Synthesis and Antibacterial Activities of 1,2,3,4,6,7,8,9-octahydro-1,3,7,9-tetra phenyl 5-pyrrolo-2,4,6,8-tetraoxo-10*H*, 5*H* pyrido[2,3-*d*; 6,5-*d*']dipyrimidine

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Abstract

Treatment of 1,3-diarylbarbituric acid with pyrrole-2-carbaldehyde and liquor ammonia in anhydrous ethanol for two hours afforded the requisite 1,2,3,4,6,7,8,9-octahydro-1,3,7,9-tetra phenyl 5-pyrrolo-2,4,6,8-tetraoxo-10*H*, 5*H* pyrido[2,3-*d*; 6,5-*d*']dipyrimidine. All the synthesized compounds have also been screened for their antibacterial activities against *Salmonella typhi* and *Aeromonas hydrophila.*

Introduction

The development of new methods for the synthesis of nitrogen heterocycles especially biannulated pyridines has been given considerable importance in Organic Chemistry. Pioneering work on the synthesis of these heterocycles were done by various routes,[1-6] owing to their broad spectrum of physiological and pharmacological properties, biomimetic oxidations[7,8], antibacterial[9,10], antineoplastic activities[11] etc. Barbituric acid has been employed as starting material for preparation of many heterocycles bearing pyrimidine nucleus.[12-14] However, earlier methods, fail to provide the pyrido[2,3-*d*; 6,5-*d*']dipyrimidines with a substituent at C_5 position. This paper incorporates the synthesize of newer pyrido[2,3-*d*;6,5-*d*'] dipyrimidine derivatives of such kind by the reaction of 1,3-diarylbarbituric acids with an amine and an aldehyde in absolute ethanol at reflux temperature for two hrs.

Experimental

Thin layer chromatography was used to access the reactions and purity of products. Mps. were determined on a Boetius Microheating Table and Mettler-FP5 melting apparatus and are uncorrected. IR spectra were obtained on Shimadzu–8201FT IR instrument as KBr pellets and only noteworthy absorption levels (cm^{-1}) are listed. ^{1}H-NMR spectra were recorded on Varian AMX-400 MHz spectrometer in $CDCl_3$ solution; chemical shifts are expressed in ppm (δ) relative TMS, coupling constants (J) in Hz and signal multiplicities are represented by s(singlet) and m(multiplet). Mass spectra were determined on a Jeol SX- 102 mass spectrometer. CHN analyses were carried out on carlo Erba 106 and Perkin-Elmer Model 240 analysers. The starting substrates have been prepared by earlier reported methods.[15,16]

General Procedure for the Synthesis of pyrido dipyrimidines

Respective 1,3-Diphenyl barbituric acid (**1a-e**, 0.002 mole), pyrrole-2-carbaldehyde (0.001 mole) directly purchased from Merck Company and liquor ammonia (2 mL) after necessary purification in anhydrous ethanol (50 ml), were refluxed for about 2 hrs. After the completion of reaction, inferred through TLC, the reaction mixture was reduced to about half of its volume and allowed to cool. The solid separated was collected and recrystallized from $CHCl_3$-MeOH(1:1) mixture.

Results and Discussion

1,3-diphenyl barbituric acid and pyrrole-2-carbaldehyde in liquor ammonia were refluxed in anhydrous ethanol for 2 hours. After the completion of the reaction, inferred through TLC studies, the volume was reduced to half and allowed to cool slowly. The separated solid was washed well and recrystallized with chloroform/methanol(1:1). The yield was 74 % and its melting point above 300 °C. The IR spectrum showed strong absorption bands at 1683 cm^{-1} for –N-CO-C groups, 1654 cm^{-1} for N-CO-N groups and 3100 – 3350 cm^{-1} for –NH groups. The ^{1}H-NMR spectrum revealed a sharp singlet at δ 6.6 for the C_5 methine proton. The other singlets at δ 8.5 and δ 13.1 were attributed to pyrrole –NH and pyrido –NH respectively. All the other twenty-three aromatic proton resonances exhibited their absorptions between δ 7.2 – 7.6 as an unresolved multiplet. The mass spectrum indicated the molecular ion peak at m/z 618. The elemental analysis further corroborated with the molecular formula $C_{37}H_{26}N_6O_4$. All the above spectral and analytical data support the structure of **3a** as 1,2,3,4,6,7,8,9-octahydro-1,3,7,9-tetra phenyl 5-pyrrolo-2,4,6,8-tetraoxo-10*H*, 5*H* pyrido[2,3-*d*; 6,5-*d*']dipyrimidine.

The plausible mechanistic pathway is shown in scheme I. The reaction may be proceeded via the formation of a bis-product through the Micheal addition of 1,3-diarylbarbituric acid to the 5-arylidine-1,3-diarylbarbituric acid which further react with liquor ammonia to give the final product. Similar series of compounds were prepared using (**1b-f**) as the starting substrates (Table I).

Table I - Physical and spectral data of compounds **3a-f**

Compd	m.p. °C	Yield (%)	Mol. formula (Mol. Wt)	Calcd % (Found)			^{1}H NMR
				C	H	N	
3a	>300	74	$C_{37}H_{26}N_6O_4$ (618.64)	71.83 (71.75)	4.24 (4.17)	13.58 (13.51)	δ 6.6(s, C_5-H), δ 7.2-7.6(m, 23H,Ar-H), δ 8.5(s, 1H, pyrrole –NH), δ 13.1(s, 1H, pyrido – NH)
3b	280	83	$C_{41}H_{34}N_6O_4$ (674.75)	72.98 (72.87)	5.08 (5.01)	12.46 (12.52)	δ 2.6(s, 12H, 4xCH_3), δ 6.6(s, 1H, C_5-H), δ 7.1-7.7(m, 19H, Ar-H), δ 8.6(s, 1H, pyrrole –NH), δ 13.2(s, 1H, pyrido –NH)
3c	>300	81	$C_{41}H_{34}N_6O_4$ (674.75)	72.98 (72.93)	5.08 (4.97)	12.46 (12.41)	δ 2.3(s, 12H, 4xCH_3), δ 6.3(s, 1H, C_5-H), δ 7.1-7.9(m, 19H, Ar-H), δ 8.5(s, 1H, pyrrole –NH), δ 13.1(s, 1H, pyrido –NH)
3d	decomp 260	69	$C_{41}H_{34}N_6O_8$ (738.74)	66.66 (66.70)	4.64 (4.58)	11.38 (11.42)	δ 3.8(s, 12H, 4xOCH_3), δ 6.3(s, 1H, C_5-H), δ 6.9-7.8(m, 19H, Ar-H), δ 8.2(s, 1H, pyrrole –NH), δ 12.8(s, 1H, pyrido –NH)
3e	decomp 285	72	$C_{41}H_{34}N_6O_8$ (738.74)	66.66 (66.58)	4.64 (4.56)	11.38 (11.32)	δ 4.0(s, 12H, 4xOCH_3), δ 6.5(s, 1H, C_5-H), δ 7.0-7.9(m, 19H, Ar-H), δ 9.01(s, 1H, pyrrole –NH), δ 12.4(s, 1H, pyrido –NH)
3f	202	63	$C_{37}H_{22}Cl_4N_6O_4$ (756.42)	58.75 (58.68)	2.93 (2.86)	11.11 (11.04)	δ 6.9(s, C_5-H), δ 7.1-8.1(m, 19H,Ar-H), δ 9.0(s, 1H, pyrrole –NH), δ 13.3(s, 1H, pyrido – NH)

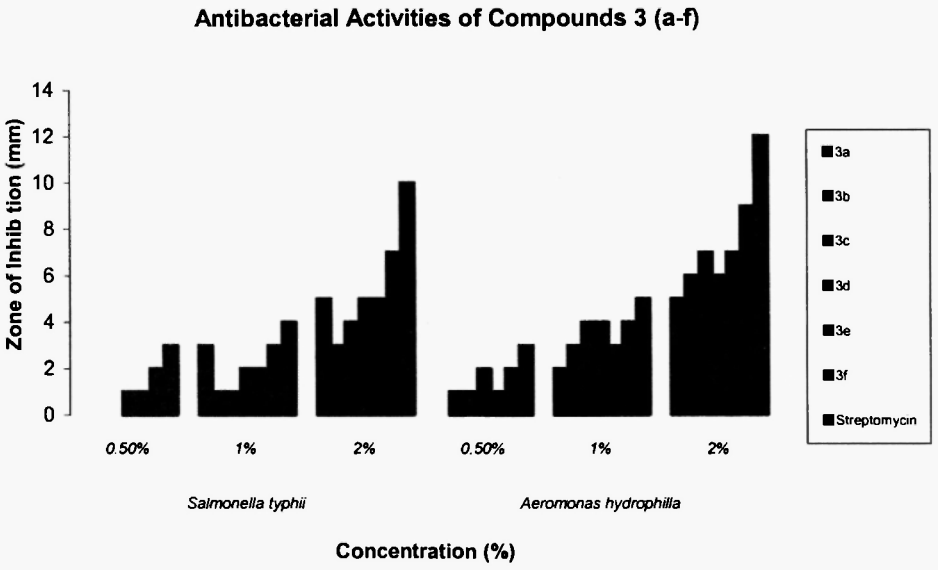
Antibacterial Studies

Antibacterial screening for the *in vitro* growth inhibitory activity against *Salmonella typhi* and *Aeromonas hydrophilla* were done for the compounds by using the disc diffusion method.[17,18] Bacteria were cultured in nutrient agar medium and used as inoculum for study. Bacterial cells were swabbed on to nutrient agar medium [prepared from NaCl (5.0 g), Peptone (5.0 g), beef extract powder (3.0 g), yeast extract powder (3.0 g), Agar (20.0 g) in 100 ml distilled water; pH = 7.5 ± 0.2)] in petri plates. The compounds to be tested were dissolved in chloroform to a final concentration of 0.5 %, 1 % and 2 % and soaked in filter paper discs of 5 mm diameter and 1 mm thickness. These discs were placed on the already seeded plates and incubated at 35 ± 2 ° C for 24 hours. The diameter (mm) of the inhibition zone around each disc was measured after 24 hours and results are listed in Table II and a graph is plotted between concentration and zone of inhibition (Fig.I). Streptomycin was used as standard.

Table 2. Antibacterial Activity of Compounds **3a-f**

Compound	Diameter of inhibition zone in mm					
	Salmonella typhii			*Aeromonos hydrophilla*		
	0.5 %	1%	2%	0.5 %	1%	2%
3a	-	3	5	-	2	5
3b	-	1	3	1	3	6
3c	-	1	4	1	4	7
3d	1	2	5	2	4	6
3e	1	2	5	1	3	7
3f	2	3	7	2	4	9
Streptomycin	3	4	10	3	5	12

Fig.I



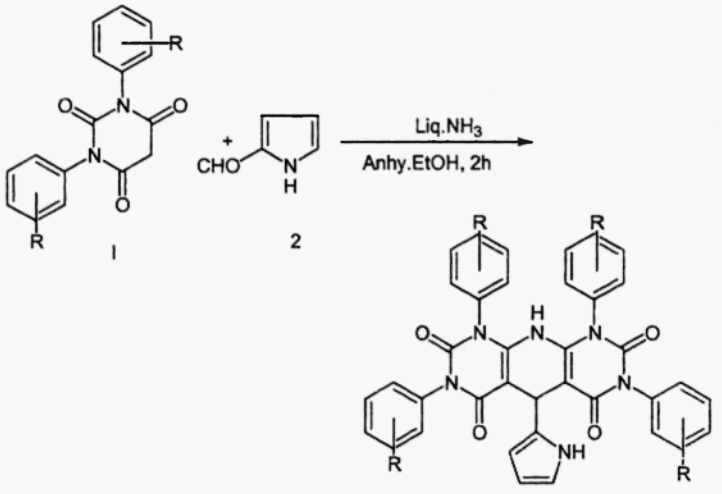
All compounds exhibited good activity against both the species of bacteria. From the graphical observation,

i. the toxicity increases with increase in concentration of test solution containing new compounds.

ii. all the compounds are active except at lower percentage and they did not reach the effectiveness of conventional bacterostatic streptomycin.

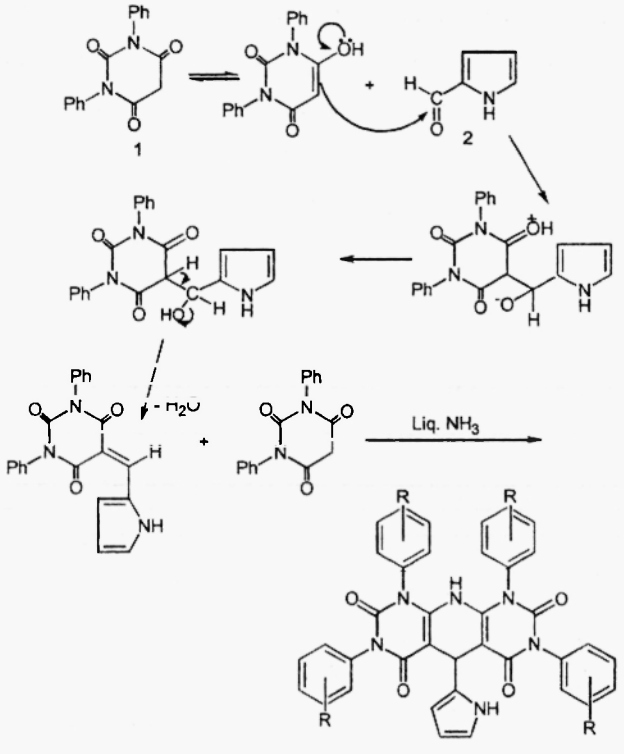
iii. The variation in effectiveness of different compounds against different organisms depend either on impermeability of cells of the microbes or diffusion in ribosomes of microbial cells.[19]

Scheme I



a) R = H b) R = 2-CH_3 c) R = 4-CH_3 d) R = 2-OCH_3 e) R = 4-OCH_3 f) R = 4-Cl

The Mechanism



Conclusion

In conclusion, we have demonstrated the synthesis of newer derivatives of pyrido dithiopyrimidines in a one-pot method through Micheal addition. The antibacterial studies of all compounds show their biological importance.

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