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Novel essential amino acid-sulfanilamide hybrid as safe anti-ulcerogenic agent with anti-*helicobacter pylori* activity

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ABSTRACT

A novel and safe essential amino acid (Leucine) incorporating sulfanilamide was synthesized, and evaluated for its anti-ulcerogenic activity and *in vitro* anti-*Helicobacter pylori* activity. The new molecule showed a dose dependent activity against absolute ethanol-induced ulcer in rats, it produced percent protection of control ulcer by 66.7 at dose 100 mg/kg. In addition it showed a potent anti-*Helicobacter pylori* activity *in vitro* against 7 clinically isolated strains. The minimum inhibitory concentration (MIC) ranged from 12.5 to 50 μg/ml. The preliminary safety studies and toxicity profile are optimistic and encouraging.

1. Introduction

Peptic ulcer (PU) is a major health problem which concerns the medical community all over the world. It is known that the major causative factor of a number of gastric pathologies including gastritis, peptic ulcers and certain gastric cancers is the *Helicobacter pylori* (*H. pylori*), which is well-known Gram −ve bacterial human pathogen that is responsible for type B gastritis of the stomach and can lead to duodenal ulcers and even gastric cancer. This species is typically treated with bismuth salts in combination with antibiotics (Iwahi et al., 1991). Inflammation, injury and infection with *H. pylori* are the main causative factors. In spite of the substantial progress in many aspects of basic and clinical research, no clear, safe remedy is available (Liou et al., 2016; Wang et al., 2015; Newman, 2008; Chimenti et al., 2007; Newman et al., 2003; Pelish et al., 2001; Sorba et al., 2001).

Sulfonamide derivatives showed many biological activities; early and recent researchers have suggested that sulfonamides are useful for the treatment of some staphylococci infections, especially against urinary infections (Blass, 2016; Bartzatt et al., 2010; Altoparlak et al., 2004). It was reported that they showed the highest inhibitory effect on gram positive bacteria, i.e. *Staphylococcus aureus*, *Nocardia asteroides*, *N. farcinia* and *Bacillus subtilis*. However, sulfonamide derivatives were also reported in treatment of Chagas disease, they showed *in vitro* activity against two strains of *Trypanosoma cruzi* (Bocanegra-Garcia et al., 2012; Genç et al., 2008).

Furthermore, sulfonamide derivatives were used as hypoglycemic agent. Sulfonamide derivatives have several clinical applications against inflammatory bowel syndrome and other related ailments in addition to their tendency to accumulate in hypoxic tumors (Ahmadi et al., 2016) (Dubois et al., 2009; Cecchi et al., 2005; Huang et al., 2001).

Sulfa drugs are well known inhibitors of dihydrofolate reductase (Bush et al., 1982). Moreover, several literatures reviews mentioned their ability to selectively inhibit the different carbonic anhydrase isoforms (Supuran, 2012). Recently, some new sulfonamide derivatives with remarkable antitumor activity were prepared in laboratory (IC_{50} 2.5–5.5 μg/mL) (Bourais et al., 2017) (Alafeefy et al., 2013, 2012).

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These findings prompted us to hypothesize that small molecule comprising both sulfanilamide and an essential amino acid would have a beneficial effect in combating many such horrible diseases and at the same time supplying such necessary components. In this regard we synthesized sulfanilamide derivative of leucine and explored its activity against peptic ulcer, *H. pylori* and its effect on liver and kidney functions.

2. Experimental

2.1. Synthesis

2.1.1. 4-Methyl-2-[2-oxo-2-(4-sulfamoylphenylamino) ethylamino] pentanoic acid(**5**)

2-Chloroacetyl chloride (1.12 g, 0.01 mol) was added drop wise with vigorous stirring to a cold suspension of sulfanilamide (1.72 g, 0.01 mol) in 10 ml dichloromethane containing 2 drops triethylamine. Stirring was continued for 1 h and the separated solid was filtered, washed with ether, dried and crystallized from aqueous-ethanol.

Yield, 69%; m.p. 270–272 °C; ^{1}H NMR (DMSO-d_6): δ 0.90 (d, 6H, J = *12.0* Hz, 2CH_3), 1.49 (m, 1H, C*H*), 1.80 (t, 2H, J = *9.0* Hz, CH_2), 2.51 (s, 1H, NH, D_2O exchange.), 3.26 (s, 2H, CH_2), 3.49 (t, 1H, J = *8.5* Hz, C*H*), 4.21 (s, 2H, NH_2, D_2O exchange.), 7.30 (s, 1H, N*H*, D_2O exchange.), 7.60 (d, 2H, J = 7.5 Hz, Ar-*H*), 7.79 (d, 2H, J = 7.67 Hz, Ar-*H*), 10.55 (s, 1H, O*H*, D_2O exchange). ^{13}C NMR: δ 22.6 (2CH_3), 24.3 (*C*H), 41.1 (*C*H_2), 50.2 (*C*H_2), 59.4 (*C*H), 118.6, 126.7, 138.4, 141.4 (Ar-*C*), 169.0, 174.7 (2*C* = O). MS (EI): *m*/*z* 343 [M^+, %]. Anal. ($C_{14}H_{21}N_3O_5S$) C, H, N.

or identification of the compound spectroscopic instruments were used such as; m.p, ^{1}H NMR, ^{13}C NMR Later on the chiral parameters such as the chiral strength, the symmetry of response as the chiral wave vector, optical activity, configuration will be determined.

2.2. Biological activity

2.2.1. Animals

Swiss albino mice of both sex (26–30 g) and male Wistar rats (180–200 g) were purchased from the animal house of King Saud University, KSA. Animals were housed in standard polypropylene cages with wire mesh top and maintained under standard conditions (temperature 23 ± 1.0 °C, humidity 55 ± 10%, 12 h light/12 h dark cycle). They fed with a standard pellet diet with water *ad libitum* and were allowed to adapt to the laboratory environment for one week before experimentation.

2.2.2. Determination of median lethal dose (LD_{50})

The oral median lethal dose (LD_{50}) of the target compound was determined as described by (Lorke, 1983). Swiss albino mice in groups of six, received one of 50, 100, 500, or 1000 mg/kg doses of the target compound. Control animals were received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded.

2.2.3. Antiulcerogenic activity

Evaluation of the anti-ulcerogenic activity was carried out using absolute ethanol-induced ulcer model as described by (Bighettia et al., 2005). Thirty male Wistar rats were divided into 5 groups each of 6 rats. Group 1 received the vehicle and served as control, group 2 received ranitidine (100 mg/kg) and served as standard, groups 3, 4 and 5 received the synthesized compound at doses 25, 50 and 100 mg/kg respectively.

Rats of all groups were fasted for 24 h then all medications were administered orally. One hour after treatment, the animals received an oral dose of absolute ethanol (1 mL/200 g) and then sacrificed one hour later, by ether inhalation, the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water.

Number of lesions in the glandular part of the stomach were measured under an illuminated magnifying microscope (10×). Long lesions were counted and their lengths were measured. Petechial lesions were counted, and then each five petechial lesions were taken as 1 mm of ulcer.

The lesion scores: the mucosal lesions were quantified by the scoring system (0–5) 0 = no damage, 1 = Local edema and inflammation without ulcers; 2 = One ulcer without inflammation; 3 = one to two ulcers with inflammation & lesion diameter <1 cm; 4 = More than two ulcers with lesion diameter 1–2 cm; 5 = Sever ulceration with lesion diameter >2 cm (Morris et al., 1989).

Ulcer index: To calculate the ulcer index (mm), the sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number. The curative ratio was determined according to the formula:

$$\% \text{ Protection of control ulcer} = \text{Control UI} - \text{Test UI} / \text{Control UI} \times 100$$

2.3. Effect on liver and kidney functions

Male Wister rats were divided into 2 equal groups each of 10 rats. The 1st group was left as a control and administrated the vehicle orally, while the 2nd group was orally administrated the synthesized compound in a dose of 100 mg/kg for 15 days. After the examination period, 6 h after the last dose blood samples were collected from the orbital plexus of rats. Samples were left to clot at room temperature for 30 min then centrifuged at 1000 rpm for 20 min.

The collected sera were used for determination of the activity of both (AST) aspirate aminotransferase and (ALT) alanine aminotransferase as liver markers. In addition, levels of blood urea, serum creatinine were also estimated as kidney markers (Awaad et al., 2013).

2.4. In-vitro anti-Helicobacter pylori activity

2.4.1. Bacterial isolates

A total of seven clinical isolates of *H. pylori* were isolated from 19 biopsies received from patients diagnosed with gastritis or peptic ulcer disease at Al-Kasr Al-Ainy hospital, Cairo, Egypt. Clinical isolates were symbolized from KA1 to KA7. Isolates were grown in Brucella agar plates (Difco, Detroit, Michigan, USA) containing 10% v/v sheep serum at 37 °C.

Identification was carried out using Gram stain and catalase, oxidase and urea hydrolysis activities. *Helicobacter pylori* ATCC 43504 was used as control.

2.4.2. Determination of anti-Helicobacter pylori activity

Determination of the amino acid-sulfanilamide hybrid activity against *H. pylori* was carried out using disk diffusion method described by McNulty et al. (2002). The amino acid-sulfanilamide hybrid compound was dissolved in 2% Tween (v/v), in order to obtain final concentration of 2 mg/ml.

Sterile 6 mm disks utilized were imbibed in 1 mL of compound solution and were deposited on the surface of the plate of Mueller–Hinton agar with 10% sheep blood inoculated with *H. pylori*, in a suspension of 6×10^8 CFU/mL (McFarland turbidity standard 2), using amoxicillin (30 μg) and erythromycin (15 μg) as the standard antibiotics. The plate was incubated at 37 °C under microaerophilic conditions in an atmosphere of 5–15% O_2 and 5–10% CO_2 for 48–72 h.

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