Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [12]: 51-58
[http://scholarsresearchlibrary.com/archive.html]

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Microwave Assisted Synthesis of 2 Amino-4, 5diphenyl-1-Substituted-1 H-Pyrrole-3-Carbonitrile for Anti-Inflammatory and Antifungal Activity

Vyankatesh D*, Swapnali T, Rahul A, Shrinivas M and Chandrakant M

Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon, Tal, Walwa, Dist. Sangli Maharashtra, India

***Corresponding author:** Vyankatesh RD, Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon, Maharashtra, India. Tel: +91 7058563964; E-mail:* vyankat87@gmail.com

---

ABSTRACT

Heterocyclic molecules represent the most utilized scaffolds for the discovery of novel synthetic drugs. As reported in recent communications, the pyrrole moiety can be found both in natural and synthetic pharmaceutical products. Pyrroles have been reported to play an important role as antibacterial, antiviral and anti-inflammatory agent. An efficient synthesis by Microwave assisted process of pyrrole derivatives by the Paal-Knorr Condensation of benzoin with primary aromatic amines in refluxing ethanol resulted in the formation of α-amino ketone intermediates, which were condensed without isolation, with Malononitrile. Various derivatives such as 2-amino-4, 5-diphenylpyrrole-3-carbonitriles (1a-1d) were prepared. The synthesis of pyrrole derivatives are formed by direct reaction of a heterocyclic compound with substituted anilines using microwave irradiation. This optimized method was used for the synthesis of a series of four substituents of pyrrole derivatives. In-vitro anti-inflammatory activity of synthesized compounds was analyzed by using spectroscopic quantification by protein denaturation method. The synthesized compounds were confirmed through spectral characterization using IR and polymorphism study. The synthesized pyrrole derivatives show promising in vitro anti-inflammatory and antifungal activity.

Keywords: Microwave irradiation, Pyrrole derivative, Anti-inflammatory activity, Antifungal activity.

INTRODUCTION

Heterocyclic chemistry is currently experiencing a renaissance because of the interest in heterocyclic scaffolds as templates for combinatorial chemistry. As heteroaromatic compounds are present in many natural products and are the constituents of numerous therapeutic agents, they represent ideal drug like structures for the elaboration of an increase in molecular diversity. Thus, the availability of simple synthetic procedures that enable the preparation of different heterocycles with functional stable groups as substituents is an important task for organic and medicinal chemists. The Paal–Knorr cyclo condensation of benzoin with aniline and other nitrogen derivatives is a well-established and valuable tool for the preparation of Pyrroles and related heterocycles [1]. Pyrrole is most significant heterocyclic structure scaffold present in lager number of biologically active molecules with wide range of application in medicinal chemistry. Besides its pharmacological activity, pyrrole derivative is play crucial role in material sciences. The traditional method relies on the cyclization of amines with ketones or diketones discovered by Knorr and Paal in 1880s [2]. Pyrrole derivatives are considerable attention of synthetic importance and extensively used in drug discovery and pharmacological activity such as anti-inflammatory activity [3]. Literature reviews prove the synthetic utility of MORE chemistry in routine organic synthesis. It can be termed as 'e-chemistry' because it is easy, effective, economical and eco-friendly and is believed to be a step towards green chemistry. Under the framework of "Green Chemistry" we were interested in developing a rapid, microwave-assisted protocol for the synthesis of 2 Amino-4, 5-diphenyl-1-Substituted-1-H-Pyrrole-3-Carbonitrile. In continuation to our earlier work on synthesis of bioactive heterocycles, we report a simple microwave protocol for synthesis of 2 Amino-4, 5-diphenyl-1-Substituted-1-H-Pyrrole-3-Carbonitrile.form Benzoin and substituted aniline [4].

EXPERIMENTAL PROCEDURE

Synthesis of 2-amino-4, 5-diphenyl-1-substituted -1H-pyrrole-3 carbonitrile [1a-d]

A mixture of benzoin (2.12 g, 0.01 mol), the amine like [a] p- nitroacetanilied(1.72 g, 1.04 mol), [b] N,N-Dimethylaniline (1.21 g,1 mol) and [c] o- Anisidine (1.38 g, 0.9 mol),[d] Aniline(1.72 g 0.01 mol), and conc. HCl (6–8 drops) in ethanol (40 ml) was fired in a microwave at 240 W for 25 min. Then cooled and Malononitrile (1.66 mg, 0.01 mol) was added, followed by a catalytic amount (1.5 ml) of pyridine portion wise and again refluxed as above, till solid formed. The solid residue was recrystallized from methanol to give compounds 2-amino-4, 5-diphenyl-1-substituted -1H-pyrrole-3 carbonitrile [1a-1d]. Melting point and % yields were recorded.

Synthesis of 2-amino-N-(4-nitrophenyl) acetamide-4, 5-diphenyl-1H- pyrrole-3-carbonitrile (1a) IR (cm^{-1})

3356(NH Str), 3057(Aro C-H), 2350(Aryl nitro), 1710(C=O), 1681(C=N), 1446(C-N). 1H NMR (300 MHz, DMSO-d6): 7.32 (m, 4H, ArH), 7.48 (m, 4H, ArH) 7.22(s, 2H, ArH), 7.1(s, 2H, ArH), 6.6 (s, 2H, ArH), 4.0 (s 1H, NH_2), 2.85 (d, 6H, CH_3). MS (70 eV): m/ z ¼ 437.46 [Mþ]. Anal. Calcd for $C_{25}H_{19}N_5O_3$: C, 68.41 H, 4.44; N, 15.89. Found: C, 67.91; H, 7.44; N, 18.89.

Synthesis of 2-amino-1-(1,1-dimethyl-1-pyridine-4-yl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile IR (cm^{-1})

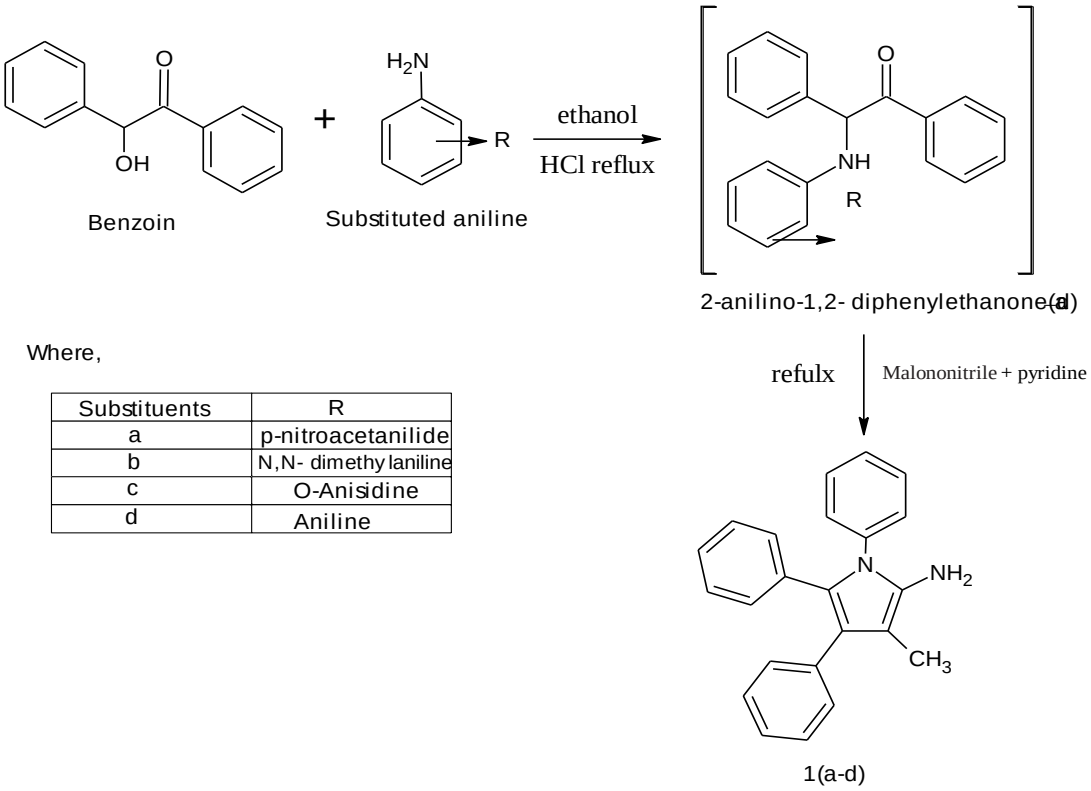
3356(NH Str), 3022(Aro C-H), 2350(Aryl nitro), 1775(C=O), 1680(C=N), 1450(C-N), 1H NMR (300 MHz, DMSO-d6): 7.32 (m, 4H, ArH), 7.48 (m, 4H, ArH) 7.22(s, 2H, ArH), 7.1(s, 2H, ArH), 6.6 (s, 2H, ArH), 4.0 (s 1H, NH_2), 2.85 (d, 6H, CH_3). MS (70 eV): m/ z ¼ 366.47 [Mþ_] Anal. Calcd for $C_{24}H_{22}N_4$: C, 73.47; H, 6.87; N, 13.36. Found: C, 13.36; H, 7.44; N, 18.89.

Synthesis of 2-amino-1-(4-amino-3-methoxyphenyl)-4, 5-diphenyl-1H-pyrrole-3-carbonitrile IR (cm^{-1})

3310(NH Str), 3030(Aro C-H), 2215(Aryl nitro), 1650(C=O), 1680(C=N), 1434(C-N), 1H NMR (300 MHz, DMSO-d6): 7.32 (m, 4H, ArH), 7.48 (m, 4H, ArH) 7.22(s, 2H, ArH), 7.1(s, 2H, ArH), 6.5-6.6 (s, 2H, ArH), 4.0 (s 2H, NH_2), 3.73 (s, 3H, CH_3). MS (70 eV): m/ z ¼ 380.45 [Mþ_]. Anal. Calcd for $C_{24}H_{20}N_4O$: C, 73.07; H, 5.81; N, 13.69. Found: C, 67.91; H, 7.44; N, 18.89.

Synthesis of 2-amino-1-(3-aminophenyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile IR (cm^{-1})

3418(NH Str), 3030(Aro C-H), 2350(Aryl nitro), 1710(C=O), 1678(C=N), 1449(C-N), 1H NMR (300 MHz, DMSO-d6): 7.32 (m, 4H, ArH), 7.48 (m, 4H, ArH) 7.22(s, 2H, ArH), 7.1(s, 2H, ArH), 6.5-7.0 (s, 4H, ArH), 4.0 (s 1H, NH_3). MS (70 eV): m/ z ¼ 350.42 [Mþ_]. Anal. Calcd for $C_{23}H_{18}N_4$: C, 72.99; H, 6.22; N, 13.73. Found: C, 67.91; H, 7.44; N, 18.89.



Where,

Substituents	R
a	p-nitroacetanilide
b	N,N- dimethy laniline
c	O-Anisidine
d	Aniline

Figure 1: Synthesis of 2-amino-1-(3-aminophenyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile

Table 1: Properties of Synthesized Compound. [1a -1d]

Name	Molecular formula	Rf value	% yield	Melting Point °C
1a	$C_{25}H_{19}N_5O_3$	0.7	77.21	142-144 °C
1b	$C_{24}H_{22}N_4$	0.75	63.26	116-120°C
1c	$C_{24}H_{20}N_4O$	0.95	79.75	100-102°C
1d	$C_{23}H_{18}N_4$	0.73	82.63	140-142°C

Biological evaluation

***In vitro* anti-inflammatory activity**

- Control DMSO
- Standard Diclofenac sodium (50 µg/ml)
- Test compound 50 µg/ml, 100 µg/ml.
- Instrument used UV spectrophotometer.

Evaluation of in vitro anti-inflammatory activity by protein denaturation

The mixture (10 ml) consisted of 0.4 ml of egg albumin (from fresh hen's egg), 5.6 ml of phosphate buffered solution (PBS, pH 6.4) and 4 ml of varying concentration of test samples so that final concentration become 50 µg/ml,100 µg/ml. Similar volume of DMSO served as control. Then the mixtures were incubated at (37°C ± 2) for 15 min. and then heated at 70°C for 5min. After cooling, their absorbance was measured at 660 nm (JASCO UV Spectrophotometer) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of 50 µg/ml, 100 µg/ml were used as reference drug and treated similarly for determination of absorbance and viscosity. The % inhibition of protein denaturation was calculated by using the following formula [5,6].

$$\%\ inhibition\ protein\ denaturation: \frac{Absorbance\ of\ \ control\ \ -\ \ Absorbance\ of\ \ test}{Absorbanceof\ \ control} \times 100$$

Antifungal activity

The antifungal activity was performed *in vitro* by agar well diffusion method against *C. albicans* and *A. Niger* using Fluconazole as standard. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 ml) of inoculum was introduced to molten PDA and poured into a petri dish. After solidification, the appropriate wells were made on agar plate by using corn borer (size 6.0 mm). Plates were incubated for 24–48 h at 28°C. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions [7].

Results and Discussion

Experimental methods

1. Synthesis of 2-amino-N-(4-nitrophenyl) acetamide-4, 5-diphenyl-1*H*- pyrrole-3-carbonitrile (1a)
2. Synthesis of 2-amino-1-(1,1-dimethyl-1-pyridine-4-yl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (1b)
3. Synthesis of 2-amino-1-(4-amino-3-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile (1c)

4. Synthesis of 2-amino-1-(3-aminophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile (1d)

The step 1 reaction afforded the yield of product 1(a-d) in the range of 63-82% and time taken by this method is 30 min-1hr. IR, MASS, NMR Spectra studies the structural elucidation of the synthesized compounds was done by the interpretation of IR, MASS and NMR spectra's. All the compounds show satisfactory IR, MASS and NMR.

Pharmacological studies

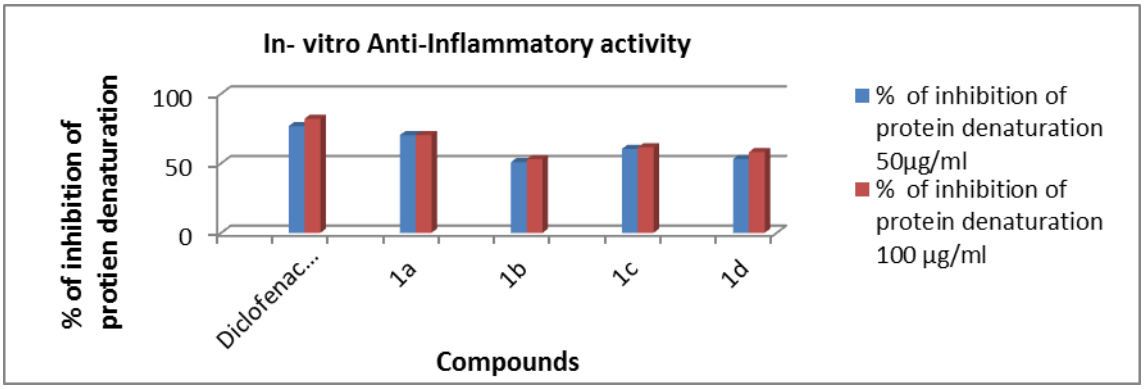


Figure 1: The anti-inflammatory activities of synthesized compounds 1(a-d)

Table 2: The anti-inflammatory activities of synthesized compounds 1(a-d)

Compounds	% of inhibition of protein denaturation		Viscosity (cps)	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Diclofenac sodium	76.7	82.01	0.69	0.72
1a	70.12	70.25	0.72	0.75
1b	50.79	52.95	0.65	0.68
1c	60.29	61.36	0.85	0.89
1d	53.06	58	0.71	0.73
Note: Viscosity of control = 1.42 cps				

Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Production of auto antigens in certain arthritic disease may be due to denaturation of proteins *in vivo*. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. It has been reported that one of the features of several non-

steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH [8].

This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation. In the present study, the relatively high viscosity of control dispersion substantiated this fact. Ability of pyrrole derivatives to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity [9].

Table 3: Antifungal activity of synthesized compounds.

Compounds	**Minimum inhibitory concentration (MIC) (µg/ml)**	
	C. albicans	***A. niger***
1a	9	65.1
1b	65.1	1
1c	15	1
1d	1	1
Fluconazole	16	8

The results are reported in Table 3. The antifungal activity showed that MIC of some of the compounds like 1b, 1c and 1d less than that of the standard, underlining their potential against these fungi.

CONCLUSION

Substituted pyrrole derivatives were synthesized and to screen the synthesized compounds for *in vitro* anti-inflammatory activity. The compounds planned for synthesis were prepared under available laboratory conditions and purity of the compounds were checked by melting point and Rf value and structural confirmation is done by IR and 1H NMR data. Amongst all synthesized compounds 1a, and 1c exhibited significant *in vitro* anti-inflammatory activity with percent protection of for 50 µg/ml is 70.12 and 60.29 and for 100 µg/ml is 82.01 and 61.36 respectively as compared to standard and the standard drug diclofenac sodium exhibited MIC for 50 µg/ml value is 76.70 µg/ml and 80 µg/ml value is 82.01%. Amongst all synthesized compounds exhibited good anti-inflammatory activity with MIC as compared to standard.

REFERENCES

1. Giacomo, M., Bond dissociation enthalpies of polyphenols: The importance of cooperative effects. *Eur. J. Org. Chem*., **2005.** 24:5277-5288.
2. Rosario, AV., et al. Synthesis and anti-inflammatory evaluation of novel benzimidazole and imidazopyridine derivatives, *Med. chem. Let*, **2012.** 2(24): 2-6.
3. Khulpe, PB., and Mohite, SK., Synthesis and characterization of 5, 6 diphenyl pyrrole derivatives and it's pharmacological profile, *J. Cur. Pharma*. **2014.** 4(3): 1181-1185.
4. Malhotra, P., Pattan, S., and Nikalje, AP., Microwave assisted synthesis and antiinflammatory activity of 3, 5-diaryl substituted-2-pyrazolines, *Inter. J. Pharma. Pharma. Sci*, **2010.** 2(2): 21-26.
5. Banerjee, M., Immunohistochemical evaluation of p63, E-cadherin, collagen I and III expression in lower limb wound healing under honey. *J. chem*., 2011, 4, 2, 413-417.
6. Pawar PY, Trivedi VV, *Cur. Pharma. Res*, **2014.** 4(2): 1117-1123.
7. Gavarkar PS, Somani RR, Synthesis of novel azole heterocycles with their anti-tubercular and antifungal evaluation. *int. j. chem. sci*, **2015.**13(1): 432-440.
8. Idhayadhulla A, Synthesis and antimicrobial activity of some new pyrrole derivatives, *Bull. Chem. Soc. Ethiop*, **2012.** 26(3): 429-435.
9. Maddila, S., et al. *J. Saudi. Chem. Soci*., **2012.** 20(1): S306-S312.