Research Article

Analgesic and Anti-inflammatory Activity of some Novel Pyrazolo[3,4-c] pyrazoles

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ABSTRACT

Present work reports the development of a novel, one-pot protocol for the rapid microwave assisted synthesis of pyrazolopyrazoles. The structures of synthesized compounds are in agreement with IR, NMR, and MASS Spectral data. A series of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]-pyrazoles (IIIa-f) was prepared by the reaction between 2,4-dinitrophenyl hydrazine, 5-methyl-2,4-dihydro- 3H-pyrazol-3-one (II) and different aldehydes to microwave irradiation. Compound II was obtained by cyclization of ethylacetoacetateate (I) with hydrazine hydrate by stirring in absolute ethanol. Microwaves used to heat the reaction mixture in the step II of the proposed reaction. All the compounds were synthesized with good yield. Synthesized compounds exhibited analgesic and anti-inflammatory activity.

Keywords: Pyrazolo-pyrazoles, Microwaves, Analgesic, Antiinflammatory.

INTRODUCTION

Industrial chemistry in the new millennium is widely adopting the concept of "Green Chemistry" to meet the fundamental scientific challenges of protecting the human health and environment while maintaining commercial viability.

The emerging area of green chemistry envisages minimum hazard as the performance criteria while designing new chemical processes, one of the thrust areas for achieving the target is to explore alternative reaction condition and the media to accomplish the desired chemical transformation with minimized by products or waste as well as eliminates the use of conventional organic solvents.

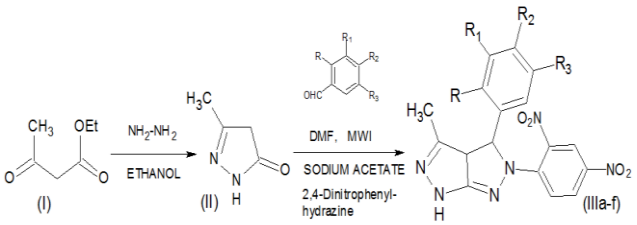
The microwaves couple directly with the molecules that are heating, leading to a rapid rise in temperature.

The result is an instantaneous heating of anything that will react to dipole rotation or ionic conduction, the two fundamental mechanisms for transferring energy from microwaves to the substance being heated.

The pharmaceutical importance of pyrazoline compounds, which are of our interest, lies in the fact that they can be effectively utilized as antibacterial[1-5], antifungal[1,3-5], anti-inflammatory[6], antitubercular[7], analgesic[8], insecticidal[9], antiparasitic[10] and antiviral[11] agents.

Some of these compounds have also anticonvulsant[12] cardiovascular[13] and anticancer[14] properties.

The present studies were performed with the objectives: microwave assisted synthesis[15] of new series of pyrazoles, characterization by spectral methods viz. IR spectra, NMR spectra, Mass spectra and screening of the analgesic and anti-inflammatory activity.



Scheme

Compound	R	R_1	R_2	R_3
IIIa	H	H	H	H
IIIb	Cl	H	H	H
IIIc	OH	H	H	H
IIId	Cl	H	Cl	H
IIIe	H	H	$N(CH_3)_2$	H
IIIf	H	OCH_3	OCH_3	OCH_3

MATERIALS AND METHODS

Experimental

All reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in well dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary. Catalyst's scientific microwave synthesizer (CATA-R, 32 liter, 850 W, 2450 MHz) was used for synthesis. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystalization and purity was checked by micro TLC.

The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pellet. ^{1}H NMR spectra were recorded in a BRUKER DPX-400MHz spectrometer using TMS as internal standard. GCMS spectra were recorded in SHIMADZU QP 50000. Elemental analysis for C, H and N were performed on a PERKIN ELMER 240 C elemental analyzer and were found to be within ± 0.4 % of the theoretical values. The prior permission of animal ethics committee of the working place was taken prior to conducting activity on animals. The synthesized compounds exhibited analgesic and anti-inflammatory activities.

Synthesis

Synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (conventional method)

Ethylacetoacetate (1.3g, 0.01mol) was placed in a conical flask and stirred magnetically during the slow dropwise addition of solution of hydrazine hydrate (98%,0.5 ml, 0.01 mol) in absolute ethanol (1ml) and temperature of about 60°C was maintained, a crystalline deposit was separated. After stirring for 1 h at room temp, the reaction mixture was cooled in an ice bath to complete recrystalisation, filtered, washed with ice-cold ethanol, dried, m.p.222°C. Yield 0.88g, 90%.

General Procedure

Synthesis of 5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IIIa-f)

A mixture of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (0.98g, 0.01mol), Aromatic aldehyde (0.01 mol), 2,4-dinitrophenylhydrazine (1.98g, 0.01 mol), was dissolved in DMF (40ml) in presence of anhydrous sodium acetate (0.82g, 0.01mol) and irradiated under microwaves (490 W) for 8 mins. Reaction completion was monitored by TLC (mobile phase: benzene), mixture was cooled, poured in ice water; crude product thus obtained was washed with cold ethanol. Yield and melting point were noted.

Spectral Data

4-Phenyl-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIa): IR (KBr, cm^{-1}): 3287 (N-H), 1585 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.5 (d, 1H, C3a -H), 7.8 (d, 1H, C4-H), 7.2-7.5 (m, 8H, Ar-H), 6.6(s, 1H, N-H), 2.4 (s, 3H, -CH_3). M/e: 366; Anal. ($C_{17}H_{14}N_6O_4$) Found: C, 55.71; H, 3.84; N, 22.93 Calculated : C, 55.73; H, 3.85; N, 22.94%.

4-(2-Chlorophenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo--[3,4-c] pyrazole (IIIb): IR (KBr, cm^{-1}): 3262 (N-H), 1520 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.7 (d, 1H, C3a-H), 6.9 (d, 1H, C4-H), 7.41-7.54 (m, 7H, Ar-H), 6.2 (s, 1H, N-H), 2.43 (s, 3H, CH_3). M/e: 400; Anal. ($C_{17}H_{13}ClN_6O_4$) Found: C, 50.55; H, 3.12; N, 21.65. Calculated : C, 50.95; H, 3.27; N, 20.97%.

4-(2-Hydroxyphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c] pyrazole (IIIc): IR (KBr, cm^{-1}): 3428 (OH and N-H), 1509 (C=N). ^{1}H NMR ($CDCl_3$) δ: 9.1 (s, 1H, OH), 8.6 (d, 1H, C3a-H), 7.3-7.5 (m, 7H, Ar-H), 7.0 (d, 1H, C4-H), 6.3 (s, 1H, N-H), 2.5 (s, 3H, CH_3). M/e: 382; Anal. ($C_{17}H_{14}N_6O_5$) Found: C, 49.32; H, 3.68; N, 20.46 Calculated : C, 49.04; H, 3.87; N, 20.18%.

4-(2,4-Dichlorophenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c] pyrazole (IIId): IR (KBr, cm^{-1}): 3281 (N-H), 1581 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.0 (d, 1H, C3a-H), 7.2-7.5 (m, 6H, Ar-H), 6.8 (d, 1H, C4-H), 6.2 (s, 1H, N-H), 2.5 (s, 3H, CH_3). M/e: 435; Anal. ($C_{17}H_{12}Cl_2N_6O_4$) Found: C, 46.80; H, 2.54; N, 19.10 Calculated : C, 46.91; H, 2.78; N, 19.31%.

4-(4-Diaminomethylphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIe): IR (KBr, cm^{-1}): 3281 (N-H), 1507 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.07 (d, 1H, C3a-H), 7.2 (d, 1H, C4-H), 7.37-7.45 (m, 7H, Ar-H), 6.5(s, 1H, N-H), 3.1 (s, 6H,-N (CH_3)$_2$), 2.5 (s, 3H, CH_3). M/e: 409; Anal. ($C_{19}H_{19}N_7O_4$) Found: C, 55.60; H, 4.32; N, 23.80 Calculated : C, 55.74; H, 4.68; N, 23.95%.

4-(3,4,5-Trimethoxyphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIf): IR (KBr, cm^{-1}) 3268 (N-H), 1586 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.07 (d, 1H, C3a-H), 6.9 (d, 1H, C4-H), 7.39-7.56 (m, 5H, Ar-H), 6.2 (s, 1H, N-H), 3.1 (s, 9H, -(OCH_3)$_3$), 2.5 (s, 3H, CH_3). M/e: 456; Anal. ($C_{20}H_{20}N_6O_7$) Found: C, 52.46; H, 4.10; N, 18.24; Calculated : C, 52.63; H, 4.41; N, 18.41%.

RESULTS AND DISCUSSION

The physical data of synthesized **compounds (IIIa-f)** is given in **Table 1**, analgesic activity is given in **Table 2** and antiinflammatory activity is given in **Table 3.**

Analgesic Activity

The synthesized compounds were assessed for the analgesic activity using Wistar Albino mice of either sex. Analgesic activity was measured by acetic acid induced writhings method.[16,17] Control group received vehicle (1 mL, 0.25 % CMC solution).

Standard drug used was aspirin (100 mg/kg). Six groups of animals were pretreated with the synthesized compounds and two groups were pretreated with standard and vehicle, respectively. Under similar conditions, after 0.5 h they were injected with 1 % (v/v) acetic acid (1 mL/100 g body weight, i.p.) and number of abdominal contractions, trunk twist response and extension of hind limbs as well as number of animals showing such response during 5 min were recorded. Mean writhings scores in all groups were calculated. Activity studies revealed that the compound IIIc with 2-hydroxyphenyl, compound IIIe with 4-dimethylaminophenyl and compound IIIf with 3,4,5-trimethoxyphenyl moieties were found to possess significant analgesic activity when compared with the standard drug.

Table 1: Physical data of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IIIa-f)

Compound	R	R_1	R_2	R_3	Reaction time (minutes)	Recrystallization Solvent	% Yield	m. p. (°C)	Molecular Formula	Molecular Weight	*Rf Value
IIIa	H	H	H	H	8	Ethanol	68	226-227	$C_{17}H_{14}N_6O_4$	366.332	0.66
IIIb	Cl	H	H	H	8	Glacial acetic acid + Ethanol(1:1)	72	215-216	$C_{17}H_{13}ClN_6O_4$	400.777	0.44
IIIc	OH	H	H	H	8	Glacial acetic acid	69	206-208	$C_{17}H_{14}N_6O_5$	382.331	0.62
IIId	Cl	H	Cl	H	8	Glacial acetic acid	79	223-224	$C_{17}H_{12}Cl_2N_6O_4$	435.222	0.60
IIIe	H	H	$N(CH_3)_2$	H	8	Ethanol	74	244-246	$C_{19}H_{19}N_7O_4$	409.399	0.44
IIIf	H	OCH_3	OCH_3	OCH_3	8	Glacial acetic acid	80	234-236	$C_{20}H_{20}N_6O_7$	456.409	0.53

* R_f value was determined in benzene: acetone (1:1)

Table 2: Analgesic Activity of Compounds (IIIa-f)

S. No.	Design of Treatment (Groups)	Dose (mg/kg, p.o.)	Number of writhings in 5 minutes	% Inhibition
1	Control (CMC, 0.25%,1ml)	-	180.00 ± 0.607	-
2	Aspirin	100	41.83 ± 0.477**	76.76
3	Compound IIIa	146	79.43 ± 0.600**	55.65
4	Compound IIIb	125	65.50 ± 0.763**	63.61
5	Compound IIIc	138	76.00 ± 0.577**	57.77
6	Compound IIId	128	58.67 ± 0.881**	67.40
7	Compound IIIe	133	69.71 ± 0.666**	61.29
8	Compound IIIf	131	71.17± 0.600**	60.46

Values are expressed as mean ± SEM, N=6; When compared with control, *= P< 0.05, **= P< 0.01, **= P< 0.001

(One way ANOVA followed by Dunnett's multiple comparison test)

Table 3: Anti-inflammatory Activity of Compounds (IIIa-f)

S. No.	Design of Treatment (Groups)	Dose (mg/kg, p.o.)	Change in paw edema at the end of 3h (mm)	% Inhibition
1	Control (CMC, 0.25%,1ml)	-	0.85 ± 0.0067	-
2	Indomethacin	10	0.22 ± 0.0060**	74.11
3	Compound IIIa	146	0.54 ± 0.0057**	36.47
4	Compound IIIb	125	0.39 ± 0.0066**	54.11
5	Compound IIIc	138	0.49 ± 0.0076**	42.35
6	Compound IIId	128	0.33 ± 0.0135**	61.17
7	Compound IIIe	133	0.42 ± 0.0060**	50.59
8	Compound IIIf	131	0.45 ± 0.0049**	47.06

Values are expressed as mean ± SEM, N=6; When compared with control,*= P< 0.05, **= P< 0.01,

**= P< 0.001 (One way ANOVA followed by Dennett's multiple comparison test)

Anti-Inflammatory Activity

The newly synthesized compounds were evaluated for their anti-inflammatory activity in Male Albino Wistar rats (150-200 gm).[18,19] Carrageenan (Sigma, St. Louis, USA) was used in the study and Indomethacin (Recon Ltd, Bangalore) was used as the standard drug.

Activity study reveals that Compound IIId and Compound IIIb when compared with control group, corresponds to 61% and 54% inhibition respectively. Whereas, standard drug Indomethacin showed 74.11% inhibition. So, Compound IIId and compound IIIb showed very significant anti-inflammatory activity. Compound IIIe and compound IIIf showed change in paw oedema, which corresponds to 50% and 47% inhibition respectively.

Compound IIIa and compound IIIc showed change in paw edema which corresponds to 36% and 42% inhibition respectively.

CONCLUSION

Microwave assisted synthesis of pyrazolopyrazoles is entirely possible.

The yield is almost quantitative.

The structures of synthesized compounds were in agreement with IR, NMR, and MASS Spectral data.

The present method is mild, exceedingly efficient, very rapid and especially eco-friendly.

All the compounds were synthesized with good yield (68-80%).

All the synthesized compounds exhibited analgesic and anti-inflammatory activities. Compound IIIc, compound IIIe and compound IIIf were found to possess significant analgesic activity and Compound IIIb and compound IIId showed very significant anti-inflammatory activity when compared with the standard drug.

The synthesized compounds are believed to exert various other activities such as antibacterial, antifungal, anticonvulsant and CNS depressant.

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