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Synthesis, acute toxicity and modeling docking studies of azo compound derived from sulphonamide and pyrimidine derivative

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ABSTRACT

A new azo pyrimidine derivative compound was synthesized by reaction of 6-chloropyrimidine-2,4-diamine with 4-aminobenzenesulfonamide via diazotization to give novel 4-[(Z)-(2,4-diamino-6-chloropyrimidin-5-yl)diazenyl]benzene- sulfonamide in good yield. Characterization of synthesized compound was carried by elemental analysis, I R, ^{1}H and ^{13}C NMR spectroscopy. The toxicity of new compound was assayed via the determination of their LD_{50} value by using Dixon's up and down method. Studied compound was found to have an LD_{50} of 718.6 mg/kg of body weight. Molecular modeling studies were performed, showing the hydrogen bindings and hydrophobic interactions.

Key words*:* Pyrimidine derivative, Azo compound, Sulphonamide drug, Acute toxicity, Molecular modiling

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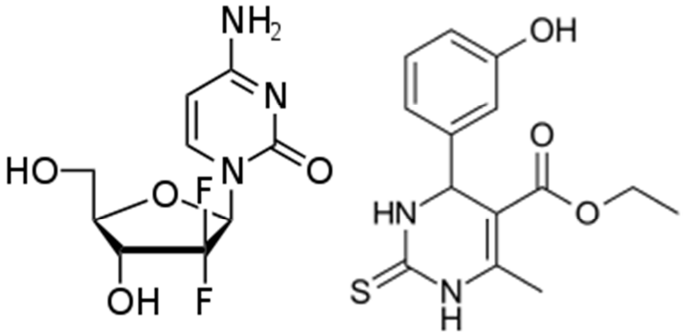
INTRODUCTION

The chemistry of Pyrimidines has been of interest to many researchers including us due to their various biological activities. Pyrimidine is an important heterocyclic moiety which is present in various biomolecules like DNA and RNA. Various pyrimidine derivatives possess diverse biological and pharmacological properties such as antibacterial [1], antifungal [2], antitumor [3,4], anticancer and HIV inhibitors [5].

Pyrimidine compounds are also used as hypnotic drugs for the nervous system [6]. Gemcitabine **1**, a pyrimidine antimetabolite, is an approved drug in the U.S. for pancreatic cancer and also in combination for certain lung cancer patients

[Figure1] [7]. Further, monastrol **2** is another model of pyrimidine derivative [8] as inhibitor of kinesin Eg 5 that interact with microtubuline and then causes mitotic arrest [9].



Gemcitabine (1] **Monastrol (2]**

Figure 1. Some of bioactive pyrimidine derivatives

The pyrimidine azo moieties have antifungal and antibacterial activities [10] and are used in the determination of trace metals in food and drinking water [11].

In the present work we describe here the synthesis, characterization, *invivo* acute toxicity and molecular modeling were studies of azo pyrimidine derivative by reaction of pyrimidine derivative with sulphonamide

MATERIALS AND METHODS

a-Physical measurements

Infrared spectra (IR0 were recorded as KBr discs in the range of 4000-400 cm^{-1} using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the department of Chemistry, College of Education for pure sciences, University of Basrah, Iraq. ^{1}H, ^{13}C NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz university,Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

b- Acute toxicity (LD50]

Animals. All experiments were performed on 10-14- weak old male and female ratus-ratus/rats weighing 200-250 gm at the time of treatment by using up-and-down method, Dixon 1980 [12].

Male and female rats were injected intraperitonially with different doses of the Amoxicillin derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final result (response-dead (X] or non response alive (O], then the following equation was applied $LD_{50} = XF + Kd$.

The estimate of LD_{50} is XF + Kd, where (XF] is the final test level and (K] is the interval between dose levels. (d] is the tabulated value (Table 1].

Table (1] Shows Dixon values. Dixon (1980]

	K represented serial tests started with :-				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests started with :-				

D_{50} = X*f*+Kd

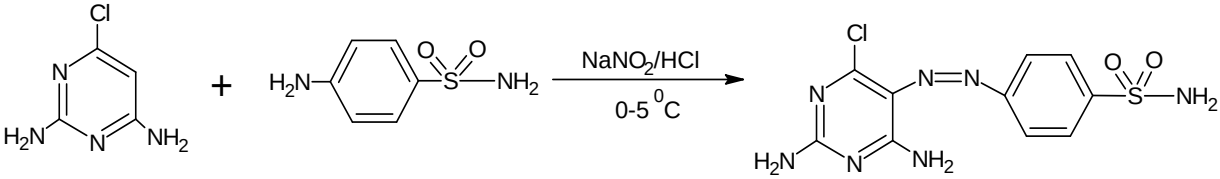
LD_{50} = Median Lethal Dose
xf = Last dose used in the experiment
k = Factor of change from the table
d = Difference between doses.

c- Synthesis and Characterization of Novel azo compound

4-[(*Z*]-(2,4-diamino-6-chloropyrimidin-5-yl]diazenyl]benzenesulfonamide

A solution of substituted aniline (sulphanamide drug] (0.14g; 0.84 mmol] in 6 N HCl (4:0 mL] was cooled to 0 – 5 ^{0}C, and then $NaNO_2$ (0.058 g; 0.84 mmol] in water (2:0 mL] was added dropwise with stirring. After the addition was completed, the solution was stirred for another 15 min and checked by iodine-starch paper. Urea (50 mg] was added to destroy the excess of HNO_2. The diazonium salt solution was then poured onto a solution of 6-chloropyrimidine-2,4-diamine (0.11g, 0.76 mmol] in water and stirred for 30 min. Potassium acetate (0.485 g] was then added, and the mixture was stirred for 16 h at room temperature. The resulting precipitate was filtered, washed with water and dried in a vacuum desiccators over P_2O_5 to give the desired azo-pyrimidine derivative. Scheme (1].

Yield; 82% , M.P.= 146-148^{0}C. FT-IR(KBr, cm^{-1}], 3340,3155(N-H,sym.,asym]; 1664(C=N]; 1610(C=C]; 1577(N-H, bending]; 1384(N=N]; 1022(C-Cl]. ^{1}H NMR(DMSO-d_6]; 7.29-7.96(m,4H-Ar-H]; 4.03(s,2H- NH_2]. ^{13}C NMR(DMSO-d_6]; 173(C-pyr.], 129-118(C-Ar]. *Anal*. for $C_{10}H_{10}N_7O_2SCl$ (M.wt 327.5]: Calc. C, 36.69; H, 3.05; N, 29.92; Found: C,36.97; H,
3.15 ; N, 30.11.



Scheme 1: Synthesis of new azo compound derived from sulphanamide and pyrimidine derivative

RESULTS AND DISCUSSION

a- Chemistry

Isolated yields, melting point, color and spectral data IR and ^{1}H and ^{13}C NMR of synthesized compound was reported. The present work describes the synthesis of new azo compound derived from amoxicillin and aldehyde to produce bioactive Schiff base, thus, the reaction of 6-chloropyrimidine-2,4-diamine with 4-aminobenzenesulfonamide at 1:1 mole ratio in the presence of $NaNO_2$ and HCl first at 0 – 5 ^{0}C, then at ambient temperature for 15 h, afforded, after purification the new pyrimidine derivative compound in good yield. IR spectra for synthesized compound displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The IR spectra of new prepared compound show strong and broad bands in the rang 3340 and 3155 cm-^{1} due to ν(N-H] symmetrical and asymmetrical stretching vibration. The IR spectra of synthesized compound displays band at 1664 cm^{-1} is due to (C=N] stretching vibration in pyrimidine ring . The bands at 1610 , 1577 and 1384 were assigned to ν (C=C], aromatic, ν (N–H] bending and ν (N=N] cm^{-1} , diazo respectively. That band at 1022 cm^{-1} is due to ν (C-Cl] (13].

The structure of synthesized compound was assigned on the basis of their ^{1}H, ^{13}C NMR spectra. The ^{1}H NMR of new compound showed similar patterns for the aromatic and pyrimidine protons and carbon atoms. The aromatic protons of compound appeared within the range 7.29-7.96 ppm. It is worthy to note that the protons of NH_2 groups resonate as a singlet at 4.03 and 7.10 ppm (14]. In the ^{13}C NMR spectra of synthesized compound, the resonance at 173 ppm was assigned to C –N of pyrimidine ring, while the resonances at m =129-118 ppm were attributed to C-Ar (13].

b- Median lethal dose (LD50]

Determination of the 50% of lethal dose (LD50] of the studied compound *in- vivo* was detected in the rats by using the "up-and-down" procedure described by (Dixon, 1980] (9]. In the experiment we using 10 animals of white rats 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (500, 550, 600, 650,700 and 750] mg/k.g b.w] in 0.1 ml (Dimethyl sulphoxide] DMSO, were administered and chosen with equal spacing (concentrations] between doses. Mortality was recorded after 24 hrs that each one animal treated with one dose and after 24 hrs was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being (OOXX] and according for Dixon value was get and the LD50 was determined according to the formula employed by Dixon (1980].

LD50 =X*f* + Kd

LD50 = 700 + 0.372] x 50
LD50 = 718.6 mg / kg b.w
1/10 LD50= 71.86 mg / kg (1 kg = 6 rats] Depending on the weight rat about 175 gram].
1/10 LD50= 11.976 mg /rat Depending on the weight rat 175 gram.

c. Molecular modeling analysis

The molecular docking was performed using SYBYL-X 1.1 and the docking results were shown by PyMOL (15]. Our molecular docking analysis of the new analogues based on the modeling study which was performed to understand the binding mode of these analogues with the aspartate aminotransferase (ATT] of *E. coli* (16] binding pocket (PDB code: 1ahg] (17].

Compound **1** has been selected for the docking modeling study, since its binding energy score -8.4, with indicating a selectivity of substituted pyrimidine ringin its binding to the enzyme pocket (Figure 2]. As shown in figure, the aromatic ring of **1** was fitted into an aromatic rich subpocket surrounded by the aromatic side chain of Tyr 256 and Tyr 65, in addition to three hydrogen bondings. The synthesized molecule was located in the middle of the binding pocket, anchoring the oxygen atom of the SO_2 group in a favourable position for hydrogen bonding with the OH group of Tyr214, in addition to a hydrogen bonding between the oxygen atom of NH2 group at C-6 of the pyrimidine ring with OH of Tyr65 of the aspartate aminotransferase (AAT] enzyme as well as the hydrogen bond between NH_2 group of the Ser285 and NH_2 group at C-2 of the pyrimidine moiety. Overall, the combination of hydrophobic interaction and □-stacking appears to govern the binding of **1** with AAT of *E. coli*.

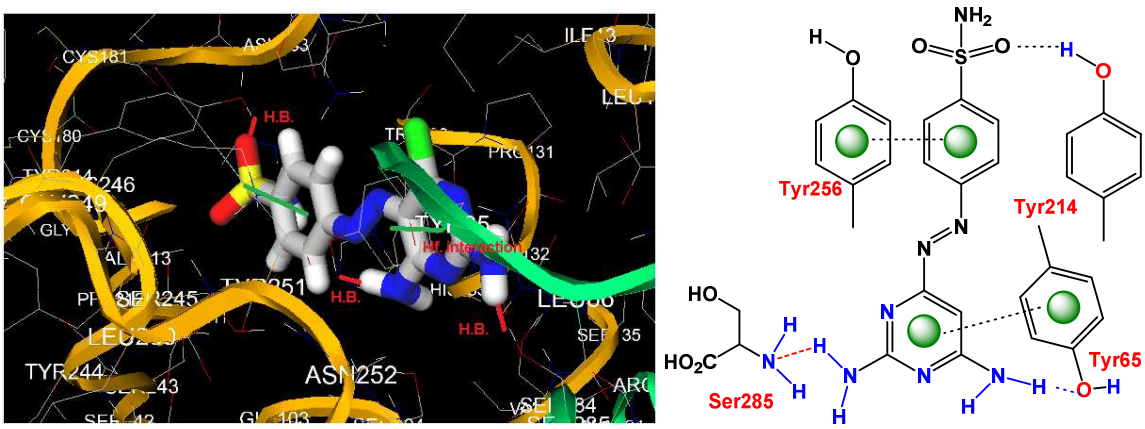


Figure 2. Docked conformation of 1 showing three hydrogen bonds: Tyr214 with oxygen atom of SO_2 group, Tyr65 with NH_2 group at C-6 of the pyrimidine backbone and NH_2 group of Ser285with NH_2 group at C-2 of the pyrimidine moiety. It also exhibits hydrophobic interactions between phenyl ring of Tyr256 and aromatic ring of the phenyl sulfonamide moiety as well as pyrimidine ring with aromatic ring of Tyr65 of AAT of *E. coli* enzyme residues

CONCLUSION

In conclusion, the present study reported the synthesis of new pyrimidine analogue namely 4-[(*Z*]-(2,4-diamino-6-chloropyrimidin-5-yl]diazenyl]benzenesulfonamide which revealed moderate *in vivo* toxic effects by LD_{50}measurment. In addition, Molecular modeling studies were performed, showing the hydrogen bindings and hydrophobic interactions.

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