International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 11 (7): 840-845, 2015
ISSN 1811-7775

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.840.845

Biological Evaluations of some Synthesized Pyrimidothieno [2,3-b] Pyrimidine Candidates as Antiulcer Agents

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ARTICLE INFO
Article History:
Received: May 25, 2015
Accepted: August 28, 2015

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ABSTRACT
Here the report antiulcer activity of some 2,6-bis substituted pyrimidothienopyridine (1-9). Eighteen pyrimidothienopyridine derivatives were synthesized and screened as analgesic, anticonvulsant and antiparkinsonian agent before. Many pyrimidines derivatives were synthesized and showed wide diversity of excellent biological activities, the most interesting one amongest these activities the antiulcer activates. It was proven that the antiulcer activities of many pyrimidines derivatives were due to their proton Pump Inhibitor Activities (PPI). Herein and due to structure similarity between the compounds in study and some pyrimidines derivatives showed potent antiulcer activities, the antiulcer activities of these compounds were evaluated using pyloric ligation ulcer model. All the tested compounds showed potent antiulcerogenic activities and the potency descending order was 6b, 6a, 5b, 5a, 7b, 7a, 9b, 9a, 8b, 8a, 2b, 2a, 3b, 3a, 4b, 4a, 1b and 1a. To specify the accurate mechanism of antiulcer activity, many animal models were used the only one that give fruit results was H+/K+-ATPase inhibition in membrane vesicles of stomach mucosa that proved all antiulcer activities of the tested compounds accomplished via H+/K+-ATPase (proton pump) inhibition activities.

Key words: 2,6-bis[pyrimidothienopyridine]pyridine, 2,6-bis [triazinothienopyridine] pyridine, antiulcer activity

INTRODUCTION

The thienopyridines are prodrugs and metabolized in the liver to active metabolites that are non-competitive antagonists of the platelet adenosine diphosphate receptor, P2Y12 (Kam and Nethery, 2003). Thienopyridine derivatives are heterocyclic system and have high pharmacological activity and they have found practical application in medicine as anti-inflammatory and analgesic activities (Madhusudana *et al*., 2012). Additionally, several types of thiophene and their derivatives were reported to possess cytotoxicity (Pinney *et al*., 1999; Romagnoli *et al*., 2006, 2010). One the other hand, antitumor activity of some, thiazole, thiophene and pyridine derivatives were reported (Ghorab and Al-Said, 2012). Recently, some of heterocyclic system incorporated with thiophene ring has been reported as (Abdalla *et al*., 2014; Amr *et al*., 2010; Hossan *et al*., 2012; Assy *et al*., 2013). In view of these observations, we have herein synthesized of some pyrimidothieno[2,3-b]pyrimidine candidates for their evaluation as antiulcerogenic agents.

MATERIALS AND METHODS

Chemistry: All the tested compounds were confirmed by physical and spectroscopic evidences according to the previously reported procedures (Amr *et al*., 2003).

Pharmacological screening

Anti-ulcer activity: Male rats (140 and 175 g) were selected for pyloric ligation ulcer model and they are divided into eleven groups consisting of six animals each and were fasted overnight. One group received normal saline 2 mL kg^{-1} (negative control). The second group received omeprazole 40 mg kg^{-1} (positive control) and the other groups received test compounds (100 mg kg^{-1}) by oral route 30 min prior to pyloric ligation. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The gastric contents were centrifuged at 1000 rpm for 10 min. One milliliter of the supernatant liquid was pipetted out and diluted to 10 mL with distilled water. The solution was titrated against 0.01 N sodium hydroxide solution using Topfer's reagent as indicator to the end point when the solution turned to orange color. The volume of sodium hydroxide consumed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of sodium hydroxide solution required was noted and total acidity calculated. After opening the stomach the ulcer index was calculated. The results are expressed as Mean±SEM. The difference between groups was determined using the one way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ was considered significant (Table 1) (Kulkarni, 1999).

Table 1: Anti-ulcer activities of the tested compounds (1-9)

Compound No.	Ulcer index	Free acid (mEq L^{-1})	Total acid (mEq L^{-1})
Control	4.50	3.28	11.14
Omeprazole	3.31	0.43	0.33
1a	0.10	0.29	0.24
1b	0.10	0.29	0.24
2a	0.10	0.25	0.22
2b	0.10	0.24	0.21
3a	0.10	0.26	0.22
3b	0.10	0.25	0.22
4a	0.10	0.28	0.23
4b	0.10	0.28	0.23
5a	0.10	0.15	0.12
5b	0.10	0.14	0.11
6a	0.10	0.14	0.11
6b	0.10	0.2	0.10
7a	0.10	0.17	0.14
7b	0.10	0.18	0.15
8a	0.10	0.21	0.21
8b	0.10	0.20	0.17
9a	0.10	0.20	0.17
9b	0.10	0.19	0.16

H+/K+-ATPase (proton pump) inhibition

Procedure for H+/K+-ATPase inhibition in membrane vesicles of stomach mucosa: Membrane vesicles containing H+/K+-ATPase are prepared from pig stomachs obtained from the local slaughter house (Ljungstrom *et al*., 1984). Pigs are fasted overnight before slaughter. The gastric mucosa of four stomachs is rinsed with cold saturated NaCl solution for 3-5 min. The superficial cells, cell debris plus the mucus are wiped off with the edge of a plastic ruler and with paper towels. The mucosa is scraped off. About 100 g scrapings are divided into portions of 10 g and homogenized in 0.25 M sucrose with seven strokes in a Potter-Elvehjem Teflon-glass homogenizer. The total volume is 600 mL which is centrifuged at 20000×g for 40 min. The pellet is discarded. The supernatant is centrifuged at 75000×g for 1 h. The resulting microsomal pellet is homogenized in 30 mL 0.25 M sucrose. Aliquots of 15 mL are transferred to 100 mL centrifuge tubes and layered on top of step gradients, from the bottom comprising 25 mL 37% sucrose (w/v) and 45 mL 7.5% Ficoll (w/v) in 0.25 M sucrose. The tubes are centrifuged at 75 000×g for 1 h in a 6×100 mL ME angle rotor at 4°C. The gradient is then fractionated by pumping Fluoroinert 70 through narrow tubing in a fractionating cap down to the bottom of the tube. Fractions are collected from top through a center hole in the fractionating cap. The yield of vesicles in a typical preparation is about 50-75 mg protein. In order to maintain a stable vesicular structure for a long period of time, the vesicles are frozen at -70°C under nitrogen.

They can then be kept for several months without decrease of H+/K+-ATPase activity. The ATPase activity is measured at 37°C as the release of inorganic phosphate (Pi) from ATP. The test drug and the standard (omeprazole) are pre-incubated in concentrations of 0.01 to 100.0 μM in enzyme containing buffers in parallel at pH 6.0 and 7.4 for 30 min at 37°C. Then, the medium of pH 6.0 is adjusted with HEPES/Tris buffer to pH 7.4. The enzyme reaction is started by addition of nigericin and Tris/ATP. The total reaction volume is 1 mL, containing 20 μg vesicular protein, 4 mM MgCl2, 10 mM KCl, 20 μM nigericin, 2 mM Tris-ATP, 10 mM HEPES and additionally 2 mM Pipes for the pre-incubation medium at pH 6.0. After 4 min at 37°C, the reaction is stopped by the addition of 10 mL of 50% trichloroacetic acid. The denaturated protein is spun down and the Pi content is determined according to LeBel *et al*. (1978) based on the reduction of a phosphomolybdate complex by p-methyl-aminophenol sulfate in a copper acetate buffer or according to Carter and Karl (1982) based on the reaction of phosphomolybdate with the basic dye malachite green. IC_{50} values are calculated by probit analysis, whereby 0% corresponds to 4 mM $MgCl_2$+dependent and 100% to 4 mM $MgCl_2$+ plus 10 mM K+dependent ATP hydrolysis. The IC_{50} values of the test compound at different pH values are compared with IC_{50} values of the standard. Statistical differences ($p<0.05$) are calculated by Student's t-test (Table 2).

RESULTS AND DISCUSSION

Chemistry: Herein a series of pyrimidothienopyridine heterocyclic derivatives (1-9) (Fig. 1 and 2) were synthesized

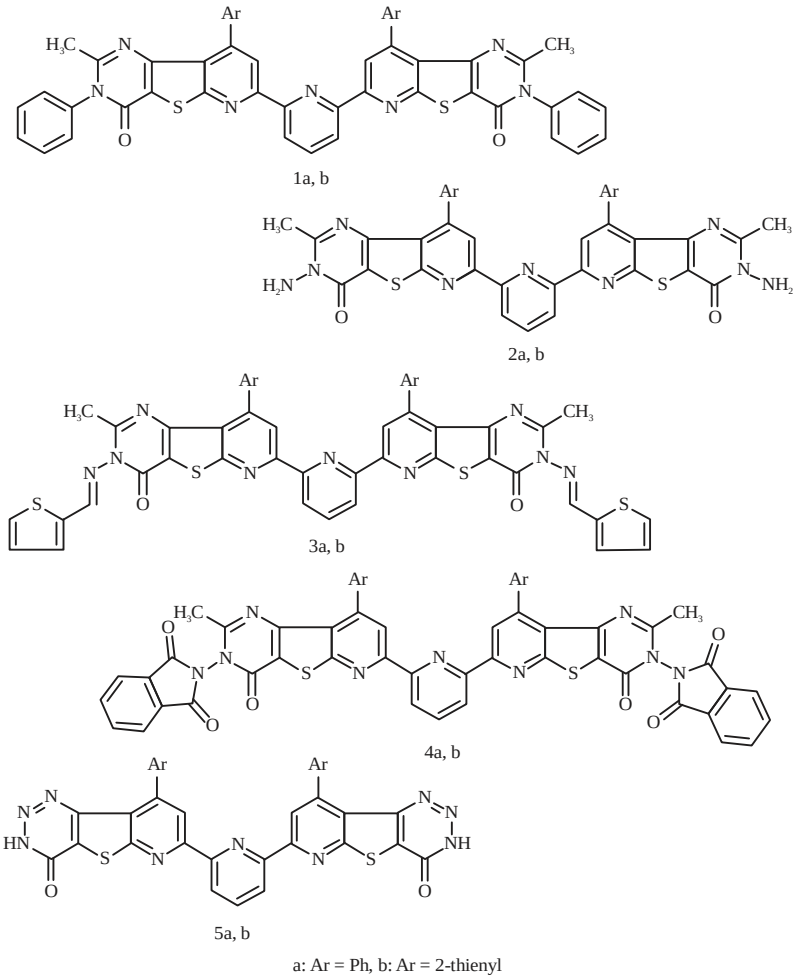

Fig. 1: Chemical structure for the tested compounds 1-5

Table 2: H+/K+-ATPase inhibition in membrane vesicles of stomach mucosa of the tested compounds (1-9)

Compound No.	IC_{50} (µM)
Control	
Omeprazole	66
1a	32.4±0.009
1b	34.3±0.009
2a	21.34±0.008
2b	20.12±0.009
3a	24.32±0.008
3b	22.33±0.007
4a	28.76±0.009
4b	26.65±0.009
5a	11.23±0.003
5b	10.34±0.004
6a	9.91±0.005
6b	9.80±0.006
7a	17.46±0.006
7b	17.23±0.007
8a	19.50±0.009
8b	19.20±0.009
9a	18.28±0.007
9b	17.99±0.008

and illustrated by physical, chemical and spectroscopic evidences before and screened as analgesic, anticonvulsant and antiparkinsonian agents (Amr *et al*., 2003). In this study, we report the activities of these compounds as antiulcerogenic agents.

Pharmacology: Pyrazolo, imidazolo and triazolopyrimidines were prepared and evaluated for cytoprotective antiulcer activity. Among them, 4-methoxy-6-methyl-2-(1H-pyrazol-1-yl)pyrimidine showed potent inhibition of the HCl-ethanol-induced and water-immersion stress-induced ulcers in rats, as well as low acute toxicity (Ikeda *et al*., 1996; Terashima *et al*., 1995). Some of substituted quinazoline and pyrimidinethione derivatives were synthesized and tested for antiulcer activity against pylorus ligation-induced, aspirin induced and ethanol induced ulcer in rat model. The compounds were scrammed for their antiulcer activity: some of these compounds showed higher activity than omeprazole used as standard (Patil *et al*., 2010; Kodhati *et al*., 2013). A series of substituted 2-(pyrimidinylsulfinyl) benzamidazoles derivatives were evaluated against antiulcer and antisecretory activity as a inhibition of gastric H^+/K^+-ATPase by induction of gastric ulcerations experimentally in male Wister rats according to the

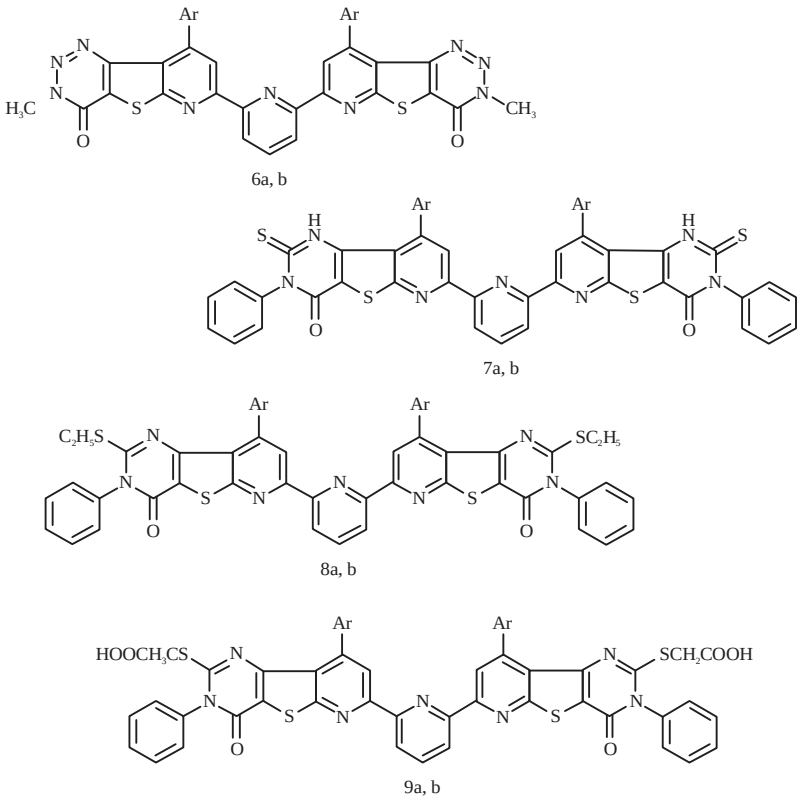

Fig. 2: Chemical structure for the tested compounds 6-9

reported method (Khan and Asnani, 2011). Many of synthesized heterocyclic derivatives containing thiazole, thiophene, pyridine and pyran derivatives were evaluated as anti-ulcer agents (Mohareb *et al*., 2015). On the other hand, some of arylthiomethylpyridine showed gastroprotective activity against 96% ethanol-induced gastric lesions greater than omeprazole. In addition, pyridine derivatives are known to possess antiulcers activities (Evangelista *et al*., 1988; Cho *et al*., 2001; Katsura *et al*., 1992).

From Table 1 and 2, all the tested compounds showed potent antiulcerogenic activities and in searching for their mechanism of action, it was founded that these compounds exerts their antiulcer activities via inhibiton of H^+/K^+-ATPase. 2,6-bis-(2-methyl-4-oxo-3,9-diphenyl-3,4-dihydropyrido [3',2':4,5]-thieno[3,2-d]pyrimidin-7-yl)pyridine (1a,) and 2,6-bis(2-methyl-4-oxo-3-phenyl-9-(2-thienyl)-3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-7-yl)pyridine (1b) showed anti-ulcerogenic activities but they still the least potent compounds, where replacing the N-phenyl radicles with N-amino greatly increases the antiulcerogenic activities as for compounds 2b and 2a.

Condensation of compounds 2b and 2a with thiophene aldehydes afforded the schiff's bases 3b and 3a that were less active than their starting 2b and 2a, respectively as antiulcer agents. The same thing happened when condensed compounds 2b and 2a with phthalic anhydride afforded compounds 4b and 4a respectively that were less actives as antiulcerogenic agent than compounds 2b and 2a. Due to stearic hindered factors compounds 4a,b were less active than compounds 3b,a. Replacing the pyrimidine moieties of derivatives 2b and 2a with triazinone ones as in compounds 5b and 5a greatly increases the antiulcerogenic activities which probably could attributed to the more basic centers that could undergoes efficient strong hydrogen bonding with H+/K+-ATPase. The later concept confirmed via methylation of derivatives 5b and 5a that afforded the more potent antiulcer N-methyl analogues 6b and 6a.

The pyrimidinethiones 7b and 7a where more active than their congeneers containing no sulfur (derivatives 1-4) due to their increasing abilities to form hydrogen bonding with the H+/K+-ATPase. Involvement of the thione in a such structure that decreasing their abilities to form hydrogen bonding with the H+/K+-ATPase decreasing their binding forces leads to decreasing the antiulcerogenic activities as in derivatives 8a,b and 9a,b but still derivatives 9a,b more active than derivatives 8a,b because it contains carboxy group for binding to H+/K+-ATPase.

All the tested compounds have a proton pump inhibitor activities and its was worth mentioned that these compounds were more active than positive control.

CONCLUSION

All the tested compounds showed potent antiulcerogenic activities and the potency descending order was 6b, 6a, 5b, 5a, 7b, 7a, 9b, 9a, 8b, 8a, 2b, 2a, 3b, 3a, 4b, 4a, 1b and 1a. The tested agents exert their antiulcerogenic activities via a proton pump inhibitor activities.

Studying the structural activities relationship of the tested agents calumniated on the following assumptions:

- Triazine polycyclic provides the highest activities
- In triazine polycyclic the free amino ones is the most active ones
- The pyrimidones were less active than the triazine polycyclic
- In the pyrimidones the thiones more active than the thioacetic acid and the later more active than the ethyl thiol
- Thienyl substituents provides more activity than the phenyl one
- N-N bond increases the activity, where the free amino group has higher activities than that involved in aliphatic bond and the later more active than that involved in cyclic ring structure
- Dihydropyrido provides more activity than the oxazine ones
- Amide increases the activity more than the ethyl ester
- Thiones increases the activity more than the ketonic ones

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-172.

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