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Original article

Synthesis and evaluation of analgesic, behavioral effects and chronic toxicity of the new 3,5-diaminopyrazole and its precursor the thiocyanoacetamide

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ABSTRACT

This study aimed to explore the analgesic, antioxidant, behavioral and toxicological effects of 3,5-diaminopyrazole and thiocyanoacetamide. Caffeine was used as reference drug whose effects are known after oral treatment with an efficient dose (10 mg/kg/day) for 30 days. The preliminary bioassays indicated that both compounds at this dose have strong antioxidant capacities and present highly analgesic effects. The behavioral study showed an activation of the rat memory by thiocyanoacetamide. This molecule caused a phobia state to open areas in the elevated plus maze and specifically agoraphobia in the open field with a lack in the development of the exploratory capacity. 3,5-Diaminopyrazole caused memory troubles in rats that forgot the pathway to the exit from the maze, and induced an anxiety state revealed by immobility in closed arms of the elevated plus maze. All these observations were compared to the treatment by the known analgesic, caffeine, which increased the state of vigilance of the rats and developed their exploratory capacity. The chronic treatment with the investigated compounds showed no sign of toxicity with the absence of effect on the body and organ weights, blood count, kidney and liver function and histology. 3,5-Diaminopyrazole and thiocyanoacetamide have potent antioxidant and analgesic activities that are higher than caffeine with a safety profile. The chronic treatment by thiocyanoacetamide activated the memory and caused an emotional state of agoraphobia, but 3,5-diaminopyrazole caused a memory impairment and an emotional state of anxiety. Thus, the present study warrants further investigations involving these novel molecules for a possible development of new strong analgesic and antioxidant drugs which have an effect on the memory capacity.

1. Introduction

Pyrazoles consist of a doubly unsaturated 5-membered ring containing two nitrogen atoms (positions 1 and 2 of the ring) [1]. Since the introduction of antipyrine, great attention has been focused on pyrazole derivatives as potent anti-inflammatory, analgesic and antipyretic agents [2–4]. The synthesis of pyrazole derivatives remains of a great interest owing to their wide applications in agrochemical and pharmaceutical industries due to their herbicidal, insecticidal, analgesic, antipyretic, anti-inflammatory, anticancer, antituberculosis, antihypertensive, antifungal, antidepressant and antimicrobial properties [5–10].

Thioamide is a functional group with the general structure R-CS-NR'R" (which can be R=C_6H_5, R'=CN and R"=NH— C_6H_5, NH—C— C_6H_{11} or NH—PCl—C_6H_5) [1]. It includes the major drugs for the treatment of thyrotoxicosis and hyperthyroidism by inhibiting of the enzyme thyroid peroxidase in the thyroid [11]. Thioamide derivatives, such as thiocyanoacetamide, were used in the chemical synthesis of 3,5-diaminopyrazole [1]. The biological and behavioral effects of these new derivatives have not yet been studied.

Caffeine is the world most widely consumed psychoactive drug. It is an adenosine receptor antagonist known to cause anxiety-like

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behaviors by increasing the plasma rennin activity as well as elevating the plasma catecholamine levels and it develops a vigilance state [12]. Caffeine can have both positive and negative health effects and used to prevent apnoea of prematurity or pain and can cause insomnia or sleep disruption [13].

In medicinal chemistry since the discovery of pyrazole, several chemical studies based on multiple synthetic procedures have been carried and led to much interest owing to its diverse therapeutic applications. Pyrazole derivative drugs are still used clinically in the treatment of depression. Because of their side effects, the medicinal chemists have focused on the discovery of new antidepressant agents with enhanced pharmacological activity and limited toxicity via the structural modification of the hydrazine group [14,15]. This work is investigating the analgesic, antioxidant, behavioral and toxicological effects of two newly synthesized molecules, 3,5-diaminopyrazole and its chemical precursor, thiocyanoacetamide, and compares them to caffeine whose effects are already known.

2. Material and methods

2.1. Animals

Six-week-old healthy male Wistar rats were housed by pairs in cages (25 × 50 cm) and maintained at 23 °C, 12/12-h light/dark cycle under specific pathogen-free conditions. The rats were allowed to acclimatize in the experimental medicine unit for a period of one week before the beginning of the study. During the experiment period, they received a commercial pellet diet (Industrial Society of Food, Sfax, Tunisia) and water *ad libitum*. Rats weighing 200 g were used for the experiments. All experimental procedures were approved by the Ethics Committee of the School of Medicine of Tunis according to the standards of the International Council for Laboratory Animal Science (ICLAS).

2.2. Synthesis of thiocyanoacetamide (2-cyano-2-p-nitrophenyl-N-benzylthioamide)

Under a nitrogen atmosphere 5.6 g-% of *t*-BuOK dissolved in tetrahydrofuran was mixed dropwise with 11.7 g-% of *p*-nitrobenzylcyanide (purchased from Sigma –Aldrich) dissolved in tetrahydrofuran. The mixture was stirred for 40 min and then, 11.8 g-% of benzyl-isothiocyanat in tetrahydrofuran was slowly added dropwise. The mixture was stirred for 3 h and then hydrolyzed with an aqueous solution of HCl. The mixture was then extracted twice with 30 mL of chloroform and dried *in vacuo*. The purification was done by a recrystallization using ether (Fig. 1).

The purity of the formed product (black crystals) was checked by the melting point at 124 °C and by the High-Performance Liquid Chromatography (HPLC) in the following conditions: Column RP-18, the wave length UV detector set at 250 nm, mobile phase (water/acetonitril) was pumped at a flow rate of 2 mL/min in a gradient mode as follows: 100% ultra-pure water for 0.01 min, 75% for 3.1 min, 66% for 9.1 min, 0% for 20 min and 100% for 22 min. The obtained chromatogram showed one peak at 4.5 min corresponding to thiocyanoacetamide; this confirmed the purity of the product and the efficacy of the chemical synthesis method. The molecular structure was identified as follows:

IR ($CHCl_3$, ν cm^{-1}): NH=3311, –SH=2540, CN=2234.

^1H NMR ($CDCl_3$, δ ppm): 3.1 (s, —CH_2), 6.3 (s, —NH) and 8.8–9.9 (m, H_{arom}).

^{13}C NMR ($CDCl_3$, δ ppm): 32 (—H_2C—); 108 (—CN); 123–155 (C_{arom}); 160.87 (S=C—).

GC–MS *m/z*: 310 (M^+), 194 (C_6H_5—H_2C—HN—CH=S), 162 ($O_2NC_6H_5$—CH—CN), 116 (C_6H_5—H_2C—HN—CH), 91 (C_6H_5—H_2C) and 77 (C_6H_5)

2.3. Synthesis of 3,5-diaminopyrazole (N^3-benzyl-4-(4-nitrophenyl)-1H-pyrazole-3,5-diamine)

0.20 g of hydrazine was added to 2 g of thiocyanoacetamide in ethanol (30 mL) and the reaction mixture was stirred at room temperature for 48 h. The solvent was then evaporated from the main reaction mixture. The residue obtained was recrystallized with ether and then the solid filtered off (Fig. 1).

The purity of the product formed (black crystal) was verified by the melting point at 135 °C and by the High- Performance Liquid Chromatography (HPLC) in the same conditions as described before (paragraph 2.2.1). The obtained chromatogram showed one peak at 15 min corresponding to 3,5-diaminopyrazole.

The molecular structure was identified as follows:

IR ($CHCl_3$, ν cm^{-1}): NH=3311, —C=C=1558, C=N=1558.

^1H NMR (DMSO-d_6, $MeOD_4$, δ ppm): 3.5 (m, CH_2); 6.9 (s, H_2N—C=); 6.9–7.9 (m, H_{arom}) and 8.3 (s, —NH).

^{13}C NMR (DMSO-d_6, $MeOD_4$, δ ppm) 39 (CH_2); 112 (—C=C of pyrazolic ring), 120–140 (C_{arom}) and 158 (C=N of pyrazolic ring).

GC–MS *m/z*: 310 (M^+), 281 (C_6H_5—H_2C—HN—C—$C(C_6H_5NO_2)$—C=C—NH_2), 167 ($C_6H_5NO_2$—C=C—NH_2), 91 (C_6H_5—H_2C) and 77 (C_6H_5) and 57 (N—NH—C—NH_2)

2.4. Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was based on the method reported by Blois [16]. 3,5-diaminopyrazole, thiocyanoacetamide or caffeine was

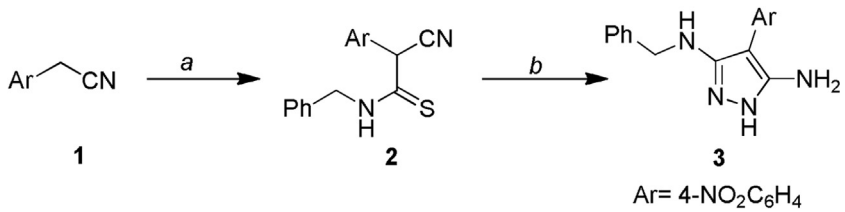

Fig. 1. Synthesis of N^3-benzyl-4-(4-nitrophenyl)-1H-pyrazole-3,5-diamine. Reagents and conditions: (*a*): *t*-BuOK, THF/*p*-Nitrobenzylcyanide, THF/benzylisothiocyanate, THF/H_2O/H^+, (*b*): Thiocyanoacetamide, EtOH/hydrazine hydrate, 48 h/H_2S.