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Antiepileptic and Antimicrobial Activities of Novel 1-(unsubstituted/substituted)-3,5-dimethyl-1*H*-pyrazole Derivatives

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Abstract: A series of 1*H*-pyrazole derivatives **(1a-f)** were synthesized by cycloaddition of acetyl acetone with respective hydrazine derivatives in ether in the presence of sodium hydroxide to generate the corresponding pyrazole derivatives. The synthesized compounds were confirmed by melting point and TLC. The structure of synthesized compounds was established by elemental analysis and various analytical techniques such as IR and ^{1}HNMR spectral studies. All the newly synthesized compounds were evaluated for their antiepileptic and antimicrobial activities.
Keywords: Pyrazoles, Antiepileptic, Antibacterial, Antifungal

Introduction

The pyrazole ring system is a five-membered heterocyclic ring structure composed of two nitrogen atoms and used in the synthesis of pharmaceuticals. The pyrazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the past few years, the therapeutic interest of pyrazole derivatives in pharmaceutical and medicinal field has been given a great attention to the medicinal chemist. Literature survey reveals that pyrazole derivatives are well known to have antibacterial[1], antifungal[2], anti-tubercular[3], anticancer[4], analgesic[5], anti-inflammatory[5], antipyretic[6], anticonvulsant[7], antidepressant[8], muscle relaxing[9], anti-ulcer[10], antiarrhythmic[11] and antidiabetic[12] activities. In recent years, the extensive studies have been focused on pyrazole derivatives because of their diverse chemical reactivity, accessibility and wide range of biological activities. Thus, in this present work, a series of novel pyrazole derivatives were synthesized and tested for their antiepileptic and antimicrobial activities.

Experimental

Melting points were determined using on veego melting point apparatus and were uncorrected. The purity of the compounds was confirmed by TLC using silica gel G as stationary phase and chloroform-methanol (9:1) as eluent. IR spectra in υ_{max} (cm^{-1}) were recorded on Shimazdu 8400 series FT-IR Spectrophotometer using KBr disc technique. ^{1}H NMR

spectra were obtained on Bruker AMX spectrophotometer at 300 MHz in DMSO-d_6. Chemical shifts were reported in δ units (ppm) relative to an internal standard of tetramethysilane. Elemental analysis determinations for final compounds were performed on Carlo Erba 108 and the analyses. The animal study was approved by the animal ethical committee (AKCP/CPCSEA/509/F(2)/2008).

Synthesis of novel pyrazole derivatives

The respective hydrazine derivative (0.2 mol) was dissolved in 10% NaOH and cooled in an ice bath at 15 ^{0}C. Acetyl acetone (0.2 mol) was added drop wise to the above solution and stirred for 1h. To this mixture water (75 mL) and ether (50 mL) were added and shaken well in separating funnel. The aqueous layer was extracted with four successive quantities of ether (15 mL). Then the collected ether extract was combined and excess of ether was removed by distillation gave the corresponding pyrazole derivatives and recrystallized from ethanol[13]. The physical data of the compounds were recorded in Table 1 and the spectral data were tabulated in Table 2.

Anti-epileptic activity

The anti-epileptic activity was carried out by maximal electrical shock induced convulsion method[14]. Albino mice of either sex (20 – 30 g) were used for this study. Mice were treated with newly synthesized pyrazole derivatives (25 mg/mL, i.p.), standard drug phenytoin (25 mg/mL, i.p.) and control (2% w/v Tween 80). After 30 min, the animals were subjected to electro shock through ear electrodes of 150 mA for 0.2 sec by electroconvulsiometer and the duration of time for extensor response was noted. The statistical significance of the difference in the mean values was calculated by students't' test[15] and tabulated in Table 3.

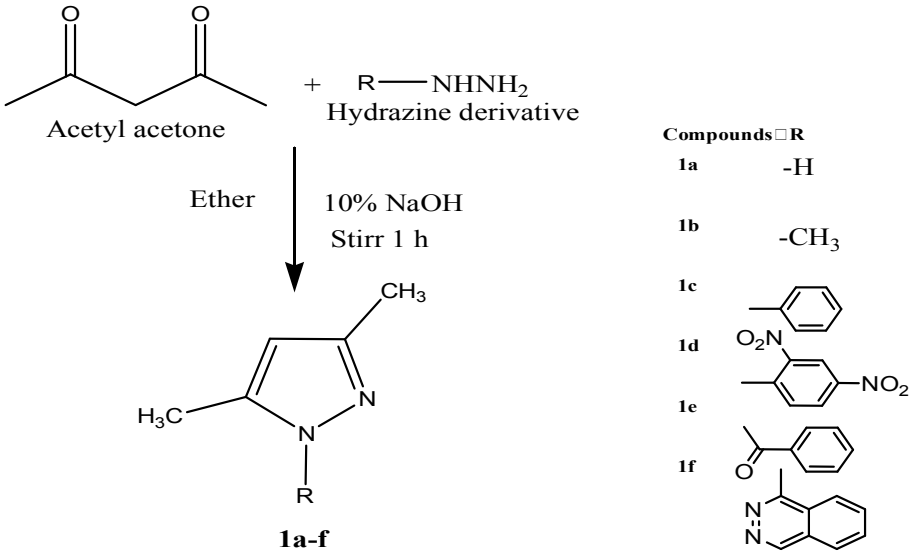
Test microorganisms

Gram positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, Gram negative organisms such as *Salmonella typhi*, *Pseudomonas auregenosa* and fungus *Candida albicans* were used for this study. All the bacterial cultures were procured from microbiology lab, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India.

Antibacterial activity

All the synthesized compounds were dissolved in 2 % v/v Tween 80 at a concentration of 100 mcg/mL. The antibacterial activity was performed by cup-plate method[16]. The respective bacterial culture was spread (swabbed) into the nutrient agar plates for uniform distribution of colonies. Using a sterile cork borer, 8 mm wide well was made on each agar plates. All the synthesized compounds (100 mcg/mL) were poured into each wells using a sterile micropipette and ofloxacin (100 mcg/mL) were used as standard. The plates were incubated for 24 h at 37 ^{o}C. After incubation, the zone of inhibition was measured and the values were tabulated in Table 4. All the experiments were done in triplicate.

Scheme 1



1a-f

1-(unsubstituted/substituted)-3,5-dimethyl-1*H*-pyrazole

Antifungal activity

The antifungal activity was tested against *Candida albicans* by cup plate method[16]. All the synthesized compounds were dissolved in 2 % v/v Tween 80 at a concentration of 100 mcg/mL. The fungal culture was spread (swabbed) into the sabouraud dextrose agar plates for uniform distribution of colonies. Using a sterile cork borer, 8 mm wide well was made on each agar plates. All the synthesized compounds (100 mcg/mL) were poured into each wells using a sterile micropipette and ketoconazole (100 mcg/mL) were used as standard. The plates were incubated for 48 h at 27 ^{o}C. After incubation, the zone of inhibition was measured and the values were tabulated in Table 4. All the experiments were done in triplicate.

Results and Discussion

A series of novel pyrazole derivatives were synthesized by the cycloaddition of acetyl acetone with the respective hydrazine derivatives. The sequence of reaction for the formation of novel pyrazole derivatives is outlined in scheme 1. The melting point and R_f values of the synthesized compound confirmed the formation of novel compounds. All the spectral data elucidated the structure of the synthesized pyrazole derivatives. Elemental analysis indicated by the symbols of the elements is determined and showed close value within ± 0.4% of theoretical values.

Anti-epileptic activity

The reduction in the time of extensor phase was taken for anti-epileptic activity. The compounds 1a, 1b, 1c and 1d showed highly significant anti-epileptic activity where as compounds 1e and 1f showed significant anti-epileptic activity at a dose of 25 mg/mL, i.p. when compared to the standard drug phenytoin 25 mg/mL.

Antibacterial and antifungal activities

All synthesized novel pyrazoles were tested against Gram positive organisms such as *S. aureus*, *S. pyogenes* and Gram negative organisms such as *S. typhi*, *P. auregenosa* and fungus *C. albicans*. All the compounds showed mild to moderate antibacterial and antifungal activities at a concentration of 100 mcg/mL when compared with ofloxacin (100 mcg/mL) and ketoconazole (100 mcg/mL) respectively.

Table 1. Physical data of novel pyrazole derivatives

Comp. code	Molecular formula	M.W.	m.p.(^{0}C)	R_f value	% yield	Elemental analysis % calculated (%found)			
						C	H	N	O
1a	$C_5H_8N_2$	96.13	88	0.951	73.35	62.47 (62.42)	8.39 (8.42)	29.14 (29.12)	-
1b	$C_6H_{10}N_2$	110.16	96	0872	62.46	65.42 (65.40)	9.15 (9.16)	25.43 (25.40)	-
1c	$C_{11}H_{12}N_2$	172.23	92	0.910	64.50	76.71 (76.74)	7.02 (7.06)	16.27 (16.24)	-
1d	$C_{11}H_{10}N_4O_4$	262.22	106	0.841	61.23	50.38 (50.32)	3.84 (3.88)	21.37 (21.34)	24.41 (24.44)
1e	$C_{12}H_{12}N_2O$	200.24	122	0.896	56.98	71.98 (71.96)	6.04 (6.08)	13.99 (13.96)	7.99 (7.96)
1f	$C_{13}H_{12}N_4$	224.26	116	0.864	58.24	69.62 (69.60)	5.39 (5.36)	24.98 (24.96)	-

Comp. code – Compound code, M.W. – Molecular weight, m.p. - Melting point

Table 2. Spectral data of novel pyrazole derivatives

Compound code	IR (KBr disc) υ_{max} (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
1a	3384 (NH), 1620.45 (C=N), 1512.22 (N-N), 1476 (CH_3)	2.34 (s, CH_3), 2.72 (s, CH_3), 5.86 (s, CH pyrazole), 13.4 (s, NH)
1b	1618.22 (C=N), 1515.82 (N-N), 1472.44 (CH_3)	2.86 (s, CH_3), 3.82 (s, NCH_3), 5.74 (s, CH pyrazole)
1c	1615.75 (C=N), 1505.82 (N-N), 1468 (CH_3)	2.62 (s, CH_3), 5.96 (s, CH pyrazole), 7.43-7.82 (m, ArH)
1d	1611.90 (C=N), 1520 (N-N), 1462.38 (CH_3), 1611.90 (Ar-NO_2)	2.78 (s, CH_3), 6.06 (s, CH pyrazole), 7.68-8.26 (m, ArH)
1e	1624 (C=N), 1522.40 (N-N), 1470 (CH_3), 1664 (C=O),	2.92 (s, CH_3), 5.96 (s, CH pyrazole), 7.98-9.16 (m, ArH)
1f	1618.52 (C=N), 1512.22 (N-N), 1476 (CH_3)	2.88 (s, CH_3), 5.66 (s, CH pyrazole), 7.84-9.42 (m, ArH)

Table 3. Anticonvulsant activity of novel pyrazole derivatives

Compound code	Duration of time (Mean ± SEM, sec)			% Protection
	Extensor	Clonus	Stupor	
Control	16.22 ± 0.12	22.64 ± 0.44	44.62 ± 5.42	-
Standard drug	3.12 ± 0.026**	11.32 ± 0.32**	14.54 ± 4.26**	80.76
1a	6.12 ± 0.24**	16.52 ± 0.12**	19.6 ± 4.74**	62.27
1b	5.94 ± 0.18**	17.98 ± 0.70**	22.2 ± 3.44**	63.38
1c	4.80 ± 0.16**	14.50 ± 0.36**	17.4 ± 2.16**	70.41
1d	7.46 ± 0.09**	15.12 ± 0.44**	18.5 ± 2.64**	54.01
1e	10.94 ± 0.22*	18.34 ± 0.56*	32.0 ± 2.82*	32.55
1f	11.67 ± 0.46*	17.45 ± 0.78*	38.18 ± 6.86*	28.05

Values are Mean ± SEM, *P < 0.05 and **P < 0.01 statistically significant from control group (n=6)

Table 4. Anti-bacterial and Antifungal activities of novel pyrazole derivatives

Microorganisms	Diameter of Zone of inhibition (mm)						
	Standard	1a	1b	1c	1d	1e	1f
S. aureus	20	15	10	13	15	12	13
S. pyogenes	21	15	12	11	09	08	11
S. typhi	18	14	09	10	06	12	14
P. aurgenosa	22	16	12	14	15	11	10
C. albicans	19	17	14	13	16	10	10

n = 3

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