavage feeding. Writhing was induced 1h later by intraperitoneal injection of 10 mL kg^{-1} of 0.6% acetic acid in distilled water (Ghosh, 1984). The number of writhes was counted for 15 min immediately after the acetic acid injection. The percentage of protection was calculated. The formula for computing percent protection/inhibition is number of writhes in the control group minus number of writhes in the drug group divided by number of writhes in the control group times 100%. The analgesic activity was compared with Diclofenac sodium (2.5 mg kg^{-1}) and the results are given in Table 2.

The results show that the analgesic activity of compound 5 g (50 mg kg^{-1}) is comparable to that of standard drug diclofenac sodium (2.5 mg kg^{-1}). Compounds 5a, 5b and 5h have also exhibited promising analgesic activity. The exact structural activity relationship is not clear from our studies. Diclofenac sodium, the standard drug used in this study contains 3-chlorophenyl moiety. The compound 5 g, 2-[(1-Phenyl-1H-pyrazolo [3,4-d] pyrimidin-4-yl)oxy]-N'-(4-(4-chlorophenyl)-1,3-thiazol-2-yl)acetohydrazide also contains 4-chlorophenyl moiety which is identical to 3-chlorophenyl moiety. Therefore the higher activity of 5 g compared to other compounds may be due to the presence of 4-chlorophenyl moiety. The compound 5 g can be recommended for further studies. Since this result is based on screening test only, further studies using larger samples have to be done for obtaining conclusive results.

Conclusions

Some new 2-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy]-*N*'-(4-aryl-1,3-thiazol-2-yl) acetohydrazide derivatives have been prepared and screened for analgesic activity. Among the tested compounds 5g, 2-[(1-Phenyl-1H-pyrazolo [3,4-d] pyrimidin-4-yl)oxy]-N'-(4-(4-chlorophenyl)-1,3-thiazol-2-yl)acetohydrazide exhibited promising analgesic activity which and can be recommended for further studies. The compounds 5a, 5b and 5h also exhibited a moderate analgesic activity.

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