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Preparation of new mefenamic acid compounds and evaluation the biological activities of their derivatives

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ABSTRACT

Ponstane Fort, a famous pharmaceutical drug has many medicinal uses. Recently, it has been used in different chemical reactions in order to improve its biological effectiveness. Three chemical compounds have been prepared as derivatives of Mefenamic acid by certain steps included adding thiosemicarbazide to Mefenamic acid with a catalyst of H_2SO_4 to obtain a Mefenamic derivative C1; mixing a certain amount of C1 with 4-carboxybenzaldehyde to obtain compound C2; dissolving C2 at various reagents by diazotization reaction to yield Mefenamic derivative C3. All formatted compounds had been monitored through FTIR-Spectra, ^{1}H.NMR-Spectra, Mass-Spectra and melting point. This method has high target product productivity, simple process procedures, mild reaction conditions**,** simple purifying process, high product purity and suitability for industrialized production**.** So that the production cost is further reduced. By using the agar well diffusion method, all prepared three compounds showed good antibacterial activity against studied Gram-positive and Gram-negative bacteria, however, C3 showed the highest activity. A skin test for Delayed Hypersensitivity was done to know if there are allergic reactions for the created compounds, while the test for Killing Mice has been done for testing mortality rate in animals. The compounds have a high cellular immune response through in experimental animals.

Keywords: Mefenamic acid, Thiadiazole, Schiff base, Hypersensitivity, Antibacterial activity, Heterocyclic rings, Azo compounds.
Article type: Research Article.

INTRODUCTION

A cyclic compound with two different atoms, at least, as members of its ring is known as a heterocyclic compound. Heterocyclic chemistry is the area of organic chemistry that deals with synthesis, characteristics, and uses of heterocyclic. The bulk pharmaceuticals, most biomass (cellulose and related materials), all nucleic acids, and several natural and manmade colours are heterocyclic compounds. In addition, heterocyclic compound having interesting anticancer capabilities, also playing a key role in the development of anticancer medications (Sabah *et al.* 2020; Ayoub *et al.* 2021; Aljeboree *et al.* 2023; Sabah *et al.* 2023; Al-Shik *et al.* 2023). In relation to a variety of settings, including antibacterial, antiviral, and anticancer activities, Schiff bases have been studied (Cimolai 2013; Zamil *et al.* 2020). They have also been taken into consideration to prevent the aggregation of amyloid. When an amine, such as the terminal group of a lysine residue, reversibly interacts with an aldehyde or ketone of a cofactor or substrate, the resulting intermediate is known as a Schiff base. PLP, a frequent cofactor of enzymes, joins with a lysine residue to generate a Schiff base, which is then transaldiminated to the substrate. Similar to this, human rhodopsin creates a Schiff base with the cofactor retinal (Vitaku *et al.* 2014; Aljamali 2019). The biological properties of azo compounds, which include antibacterial, anti-inflammatory, anthelmintic, antiviral, and anticancer effects, have generated growing interest in their research. Examples of common azo dye

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compounds that are also medications are sulfasalazine, used to treat inflammatory bowel disease, and the analgesic Formazan- mefenamate derivative (Kibrom & Mulugeta 2022; Sahilu *et al.* 2022). A five-membered heterocyclic molecule with one sulphur and two nitrogen atoms, thiadiazoles are a subfamily of usual chemicals. Because of the two double bonds and the lone pair of sulphur, they are aromatic rings with a wide range of biological activity, including antibacterial, anticancer, antiphrastic, and antiviral effects. High lip solubility, enhanced pharmacokinetics, and increased biological activity are all caused by the presence of sulfur atoms in molecules containing thiadiazole rings (Sivaev & Bregadze's research 2014; Mustafa & Mushin 2019). Azo groups are amongst the most kinds, classes of chromophores with various pharmaceutical applications of drugs like anticancer, antifungal, antiviral, anti-inflammatory and anti-bacterial (Mezgebe & Mulugeta 2022).

MATERIALS AND METHODS

Chemical study

Three chemical compounds have been prepared as depending on to the Schemes:

Compound C_1: 5-(2-((2,3-dimethylphenyl) amino) phenyl)-1,3,4-thiadiazol-2-amine.

Equal moles of Mefenamic acid and thiosemicarbazide were mixed in 20 mL ethanol and sulfuric acid 5.4 mL, refluxing for 15 h. Then, the compound C_1 precipitate was filtered and dried. Yield 87%, M.P 115-120 °C, Rf 0.7.

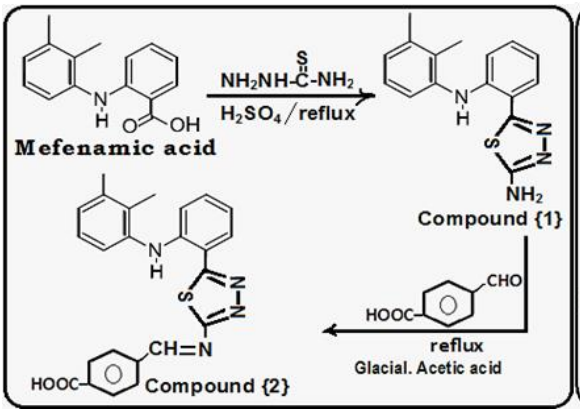
Compound C_2: 4-(((5-(2-((2,3-dimethylphenyl) amino) phenyl)-1,3,4-thiadiazol-2-yl) imino) methyl) benzoic acid.

For a 5-hour reaction, 0.01 mole of C_1 was dissolved in 20 mL ethanol with 0.01 mole 4-Carboxybenzaldehyde, along with 1-2 drops of glacial acetic acid (GAA). To dry and evaporate the product and get it ready for crystallization, absolute methanol was utilized compound C_2. Yield 80%, M.P 123-127 °C, Rf 0.8.

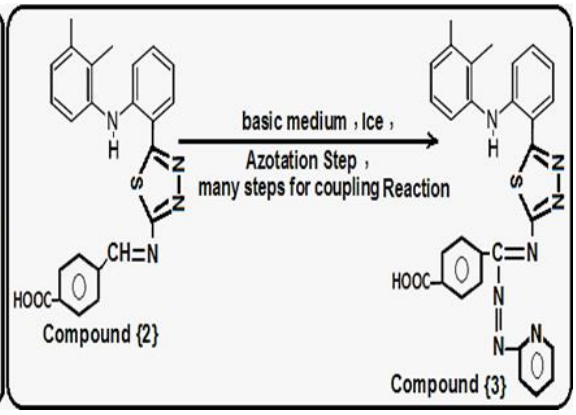
CompoundC_3:4-((1E)-((5-(2-((2,3-dimethylphenyl)amino)phenyl)-1,3,4-thiadiazol-2-yl)imino)(pyridin-2-yldiazenyl)methyl)benzoic acid.

Basic solution of Schiff base C_2 (dissolved C_2 in 5% sodium hydroxide) for 5 min, then addition this mixture to azo salt from (2-aminopyridine) stirring was continued after 24 h, filtration, dried, recrystallization, to yield formazan (coloured compounds). The solid precipitate was filtered, washed, dried and recrystallized from ethanol to give a new compound C_3. Yield 77%, MP 135-140 °C, Rf 0.6.

Finally, all generated compounds were subjected to thin layer chromatography (TLC).



Scheme 1: Preparation of Compounds C_1 & C_2.



Scheme 2: Preparation of Compound C_3.

Biological investigation

Antibacterial activity test

For evaluation of antibacterial activity of the chemical compounds created in the current study, Agar-well diffusion method was used with four bacterial species included *Shigella flexeneri* and *Proteus mirabilis* (Gram negative), *Staphylococcus aureus* and *Enterococcus faecalis* (Gram positive) each chemical compound was used in five concentrations including 5, 10, 20, 30, 40 and 50 µg mL^{-1}. By using DMSO as a solvent, an inoculum bacterial suspension was streaked on a Muller-Hinton agar. A hole with a diameter of 9 mm was punched by a sterile cork borer (No. 6) on the inoculated streaked media. 200 µL of each concentration of a chemical compound

was introduced into each hole. Pre-diffusion time was allowed for one hour, afterward the plates were incubated at 37 °C for 18 h. Diameter of inhibition zone was measured in mL (Al Mousawi *et al.* 2022).

Experimental Design

Skin test for delayed hypersensitivity

Thirty male rabbits, *Oryctalagus cuniculus*, 2-3 month olds, were chosen to receive intradermal injections of mefenamate derivatives at three different concentrations (10, 20 and 30 μg mL^{-1}), which were first dissolved in DMSO (dimethyl sulfoxide) as the solvent. After shaving the morphological zone of the hair, 27 rabbits were used as the test group (three animals for each of the three concentrations of each compound), while the other three were the control group, who received the same injections of sterile normal saline (Prasad & Arena 2013; Alejandro & Rafael 2021). This approach was unique to this study. Positive outcomes were found employing different calibration methods. The rabbit's double fold thickness was assessed after 24, 48 and 72 hours, and the results showed erythema, indurations, and necrosis at the injection site.

Killing test in mice

This test was carried out to investigate the chemical compounds lethal effects. The tail vein of 30 males Swiss mice, weighting between 18 and 22 g were injected with 0.5 mL of various concentrations of these compounds after being pulled out. After 24 to 72 hours, the favourable outcome was noted. Animal deaths served as a sign that this test had produced positive results **(**Abdullabass & Jawad 2020**)**

RESULTS AND DISCUSSION

Investigating spectra

FT-IR (ν, cm^{-1}): spectrum of compounds C_1, C_2 and C_3 exhibited absorption bands as follows (Amooshahi *et al.* 2022):

Compound C_1: appearance absorption bands for FTIR (ν, cm^{-1}): 3350-3145 (NH_2, NH), 3007 (C-H arom.), 2931-2812(C-H aliph.), 1642 (C═N ring), 787 (C-S).

Compound C_2: appearance absorption bands: 3548 (OH), 3320 (NH), 3016 (C-H arom.), 2950-2915 (C-H aliph.), 1720 (C═O carboxylic group), 1655, 1612 (for C=N endocycle ring and imine group), 783 (C-S).

Compound C_3: Because of the NH amine group at 3340 cm^{-1} and the thiadiazole C=N endocycle absorption band at 1659 cm^{-1}, the spectra looked to have many frequencies. Frequency N-C=N group of formazan at 1635, frequency at 1430, 1490 and 1500 cm^{-1} because of azo group in N=N-C of formazan, also band at 1705 cm^{-1} for carbonyl group and 762 cm^{-1} for C-S.

H.NMR (δ, ppm, DMSO) spectrum for compounds C_1, C_2 and C_3

The signals in the ^{1}HNMR spectra appeared to be obvious indications of newly formed compounds through the elimination of certain groups and the appearance of other new groups via new signals, which point to the production of the new compounds indicated by the peaks (Sabah *et al.* 2020):

Compound C_1: ^{1}H NMR (δ ppm): 1.10, 1.28 (s, 3H, $2CH_3$), 5.35 (s, 2H, NH_2), 7.06-7.90 (m, 7H, Ar-H).

Compound C_2: ^{1}H NMR (δ ppm): 0. 99, 1.05 (s, 3H, $2CH_3$), 7.35-7.99 (m, 11H, Ar-H), 8.59 (s, 1H, CH=N), and 11.87 (s, 1H, COOH).

Compound C_3: ^{1}H NMR (δ ppm): 1.00, 1.03 (s, 3H, $2CH_3$),7.05-7.69 (m, 11H, Ar-H), 7.77-8.50 (m, 4H, pyridine ring), and 11.54 (s, 1H, COOH).

Mass–Spectra

Finally, characteristic peaks were indicated for independent fragments (Nashaan & AlRawi 2023; Nashaan *et al.* 2022) by m/z (Rel. Int. in %): the mass spectra for compound C_2: Chemical Formula: $C_{24}H_{20}N_4O_2S$ (M.Wt.= 428). Fig. 1 illustrates the characteristic peaks represented by m/z=129, 109, 231 (22%), 169 (50%), 129 (40%), which agrees with the molecular weight of the structure inspired by for compound C_2. Also, the mass spectra of compound C3: Chemical Formula: $C_{29}H_{23}N_7O_2$, (M.Wt. = 428). Fig. 2 exhibits the characteristic peaks represented by m/z=533 (10%), 521(5%), 442 (60%), 367(50%), 231 (20%), 144 (70%), 93 (100%). These data confirmed the structure inspired by for compound C_3.

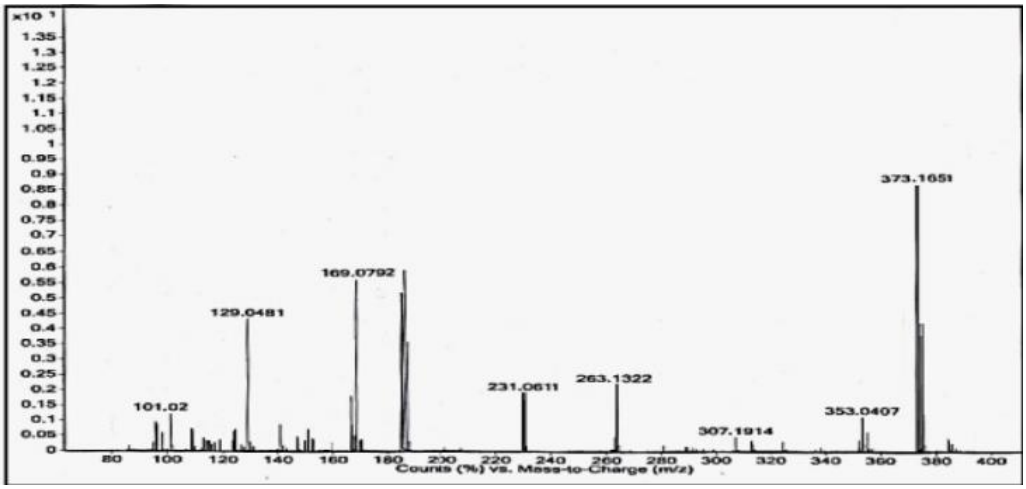


Fig. 1. Mass–spectrum of compound C_2.

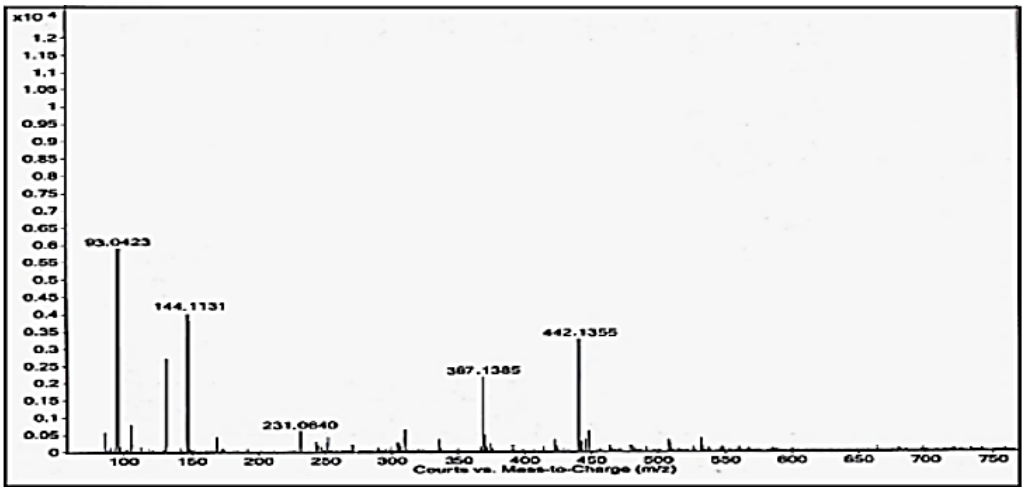


Fig. 2. Mass-spectrum of compound C_3.

Biological investigation

Antibacterial activity test

As detailed in Table 1, all assayed chemical compounds revealed inhibitory action against studied bacteria (Fig. 3). The antibacterial activity varied according to the type and concentration of chemical compounds. The C_3 showed higher antibacterial activity than the other two compounds. The C_1 and C_2 exhibited little effects, since the elevated compound concentration caused upraised antibacterial activity, as shown by the diameters of inhibition zones. This result is in agreement with many studies that documented that active groups and their antimicrobial activities upraise by the elevated concentrations (Al Sa'ady & Hussein 2020). The antibacterial activity of azo compounds may be attributed to their ability to make a damage bacterial cell wall and cell membrane as well as alteration in its permeability, or displaying an effect on a cell's metabolic pathway and interruption with cell proteins followed by inhibition in their activity which may lead to the inhibition of growth and cell death. These chemicals' antibacterial activity is dependent on the physical and chemical characteristics of the molecules.

Table 1. Inhibition zone diameters (mm) of Mefenamate derivatives at different concentrations against bacteria.

Type of Compound	Concentrations of Mefenamate derivatives (μg mL^{-1})	Inhibition zone (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*E. faecalis*	*Sh. Flexeneri*	*P. mirabilis*
C_1	5	8	0	7	0
	10	10	8	9	7
	20	15	10	14	10
	30	25	21	20	15
	40	38	35	34	31
	50	40	39	36	34
C_2	5	10	10	10	10
	10	14	12	11	8
	20	18	15	14	12
	30	25	20	18	11
	40	28	23	20	18
	50	31	26	23	22
C_3	5	10	8	10	7
	10	15	12	11	9
	20	25	20	14	14
	30	32	30	19	14
	40	39	37	34	31
	50	45	42	38	36

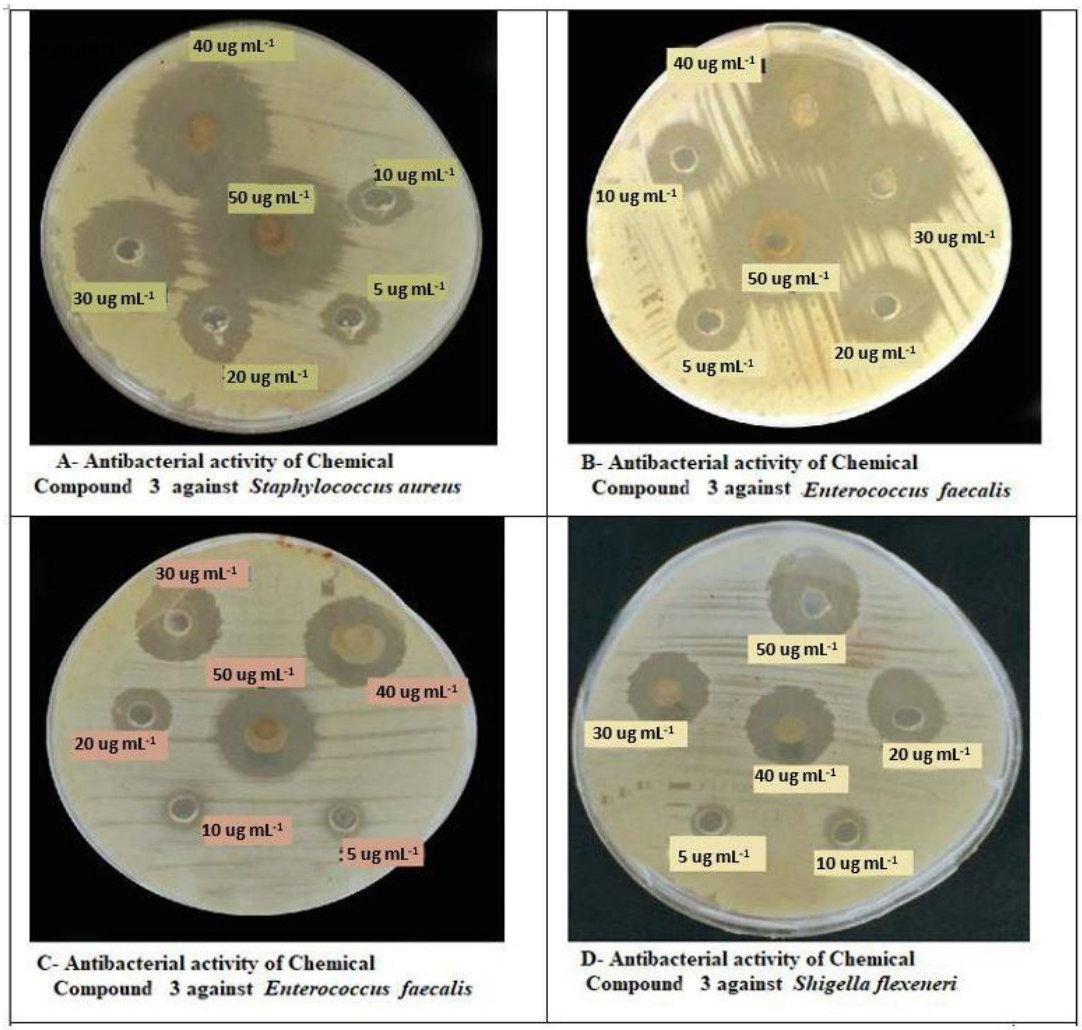


Fig. 3. Antibacterial activity of chemical compound created in the current study against different types of bacteria.

Experimental Design

Skin test for Delayed Hypersensitivity

To determine the impact of azo – imidazole compounds on the skin of experimental rabbit, a delayed type hypersensitivity test was carried out (Table 2). The Immune response was represented by the appearance of signs of cellular sensitivity that stimulate hypersensitivity of redness, thickening and necrosis 24 h after injection of these compounds. This was done since these compounds are commonly used to dye cotton, silk, and wool, because they are simple to use, affordable, and produce vibrant colours. Because they operate as antigens that might be T-dependent types through the activation of Th_1 and Th_2. These substances activated the cell mediate immune response in animals *in vivo* (Abdullabass & Jawad 2020) T-cells produce TNF that work on endothelial cells in dermal blood arteries to cause the sequential expression of the adhesion molecules during the skin test. Following infiltration of the leukocytes, primarily lymphocytes and macrophages, by these molecules at the response site after 4 hours, erythema and indurations form and peak 24-72 h later. Since the applied azo-pigments do not penetrate very deeply into the skin of the test animals, the hypersensitivity reactions might be challenging. By using various chemicals, intradermal testing might be more precise and sensitive (Anne *et al.* 2001; Kadhum 2017).

Killing test in mice

Only one animal was killed utilizing the C_3 chemical at a concentration of 30 mg mL^{-1} in our mice killing test, which may have been attributable to the animals physiological states or the ambient circumstances. The azo dyes are water-soluble and easily absorbed by the body through skin contact, inhalation, and ingestion of dust. Some of them might be utilized as food colours with no sensitizations. The outcomes of experimental sensitization of animals do not always reflect what might be anticipated in humans since humans are more sensitive than animals to some sensitizing stimuli (Patricia & Russell 2013)

Table 2. Skin delayed type hypersensitivity testing of chemical compounds in immunized rabbits.

Type of Compound	Concentrations of Mefenamate derivatives ($\mu g\ mL^{-1}$)	Skin test \ Hours		
		24	48	72
	10	-	-	-
C_1	20	E (4 mm)	-	-
	30	E (9 mm)	-	-
	10	E (8 mm)	EI (8 mm)	-
C_2	20	E (10 mm)	EI (10 mm)	-
	30	EI (11mm)	EI (11 mm)	EIN (11 mm)
	10	-	-	-
C_3	20	E (7 mm)	EI (7 mm)	EIN (7 mm)
	30	EI (12 mm)	EIN* (12 mm)	EIN (12 mm)

E: Erythema, I: Induration, N: Necrosis * Reaction area (mm) with negative result for all control groups.

CONCLUSION

The prepared Azo-Schiff base derivatives from Mefenamic acid were discovered to be stable, and some of them possess greasy or sticky strength and many of them exhibit similar activity to that of the Mefenamic drug inflammation reduction *in vitro*, since the applied azo-pigments did not penetrate very deeply into the skin of the examined animals. There may be difficulty in triggering hypersensitive reactions from the chemical substance's with highly induced a cellular immune response through the activation of Th_1 and Th_2. All assayed chemical compounds revealed inhibitory action against studied bacteria varied according to the type and concentration of chemical compound, suggesting that they may one day be used to treat a wide variety of ailments.

Conflicts of interest: none to declare.

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Ethical issue: Permission for the study was obtained from the research ethics and scientific committee of the College of Pharmacy at the University of Babylon.

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