

Synthesis, anti-bacterial and anti-cancer activities of Some Antipyrine Diazenyl Benzaldehyde Derivatives and Antipyrine-Based Heterocycles

Muna S. AL-Rawi, Huda A. Hassan, Dheefaf F. Hassan.

Department of Chemistry, College of Education For Pure Science Ibn Al- Haitham, University of Baghdad.

Abstract:

The present study envisaged utilizing 4-aminoantipyrine as key intermediate for the synthesis of some new derivatives bearing anti-bacterial and anti-cancer activities moieties viz., antipyrine diazenyl benzaldehydes 2_(a-d) which were obtained by coupling of diazotized 4-aminoantipyrine (1) with substituted benzaldehydes at 0°C (iced) temperature. The other antipyrine derivatives were containing bis heterocycles like bis thiazolidinone-antipyrine (4), bis imidazolidinone -antipyrine (5) and bis azetidinone -antipyrine (6). These compounds were prepared through the reaction between 4-aminoantipyrine and terephthalaldehyde to get (3) which were reacted with mercaptoacetic acid, glycine or chloroacetyl chloride separately to get compounds (4), (5) and (6) respectively. The structures of all compounds were established on bases of melting points, FTIR, ¹HNMR, mass spectroscopy (for some compound) and elemental analysis. These derivatives were investigated for their anti-bacterial activity against two kinds of bacteria (*G*⁻) and (*G*⁺) and compared to the Amoxicilin and Lincomycin standard drugs. Two cancer cell lines include: The human pelvic rhabdomyosarcoma (RD) and the mouse cell line (L₂₀B) were used in this study and these derivatives showed different cytotoxic activity *in vitro*.

Keywords: antipyrine, thiazolidinone, imidazolone, azetidinone, antibacterial activity, anticancer activity.

Introduction:

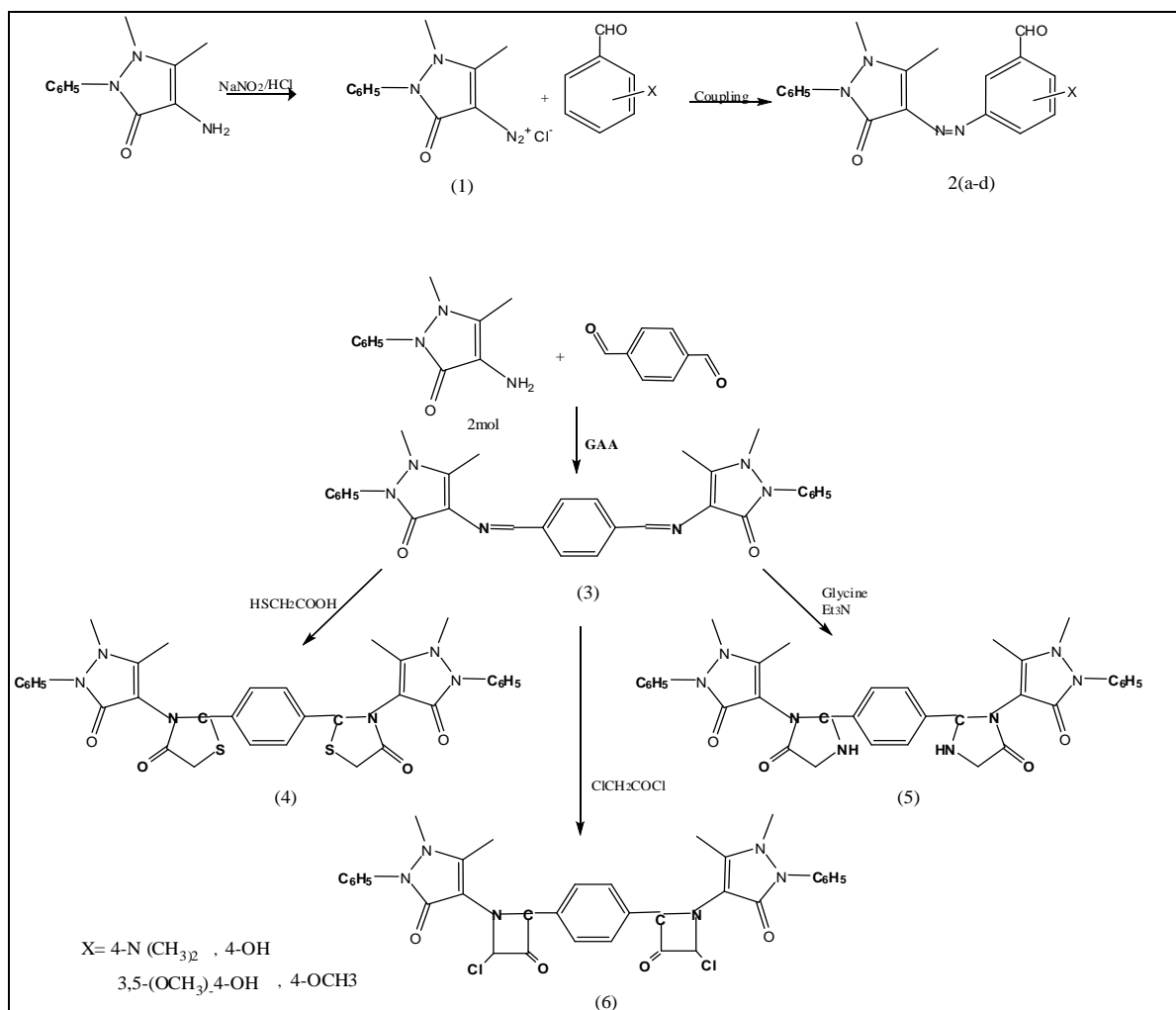
In the recent years there has been a considerable interest in the chemistry of antipyrine and its derivatives because it was the first pyrazoline-5-one derivative used as analgesic antipyretic and anti-inflammatory drug. Its derivatives as well attracted remarkable attention due to their anti-inflammatory [1], analgesic [1,2], anticancer [3], anthelmintic and insecticidal activities [4]. They also considered a significant tool for the synthesis of biologically active Schiff bases and metal complexes [5], in addition to their uses as corrosion inhibitors for copper in an acidic medium and

precursor for the synthesis of macrocyclic compounds [6,7]. Other important derivatives are antipyrine-azo compounds for their applications to polyester fabrics [8] and biological evaluation as antioxidant agents [9]. These azo compounds were prepared from 4-amionantipyrine which also used as intermediate to make poly-functionally substituted heterocyclic rings among them : azetidenone-antipyrine and imidazolidinone-antipyrine derivatives which exhibit antimicrobial activity [10,11] while thiazolidinone-antipyrine derivatives show anticancer activity[12]. Based on the above information and due to our interest in antipyrine as a biologically active pharmacophore it was thought worth while to synthesize some new heterocyclic derivatives incorporating antipyrine moiety starting from 4- amionanti -pyrine followed by their antibacterial activity and cytotoxicity effect on two cancer cell lines including: the human pelvic rhabdomyosarcoma (RD) and the mouse cell line(L₂₀B).

Experimental

A) Materials : The chemicals were supplied from GCC, Merck and Aldrich Chemicals Company and used as received .

B) Techniques : FTIR spectra were recorded using KBr discs on a Shimadzo (Ir prestige-21) FTIR spectrophotometer . ¹H NMR spectra were carried out by company : Bruker Avance (400 MHz) , origin : Switzerland and are reported in ppm(δ), DMSO was used as a solvent with TMS as an internal standard . The mass spectra recorded on MS model: 5975C VL MSD with Tripe-Axis Detector, at Central lab ,Tahran University (Iran). Uncorrected melting points were determined by using Hot-Stage, Gallen Kamp melting point apparatus. Elemental analysis (C.H.N.S) were carried out using an EuroEA Elemental Analyzer at (The Central Service Laboratory -College of Education For Pure Science Ibn Al- Haitham).The antibacterial activity was performed in Center for Market Research and Consumer Protection ,University of Baghdad .



The scheme (1) for synthesized compounds (1- 6).

General procedure

All compounds were synthesized 2_(a-d) and (3-6) according to scheme (1), and the following procedures. All known compounds gave acceptable CHN-S, FTIR and ¹HNMR spectra that matched data reported in the construct to references.

Synthesis of substituted-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) diazenyl] benzaldehyde 2_(a-d) [13]:

A stirred solution of 4- amino antipyrine (2.03g ,0.01 mol) in 2N hydrochloric acid (3 ml) was cooled below (0⁰C) in an ice-salt bath and diazotized with sodium nitrate (0.69g, 0.01 mol) in water (10 ml) with good stirring. The temperature was always kept in (0-5)⁰C. The last part of nitrite solution was add slowly and drop wise. The mixture was complete diazotization and the red color is stable for few minutes[8]. The above cold diazonium solution was added slowly to a well stirred solution to substituted benzaldehydes (0.01 mol) in ethanol(15ml) containing sodium acetate (0.86g, 0.01 mol) and the mixture was cooled in an ice salt bath for 30 minutes. The mixture was left about 2hrs at room temperature. The solid product was filtered, dried and recrystallized from ethanol to give compounds 2_(a-d).

Synthesis of Schiff bases (3): 4,4'- [(1,4-benzaldine amino) bis (N,N` - (1,5-dimethyl -2-phenyl-1H-pyrazol-3(2H)-one)].

A mixture of 4- amino antipyrine (4.06g, 0.02 mol) , terephthalaldehyde (1.34g, 0.01 mol) , in absolute ethanol (15 mL), two drops of glacial acetic acid (GAA) was added and was refluxed for 6hrs. The mixture was then allowed to cool at room temperature [5] , and the residue was filtered and dried, recrystallized from ethanol to give yellow crystals. Yield (75%), m.p = (240-242) °C .

Synthesis of compound(4): 2,2'-[(1,4-phenylene)bis(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)thiazolidin-4-one)] [12].

Compound of Schiff bases (3) (5.04 g, 0.01mol) and thioglycolic acid (0.02 mol) was refluxed in dry benzene (10 mL) for 8 hrs. The solvent was evaporated and the reaction mixture was neutralized with sodium bicarbonate solution, and the off-white solid was filtered, and recrystallized from petroleum ether (60-70)°C. Yield: 60%; m.p. = 94-96°C.

Synthesis of compound(5): 2,2'- [(1,4-phenylene) bis (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) bis(imidazol-5-one-2,1-diyl)].

A mixture of Schiff bases(3) (5.04g,0.01mol) , glycin (1.5g, 0.02 mol) and triethylamine (1mL) in ethanol(15mL) was refluxed for 9hrs. The reaction mixture was neutralized with diluted HCl and then poured into ice-cold water[11]. The precipitate was filtered off, washed with water and recrystallizes from ethanol to give yellowish brown solid (5). Yield (68%), m.p = (189-191) °C .

Synthesis of compound(6): 4,4'- [(1,4-phenylene)bis (1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one) bis (2-chloro-3-oxoazetidine-4,1-diyl)].

Chloroacetylchloride (0.02mol) in 10ml of dioxan cooled at (0-5) °C, to this flask triethylamine (0.01mol) in(10mL) dioxane was added, and compound(3) (5.04g,0.01 mol) in 10mL of dioxane was slowly added and refluxed in water bath for (12hrs) [10]. After the reaction had been completed, the reaction mixture poured into ice-cold water to allow dark yellow solid precipitate , which was filtered and dried. Yield (65%), m.p = (140-142) °C.

Antibacterial activity

Antibacterial activity of synthesized compounds 2_(a-d) - 6 was determined using a disc diffusion method 50 µl of each (*G-*) *Escherichia coli* and (*G+*) *Staphylococcus aureus* bacterial suspension of approximately (10⁸) colony cell/ml was incubated at (37) °C for 24 h, were spread on Muller-Hintone agar using sterile collon. 50 mg/ml solution of synthesized compounds in DMSO, then inhibition zone diameter (mm) were measured table (1). The Antibacterial activity of synthesized compounds comparison with common antibiotic, Amoxicilin and Lincomycin.

Table 1 : antibacterial activity of the synthesized compounds 2_(a,b) - 6

Co mp. no.	Zone of inhibition(mm)		Comp. no.	Zone of inhibition(mm)	
	<i>Esherichia coli</i> (G-)	<i>Staphylococcus aureus</i> (G+)		<i>Esherichia coli</i> (G-)	<i>Staphylococcus aureus</i> (G+)
2a	+	++	4	-	+
2b	-	-	5	-	++
2c	-	++	6	+	+
2d	-	+	Amoxicilin	-	+++
3	+	++	Lincomycin	-	++

(0-3)mm = - , (6-9)mm = + , (10-14)mm = ++, (15-18)mm = +++

Cytotoxicity Assay for synthesized compounds:

Eight compounds 2_(a-d)-6 with different concentrations were screened for their anticancer activity by using cultured cells in microtiter plate-96wells . When cells in the incubated falcon became mono-layer, the confluent monolayer was trypsinized to obtain single cell suspension. A liquot 200 µl/10⁴-10⁵ cells/well from single cell suspension then were added to whole 96 wells of the microtiter plates, which covered by parafilm. In humidified chamber at 37°C, 5% CO₂ ,the plates were incubated until the cells approach to confluence. When the cells are replete of its activity, they were exposed to three concentrations of the synthesized compounds 2_(a-d)-6 µg/ml for cell line . Aliquot of 200µl of each concentration were pipette into each well, while 200µl of maintenance medium were value- added to each well of monitoring group, then plates were locked with parrafilm and put back to the incubator. Estimation of cytotoxicity was carried out after (48) hr. Cell viability was estimated after 48 hr. of exposure by removing the medium, supplement 20 µl/well solution of MTT and incubating for 4 hr. at 37°C. The crystals lagging in the wells were solubilized by the addition of 200 µl/well of (DMSO) directly incubation in 37°C for 15 min with shaking. The absorbance was measured on a microplate reader at (620) nm . The rate of inhibition of cell growth was counted according to follow equation :

$$\text{Inhibition rate} = \frac{\text{mean of control} - \text{mean of treatment}}{\text{mean of control}} \times 100$$

Theis compounds 2_(a-d)-6 were examend their anticancer activity in two cell lines: human pelvic rhabdomyosarcoma (RD) and mice intestines carcinoma cell line (L_{20b}) according to the method described by Freshney[14] . Results are expressed in percentage table (2) . All compounds except [3] showed inhibition for both cell lines , while compound [4] exhibit inhibition more than 40% inhibition for human pelvic rhabdomyosarcoma and mice intestines carcinoma cell line.

Table (2):The inhibition of cells growth of synthesized compounds_{2(a-d)}-6 μ l/well.

Comp.	inhibition of cells growth for RD%	inhibition of cells growth for L ₂₀ b%
2a	45	15.4
2b	39	37.7
2c	41.1	29.5
2d	31.5	25.9
3	-	-
4	42.2	40.7
5	33	26.9
6	26	19.6

Results and discussion :

Azo compound and heterocyclic compounds are very important organic compound having wide spectrum of biological activities. Diazonium coupling reactions are typical electrophilic aromatic substitutions in which the positively charged diazonium ion in the electrophile that interact with the electron-rich ring of substituted benzaldehyd.

The new antipyrene diazenyl benzaldehydes _{2(a-d)} were prepared by coupling of diazotized 4-aminoantipyrene (1) with substituted benzaldehydes at 0-4°C. The novel compounds _{2(a-d)} were identified by their melting points, C.H.N. analysis, FTIR and ¹H NMR spectroscopy. The characteristic FTIR absorption bands of compounds _{2(a-d)} showed the demise of two absorption bands due to NH₂ stretching of 4-aminoantipyrene together with the presence of a band around (1489 -1456)cm⁻¹ which is due to the ν N=N group and two sharp peaks of carbonyl stretching of pyrazole ring, and aldehyde group around (1674-1683) cm⁻¹ and (1716-1708)cm⁻¹, respectively. It also shows a stretching broad band around 3390 cm⁻¹ due to the intermolecular hydrogen bonding of ν O-H .

Compound (2a): 3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl]-4-dimethylaminobenzaldehyd: Yield: 70% ; m.p. = 115-117°C, Light Orange ; IR(KBr) cm⁻¹: 1705 (C=O) aldehyde, 1683 (C=O) pyrazol ring, 1483 (N=N), 1132N(CH₃)₂; ¹H NMR (DMSO) δ ppm: 9.65 (s, 1H, CHO), 6.76-7.67 (m, 8 H, ArH), 3.39 (s, 6 H, N(CH₃)₂), 3.02 (s, 3 H, N-CH₃), 2.49 (s, 3H, CH₃), Anal. Calcd. for C₂₀H₂₁N₅O₂: C, 66.11; H, 5.78; N, 19.28. Found: C, 66.82; H, 5.05; N, 18.87.

Compound (2b): 3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl]-4-hydroxybenzaldehyd: Yield: 58%; m.p. = 124-126°C, Yellowish Brown ; IR(KBr) cm⁻¹: 1708 (C=O) aldehyde, 1697 (C=O) pyrazol ring, 1469(N=N), 3246(OH); ¹H NMR (DMSO) δ ppm: 9.77 (s, 1H, CHO), 9.40 (s, 1H,OH), 7.81-6.94 (m, 8 H, ArH), 3.43 (s, 3 H, N-CH₃), 2.46 (s, 3 H, CH₃), Mass spectrum of compound (2b) showed M+1=336, the peaks at m/z =307, 263, 214, 188, 167, 146, and 119 of characteristic fragmentation of the this compound. Also the fragments at m/z = 77, and 56 for the aromaticity of compound (2b) . Anal. Calcd. for C₁₈H₁₆N₄O₃ :C, 64.28; H, 4.76; N, 16.66.

Found: C, 64.98; H, 4.23; N, 16.04.

Compound (2c): 2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl]-4-hydroxyl-3,5-dimethoxybenzaldehyde: Yield: 60%; m.p. = 159-160°C, Dark orange; IR(KBr) cm^{-1} : 1714 (C=O) aldehyde, 1697 (C=O) pyrazol ring, 1456 (N=N), 3390 (OH), 842(OCH₃); ¹H NMR (DMSO) δ ppm: 9.78 (s, 1H, CHO), 9.39 (s, 1H,OH), 7.22-7.68 (m, 6 H, ArH), 3.85 (s, 3 H, N-CH₃), 3.14-3.44(m,6H,OCH₃), 2.40 (s, 3 H, CH₃), Anal. Calcd. for C₂₀H₂₀N₄O₅ : C, 60.60; H, 5.05; N, 14.14. Found: C, 61.09; H, 4.83; N, 14.74.

Compound (2d): 3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl]-4-methoxybenzaldehyde Yield: 66%; m.p. = 128-130°C, Green- yellow; IR(KBr) cm^{-1} : 1716 (C=O) aldehyde, 1674 (C=O) pyrazol ring, 1471 (N=N), 3390 (OH), 842 (OCH₃); ¹H NMR (DMSO) δ ppm: 9.30 (s, 1H, CHO), 7.55-7.27 (m, 8 H, ArH), 3.85 (s,3H,OCH₃), 3.58 (s, 3 H, N-CH₃), 2.60 (s, 3 H, CH₃), Anal. Calcd. for C₁₉H₁₈N₄O₃: C, 65.14; H, 5.14; N, 16.00. Found: C, 65.89; H, 4.73; N, 15.74.

The novel symmetrical Schiff base (3) was synthesized by refluxing of two moles of 4- aminoantipyrine with one mole of terephthalaldehyde in absolute ethanol with few drops of glacial acetic acid (GAA). This compound (3) was identified by melting point, FTIR and ¹H NMR spectroscopy. FTIR absorption spectra showed the demise of absorption bands due to NH₂ and C=O groups of the starting materials together with presence of new absorption band in the region (1649) cm^{-1} which is due to azomethine group(C=N stretching), peak at 1595 cm^{-1} due to C=C of pyrazole ring and stretching band at 1718 cm^{-1} for C=O of pyrazole ring. Finally, a stretching broad band around 3392 cm^{-1} assigned to the intermolecular hydrogen bonding of ν O-H band. ¹H NMR (DMSO) δ ppm: 7.86-7.36 (m, 14H, ArH), 3.22 (s,2H, CH=N), 3.30 (s, 6 H, N-CH₃), 1.99 (s, 6H, CH₃),

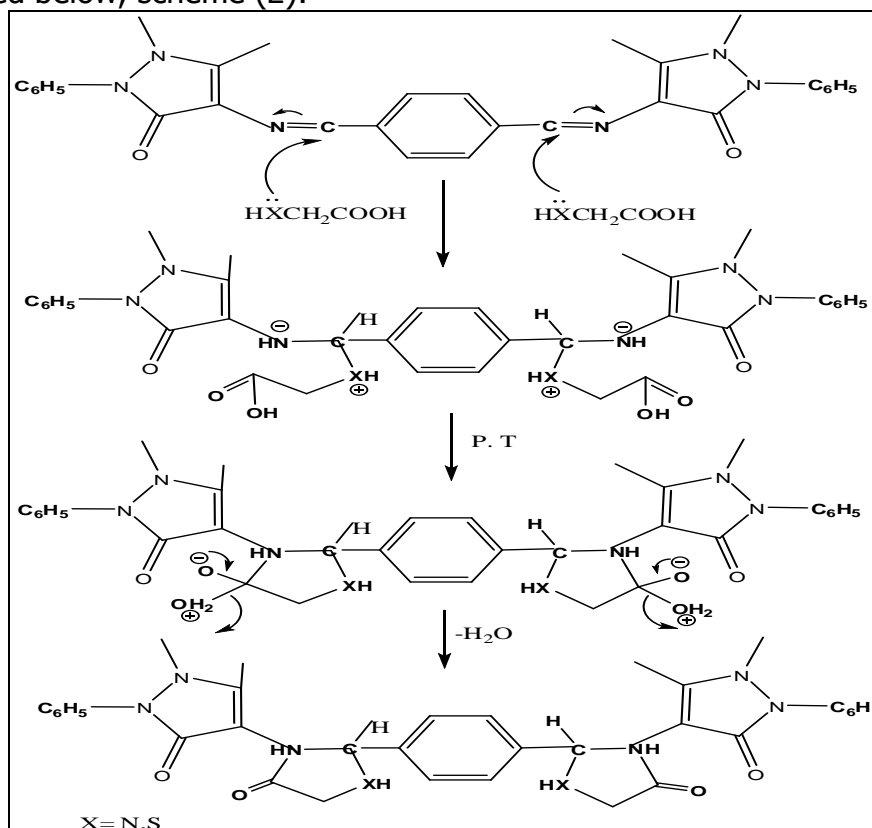
Thiazolidin-4-one derivative (4) was synthesized by refluxing schiff base (3) with two mole of thioglycolic acid was refluxed in dry benzene for 8 hrs. The new compound (4) was characterized by melting points , C.H.N-S analysis , FTIR and, ¹H NMR spectroscopy. The FT-IR spectra for compound (4) showed the disappearance of a stretching band of imine groups and the appearance of a stretching band of carbonyl group of thiazolidinone moiety around (1712-1716) cm^{-1} and C-S groups around (890) cm^{-1} , this was the most characteristic evidence for the success of cyclization step. ¹H NMR (DMSO) δ ppm: 7.51-8.30(d,4H,Ph),7.00-6.69 (m, 10 H, ArH), 5.91 (s, 2 H, CH -N), 4.92 (s,4H,CH₂-S),3.96 (s,6H,N-CH₃), 2.05 (s, 6 H, CH₃). Anal. Calcd. for compound (4) C₃₄H₃₂O₄N₆S₂: C, 61.81; H, 4.84; N, 12.72;S,10.90 Found: C, 61.98; H, 4.25; N, 12.32;S,9.89.

The novel imidazolidinone derivative (5) was synthesized by refluxing Schiff base (3) with two mole of glycine and triethylamine in ethanol for 9hrs. This compound identified by melting point, C.H.N. analysis, FTIR and ¹H NMR spectroscopy. The FTIR spectrum of compound (5) showed bands in the range (3226-3294) cm^{-1} which is assigned to asymmetric and symmetric bands of NH group. A significant band at 1710 cm^{-1} which could be assigned to stretching vibration of the carbonyl of imidazolidinone ring, they also showed the appearance absorption band due to ν O-H group tautomeric for synthesized compound (5). ¹H NMR (DMSO) δ ppm: 9.64 (s,1H ,taut.OH) ,

6.99-7.63 (m, 10 H, ArH), 7.87-8.47 (d,4H,Ph), 4.56 (s,2H,CH₂-N), 4.00 (s, 1 H, NH-CH), ,3.34 (s,3H,N-CH₃), 2.37 (s, 3 H, CH₃). Anal. Calcd. for compound (5) C₃₄H₃₄O₄N₈: C, 66.01; H, 5.50; N, 18.12. Found: C, 65.83; H, 4.95; N, 17.85.

The Schiff base compound (3) was reacted with chloroacetyl chloride followed by the addition of triethylamine under reflux in water bath to yield the corresponding azetidinone derivative (6). The structure of the azetidinone derivative (6) was identified by their melting point, C.H.N analysis, FTIR and ¹HNMR spectroscopy. The FT-IR spectra of compound (6) showed the presence of the characteristic absorption band in region (1651-1660) cm⁻¹ due to stretching vibration of C=O group of azetidine ring. ¹HNMR (DMSO) δ ppm: 9.62(s,1H,taut.OH), 7.40-7.55 (m, 10 H, ArH), 7.89 (d,4H,Ph), 4.37 (s, 2 H, CH -N), 3.57 (s,6H,N-CH₃), 2.51 (s, 6 H, CH₃). Anal. Calcd. for compound (6) C₃₄H₂₈O₄N₆Cl₂: C, 62.10; H, 4.60; N, 12.78. Found: C, 62.99; H, 4.51; N, 12.05.

The suggested mechanism to obtain the target product (4) and (5) is outlined below, scheme (2):



Scheme 2: Mechanism for the synthesis of compounds(4) or(5)

References:

- 1- I. Mohanram and J. Meshram, "Synthesis and Biological Activities of 4- Amino antipyrene Derivatives Derived from Betti-Type Reaction", Hindawi Publishing Corporation ISRN Organic Chemistry, Volume 2014, Article ID 639392, 7 pages.
- 2- A. A. Fadda and K. M. Elattar, "Design and Synthesis of Some Enamino nitrile Derivatives of Antipyrene as Potential Novel Anti-Inflammatory and Analgesic Agents", Journal of Biosciences and Medicines, 3,2015, pp. 114-123.

- 3- D. Premnath, P. Mosae Selvakumar, P. Ravichandiran , G. Tamil Selvan, M. Indiraleka , J. Jannet Vennila," Synthesis and spectroscopic characterization of fluorescent 4-aminoantipyrine analogues: Molecular docking and in vitro cytotoxicity studies", *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 153, 2015, pp. 118–123.
- 4- M. Himaja, R. Kailash, K.V. Anish, M.V. Ramana and A.A. Karigar, "Synthesis And Evaluation of Anthelmintic And Insecticidal Activities of 4-Amino-Antipyrine Derivatives of Amino Acids And Peptides", *Journal of Pharmaceutical and Scientific Innovation*, 1 (1), 2012, pp. 67-70.
- 5- P. Deshmukh, P. Kumar Soni, A. Kankoriya, A. K. Halve and R. Dixit," 4-Aminoantipyrine: A Significant Tool for the Synthesis of Biologically Active Schiff Bases and Metal Complexes", *Int. J. Pharm. Sci. Rev. Res.*, 34(1), Article No. 26, 2015, pp.162-170.
- 6- A. M. Eldesoky, M. A. Diab, A. A. El-Bindary, A. Z. El-Sonbati, H. A. Seyam, " Some antipyrine derivatives as corrosion inhibitors for copper in acidic medium: Experimental and quantum chemical molecular dynamics approach", *J. Mater. Environ. Sci.*, 6 (8), 2015, pp. 2148-2165.
- 7- M. Muthukkumar, M. Malathy and R. Rajavel," Synthesis and spectral characterization of new mononuclear Schiff base complexes derived from 4-aminoantipyrine, 2, 3-butadione and o-phenylenediamine", *Der Chemica Sinica*, 6 (11), 2015, pp.12-20.
- 8- A. A. Fadda and K. M. Elattar, "Synthesis of Novel Azo Disperse dyes Derived from 4-Aminoantipyrine and their Applications to Polyester Fabrics", *American Journal of Organic Chemistry*, 2 (3), 2012, pp. 52-57.
- 9- A. A. Fadda and K. M. Elattar, " Efficient and Convenient Route for the Synthesis of Some New Antipyrinyl Monoazo Dyes: Application to Polyester Fibers and Biological Evaluation", *Hindawi Publishing Corporation Journal of Chemistry*, Volume 2013, Article ID 928106, 10 pages.
- 10- A. K. Yadav, I. Shukla, C. R. Sahu and S. R.Sahu," Synthesis and Antimicrobial Evaluation of some novel 4-[4-(3-chloro-2oxo-4-substituted-phenylazetidid-1-yl)phenylamino]-1,2-dihydro-2,3-dimethyl-1-phenyl pyrazole-5-one Indo", *American Journal of Pharmaceutical Research*, 2 (8), 2012, pp. 800-805.
- 11- H. A. Elhady, H. M. Aly and N. M. Saleh," Syntheses and antimicrobial activity of new benzofuran, pyrrole, imidazole and thioxoimidazolidin incorporating antipyrine moiety, *International Journal of Advanced Research*, Volume 2, Issue 3, 2014, pp. 806-816.
- 12- M. Nataliya , R. Rostyslav , I. Iryna, Yu.Olga , P.Liliya, B. Roman, S.Lesya, and S.Rostyslav ,"Putative anticancer potential of novel 4-thiazolidinone derivatives: cytotoxicity toward rat C6 glioma *in vitro* and correlation of general toxicity with the balance of free radical oxidation in rats", *Croatian Medical Journal*, 2016, 57(2),pp.151–163.
- 13- O.A. Adegoke and O.E. Umoh, "A new approach to the spectrophotometric determination of metronidazole and tinidazole using p-dimethylamino - benzaldehyde", *Acta Pharm.* 59, 2009, pp.407-419.
- 14- R.I. Freshney, "Culture of Animal Cells: A manual of Basic Technique and Specialized Applications," 6th Edition, Wiley: New York, 2010.