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Synthesis and biological evaluation of novel pyridazine analogues (3-aldehyde-1-phenylpyridazine derivatives)

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ABSTRACT

Five new pyridazine compounds were synthesized. The usefulness of IR, ^{1}HNMR and ^{13}CNMR spectroscopy for the structural analysis of the new compounds is well established. The differences in molecular structure between one absorbing compound and another affected a shift in absorption wavelength (λmax). The structure-activity relationship among the pyridazine derivatives was measured against some pathogens. Compound 3e was found to be a more active drug than the standard antibiotic, streptomycin SO_4.

Keywords: Biological evaluation, novel, pyridazine derivatives, antimicrobial agents.

INTRODUCTION

Pyradazine has no household use. It is mainly used in research and pharmaceuticals as building blocks for more complex compounds (Bourguignon et al., 2006). Interest in pyridazine derivatives has remained unabated due to their biological activities as antiplatelet agents (Coetho et al., 2004), antimicrobial agents (Deeb et al., 2004), anti-inflamatory agent (Webster, 2002) and anti-depresant (Nemmeroff, 1988). Attempts have been made by Chemists to synthesize and characterize compounds containing pyridazine functionality in order to explore the usefulness of this heterocyclic template. Generally, the presence of a pyridazine structure in a bioactive molecule confers good bioavailability, especially with the central nervous system (CNS). The purpose of this study was to synthesize, evaluate the biological importance of 3-aldehyde-1-phenylpyridazine derivatives (3a-e) as presented in scheme 1 and examine structure-activity relationship.

MATERIALS AND METHODS

All chemicals were obtained from Chemistry Department, Kogi State University and were used without further purification. The melting points (m.p) were determined on a SMP3 melting point apparatus and were reported in °C uncorrected. Column Chromatography was performed on Scharlan Silica gel 60 (70-230 mesh). Elemental analysis was performed using a Perkin-Elmer 2400 II CHN Analyser (Paperno et al, 1997).

The infrared (IR) spectra were recorded in CM^{-1} on a Bulk Scientific 500 spectrophotometer. The ^{1}H and ^{13}C-NMR spectra were recorded on a varian Germini 2000 spectrophotometer operating 200 and 50 MH_z respectively. Chemical shifts were recorded as б values in PPM referenced to the solvent. HPLC separations were performed on a Bulk Scientific 500 apparatus using a reverse phase Lichrospher 100 RP-18 (5 M) column, at room temperature (eluent: methanol/water 8:2, v/v). Antimicrobia activity tests were carried out at the microbiology Laboratory Faculty of Natural Sciences Kogi State University. The

compounds (3a-e) were prepared according to the method of Dilek et al. (2006) and Alberto et al. (2003).

3-aldehyde-1-phenylpyridazine (3a)

This compound was prepared by refluxing an equimolar mixture of furfural (1.7 ml, 0.02 mol) and phenylhydrazine 92.89 g, 0.02 mol) in toluene (20 ml) for four hours. The solvent (toluene) was removed by evaporation and the oily residue was treated with diethyl ether overnight. The crude product formed was crystallized from acetic acid to give (58%) of 3a. m.p:356 °C-358 °C. IR:1714 (C=O), 3475 (NH-Ar), 1670 (C-N), 886 (C-H-Ar), 1391 (C-H bending), 1600 (C=C-Ar), 2675.60 (C-H stretchers). ^{1}H NMR (200 MH_z): 7.798 (s.Ar-H), 10.724 (s.NH), ^{13}CNMR (50 MH_z): 1842(s,C=O), 180.74 (s. C3), 169.82 (s.C4), 186.32 (s.C5) 125.34 (s.C6). 148.72-136.40 (m. Ar.C)

3-aldehyde-1,5,6,-triphenylpyridazine (3b)

The general procedure as in 3a was applied by reacting compound 1b with 2b. This was refluxed for 4 hrs, resulting in (55%) of 3b. mp: 403-406 °C. IR: 3452 (NH-Ar), 1718 (C=O), 882 (C-H-Ar), 1684 (C-N), 1606 (C=C-Ar) ^{1}H NMR (200 MH_z): 7.890 (s.Ar-H), 10.926 (s.N-H), ^{13}CNMR (50 MH_z): 186.48 (m.C=O), 181.62 (s.C3) 169.70 (s.C4), 190.40 (s.C5), 140.28 (C6) 152.68-147.10 (m.Ar.C).

3-aldehyde-1-orthoanizidine-5-hydroxy-pyridazine (3c)

Compound 3c was prepared by reacting compound 1c with 2c. Refluxing was for 4 hrs. This yielded (60%) of 3c mp: 368-371 °C. IR: 1715 (C=O), 3478 (NH-Ar) 1672 (C-N), 886 (C-H-Ar), 3300 (OH), 3450 (NH_2) ^{1}HNMR (200 MH_z): 4.79(s,OCH_3), 5.890(s.NH_2), 10.340 (s.N-H), 7.635 (s.Ar-H) ^{13}CNMR (50 MH_z): 183.80 (s.C=O), 181.71 (sC_3) 167.71 (s.C4), 184.38 (s.C5), 123.38 (s.C6), 147.42-138.39 (m.Ar.C).

3-aldehyde-1-aniline-5,6-dihydroxy pyridazine (3d)

Compound 3d was prepared according to the general procedure by the reaction of 1d and 2d with a reflux time of 4 hrs. This gave a yield of 65%. mp: 370-373 °C. IR: 1720 (C=O), 3478 (NH-Ar), 1680 (C-N), 888 (C-H-Ar), 3402 (NH_2), 3350 (OH). ^{1}HNMR (200 MH_z): 5.920 (s.NH_2), 10.410 (s.N-H), 7.813 (s.Ar-H). ^{13}CNMR (50 MH_z). 182.81 (s.C=O), 182.92 (s.C_3) 167.83 (s.C_4), 190.48 (s.C5), 127.39 (s.C6) 149.68-139.47 (m. Ar. C).

3-aldehyde-5-hydroxy-1-otho-toluidine-6-prenylpyradazine (3e)

Compound 3e was prepared according to the general procedure by the reaction of 1e and 2e with a reflux time of 4hr. This gave a yield of 67%. mp: 382-385 $^{\circ}$C. IR: 1723 (C=O), 3476 (NH-Ar), 1683 (C-N) 884 (C-H-Ar), 3459 (NH_2), 3353 (OH), 1381 (-C=C). ^{1}HNMR (200 MH_z): 6.830 (s.NH_2), 9.894 (s.N-H), 7.481 (s.Ar.H), 2.42 (s.CH_3) ^{13}CNMR (50MH_z): 182.70(s.C=O), 182.96 (s.C_3), 167.90 (s.C_4), 190.50 (s.C5), 124.91 (s.C6) 148.71-146.16 (m.Ar.C).

Antimicrobial activity test

The antimicrobial screening method employed was the Agar-Dilution streak methods of Akpulu et al. *(*1994) and Peter et al. (2002). The antimicrobial activity was measured by determining the concentration of agent needed to inhibit the growth of test microorganisms.

Clinical isolates, including *Pseudomonas* species, *Escherichia* species, *Citrobacter* species, *Salmonella* species, *Shigella* species, *M. Spegmatis* species, *Klebsiella* species and *S. aureus* species, were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD).

The Agar and Pyridazine sample were poured into sterile dishes, allowed to set and inoculated with the organism by streaking and incubated at 37 °C for 24 hrs in duplicate. Control plates which contained no Pyridazine sample to ensure viability and for comparison were similarly inoculated and incubated. After incubation, susceptibility and inhibition were measured by absence of growth.

RESULTS

The structures of compounds 3a-e were confirmed by elemental analysis, IR, ^{1}HNMR and ^{13}CNMR data obtained. The spectral analysis of the compounds (3a-e) confirmed the characteristic functional groups, the types

of bonds present, protons and carbon atoms present.

From scheme 1, it is believed that the reaction starts with the interaction of the nitrogen atom bearing R_2 radical in phenylhydrazine and C5 of the furan-2-aldehyde (Dilek et al., 2006). This suggests that the interaction of C5 and nitrogen constitutes the first stage of the reaction.

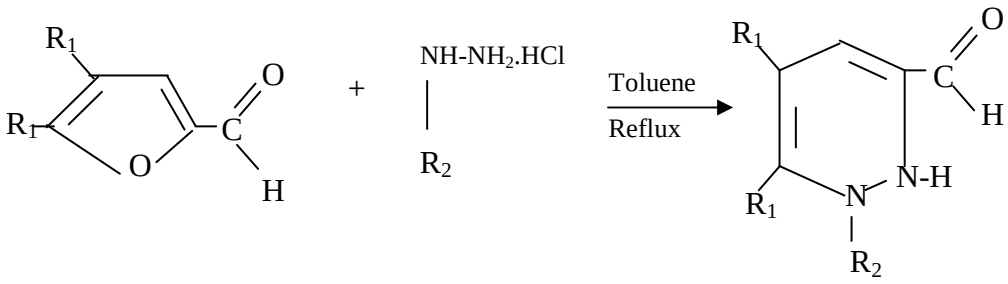
From Table 1, it is evident that compound 3a absorbed at 360 nm, while pyridazine molecule absorbed at 246 nm. This bathochromic shift may be assigned to substituent groups attached to the pyridazine ring in compound 3a, that is the phenyl group at postion-1 and the carbonyl group at position 3.

Compound, 3b-e absorbed in the range 367-390 nm. This powerful bathochromic shift is probably due to high molecular weight of the compounds and the presence of a prenyl group in compound 3e which increased the unsaturation of the compound.

The results of the biological tests are as presented in Table 2.

Table 1: Some physical characteristics of synthesized compounds.

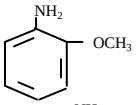
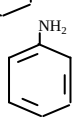
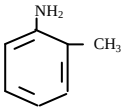
Compound	λmax (nm)	Melting point (°C)
Pyridazine	246	-8
3a	360	356-358
3b	381	403-406
3c	367	368-371
3d	372	370-373
3e	390	382-385



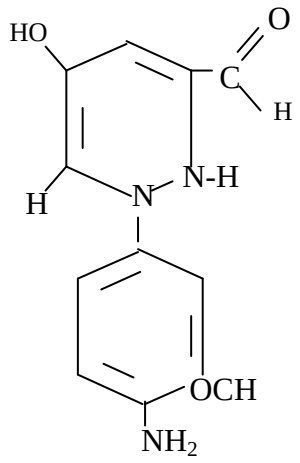
	1	2	3	
1	R_1	R_2	R_1	R_2
a	H	Ph	H	ph
b	Ph	Ph	Ph	Ph
c	OH,H	o-anizidin	O H	o-anizidin
d	OHOH	aniline	OH	aniline
e	OH, Prenyl group	o-toluidine	OH, Prenyl	o-toluidine

Scheme 1: Synthesis of pyridazine-3-al derivatives.

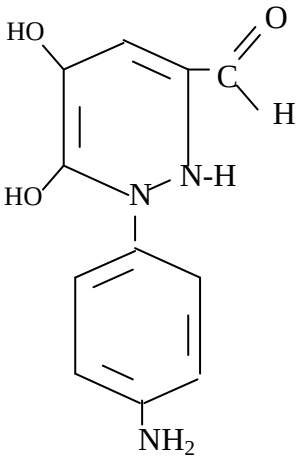
Table 2: Structure and antibacterial activity (MIC) of pyridazine derivatives against some pathogens.

Pyridazine derivative	Substituents			Pathogens (MIC (µg/ml)							
	R_1 position 5	R_1 position 6	R_2	*K.s*	P.s	Sh.s	Ec	S.s	C.s	S.a	M.s
3-aldehyde-1-phenylpyridazine	H	H	Ph	4.93	2.78	4.20	3.16	3.94	3.43	4.26	5.13
3-aldehyde-1,5,6-triphenylpyridazine	Ph	Ph	Ph	4.74	2.67	3.90	2.94	3.52	3.35	4.12	4.96
3-aldehyde-1-othoanizidine-5-hydroxypyridazine	OH	H		3.90	4.38	3.48	7.96	3.47	2.98	3.40	4.53
3-aldehyde-1-aniline-5,6-dihydroxypyridazine	OH	OH		3.85	1.32	3.24	1.84	3.36	2.78	3.23	4.20
3-aldehyde-5-hydroxy-1-otho-toluidine-6-prenyl(pentene) pyridazine	OH	Prenyl		3.59	0.73	3.12	0.96	2.17	1.53	3.19	3.96
Streptomycin SO_4				3.18	4.32	3.65	5.90	4.48	5.28	2.74	3.42

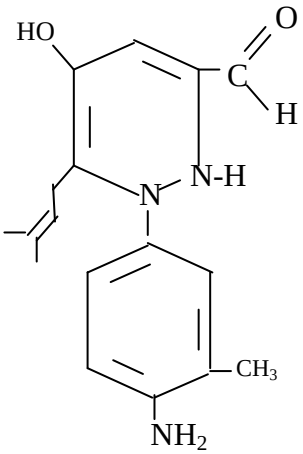
K.s: *Klebsiella* species; P.s: Pseudomonas species; Sh.s: Shigella species; Ec: E. coli; S.s: Salmonela sp; C.s: Citrobacter sp; S.a: S. aureus; M.s: M. smegmatis;



3c



3d



3e

DISCUSSION

Table 1 supports the fact that relatively minor differences in molecular structure between one absorbing substance and another may bring about a shift in absorption wavelength, λmax. As one and more unsaturated linkages occur in the molecule, so Δmax is displaced towards longer wavelength. This shows that structural factors determine whether or not a molecule will absorb in the visible. They also decide where such absorption will occur (Abrahart, 1968). The high melting point of the synthesized compounds (3a-e) is an indication of stability of the compounds.

The structure activity relationship among the pyridazine derivatives were measured against eight pathogens (Okwute and Mitscher, 1992). The activity of the

pyridazine compounds against the eight bacterial strains are provided in table 2. The activity of the compounds varies depending on structure. From table 2, all the compounds were active on the eight microorganisms. Compound 3e showed the greatest activity of 0.73 µg/ml against *Pseudomonas* species, 0.96 µg/ml against *Escherichia coli*, 1.53 µg/ml against *Citrobacter* species and 2.17 µg/ml against *salmonella* species. This table also revealed compounds 3e to be a more active drug on *pseudomonas* species, *shigella* species, *Escherichia coli*, *Salmonella* species and *Citrobecter* species than the standard antibiotic, streptomycin SO_4.

Generally, all the compounds showed activity between 0.73 µg/ml against *pseudomonas* species and 5.13 µg/ml against *M. spegmatis.* Interestingly, compound 3c and 3d also showed remarkable activity on all the organisms. Examination of all the compounds revealed some important structural requirements for the antibacterial activity.

The presence of prenyl, hydroxyl and amino groups probably accounts for the higher activity of compounds 3c, 3d and 3e shown below.

The similarity on the activity of compounds 3c and 3d on all the microorganisms could be attributed to the presence of OH group on the pyridazine ring of the compounds but the presence of two hydroxyl groups on compound 3d might be responsible for its higher activity.

All the compounds possess OH group except compounds 3a&3b. This could be responsible for their lower activity than streptomysin SO_4 against *Klebsiella* sp. *Shigella* sp, *S. aereus* and *M. smegmatis.*

The presence of prenyl group in compound 3e enhanced the activity. This tends to suggest that the mode of antibacterial action may be aided by the presence of prenyl and hydroxyl groups on the pyridazine ring and to some extent the presence of amino group on the aromatic ring. The compounds possessing these groups on both ring displayed appreciable antibacterial activity, while those which completely lack these groups exhibited decrease activity.

This suggests that the substitution partern in each of the rings is a strong contributory factor to the degree of activity of the pyridazine compounds. Hence compound 3e has a greater activity than 3d, which in turn is greater than compound 3c.

The OH group increases the lipophilic character of the pyridazine compounds that possess it. The group increases the ability of the compounds to permeat the cell-wall of the microorganism. The prenyl group enhances this permeation. Instead of the compound being traped in the bacterial that have high cell-wall lipid content, they are able to permeate the organism (compound 3e) (Okwute and Mitscher, 1992)

The exact role of the amino group is not understood but its importance to antimicrobial activity of some flavonoids has been reported (Odin et al., 2003)

The mode of action

The mechanism of action (Giovannoni et al., 2007) of the new pyridazine compounds consist of the following categories: Inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function and inhibition of protein synthesis. In gram-positive bacteria, the cell wall consist largely of a thick layer of peptidoglycan, which gives the cell rigidity and maintain a high internal osmotic pressure. In gram-negative bacteria, this layer is thinner and the internal osmotic pressure is correspondingly lower. The action of the pyridazine compounds is to block peptidoglycan synthesis, which severely weakens the cell wall. The compounds also interfere with protein synthesis by inhibiting the binding of aminoacyl tRNA to the recognition site and prevents peptide bonds from forming.

Conclusion

The high melting points indicate the stability of the synthesized compounds. The spectral data were used in the elucidation of the structures of the compounds. Structural factors affected absorbance (λmax) and the activity of the new pyridazines.

Compound 3e was found to be more active on *Pseudomonas* species, *Shigella* species, *Escherichia coli*, *Salmonella* species and *Cintobacter* species than the standard antibiotic, streptomycin SO_4. The presence of

prenyl group in the compound might be responsible for this activity.

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