

SYNTHESIS AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SOME NEW PYRIDO [4, 3-D] PYRIMIDINE DERIVATIVES

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Abstract: In this investigation, we evaluated the anti-inflammatory activity of some new pyrido[4,3-d] pyrimidine analogues, by using animal model of carrageenan-induced paw edema in mice. New series of pyrido[4,3-d] pyrimidines derivatives were prepared in order to obtain new compounds with potential anti-inflammatory effect. A total of three novel pyrido pyrimidine derivatives compounds (2.5a, 2.5b and 2.5c) were synthesized by taking 4,6-diamino-2-bromo-3-cyanopyridine as starting compounds and were characterized by different analytical technique like IR, NMR and Mass spectroscopy. The synthesized compounds were screened for their anti-inflammatory potency in vivo by comparing with standard drug diclofenac sodium. It has been observed that the maximum anti-inflammatory activity was recorded by the compound 2.5a having 65.38% percentage protection of edema followed by compound 2.5b (60.25%), 2.5c (50%) and Diclofenac sodium registered 64.10%. It has been found that the extent of increase in paw volume reduced significantly up to 3 hrs when compared with solvent control.

Key words: Heterocyclic compound, Pyrido pyrimidine, Anti-inflammatory activity, Spectral analysis.

1. INTRODUCTION

Inflammatory reaction, typically characterized by redness, swelling, heat, and pain, is one of the most important host defense mechanisms against invading pathogens. However, persistent or over-inflammation leads to tissue damage and possibly the failure of organs.¹

The classical non-steroidal anti-inflammatory drugs (NSAIDs) are useful for the treatment of inflammation, pain and fever. The actions of NSAIDs are based on the inhibition of the enzyme cyclooxygenase (COX), which catalyzes the metabolism arachidonic acid (AA) to prostaglandin H₂ (PGH₂). PGH₂ is further metabolized to prostanoids, prostaglandins (PGs) and thromboxane-A₂ (TXA₂). Various physiological effects of PGs include acute and chronic inflammatory reactions, blood pressure (BP) change, platelet aggregation, induction of labor and intensification of pain and fever. However, long-term usage of NSAIDs is associated with several side effects including gastrointestinal (GI) lesions, bleeding, as well as hepato- and nephro-toxicities^{2,3,4}.

In designing new drug molecules, developing safer analgesic and anti-inflammatory drugs without such side effects has recently been the goal of many researchers. Different pharmacophores with a different mode of action in the structure may lead to compounds having efficiency in biological activity. Research has been directed in recent years, where a substantial number of pyrido[3,4-d]pyrimidine have been reported to possess various types of biological activities such as antimicrobial, antifeedant, herbicidal, antihypertensive, antipyretic, antiplatelet, anticancer, anticonvulsant, antitubercular, cardiogenic and other anticipated activities^{5,6}. On the other hand, a considerable number of pyrido[3,4-d]pyrimidine derivatives are endowed with analgesic and anti-inflammatory properties^{7,8}.

Diverse pharmacological activities of the compounds containing pyridazinone moieties^{9,10,11} have prompted us to synthesize new compounds with analgesic and anti-inflammatory activities that may be potent, selective and less toxic.

Stimulated by these findings, our attention has been focused on the synthesis and evaluation of some pyrido[4,3-d] pyrimidine derivatives (2.5a-2.5c) anti-inflammatory agents using the carrageenan-induced oedema model. Indomethacin was used as reference drugs anti-inflammatory activities.

MATERIAL AND METHODS

Experimental

Chemistry: All chemicals were obtained from commercial suppliers, and used without further purification. ¹H and

¹³C NMR spectra were recorded with a Varian Gemini 200 (¹H NMR: 200 MHz, ¹³C NMR: 50 MHz) or a Bruker Advance 300 (¹H NMR: 300 MHz, ¹³C NMR: 75 MHz), using tetramethylsilane as internal standard for ¹H NMR spectra and DMSO-*d*₆ (39.5 ppm) or CDCl₃ (77.2 ppm) for ¹³C NMR spectra. Abbreviations used are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad signal. Coupling constants are expressed in Hertz. Mass spectra are obtained with a Finnigan LCQ advantage Max (ion trap) mass spectrophotometer from Thermo Finnigan, San Jose, CA, USA. Exact mass measurements are performed on a quadrupole time-of-flight mass spectrometer (Q-tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface.

Samples were infused in *i*-PrOH/H₂O (1:1) at 3 μl/min. Melting points are determined on a Buchi SMP-20 apparatus and are uncorrected. Infra-red spectra were recorded on a Perkin Elmer Spectrum RX I FT-IR System in KBr. Vibration frequencies in IR spectra are expressed in cm⁻¹. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254 nm) were used for TLC. Column chromatography was performed on ICN silica gel 63-200, 60 Å.

Synthesis of 4,6-Diamino-2-bromo-3-cyanopyridine (2.2)

HBr (*in situ* prepared from H₂SO₄ and NaBr) was bubbled for 2 hours through a solution of malononitrile **2.1** (13 g, 0.197 mol) in toluene (400 ml) at 0 °C. A light yellow precipitate was formed and the reaction mixture was then heated at 100 °C for 2 hours, with a lot of gas evolution. After cooling to room temperature, the yellow solid was filtered off and washed with toluene and air dried. The solid was mixed with water and the pH of the suspension was adjusted to 9-10 by the addition of a 33 % aqueous NH₃ solution. After stirring at room temperature for 1 hour, the mixture was filtered. Recrystallization from methanol afforded a yellow solid. After drying at 60 °C in a vacuum oven, the title compound was obtained as an orange solid (10.1 g, 48 %).

Synthesis of 3-Cyano-4, 6-diaminopyridine (2.3)

To a solution of 4,6-diamino-2-bromo-3-cyanopyridine **2.2** (7 g, 32.8 mmol) in THF/MeOH (2:1, 160 ml) was added potassium acetate (4.54 g, 32.8 mmol) and 10% Pd/C (0.925 g). The reaction mixture was stirred under a hydrogen atmosphere (pressure of 1 atm, using a balloon filled with hydrogen) at 25 °C for 4 hours. The mixture was filtered over Celite and the solvent was evaporated *in vacuo*. The solid was dissolved in dilute HCl and water. Adjustment of the solution pH to 10 (with a concentrated NaOH solution) and cooling afforded the title compound (3.94 g, 89 %) as a yellow solid.

Synthesis 4, 6-Diaminonicotinamide (2.4)

A solution of 3-cyano-4,6-diaminopyridine **2.4** (2.1 g, 15.7 mmol) in 90 % H₂SO₄ (10 ml) was stirred at 60-70 °C for 3 hours. The resulting solution was poured into a 40 % NaOH solution to give a mixture of 4,6-diaminonicotinamide as a pale yellow solid and inorganic salt (2.1 g, 85 %). An analytical pure sample was obtained by recrystallization from water to give a pale yellow solid.

Synthesis of 7-Aminopyrido[4,3-d]pyrimidin-4(3H)-one (2.5a)

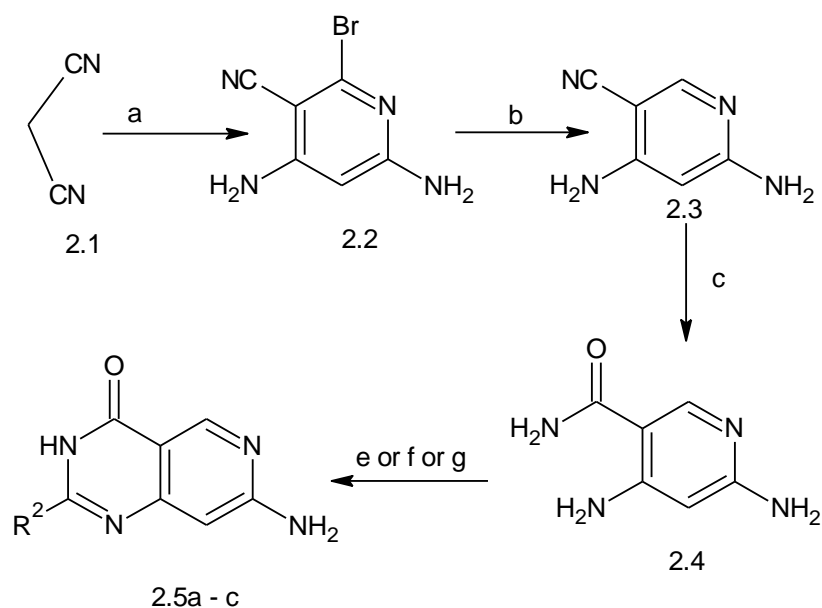
A solution of 4,6-diaminonicotinamide **2.5** (2 g, 13.1 mmol) in triethylorthoformate (60 ml) was refluxed under a nitrogen atmosphere for 24 hours. The solvents were evaporated *in vacuo* and the residue was redissolved in a hot 2 M NaOH solution (20 ml) for 30 minutes and thereafter neutralized with a 6 N HCl (6.5 ml) solution. On cooling, 7-aminopyrido[4,3-d]pyrimidin-4(3H)-one precipitated out. The yellow solid was filtered off and was dried in a vacuum oven,

Synthesis of 7-Amino-2-methylpyrido[4,3-d]pyrimidin-4(3H)-one (2.5b)

A solution of 4,6-diaminonicotinamide **2.5** (3 g, 13 mmol) in triethylorthoacetate (60 ml) was refluxed under N₂ for 24 hours. After removing the solvent, the residue was dissolved in hot 2 M NaOH (20 ml) for 30 minutes and then neutralized with 6 N HCl (6 – 7 ml). On cooling, 7-amino-2-methylpyrido[4,3-d]pyrimidin-4(3H)-one precipitates out. The yellow solid was filtered off and dried in a vacuum oven, affording the pure title compound (2.4 g, 72 %). mp > 290 °C.

Synthesis of 7-Amino-2-(methoxymethyl)pyrido[4,3-d]pyrimidin-4(3H)-one (2.5c)

To a solution of 4,6-diaminonicotinamide **2.5** (0.395 g, 2.6 mmol) in DMF (12 ml) was added methoxyacetyl chloride (0.498 ml, 5.45 mmol) and pyridine (0.525 ml, 6.46 mmol). The reaction was stirred at room temperature under a nitrogen atmosphere for 3 hours. Then, 5 N NaOH (8 ml) was added and the reaction mixture was stirred for an additional 18 hours. The reaction mixture was concentrated *in vacuo* and acidified with a concentrated HCl solution. On cooling, the product precipitates out. The pale yellow solid is collected by filtration and dried in a vacuum oven, furnishing the pure title compound (0.27 g, 50 %). mp 272-273 °C.



- a) HBr (g), 0 ° C, 2 h, toluene then 100 ° C, 2 h; b) 10 % Pd/C, H₂, KOAc, THF/MeOH (2:1 v/v), rt, 4 h; c) 90 % H₂SO₄, 80 ° C, 3 h; e) triethyl orthoformate, 140 ° C, 24 h; f) triethyl orthoacetate, 140 ° C, 24 h; g) methoxyacetyl chloride, pyridine, DMF, rt, 3 h then 5 N NaOH, rt, 24 h

Scheme -1 Synthesis of pyrido[4,3-d] pyrimidine

Characterization and spectral analysis of synthesized compounds

7-Aminopyrido[4,3-d]pyrimidin-4(3H)-one (2.5a)

% of yield (1.28 g) 60 %;

¹H NMR(200 MHz, DMSO-*d*₆, 25 °C): δ = 11.80 (br s, 1 H, NH), 8.72 (s, 1 H, H-5), 7.97 (s, 1 H, H-2), 6.75 (br s, 2H, NH₂), 6.36 (s, 1 H, H-8) ppm. ¹³C NMR (50 MHz, DMSO-*d*₆, 25 °C): δ = 163.83, 160.31, 154.90, 150.23, 149.17, 108.98, 98.93 ppm. MS: calcd for C₇H₇N₄O [M⁺H]⁺ 163.16, found 163.31.

7-Amino-2-methylpyrido[4,3-d]pyrimidin-4(3H)-one (2.5b)

% of yield (2.4 g, 72 %). mp > 290 °C.

¹H NMR (200 MHz, DMSO-*d*₆, 25 °C): δ = 11.79 (br s, 1 H, NH), 8.68 (s, 1 H, 5-H), 6.68 (brs, 2 H, NH₂), 6.28 (s, 1 H, 8-H), 2.25 (s, 3 H, 2-CH₃) ppm. ¹³C NMR (50 MHz, DMSO-*d*₆, 25 °C): δ = 163.8, 161.3, 158.4, 155.1, 150.0, 107.4, 98.3, 21.6 ppm. MS: calcd for C₈H₉N₄O [M⁺H]⁺ 177.18, found 177.30.

7-Amino-2-(methoxymethyl)pyrido[4,3-d]pyrimidin-4(3H)-one (2.5c)

% of yield (0.27g, 50 %, mp > 290 °C

¹H NMR (200 MHz, DMSO-*d*₆, 25 °C): δ = 11.70 (br s, 1 H, NH), 8.71 (s, 1 H, 5-H), 6.74 (s, 2 H, NH₂), 6.35 (s, 1 H, 8-H), 4.24 (s, 2 H, 2-CH₂), 3.33 (s, 3 H, OCH₃) ppm. ¹³C NMR (50 MHz, DMSO-*d*₆, 25 °C): δ = 164.0, 161.2, 157.9, 154.7, 150.4, 108.0, 99.0, 71.6, 58.7 ppm. IR (KBr): ν̃ = 3407, 3328, 3201, 2826, 1697, 1655, 1598, 1486, 1261, 1115 cm⁻¹. MS: calcd for C₉H₁₁N₄O₂ [M+H]⁺ 207.21, found 207.21.

In vivo anti-inflammatory activity test

The anti-inflammatory activity was assessed as suggested by Winter *et al*¹² by using carrageenan as edematogenic agent on adult albino rats of either sex weighing between 125-150g. The selected albino rats were housed in groups of six each in acrylic cages under laboratory conditions. The test compounds were administered intraperitoneally in the form of suspension with tween-40 and normal saline at a dose of 50mg/kg b.w. The Diclofenac sodium 10mg/kg was used as reference standard and administered in a similar manner. The control group received the solvent (tween-40 + normal saline) at 2ml/kg b.w. in a similar manner. All the test samples were administered 30mins before injection of carrageenan (0.1ml of 1% w/v in normal saline) into the sub-plantar region of left hind paw of each rat.

The contra-lateral paw was injected with an equal volume of saline. The increase in paw swelling (volume) was determined by plethysmometer by calculating the difference between the volumes of the mercury displaced by the two paws in ml. The increase in paw volume was measured at 1, 2 & 3 hrs after the administration of the test compounds. The percentage protection of edema was calculated at the end of 3 hrs as per the following formula.

$$\text{Percentage of protection} = \frac{P_c - P_t}{P_c} \times 100$$

Where PC = Increase in paw volume at time 't' of solvent control & Pt = Increase in paw volume at time 't' of test.

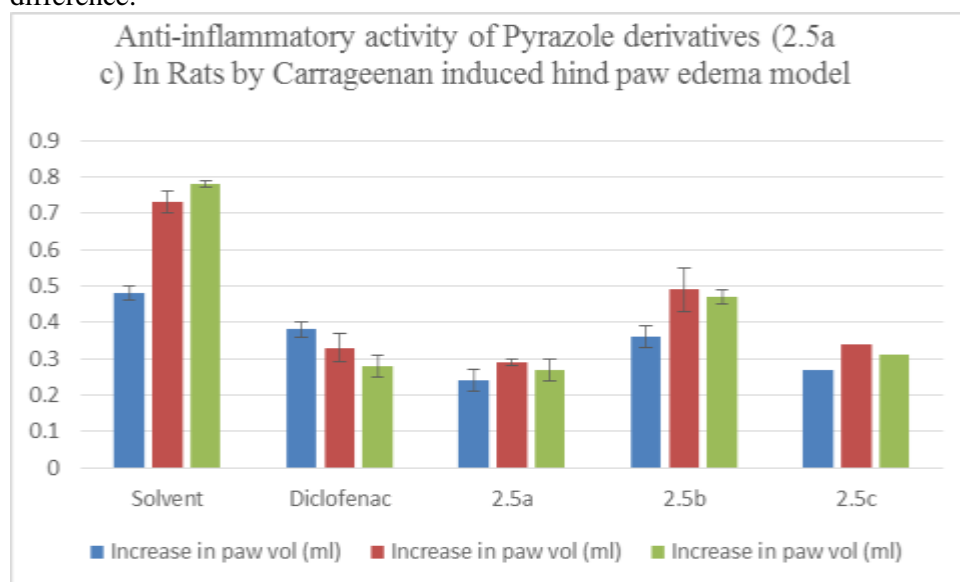
Table 3: Anti-inflammatory activity of Pyrazole derivatives (2.5a – c) In Rats by Carrageenan induced hind paw edema model

Sl. No.	Compd	Increase in paw vol (ml)			% protection at 3h
		1h	2h	3h	
1	Solvent	0.48±0.02	0.73±0.03	0.78±0.01	-
2	Diclofenac	0.38±0.02**	0.33±0.04***	0.28±0.03***	64.10
3	2.5a	0.24±0.03***	0.29±0.01***	0.27±0.03***	65.38
4	2.5b	0.36±0.03**	0.49±0.06**	0.47±0.02***	39.74
5	2.5c	0.27±0.03***	0.34±0.05***	0.31±0.05***	60.25

Values expressed as Mean±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, Dose of the test compound: 50 mg/kg.

Statistical analysis

Results were expressed as means ± s.e.m. Statistical significance was analyzed by using the one-way analysis of variance followed by Tukey's Multiple Comparison Test where p < 0.05 was accepted to be a significant difference.



RESULT AND DISCUSSION

The anti-inflammatory activity of present synthesized compounds was evaluated by carrageenan induced acute inflammatory method in rats on comparison with standard diclofenac sodium. The result revealed that all synthesized compounds shown anti-inflammatory activity (*p<0.05, **p<0.01, ***p<0.001) when compared to control and positive control group. These showed the percentage protection of edema ranges between 39.74 and 65.38 at the end of 3 hrs; whereas the standard drug Diclofenac sodium registered 64.10% (Table – 2). It has been observed that the maximum anti-inflammatory activity was recorded by the compound **2.5a** having 65.38% percentage protection of edema followed by compound **2.5b** (60.25%), **2.5c** (50%). The compound **2.5a** showed more percentage protection of edema than that of standard drug Diclofenac sodium. It has been found that the extent of increase in paw volume reduced significantly up to 3 hrs when compared with solvent control.

Conclusion

In conclusion, we reported here several pyridazinone compounds as analgesic and anti-inflammatory agents. Further investigations in the future should determine analgesic and anti-inflammatory potency for title compounds, the effect of specific substituents and their positions in the molecules, and their related toxicity.

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