



Original article

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

Amani S. Awaad^{a,*}, Ahmed M. Alafeefy^b, Fatmah A.S. Alasmary^c, Reham M. El-Meligy^d, M.E. Zain^e, Saleh I. Alqasoumi^f

^a Pharmacognosy Department, College of Pharmacy, Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia

^b Department of Chemistry, Kulliyah of Science, International Islamic University, Malaysia

^c Chemistry Department, College of Science, King Saud University, Riyadh 11362, Saudi Arabia

^d Aromatic and Medicinal Plants Department, Desert Research Center, Cairo, Egypt

^e Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

^f Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia

ARTICLE INFO

Article history:

Received 27 December 2016

Accepted 24 February 2017

Available online 27 February 2017

Keywords:

Natural products hybrid

In-vitro

Anti-*Helicobacter pylori*

Amino acid

Sulfanilamide

Anti-ulcerogenic agent

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.

Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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

* Corresponding author at: Pharmacognosy Department, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, P.O. Box 173, Riyadh 11942, Saudi Arabia.

E-mail address: amaniawaad@hotmail.com (A.S. Awaad).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

sulfonamide derivatives with remarkable antitumor activity were prepared in laboratory (IC_{50} 2.5–5.5 $\mu\text{g}/\text{mL}$) (Bourais et al., 2017) (Alafeefy et al., 2013, 2012).

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; ^1H NMR (DMSO- d_6): δ 0.90 (d, 6H, $J = 12.0$ Hz, 2CH_3), 1.49 (m, 1H, CH), 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, CH), 4.21 (s, 2H, NH_2 , D_2O exchange.), 7.30 (s, 1H, NH, 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, OH, D_2O exchange). ^{13}C NMR: δ 22.6 (2CH_3), 24.3 (CH), 41.1 (CH_2), 50.2 (CH_2), 59.4 (CH), 118.6, 126.7, 138.4, 141.4 (Ar-C), 169.0, 174.7 ($2\text{C} = \text{O}$). MS (EI): m/z 343 [M^+ , %]. Anal. ($\text{C}_{14}\text{H}_{21}\text{N}_3\text{O}_5\text{S}$) C, H, N.

or identification of the compound spectroscopic instruments were used such as; m.p., ^1H 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 $55 \pm 10\%$, 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 \times). Long lesions were counted and their lengths were measured. Petchial 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:

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

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) aspartate 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% O₂ and 5–10% CO₂ for 48–72 h.

2.4.3. Determination of the minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration (MIC) was carried out by the broth microdilution assay (European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical and Infectious, 2003). A total of 100 µL of BHI broth supplemented with 10% defibrinated sheep blood inoculated with 6×10^8 *H. pylori* (McFarland turbidity standard 2) and 100 µL of serial dilutions of amino acid-sulfanilamide hybrid compound dissolved in 2% Tween (v/v) was added to each well in the microplate, to reach final concentrations of 12.5; 25; 50; 100; 200; 400 and 800 µg/ml. The microplate was incubated at 37 °C under microaerophilic conditions in an atmosphere of 5–15% O₂ and 5–10% CO₂, for 48–72 h. After incubation, the plates were visually examined, the optical density was determined at 450 nm and each well was replicated in blood agar (Mueller–Hinton agar with 5% sheep blood), to determine the MIC.

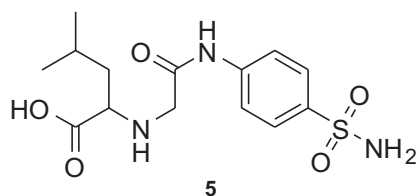


Fig. 1. Chemical structure of 4-Methyl-2-[2-oxo-2-(4-sulfamoylphenylamino)ethylamino] pentanoic acid.

3. Results and discussion

The target compound was synthesized according to a reported procedure (Saeed et al., 2015). Sulfanilamide was reacted with 2-Chloroacetyl Chloride at room temperature in dichloromethane containing triethylamine. The obtained alkyl halide was then refluxed with leucine in 1-butanol containing anhydrous potassium carbonate in the presence of catalytic amount of potassium iodide. The product was obtained in about 50% yield and purified by column chromatography. The structure was confirmed by NMR spectra. The spectrum showed the aliphatic protons at δ of 0.9 ppm, two different protons on two different tertiary carbons δ at 1.49 ppm and 3.49 ppm and the carbons bearing them at 22.6, 24.3 and 59.9 respectively. Two CH₂ groups at δ 1.80, 3.26 and their carbons appeared at 41.1, 50.2, respectively. The SO₂NH₂ protons were found at δ 4.21. Two singlets corresponding to two NH groups were seen at δ 2.51 and 7.30, respectively. One OH appeared at δ 10.55 due to the free carboxyl group of leucine. The two carbonyl groups were seen at δ 169.0 and 174.7. The two dimensional spectrum showed that the obtained structure is in accordance with the proposed one.

The compound was identified as (see Figs. 1 and 2).

3.1. Determination of median lethal dose (LD₅₀)

The target compounds in doses up to 1000 mg/kg did not produce any behavioral changes and mortality in mice. Therefore, it can be categorized as highly safe since substances possessing LD₅₀ higher than 50 mg/kg are nontoxic (Soliman et al., 2012).

3.2. Anti-ulcerogenic activity

The present results showed that the amino acid-sulfanilamide hybrid possessed a potent dose dependent anti-ulcerogenic activity.

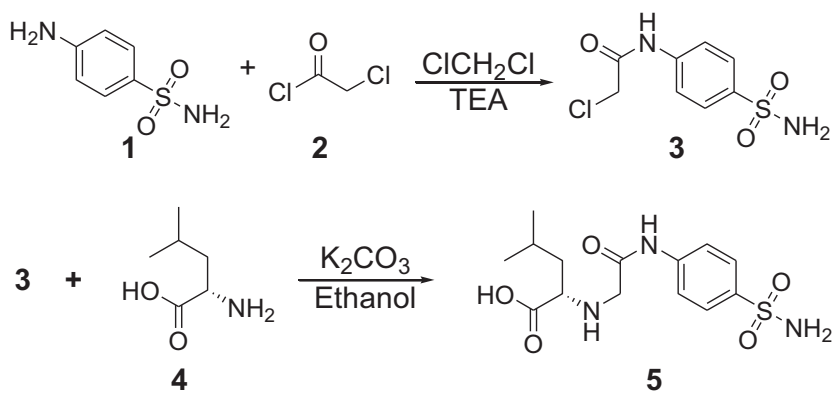


Fig. 2. Scheme for synthesizing compound 5.

Table 1

Anti-ulcerogenic effect of amino acid-sulfanilamide compound on absolute alcohol-induced ulcer in rats.

Groups	Dose mg/kg	Score	No of ulcers	Ulcer index	% Protection
Control	–	3.25	16.40 ± 0.5	10.92 ± 1.74	0
Ranitidine	100	2.2	6.9 ^{***} ± 1.05	6.0 [†] ± 1.24	45.05
Compound 5	25	2.2	11.1 [†] ± 1.79	9.1 [†] ± 1.24	16.7
Compound 5	50	1.6	8 [†] ± 1.58	4.48 [†] ± 1.08	59
Compound 5	100	1.4	6.2 ^{***} ± 1.3	3.64 ^{***} ± 0.47	66.7

Data are expressed as mean ± SD, n = 6.

[†] p ≤ 0.05.

^{**} p ≤ 0.01.

^{***} p ≤ 0.001.

Table 2
Effect of amino acid-sulfanilamide compound on liver and kidney functions of rats.

Groups (100 mg/kg)	ALT (U/l)	AST (U/l)	Blood urea (mg/dl)	Creatinine (mg/dl)
Control	42.49 ± 0.37	60.77 ± 0.37	70.50 ± 1.36	0.88 ± 0.02
Compound 5	38.67 ± 0.22	63.23 ± 0.39	65.50 ± 1.9	0.85 ± 0.02

Data are expressed as mean ± SD, n = 10.

Table 3
Activity of amino acid-sulfanilamide hybrid compound and two antibiotics against clinical isolates of *H. pylori*.

<i>H. pylori</i> strains	Inhibition zone (mm)		
	Compound 5 (2 mg)	Amoxicillin (30 µg)	Erythromycin (15 µg)
KA1	18	22	21
KA2	15	23	22
KA3	16	24	24
KA4	15	23	23
KA5	15	25	21
KA6	17	26	25
KA7	19	27	24
ATCC 43504	16	33	29

Table 4
Minimum inhibitory concentration (MIC) of amino acid-sulfanilamide hybrid compound against clinical isolates of *H. pylori*.

<i>H. pylori</i> strains	Concentration (µg/ml)
KA1	50
KA2	50
KA3	50
KA4	50
KA5	50
KA6	25
KA7	12.5
ATCC 43504	50

ity. It produced percent protection of control ulcer ranged from 16.7% to 66.7%, while the standard drug ranitidine (100 mg/kg) produced 45.05% (Table 1). The target compound (100 mg/kg) was significantly more effective than the standard in reducing ulcer index and ulcer score.

3.3. Effect on liver and kidney functions

Both liver and kidney functions were not affected as there is no significant difference between control and test group in all experiments, at the 0.05 level of probability (Table 2). These results showed that, the compound didn't reveal hepatotoxic manifestation. In addition, no apparent nephrotoxic manifestations were recorded.

3.4. In-vitro anti-*Helicobacter pylori* activity

The activity of amino acid-sulfanilamide hybrid compound against *H. pylori* was determined (Table 3). Seven isolates of *H. pylori* were obtained from 19 gastric biopsies and their susceptibility to two antibiotics and amino acid-Sulfanilamide hybrid compound was determined by disk diffusion (Table 3). All the isolates of *H. pylori* were sensitive to amino acid-Sulfanilamide hybrid compound, amoxicillin and erythromycin.

The results revealed that the highest inhibition zones, 19, 18 and 17 mm, were obtained against *H. pylori* KA7, KA1 and KA6,

respectively. On the other hand, the lowest inhibition zone 15 mm was obtained against *H. pylori* KA2, KA4 and KA5 (Table 3).

The minimum inhibitory concentration (MIC) was determined for the amino acid-sulfanilamide hybrid compound using broth dilution method (Table 4). The results showed that the lowest minimum inhibitory concentrations (12.5 and 25 µg/mg) were obtained against *H. pylori* KA7 and KA6, respectively (Table 4).

The results of the current study confirmed the concept of Chimenti et al., 2007; they mentioned that anti-*H. Pylori* activity strongly depends on the presence of an acyl function like acid, ester or acyl chloride in the 3-benzamidic group and of a halogen in the 6-position of the coumarin ring.

As conclusion compound 5 is a novel compound which showed very promising anti-ulcerogenic and anti *H. pylori* activities with no side effects on liver and kidney functions.

Acknowledgment

This project was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University under the research group number (2016/01/6235).

References

- Ahmadi, A., Khalili, M., Sohrabi, L., Delzende, N., Nahri-Niknafs, B., Ansari, F., 2016. Synthesis and evaluation of the hypoglycemic and hypolipidemic activity of novel sulfonamide-benzothiazole derivatives of benzylidene-2,4-thiazolidinedione. *Mini Rev. Med. Chem.* 29, 29.
- Alafeefy, A.M., Alqasoumi, S.I., Ashour, A.E., Masand, V., Al-Jaber, N.A., Ben Hadda, T., Mohamed, M.A., 2012. Quinazoline-tyrphostin as a new class of antitumor agents, molecular properties prediction, synthesis and biological testing. *Eur. J. Med. Chem.* 53, 133–140.
- Alafeefy, A.M., Isik, S., Abdel-Aziz, H.A., Ashour, A.E., Vullo, D., Al-Jaber, N.A., Supuran, C.T., 2013. Carbonic anhydrase inhibitors: benzenesulfonamides incorporating cyanoacrylamide moieties are low nanomolar/subnanomolar inhibitors of the tumor-associated isoforms Ix and Xii. *Bioorg. Med. Chem.* 21, 1396–1403.
- Altöparlak, U., Kadanali, A., Celebi, S., 2004. Slime factor positivity in coagulase negative staphylococci isolated from nasal samples of haemodialysis patients. *Int. J. Clin. Pract.* 58, 1112–1114.
- Awaad, A.S., Al-Jaber, N.A., Moses, J.E., El-Meligy, R.M., Zain, M.E., 2013. Antiulcerogenic activities of the extracts and isolated flavonoids of *euphorbia cuneata* vahl. *Phytother. Res.* 27, 126–130.
- Bartzatt, R., Cirillo, S.L., Cirillo, J.D., 2010. Sulfonamide agents for treatment of staphylococcus mrsa and mssa infections of the central nervous system. *Cent. Nerv. Syst. Agents Med. Chem.* 10, 84–90.
- Bighettia, A.E.A.N.M.A., Kohna, L.K., Rehdera, V.L.G., Foglioa, M.A., Possentia, A., Vilelaa, L., Carvalho, J.E., 2005. Antiulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from *Mikania laevigata* Schultz Bip. *Phytomedicine* 12, 72–77.
- Blass, B., 2016. Sulfonamide derivatives and pharmaceutical applications thereof. *ACS Med. Chem. Lett.* 7, 12–14.
- Bocanegra-García, V., Villalobos-Rocha, J.C., Nogueira-Torres, B., Lemus-Hernandez, N., Camargo-Ordóñez, A., Rosas-García, N.M., Rivera, G., Rivera, G., 2012. Synthesis and biological evaluation of new sulfonamide derivatives as potential anti-trypansomoma cruzi agents. *Med. Chem.* 8, 1039–1044.
- Bourais, I., Maliki, S., Mohammadi, H., Amine, A., 2017. Investigation of sulfonamides inhibition of carbonic anhydrase enzyme using multiphotometric and electrochemical techniques. *Enzyme Microbial Technol.* 96, 23–29.
- Bush, K., Freudenberger, J.S., Slusarchyk, D.S., Sykes, R.B., Meyers, E., 1982. Activity of sulfa drugs and dihydrofolate reductase inhibitors against candida albicans. *Experientia* 38, 436–437.
- Cecchi, A., Hulikova, A., Pastorek, J., Pastoreková, S., Scozzafava, A., Winun, J.-Y., Montero, J.-L., Supuran, C.T., 2005. Carbonic anhydrase inhibitors. Design of fluorescent sulfonamides as probes of tumor-associated carbonic anhydrase Ix

- that inhibit isozyme Ix-mediated acidification of hypoxic tumors. *J. Med. Chem.* 48, 4834–4841.
- Chimenti, F., Bizzarri, B., Bolasco, A., Secci, D., Chimenti, P., Carradori, S., Granese, A., Rivanera, D., Lilli, D., Zicari, A., Scaltrito, M.M., Sisto, F., 2007. A novel class of selective anti-helicobacter pylori agents 2-oxo-2h-chromene-3-carboxamide derivatives. *Bioorg. Med. Chem. Lett.* 17, 3065–3071.
- Dubois, L., Lieuwes, N.G., Maresca, A., Thiry, A., Supuran, C.T., Scozzafava, A., Wouters, B.G., Lambin, P., 2009. Imaging of Ca Ix with fluorescent labelled sulfonamides distinguishes hypoxic and (Re)-oxygenated cells in a xenograft tumour model. *Radiother. Oncol.* 92, 423–428.
- European Committee For Antimicrobial Susceptibility Testing Of The European Society Of Clinical, M., Infectious, D., 2003. Determination of minimum inhibitory concentrations (Mics) of antibacterial agents by broth dilution. *Clin. Microbiol. Infect.* 9, Ix–Xv.
- Genç, Y., Özkanca, R., Bekdemir, Y., 2008. Antimicrobial activity of some sulfonamide derivatives on clinical isolates of staphylococcus aureus. *Ann. Clin. Microbiol. Antimicrobials* 7, 17–117.
- Huang, Z., Lin, Z., Huang, J., 2001. A novel kind of antitumour drugs using sulfonamide as parent compound. *Eur. J. Med. Chem.* 36, 863–872.
- Iwahi, T., Satoh, H., Nakao, M., Iwasaki, T., Yamazaki, T., Kubo, K., Tamura, T., Imada, A., 1991. Lansoprazole, a novel benzimidazole proton pump inhibitor, and its related. *Antimicrob. Agents Chemother.* 35, 490–496.
- Liou, J.-M., Wu, M.-S., Lin, J.-T., 2016. Treatment of helicobacter pylori infection: where are we now? *J. Gastroenterol. Hepatol.* 31, 1918–1926.
- Lorke, D., 1983. A new approach to practical acute toxicity testing. *Arch. Toxicol.* 54, 275–287.
- McNulty, C., Group, A.T.P.H.W., Owen, R., Tompkins, D., P., Hawtin, Mccoll, K., Price, A., Smith, G., Teare, L., 2002. Helicobacter pylori susceptibility testing by disc diffusion. *J. Antimicrob. Chemother.* 49, 601–609.
- Morris, J.C., Heyman, A., Mohs, R.C., Hughes, J.P., Van Belle, G., Fillenbaum, G., Mellits, E.D., Clark, C., 1989. The consortium to establish a registry for Alzheimer's disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* 39, 1159–1165.
- Newman, D.J., 2008. Natural products as leads to potential drugs: an old process or the new hope for drug discovery? *J. Med. Chem.* 51, 2589–2599.
- Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* 66, 1022–1037.
- Pelish, H.E., Westwood, N.J., Feng, Y., Kirchhausen, T., Shair, M.D., 2001. Use of biomimetic diversity-oriented synthesis to discover galanthamine-like molecules with biological properties beyond those of the natural product. *J. Am. Chem. Soc.* 123, 6740–6741.
- Saeed, A., Mahmood, S.U., Rafiq, M., Ashraf, Z., Jabeen, F., Seo, S.Y., 2015. Iminothiazoline-sulfonamide hybrids as jack bean urease inhibitors; synthesis, kinetic mechanism and computational molecular modeling. *Chem. Biol. Drug Des.* <http://dx.doi.org/10.1111/cbdd.12675>.
- Soliman, G.A., Donia, A.R., Awaad, A.S., Alqasoumi, S.I., Yusufoglu, H., 2012. Effect of *Emex spinosa*, *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* extracts on the reproductive organs of adult male rats. *Pharm. Biol.* 50, 105–112.
- Sorba, G., Bertinaria, M., Di Stilo, A., Gasco, A., Scaltrito, M.M., Brenciaglia, M.I., Dubini, F., 2001. Anti-helicobacter pylori agents endowed with H2-antagonist properties. *Bioorg. Med. Chem. Lett.* 11, 403–406.
- Supuran, C.T., 2012. Structure-based drug discovery of carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem.* 27, 759–772.
- Wang, S., Cameron, S.A., Clinch, K., Evans, G.B., Wu, Z., Schramm, V.L., Tyler, P.C., 2015. New antibiotic candidates against helicobacter pylori. *J. Am. Chem. Soc.* 137, 14275–14280.