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SYNTHESIS AND ANTICONVULSANT ACTIVITIES OF SMALL N- SUBSTITUTED 2, 5-DIMETHYL PYRROLE AND BIPYRROLE

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A series of N-substituted 2, 5-dimethyl pyrrole and bipyrrole derivatives were synthesized by Paal-Knorr method and evaluated for anticonvulsant activity at NIH. Anticonvulsant activity was determined after intraperitoneal (i.p.) administration to mice by maximal electroshock (MES) and subcutaneous metrazol (ScMET) induced seizure method at 30, 100 and 30 mg/kg dose levels. Minimal motor impairment was determined by rotorod test at the same dose levels. Compound 7 and 10 showed trace signs of anticonvulsant protection in the primary model screens, therefore selected for reevaluation screening in the 6 Hz model. Compound 10 was found to possess anticonvulsant activity at 100 mg/kg dose level in 6 Hz test.

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1. Introduction

In recent years, antiepileptic drug development has been one of the most prominent research areas. Although several new anticonvulsants are already in clinical use, some types of seizures are still not adequately treated with current therapy and have limitations, intolerable side effects. In response to these limitations, the development of new drugs to optimally manage seizures has been strongly advocated. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.

Substitution at nitrogen in the pyrrole ring has proved the significance of the pyrrole nucleus in various biological activities as analgesic[1, 2], CNS depressant[3], antifungal[4], antimycobacterial[5, 6], anticancer[7, 8], anticonvulsant[9, 10] and anti HIV[11] activities. According to these reports, we aimed to prepare N-substituted pyrrole derivatives in order to investigate the influence of replacing the N of pyrrole ring by different substitutions on anticonvulsant activity. The structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR and elemental analysis. Anticonvulsant activities of the compounds **1-12** were examined by MES and scMet tests at NIH according to the guidelines. Compounds active in either the MES or PTZ tests have generally been efficacious in clinical trials, although inactivity in these tests does not necessarily indicate lack efficacy. An alternate electroshock test- the 6 Hz model, in which the endpoint is limbic seizure activity rather than tonic hind limb extension as in the MES test can be used[12].

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2. Results and Discussion

Results

Chemistry

It was observed that, both the reactions are successful at room temperature and the reaction conditions are mild in comparison to other reported methods. Formation of N- substituted pyrrole derivatives with aromatic amines resulted in fairly good yields as compared to aliphatic and aliphatic-aromatic amines. Aromatic amines with substitution at *para* position were found to be more reactive than substitution at *meta* or *ortho* positions. Amongst aromatic amines, the *para* substituted amines gave better yield as compared to substitution at other positions.

All the synthesized compounds have shown following characteristic peaks in IR spectra indicating the formation of product. C-N stretch in the region of 1360-1310 cm^{-1}. Aromatic and heteroaromatic ring stretch in the region of 1600-1450 cm^{-1} and 1600-1300 cm^{-1} respectively. C-H stretch for CH_3 in the region of 2962-2853 cm^{-1}. Compounds 9 and 10 have also shown characteristic asymmetric C-O-C stretch at 1247 cm^{-1} and 1240 cm^{-1} and symmetric C-O-C str. at 1043 cm^{-1} and 1022 cm^{-1} respectively.

All the synthesized compounds have shown the α-methyl protons in the pyrrole ring resonated as a singlet in the region of δ 1.96-2.17 integrating for six protons and the β-protons of the pyrrole ring was seen as broad singlet in the region of δ 5.76-5.95 integrating for two protons indicating the formation of product..

In addition, following peaks were also observed which support the formation of different N-substituted pyrroles. Compounds having substitution at the para position in the phenyl ring; the aromatic protons were appeared downfield as deshielded. In compound 6, a singlet observed at δ 2.41 was ascribed to Ar-CH_3 protons. In compounds 5, a singlet observed at δ 3.85 integrating for three methoxy protons.

Pharmacology

All the compounds were evaluated for anticonvulsant activity at NIH after intraperitoneal (i.p.) administration to mice by maximal electroshock (MES), subcutaneous metrazol (ScMET) induced seizure method and neurotoxicity test at 30, 100 and 30 mg/kg dose levels. Compound 7 showed reduction in tonic extension in scMET and compound 10 showed trace signs of anticonvulsant protection in the primary model screens, therefore selected for reevaluation screening in the 6 Hz model. Compound 10 has shown protection in 50% and 100% animals at 0.25 and 0.5 hr duration respectively at 100 mg/kg.

Conclusion

Compound 7and 10 were found to possess anticonvulsant activity hence can be used as lead to develop antiepileptic drugs. Also further substitutions on pyrrole nucleus can lead to more potent compounds.

Experimental section

Melting Points were determined on Perfit Digital Melting Point Apparatus and were uncorrected. Thin Layer Chromatography was performed on Silica gel G plates and the spots were detected in iodine chamber. UV spectra were recorded by Shimadzu double beam UV/VISIBLE spectrophotometer (uv-1700). The values of λ_{max} for each compound were recorded (Table 1). IR spectra were recorded in KBr disc on Shimadzu FTIR-8400S. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300MHz on BRUKER Spectrometer and all chemical shifts were given in ppm relative to tetramethylsilane. The elemental analyses (C, H, N) were performed using Perkin-Elmer model 240c analyzer. The chemicals were purchased from Sigma Alderich Chemical Corporation.

Compounds **1-12** were prepared according to a reported procedure based on simple Paal-Knorr method[13]. The synthetic approach to obtain N-substituted pyrrole and bipyrrole derivatives followed the reaction shown in scheme I and II. The Nitrogen estimation was carried out using Kjeldahl method and the results are comparable to analytically calculated values. The structures of the target compounds were confirmed by IR, ^{1}H NMR and elemental analysis technique. Table 1 summarizes the physical and spectral data of the synthesized compounds.

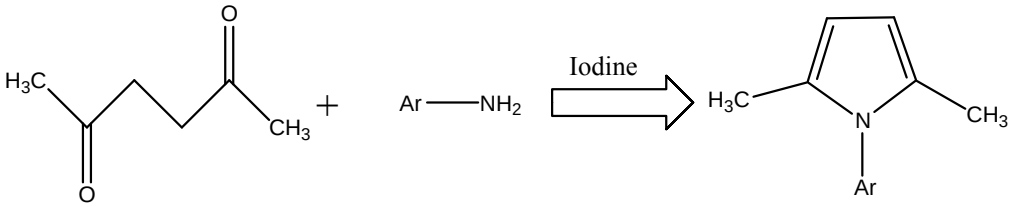
Preparation of Compounds (1-10) (Scheme I)

To a solution of the amine (0.024 mol) and hexane2, 5-dione (0.02 mol) in THF (10ml) at room temperature was added iodine (0.001 mol). The mixture was stirred at this temperature for the time period specified in Table 1. The reaction was monitored by TLC using silica gel G as stationary phase and mixtures of various organic solvents as mobile phase. An iodine vapor was used as detecting agent. Dichloromethane (50 ml) was then added to the mixture. The resulting mixture was washed successively with 5% $Na_2S_2O_3$ solution (20 ml), saturated $NaHCO_3$ solution (20 ml) and brine (20 ml). The organic layer was then dried with anhydrous sodium sulphate. It was concentrated under vacuum on rotary evaporator. The precipitate was collected and dried at room temperature. The N-substituted pyrroles were recrystallized by using Methanol: water (7:2).

Preparation of Compounds (11, 12) (Scheme II)

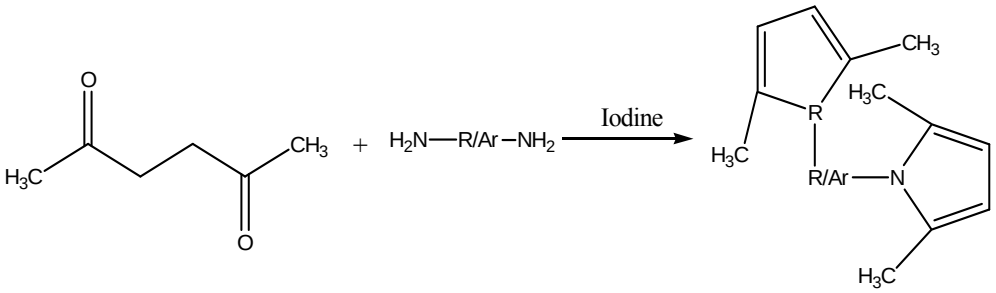
To a solution of the diamine (0.024 mol) and hexane2, 5-dione (0.02 mol) in THF (10ml) at room temperature was added iodine (0.001 mol). The mixture was further treated in the same way as it is given for compounds 1-10.

Scheme I:



Ar = Aromatic primary amine

Scheme II:



11. R = -CH_2-CH_2-

12. Ar = o-Phenylenediamine

Table 1. Melting point, Elemental analysis, log P and λ_{max} values of compounds (1-12).

No.	R/Ar	MP	Elemental analysis Calc (Found) % C	H	N	log P	λ_{max} (nm)
1	Phenyl	45-50	84.21(84.15)	7.60(7.56)	8.23 (8.14)	-0.3116	239
2	4-Nitrophenyl	125-9	66.67(66.64)	5.56(5.50)	12.96(12.89)	-0.3125	248
3	4-Bromophenyl	68-70	57.14(57.09)	4.76(4.70)	5.62 (5.59)	-0.2474	268
4	4-Fluorophenyl	38-40	76.19(76.11)	6.35(6.28)	7.40 (7.14)	0.0104	264
5	4-Methoxyphenyl	34-39	77.61(77.58)	6.47(6.41)	6.96(6.87)	0.5634	223
6	4-Methylphenyl	43-47	84.32(84.27)	8.11(8.07)	7.56 (7.48)	-0.1966	274
7	4-Iodophenyl	77-83	48.48(48.42)	4.04(3.98)	4.71 (4.56)	-0.4346	251, 231
8	1-Naphthalenyl	116-120	86.88(86.79)	6.79(6.67)	6.33 (6.20)	- 0.1817	274, 224
9	4-Chlorophenyl	46-48	70.24(70.18)	5.85(5.81)	6.82 (6.67)	0.2841	247
10	2-Hydroxyphenyl	92-95	77.00(76.79)	6.95(6.87)	7.48 (7.21)	0.5827	275
11	Ethylene	110-112	77.77(77.56)	9.25(9.08)	12.96(12.84)	-0.2000	248
12	o-Phenylene	47-49	81.81(81.78)	7.57(7.46)	10.60 (10.46)	-0.4346	287

IR and 1H-NMR data of compounds 1-12:

***1-Phenyl-2, 5-dimethylpyrrole* (1)**

% Yield: 87.72, IR (KBr): 3099, 3049, 2927, 2920, 2891, 2858, 1598, 1519, 1494, 1402, 1380, 1319, 750, 717 cm^{-1}. ^1H NMR ($CDCl_3$) (400MHz): δ 2.03 (6H, s), 5.90 (2H, s), 7.20-7.47 (5H, m)

***1-(4-Nitro phenyl)-2, 5-dimethylpyrrole* (2)**

% Yield: 89.35, IR (KBr): 3105, 3074, 2927, 2920, 2852, 1595, 1517, 1492, 1398, 1336, 854 cm^{-1}. ^1H NMR ($CDCl_3$) (400MHz): δ 2.05 (6H, s), 5.94 (2H, s), 7.33-7.38 (2H, d, *J*=8.8 Hz), 8.32-8.34 (2H, d, *J*=8.4 Hz)

***1-(4-Bromophenyl)-2, 5-dimethylpyrrole* (3)**

% Yield: 80.32, IR (KBr): 3078, 3051, 3033, 2981, 2933, 2918, 2889, 1587, 1519, 1483, 1380, 1319, 1064, 1035, 840, 547 cm^{-1}. ^1H NMR ($CDCl_3$) (400MHz): δ 2.01 (6H, s), 5.88 (2H, s), 7.05-7.58 (2H, d, *J*= 9.6 Hz), 7.55-7.85 (2H, d, *J*=6.4 Hz).

***1-(4-Fluorophenyl)-2, 5-dimethylpyrrole* (4)**

% Yield: 83.04, IR (KBr): 3073, 2977, 2921, 2894, 1514, 1506, 1438, 1384, 1323, 1222, 1091, 842 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2.01 (6H, s), 5.89 (2H, s), 7.10-7.17 (4H, m).

***1-(4-Methoxyphenyl)-2, 5-dimethylpyrrole* (5)**

% Yield: 79.10, IR (KBr): 3099, 3064, 2958, 2929, 2891, 2837, 1514, 1461, 1440, 1367, 1247, 1043, 842 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2.01 (6H, s), 3.85 (3H, s), 5.87 (2H, s), 6.94-6.97 (2H, d, *J*=8.7Hz), 7.11-7.14 (2H, d, *J*=8.4 Hz)

***1-(4-Methylphenyl)-2, 5-dimethylpyrrole* (6)**

% Yield: 92.70, IR (KBr): 3121, 3046, 2983, 2920, 2893, 2858, 1590, 1515, 1436, 1380, 1321, 1035, 827 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2.02 (6H, s), 2.41 (3H, s), 5.88 (2H, s), 7.07-7.10 (2H, d, *J*=9Hz), 7.23-7.26 (2H, d, *J*=9Hz).

***1-(4-Iodophenyl)-2, 5-dimethylpyrrole* (7)**

% Yield: 80.13, IR (KBr): 3082, 3028, 2975, 2931, 1583, 1528, 1479, 1379, 1321, 1093, 837, 545 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2.02 (6H, s), 5.89 (2H, s), 6.94-6.97 (2H, d, *J*=8.4 Hz), 7.77-7.79 (2H, d, *J*=8.4 Hz)

***1-(1-Naphthalenyl)-2,5-dimethylpyrrole* (8)**

% Yield: 89.81, IR (KBr): 3100, 2972, 2926, 2880, 1521, 1461, 1411, 1397, 1304, 748 cm^{-1}. ^1H NMR ($CDCl_3$) (400MHz): δ 1.87 (6H, s), 5.98 (2H, s), 7.10-7.12 (1H, d, *J*=8.4 Hz), 7.40-7.44 (1H, m, *J*=8.4 Hz), 7.48-7.56 (2H, m), 7.90-7.92 (2H, d, *J*=8 Hz).

***1-(4-Chlorophenyl)-2, 5-dimethylpyrrole* (9)**

% Yield: 78.04, IR (KBr): 3097, 3053, 2974, 2920, 2893, 2854, 1596, 1496, 1369, 1321, 757 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2.02 (6H, s), 5.89 (2H, s), 7.13-7.16 (2H, d, *J*=9 Hz), 7.41-7.44 (2H, d, *J*=9 Hz).

1-(2-Hydroxyphenyl)-2, 5-dimethylpyrrole (10)

% Yield: 65.72, IR (KBr): 3370, 2918, 2885, 2852, 1589, 1500, 1398, 1319, 1232, 748, 621 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 1.96 (6H, s), 5.95 (2H, s), 6.97-7.00 (1H, d, J= 7.2 Hz), 7.04-7.12 (2H, m), 7.29-7.34 (1H, d)

Bi-pyrrole-I (11)

% Yield: 76.10, IR (KBr): 3099, 2985, 2919, 2862, 1600-1400, 1306 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 2 (12 H, s), 3.92 (4H, t), 5.46-5.74 (4H, m)

Bi-pyrrole-II (12)

% Yield: 68.07, IR (KBr): 3074, 2955, 2925, 2895, 1605, 1504, 1400, 1321, 767 cm^{-1}. ^1H NMR ($CDCl_3$) (300MHz): δ 1.97 (12H, s), 5.92 (4H, m), 6.76-7.24 (4H, m)

Pharmacology:

Anticonvulsant Screening Program at the National Institute of Neurological Disorders and Stroke (NINDS, NIH)

The anticonvulsant evaluation was undertaken by the National Institute of Neurological Disorders and Stroke, Bethesda, USA according to their protocols[14] male albino mice (CF#1 strain, 18-25 g) and male albino mice were used as experimental animals. The compounds were suspended in 0.5% methylcellulose/water mixture. All the compounds were administered i.p. in a volume of 0.01 ml/g body weight of mice at 30, 100 and 300 mg/kg to one to three animals. Activity was established using the MES, scPTZ and neurotoxicity. Some selected compounds described in this study were examined for oral activity in the MES screen and 6 Hz screen which uses a threshold stimulus vs. the maximal electroshock suprathreshold stimulation[12]. The results are presented in Table 2.

Electroshock method

Maximal seizures were induced by the application of electrical current to the brain via corneal electrodes. The stimulus parameters for mice were 50mA in a pulse of 60 Hz for 200 ms. The mice were given the test drug intraperitoneally. Abolition of hind limb extensor spasm was recorded as a measure of anticonvulsant activity.

Subcutaneous metrazole seizure pattern test

A metrazole dose of 85 mg/kg administered subcutaneously to mice causes seizures in more than 97% of the animals. This is called the convulsive dose 97 (CD_{97}). The test was carried out by giving the metrazole injection approximately 10 minutes before the anticipated time of the peak anticonvulsant drug action. The animals were observed during the following four hours for the occurrence of seizures. A threshold convulsion is defined as one episode of clonic spasms which persists for at least 5 seconds. Absence of even a threshold convulsion during the period of observation is taken as the endpoint in this test.

Neurotoxicity (NT) screening

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 10 rpm. The rod diameter was 3.2 cm. Trained animals were injected intraperitoneally with the test compounds at doses of 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least 1 min in each of the three trials. The results are shown in Table 2.

Table 2. Anticonvulsant activity of the compounds 1-12.

No.	*R/Ar*	*Anticonvulsant activity (mg/kg)*		*Toxicity Screen[a, b] (mg/kg)*	
		MES[a]	**scMET[a]**	***0.5***	***4.0***
1	Phenyl	NA	NA	100 (12.5 %)	-
				300 (50 %)	-
2	4-Nitrophenyl	NA	NA	100 (12.5 %)	-
3	4-Bromophenyl	NA	NA	100 (12.5 %)	-
				300 (50%)	-
4	4-Fluorophenyl	NA	NA	300 (75 %)	300 (50%)
5	4-Methoxyphenyl	NA	NA	-	-
6	4-Methylphenyl	NA	NA	300 (50%)	-
7	4-Iodophenyl	NA	NA*	30 (25 %)	-
				100 (25 %)	-
8	1-Naphthalenyl	NA	NA	100 (12.5 %)	-
9	4-Chlorophenyl	NA	NA	-	-
10	2-Hydroxyphenyl	NA	NA	-	-
11	Ethylene	NA	NA	-	-
12	o-Phenylene	NA	NA	-	-

The animals were examined from 0.5 to 4 hr after the convulsive stimuli was applied.
[a]The primary of MES, scMET and toxicity were performed by intraperitoneal injection in mice at doses 30, 100 and 300 mg/kg.
[b]Values in parentheses in the neurotoxicity test indicate the number of animals exhibiting toxicity against the number of animals tested.
NA indicates absence of activity at the maximum dose administered (300 mg/kg).
*scMET test (30 mg/kg, 0.5 hr): Tonic extension

Table 3: Anticonvulsant activity in 6 Hz test.

ompound	*Dose (mg/kg)*	*0.25*	*0.5*	*1.0*	*2.0*
7	4-Methoxyphenyl	0/4	0/4	1/4	0/4
10	4-Iodophenyl	2/4	4/4	1/4	1/4

*The animals were examined at 0.25, 0.5, 1.0 and 2.0 hr.
*Values indicate the number of animals showing protection.

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