Research Article

Novel ١,٣,٤-Oxadiazole mannich base: Synthesis, Investigation of Anti-inflammatory activity and Ulcerogenic liability

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Abstract

In attempt to develop safer anti-inflammatory agents, ١,٣,٤-oxadiazole/ mannich base hybrid was synthesized, characterized and screened for anti-inflammatory activity. The synthesized compound ٦ showed promising anti-inflammatory activity with ٧٦.٦٧٪ reduction in paw edema thickness. Its anti-inflammatory activity represents ٩٢٪ compared to indomethacin. Histopathological investigation showed that compound ٦ exhibited safer gastric profile (UI=٣) compared to indomethacin (UI=٣٥).

Key words: ١,٣,٤-Oxadiazole, Anti-inflammatory, Ulcerogenic liability, Histopathological investigation.

Introduction

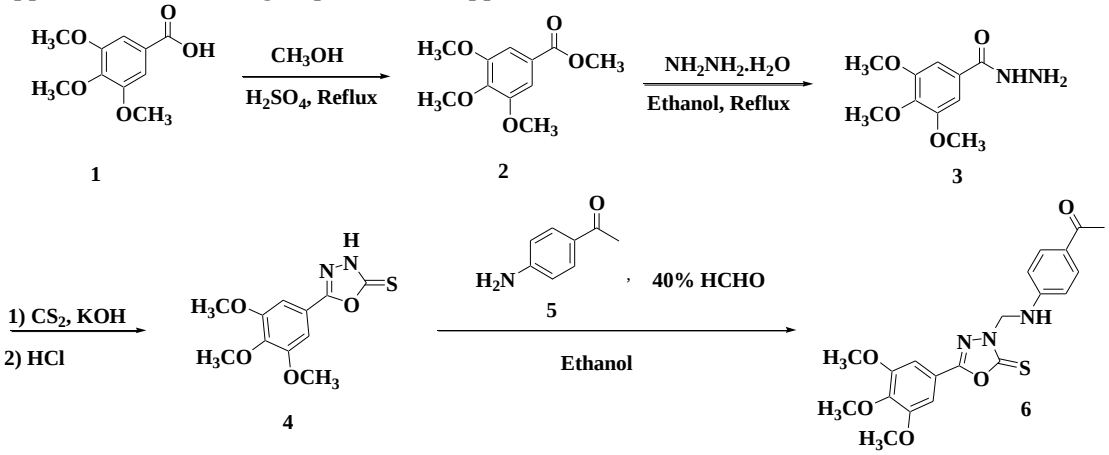
Indomethacin belongs to a class of non-steroidal anti-inflammatory drugs (NSAIDs); the most commonly used drugs for reducing pain, swelling and fever associated with inflammatory diseases.[١] NSAIDs exert their anti-inflammatory action via blocking the metabolism of arachidoinc acid into prostaglandins (PGs) through inhibition of cyclooxygenase enzymes (COX).[٢] Two isoforms of COXs are isolated; COX-١ and COX-٢.[٣] COX-١ is important for physiological processes while, COX-٢ is synthesized in response to inflammatory stimuli.[٤] NSAIDs inhibit both COXs enzyme causing local and systemic gastrointestinal toxicities due to the inhibition of cytoprotective PGs; important mediators for maintenance of integrity of gastric mucosa.[٥] It has been also reported that heterocyclic compounds containing ١,٣,٤-oxadiazole has diverse biological activities including anti-inflammatory[٦], anticancer,[٧] anti-fungal,[٨] and antibacterial activity.[٩] In addition, mannich bases are important bioactive moietes that possess diverse biological activities such as anti-inflammatory,[١٠] anticancer,[١١] antibacterial,[١٢] antifungal,[١٢] antitubercular,[١٣] analgesic,[١٤] anti-HIV,[١٣] and antipsychotic activity.[١٥] Based on aforementioned information, herein, we report the design and synthesis of novel ١,٣,٤-oxadiazole mannich base hybrid gathering the two bioactive entities in one compact structure for the purpose of synergism and in the same time offering potential bioisoster for the free carboxylic group in conventional NSAIDs that responsible for the local gastrointestinal irritation.

Results and discussion.

Chemistry

١,٣,٤-oxadiazole mannich base ٦ was prepared according to Scheme ١. Esterification of ٣,٤,٥- trimethoxybenzoic acid using usual Fischer esterification[١٦] using methanol in the presence of conc. $H_٢SO_٤$, treatment of the ester ٢ with hydrazine monohydrate ٩٥٪ afforded hydrazide ٣. Cyclization of ٣ using carbon disulfide and potassium hydroxide gives ١,٣,٤-oxadiazole derivative ٤. Heating at reflux of ١,٣,٤-oxadiazole derivative ٤ with *p*-aminoacetophenone ٥ and ٤٠٪ HCHO afforded compound ٦ which was confirmed by the appearance of a triplet signal of NH group at ٧.٩٦ ppm in ^{١}H NMR and also the

appearance of C=O group at ١٩٦.٣٢ ppm in ^{13}C NMR.



Scheme ١. Synthesis of ١,٣,٤-oxadiazole mannich base hybrid.

Biological evaluation

Anti-inflammatory activity

Anti-inflammatory activity of compound ٦ was tested using carrageenan-induced rat paw edema method.[١٧] Compound ٦ was administered via the intraperitoneal route in equimolar doses to (٠.٠٥ mol, ١٧mg/Kg) of the standard drug (indomethacin), ٣٠ min before carrageenan injection at the right hind paw of adult albino male rats. Mean changes in the paw edema thickness were recorded every hour for ٤ hours after carrageenan injection. The anti-inflammatory activity was calculated as the percentage of reduction in edema thickness induced by carrageenan and was determined using the following formula:

$$\% \text{ of edema inhibition} = \frac{(V_R - V_L)_{control} - (V_R - V_L)_{treated}}{(V_R - V_L)_{control}} \times ١٠٠$$

Where V_R represents the mean right hind paw thickness and V_L represents the mean left hind paw thickness. $(V_R - V_L)_{control}$ represents the mean increase in paw thickness in the control groups of rats. $(V_R - V_L)_{treated}$ represents the mean increase in paw thickness in rats treated with the tested compounds.

Results are expressed as % mean ± standard error of mean (SEM) and listed in Table ١. Results recorded revealed that compound ٦ had higher anti-inflammatory activity over the three hours reached maximum activity at the third hour and decreased to ٧٦.٦٧٪ at the fourth hour. It has ٩٢٪ potency relative to indomethacin.

Ulcerogenic liability

The ulcerogenic liability of the synthesized compound ٦, indomethacin, were assessed. Results were obtained from the post mortem studies of rats sacrificed ٤ h after anti-inflammatory evaluation. It exhibited safer profile compared to that of indomethacin (UI =٣٥). The results were supported with histological examination of gastric stomach of rats treated with compound ٦. (Fig. ١A), which showed normal gastric wall with no signs of inflammation or edema, while indomethacin (Fig. ١B) showed edema and infiltration of inflammatory cells. The safety profile of the designed compound may be attributed to bioisosteric replacement of carboxylic function group with ١,٣,٤-oxadiazole moiety.

Table (١). Anti-inflammatory activity exhibited by compounds ٦, ibuprofen, and indomethacin using carrageenan induced paw edema method.

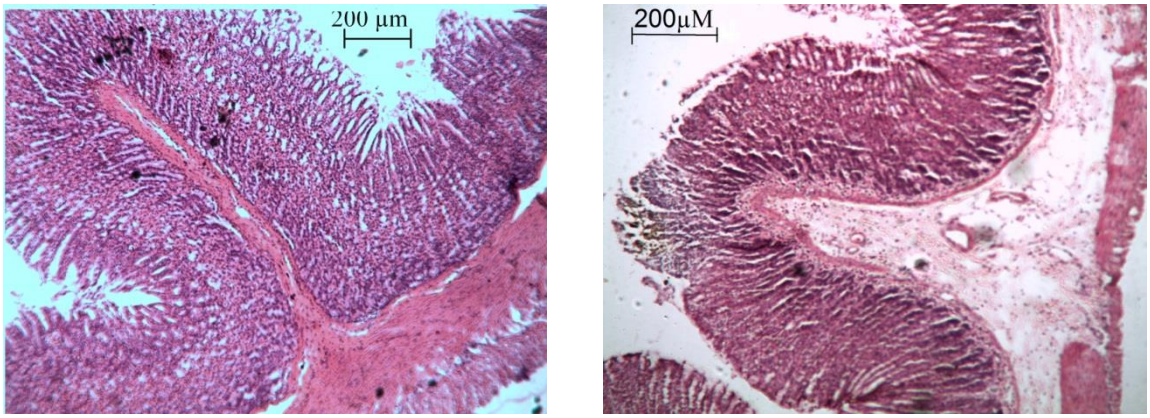
Compound	% of Edema inhibition (% mean ± standard error, n=٥)				Potency[a]	Ulcer index
	١ h	٢ h	٣ h	٤ h	٤ h	------
control	٠	٠	٠	٠	٠	------
٦	٦٦.٦٧±٢.٤٩*** *	٨٠.٠٠±٢.٩٤*** *	٩٠.٠٠±٣.١٤****	٧٦.٦٧±١.٩٣****	٩٢.٠١	٣
Indomethacin[c]	٥٤.٣٣±٥.٤٦***	٦٦.٦٧±٧.٢٧***	٨٦.٦٦±٧.٢٧***	٨٣.٣٣±٥.١٤****	١٠٠.٠٠	٣٥

[a] Potency was expressed as % of edema inhibition of the tested compounds relative to % of edema inhibition of indomethacin at ٤

[b] Ibuprofen dose = ١٠ mg/Kg, [c] Indomethacin dose= ١٧ mg/Kg

*Significantly different from control group at p= ٠.٠٥, ** significantly different from control group at p= ٠.٠١,

*** significantly different from control group at p= ٠.٠١, and **** significantly different from control group at p= ٠.٠٠١.



A B

Fig. ١. Histological examination of the stomach lining for rats treated with **A**) compound ٦ (٤x); **B**) Indomethacin (٤x).

Experimental

Chemistry

Chemicals and solvents used were of analytical grade. Progress of the reactions was monitored by thin layer chromate-graphy with ethyl acetate/ methylene chloride (١:١) as the mobile phase on pre–coated Merck silica gel ٦٠ F٢٥٤ aluminum sheets. Melting points were determined on Stuart electro-thermal melting point apparatus and were uncorrected. IR spectra were recorded on Nicolet iS٥ (ATR) FT-IR spectrometer at Minia University. ^{١}H NMR spectra were recorded on Bruker Avance III ٤٠٠ MHz and ^{١٣}C spectra were recorded on Bruker AG, Switzerland, ١٠٠ MHz. High resolution mass spectra were collected via Thermo Scientific Q Exactive™ Orbitrap mass spectrometer.

[٤-([٥-(٣,٤,٥-Trimethoxyphenyl)٢-thioxo-٢,٣-dihydro-١,٣,٤oxadiazole-٣-yl]methyl amino)phenyl]-١-ethanone (٦). Yellow solid (٥.٢ g,٦٢ % yield); mp ١٧٧-١٧٨ °C; ^{١}HNMR (DMSO-d٦); □□□ppm □□□□□□□□□t, ١H, J=٧.١ Hz, NH), ٧.٧٩ (d, ٢H, J = ٨.٧ Hz, Ar-H), ٧.٠٩ (s, ٢H, Ar-H), ٦.٩٦ (d, ٢H, J = ٨.٨ Hz, Ar-H), ٥.٥٥ (d, ٢H, J=٧.٠ Hz, CH_2), ٣.٨٦ (s, ٦H, $2OCH_3$), ٣.٧٤ (s, ٣H, OCH_3); ٢.٤٣ (s, ٣H, CH_3); ^{١٣}CNMR (DMSO-d٦) □□□ppm□□ ١٩٦.٣٢, ١٧٥.٨١, ١٥٩.٣٣, ١٥٤.١١,□١٥٠.٣٦,

١٤١.٩٤, ١٣٠.٨٨, ١٢٧.٨٣, ١١٧.٤٣, ١١٢.٧٣, ١٠٤.٠١, ٦٠.٧٣, ٥٧.٦٦, ٥٦.٦٦, ٢٦.٥٦.

Biological evaluation

Screening of anti-inflammatory activity

The anti-inflammatory activity of compound ٦ was tested using the carrageenan-induced paw edema method.[١٧] Briefly, male SD rats were randomly assigned to different groups. The rats received either control (vehicle) or equimolar dose of the tested compound by an intraperitoneal injection. Thirty minutes later, the rats were challenged by a subcutaneous injection of ٠.٠٥ mL of ١٪ solution of carrageenan into the plantar side of the left hind paw while the right paw was used as a control. The size of the paw edema was recorded every hour for ٤ h to determine the duration of action. At the end of the experiment, rats were sacrificed and the weights of the right and left paws were measured.

Ulcerogenic liability

The synthesized compound ٦ and indomethacin were evaluated for their ulcerogenic liability according to the reported procedure.[١٨] the ulcerogenic potential was evaluated after intraperitoneal administration of the tested compound under investigation. The stomachs were removed, collected, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. Examination of mucosal layer was done using magnifying lens to detect macroscopically visible lesions. The number of lesions if any was counted and recorded. Ulcers were classified into levels, level I, in which ulcer area is less than ١$mm^٢$, level II, in which ulcer area in the range ١-٣ $mm^٢$, and level III, in which ulcer area more than ٣$mm^٢$, and this rated according their areas in $mm^٢$. The following parameters were calculated according to the following formula: Ulcer index (UI)= ×(number of ulcers level I) + ٢ (number of ulcers level II) + ٣ (number of ulcers level III).

Conclusion

١,٣,٤-oxadiazole mannich base hybrid ٦ was synthesized and evaluated for its anti-inflammatory and ulcerogenic liability, the results indicated that compound ٦ exhibited promising anti-inflammatory activity compared to that of indomethacin with maximum activity at the third hour with ٩٠٪ inhibition in paw edema thickness then decrease in the anti-inflammatory activity to ٧٦٪ at the fourth hour. The anti-inflammatory activity of the tested compound represents ٩٢% that of indomethacin activity with safer gastric profile compared to indomethacin. In conclusion, mannich base of ١,٣,٤-oxadiazole offering good anti-inflammatory agent with safer profile comparted to conventional NSAIDs.

Conflict of interest

Authors declare that there is no conflict of interest in the presented research**.**

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