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Research Article

Synthesis and In-silico study of 5-oxo-1, 2-diphenyl-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate

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Abstract
In the current study, the pyrrole-3-carboxylate **(4)** derivative was synthesized and evaluated *in-silico* for potential biological activity. The synthesis was done in several steps. It started with a 1,3-dicarbonyl compound, aniline and aldehyde reacting in mild conditions. The key cyclization step formed the pyrrole core through a condensation reaction, followed by esterification to produce the desired compound **(4)**. The compound was characterized using various spectroscopic techniques, including FTIR, ESMS, ^{1}H NMR, and ^{13}C NMR. The results suggested that the synthesized compound **(4)** has potential drug-like properties, based on molecular descriptors and *in-silico* studies using ADMET profiling.

Keywords: ADMET, Synthesis, Characterization and Drug-likeness etc.

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Introduction

The World Health Organization (WHO) states that antimicrobial resistance is a major global public health concern and one of the greatest threats to humankind. It contributes significantly to mortality and chronic infections. [1]. A class of heterocyclic organic compounds known as pyrazole derivatives has a five-membered ring structure and two nitrogen atoms that are adjacent to one another. Pyrazoline, also known as pyrazol-2-one, is one such derivative. [2]. Although most pyrazol-2-one derivatives are synthetic, some naturally occurring pyrazole-related structures have been found. [3]. Although pure derivatives of pyrazol-2-one is not common in nature, there are natural sources that either have structures resembling pyrazoles or, *via* metabolic processes, yield compounds with similar biological activity. Many plants produce alkaloids with ring systems related to pyrazoles. [4]. These substances can have pharmacological properties resembling those of synthetic pyrazol-2-one derivatives and are biosynthesized from amino acids like tryptophan. The plant *Anadenanthera peregrine* is recognized for producing alkaloids that resemble pyrazoles. [5]. Family Cactaceae, it has been reported that some cactus species produce alkaloids that contain pyrazoles. [6]. The development of the pyrazole ring with a carbonyl group at the 2-position is necessary for the synthesis of pyrazol-2-one derivatives. [7]. Different synthetic strategies can be used, based on the desired pyrazole ring substitution pattern. Because of their wide range of biological activities, pyrazol-2-one derivatives are desirable subjects for pharmaceutical research. [8]. These derivatives have been studied for various therapeutic applications such as analgesic, anti-inflammatory, antimicrobial, anticancer, antidiabetic, anti-coagulant, cardio-protective and antioxidant effects [9-16]. Many pyrazol-2-one derivatives have been developed and brought to market as medications, mostly for their antipyretic, analgesic, and other medicinal properties. Several well-known pyrazol-2-one derivatives that have been or are being used as pharmaceutical drugs include one non-steroidal anti-inflammatory medication (NSAID) that reduces inflammation and pain is phenylbutazone **(1).** It does this by blocking the actions of cyclooxygenase (COX-1 and COX-2). [17]. Metamizole **(2)** modifies the action of endogenous opioid peptides and inhibits prostaglandin synthesis. Moreover, it has potent antispasmodic properties. [18]. Pain and fever are decreased by phenazone **(3)** antipyrine, which works by preventing prostaglandin production. [19] **Figure 1**.

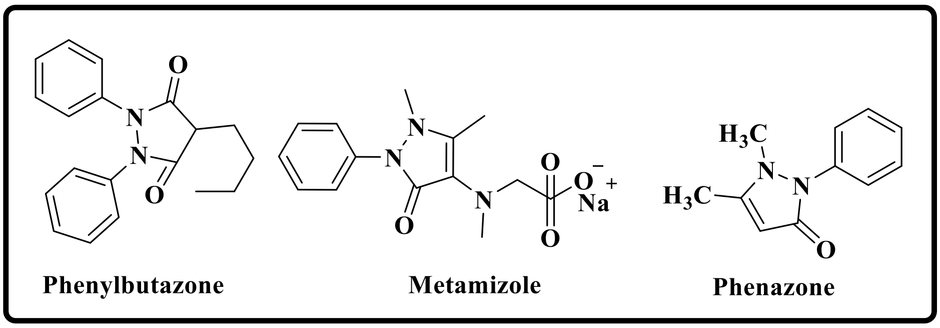


Figure 1. Some marketed drugs of pyrazol-2-one derivatives

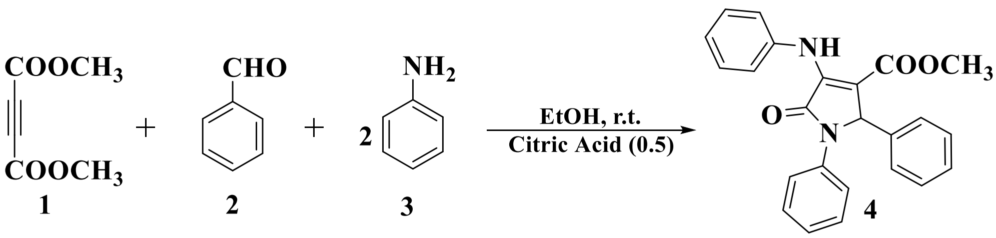
EXPERIMENTAL

Material and methods

All solvents and chemical reagents were purchased from Merck and did not further purify them during use. Fourier transform infrared spectroscopy (FTIR) analysis using a PerkinElmer Nicolet 6700 FTIR spectrometer. Bruker Avance 400 MHz UltrashieldTM spectrometer used for ^{1}H and ^{13}C NMR and dissolved the sample in DMSO-d_6. The ESI-MS spectra using ThermoElectron Corporation's ion trap LCQ Advantage Max mass spectrometry. The Stuart SMP10 melting point apparatus to ascertain the melting points of the synthesised compounds.

General procedure for the synthesis of 5-oxo-1,2-diphenyl-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate (4)

The synthesis of pyrrol-2-one derivatives, a model reaction involving DMAD (2 mmol), aniline (4 mmol) and aldehyde (2 mmol) was treated with citric acid (0.5 mL) in 1.5 mL EtOH at room temperature. The formation of pyrroline-2-one derivative obtained after 5 h in 73% yield was confirmed by comparing its melting point and spectroscopic data with the data of the same compound reported in the literature [20] (Scheme 1).



Scheme 1. Scheme for the synthesis of pyrrole-3-carboxylate

Characterization

methyl 5-oxo-1,2-diphenyl-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate (4): white solid, mp: 178 – 180ºC; IR (KBr) ν/cm^{-1} 3305, 3202, 3019, 2947, 2862, 1701, 1664, 1587, 1509, 1464, 1428, 1299, 1265, 1169, 1152, 1122, 858, 811, 732, 601; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.35 (s, 1H), 7.71 – 7.28 (m, 15H), 5.84 (s, 1H), 3.96 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.46, 163.78, 141.93, 136.98, 136.23, 131.12, 130.34, 129.03, 128.74, 128.64, 128.41, 127.33, 124.24, 123.49, 110.30, 63.01, 51.36 ppm; MS (ESI) m/z 385.5 (M+H$^+$, 100); Anal. Calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29.

The optimization of reaction conditions for the synthesis of pyrrol-2-one **(4)** was thoroughly investigated using various solvents and catalysts. Initially, DMSO with pTSA yielded only trace amounts of the product. Switching to methanol with sulfamic acid improved the yield to 69%. Further improvement was observed with methanol and citric acid, reaching a 70% yield. Ethanol with citric acid also provided a comparable yield of 73%. The combination of ethanol and water with citric acid produced a slightly lower yield of 68%, while the use of salicylic acid in the same solvent system resulted in a yield of 64%. Finally, ethanol and water with $N(Et)_3$ as the catalyst yielded 31% of the desired product. Therefore, the optimal conditions were determined to be methanol or ethanol with citric acid, providing the highest yields of pyrrol-2-one **(4)** (**Table 1**).

Table 1. Optimization of reaction conditions for the synthesis of pyrrol-2-one **(4)** **

Entry	Solvent	Catalyst	Amount (mL)	Time (h)	Yield%*
1	DMSO	pTSA	0.5	10	Trace
2	MeOH	Sulfamic acid	0.5	10	69
3	MeOH	Citric acid	0.5	05	70
4	**EtOH**	**Citric acid**	**0.5**	**05**	**73****
5	$EtOH/H_2O$	Citric acid	0.5	05	68
6	$EtOH/H_2O$	salicylic acid	10	12	64
7	$EtOH/H_2O$	$N(Et)_3$	10	12	31

*Isolated yields. **Optimized reaction condition for the synthesis of pyrrol-2-one. Reaction was done using ethanol (1.5 mL) as solvent with DMAD (2 mmol), aniline (4 mmol) and aldehyde (2 mmol) was treated and Citric acid at room temperature.

Molecular descriptors (MDs) analysis

The molecular physicochemical properties of the synthesized compound **(4)** calculated in this study using the Molinspiration Property Engine v2022.08. The formula % ABS = 109 − (0.345 × TPSA) was utilized to compute the percentage of absorption (% ABS). The following were determined: number of rotatable bonds (RB), molecular weight (MW), partition coefficient (log P), topological polar surface area (TPSA), hydrogen bond donors (OHNH), and acceptor sites (ON) of hydrogen bonds. To evaluate the drug-likeness of chemical scaffold, researchers have recently introduced several of MD's rules; Lipinski's rule of five (Ro5) has received the greatest level of acceptance. According to this rule, drug development and design (DDD) greatly reduces the likelihood of misleading results. [21].

ADMET analysis

The ADME SAR tool was employed in this study to assess the synthesized compounds' ADMET properties. The datasets that are accessible for structure-based searches targeted at finding ADMET properties are regularly updated by this server.

RESULTS AND DISCUSSION

Chemistry

The synthesis of pyrrole-3-carboxylate **(4)** reacting the 1,3-dicarbonyl compound, aniline and aldehyde in the room temperature. The synthesized compound was characterized using FTIR, ESMS, ^{1}H NMR, and ^{13}C NMR (**Figure 2-3**).

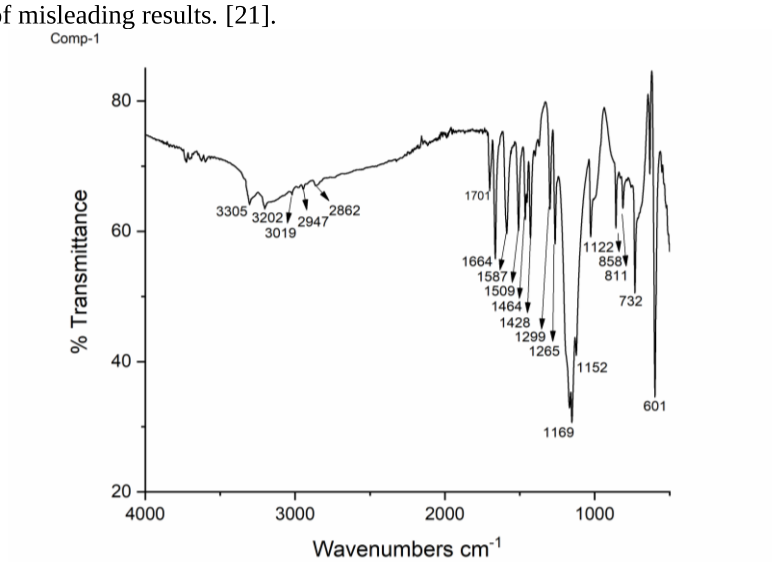


Figure 2. FTIR spectrum of pyrrole-3-carboxylate (7)

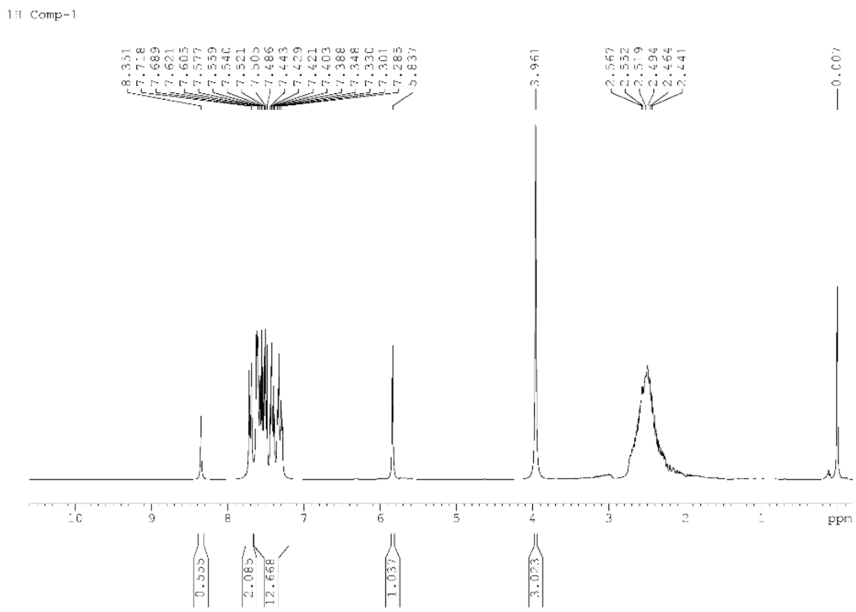


Figure 3. ^1^H NMR spectrum of pyrrole-3-carboxylate **(7)**

***In silico* drug likeness and ADMET study**

A poor pharmacokinetic profile is the reason behind many clinical trial failures. Therefore, the importance of absorption, distribution, metabolism, and excretion (ADME) research for the development of new drugs cannot be overstated [22]. The funding agencies bear a greater financial burden and waste uncompensated time when a hit molecule fails during late-stage drug development. As a result, the *in silico* ADME approach to drug discovery, which screens out unsuitable candidates early on, proves to be a time- and money-efficient one. To achieve this, an *in-silico* analysis of the synthesis of pyrrole-3-carboxylate **(4)** was conducted using the Molinspiration web tool. Most of the compounds exhibited good cellular absorption as indicated by their topological polar surface area (TPSA) of 58.64 Å^2. Additionally, their n-ROTB of ≥3 indicated good molecular flexibility, and their molecular volume (MV) ranged from 384.44 to 384.44, indicating less steric hindrance and increased cellular transportation. Finally, their logP values, a measure of molecular hydrophobicity, were found to be less than 5, which indicated good absorption, bioavailability, and hydrophobic drug-receptor interactions. Aside from this, most molecules adhered to Lipinski's rule. As a result, the compound displayed a good absorption profile that could be interpreted based on the determined ADME parameters. (**Table 2-3**).

Table 2. Bioavailability and drug-likeness of the synthesized **(4)** compound.

Drug-likeness Violations	
Filtering Rules	**Number of violations**
miLogP	4.71
TPSA	58.64
natoms	29
MW	384.44
nON	5
nOHNH	1
nviolations	0
nrotb	6
volume	348.96
ABS%	88.76

Table 3. Shows the relative ADMET profiles of the synthesized **(4)** compound.

Parameters	**Probability**
Absorption	
Human intestinal absorption (HIA+)	0.9954
Bloo-Brain Barrier (BBB+)	0.6639
Caco-2 permeability (Caco2+)	0.5894
Renal Organic Cation Transporter	0.8807
P glycoprotein substrate	0.5897

P-glycoprotein inhibitor	0.6141
Distribution	
Subcellular localization (Mitochondria)	0.6858
Metabolism	
CYP450 2C9 Substrate (Non-substrate)	0.3329
CYP450 2D6 Substrate (Non-substrate)	0.8553
CYP450 3A4 Substrate (Non-substrate)	0.5479
CYP450 1A2 Inhibitor (Inhibitor)	0.5293
CYP450 2C9 Inhibitor (Inhibitor)	0.6203
CYP450 2D6 Inhibitor (Non-inhibitor)	0.8478
CYP450 2C19 Inhibitor (Inhibitor)	0.7907
CYP450 3A4 Inhibitor (Non-inhibitor)	0.6131
CYP Inhibitory Promiscuity (High CYP Inhibitory Promiscuity)	0.7807
Human Ether-a-go-go-Related Gene Inhibition (weak inhibitor)	0.9798
Biodegradation (not ready biodegradable)	0.9666
Toxicity	
AMES Toxicity (non-AMES toxic)	0.5777
Carcinogens (non-carcinogens)	0.8554

CONCLUSION

In conclusion, the pyrrole-3-carboxylate **(4)** that was synthesized exhibits potential as a lead compound for additional biological assessment and medication development, especially for treatments that target inflammation and neuroprotection. Compound **(4)** was being evaluated *in-silico* using the admetSAR tool. Its drug-likeness and ADMET attributes demonstrated that it has positive ADMET traits.

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Conflict of interest

The authors declare no competing interests.

Author contributions

IA and *NA* planned and supervised the experiments. While *BS* synthesized and characterized the compounds and prepared the initial draft of the manuscript. *IA, CSY, AA, VKV, BS* and *ARK* were contributed for writing, reviewing, and conducting the *in-silico* study. All authors discussed the results and contributed to the final manuscript.

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