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Synthesis, characterization and biological activity of some new 1,2,4-triazine derivatives

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Abstract: The title compound 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2 H)-one)pyridine **6**, molecular formula $C_{29}H_{43}N_5O$, was obtained through a multi steps reactions using tricyclic ketone, anthrone, as starting material. All the newly synthesized compounds were characterized using spectroscopic methods such as FTIR, ^{1H}NMR, ^{13C}NMR and elemental analysis. All synthesized compounds were primary in *vitro* screened for their antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* PTCC 1023) and Gram-negative (*Escherichia coil* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027) bacteria by the drug diffusion method.
Keywords: Carbohydrazides, 1,2,4-triazine, spectral studies, synthesis, biological activity.

Introduction

Hydrazides and related compounds have been described as useful building blocks for the assembly of various heterocyclic rings [1]. A large number of aliphatic, alicyclic, aromatic and heterocyclic carbohydrazides, their derivatives and related compounds are reported to present a plethora of biological activities [2]. Thus, different carbohydrazides were found to be useful as medicaments especially in the treatment of inflammatory and autoimmune diseases, osteoarthritis, respiratory diseases, tumors, cachexia, cardiovascular diseases, fever, hemorrhage and sepsis. Carbohydrazides and related compounds exhibited antifungal [3], antiviral [4], bacteriostatic [5, 6], antiparasite [7], antituberculous [8], psychotropic, and insecticidal [9] activities. Some heterocyclic carbohydrazides are useful as antifertility agents in rats and pigeons [10]. Other carbohydrazides were reported to be components of deodorant compositions that can be used for removal of offensive odor components [11]. In the last decade numerous 1,2,4-triazine derivatives have been synthesized and screened in *vitro*/*vivo*, thus revealing their varied biochemical, biological, pharmacological or cellular activities [12]. These facts encouraged us to synthesize some new 1,2,4-triazine derivative, their derivatives in anticipation of expected interesting biological activities.

Materials and methods

Synthesis of N-anthracen-9(10H)-ylidene-4-methylpyridine-2-amine **2**

A mixture of anthrone (0.012 mol), 15 ml glacial acetic acid and 2-amino-4-methylpyridine (0.012 mol) was heated under reflux for 10 hrs. The reaction mixture was filtered off and recrystalized from ethanol.

Synthesis of 2-(anthracen-9(10H)-ylideneamino)-4-carboxylic acidpyridine **3**

Compound **2** (0.05 mol) is added to a solution of (0.05 mol) of potassium permanganate and (0.05 mol) of sodium carbonate in (25 ml) water and the mixture is heated under reflux until the color of the permanganate has disappeared (15 hrs). The reaction mixture was filtered while still hot to get rid of the MnO_2 precipitate. The cooled filtrate is acidified with sulphuric acid (20 %), the carboxylic acid precipitate is filtered off, washed with a little cold water and used without further purification.

***Synthesis of ethyl 2-(anthracen-9(10H)-ylideneamino)pyridine -4-carboxylate* 4**

A mixture of the acid **3** (0.01 mol), abs. ethanol (10 ml), and few drops of conc. sulfuric acid was refluxed for 10h, the reaction mixture was cooled to room temperature and then in the refrigerator for 5 hrs. The solid product was filtered off washed and recrystalized from ethanol.

***Synthesis of 2-(anthracen-9(10H)-ylideneamino)pyridine-4-carbohydrazide* 5**

A mixture of ester **4** (0.012 mol) and hydrazine hydrate (0.02 mol) was refluxed for 5 hrs, then abs. ethanol (15 ml) was added and refluxed for further 8 hrs. The separated precipitate was filtered and washed with cold water.

***Synthesis of 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2 H)-one)pyridine* 6**

Compound **5** (0.01 mol) and chloroacetamide (0.01 mol) were mixed together in (20 ml) abs. ethanol. The reaction mixture was refluxed for 24 hrs, the solvent was reduced to one third its volume under reduced pressure. The crude product was obtained by filtration, washed with water and recrystalized from chloroform.

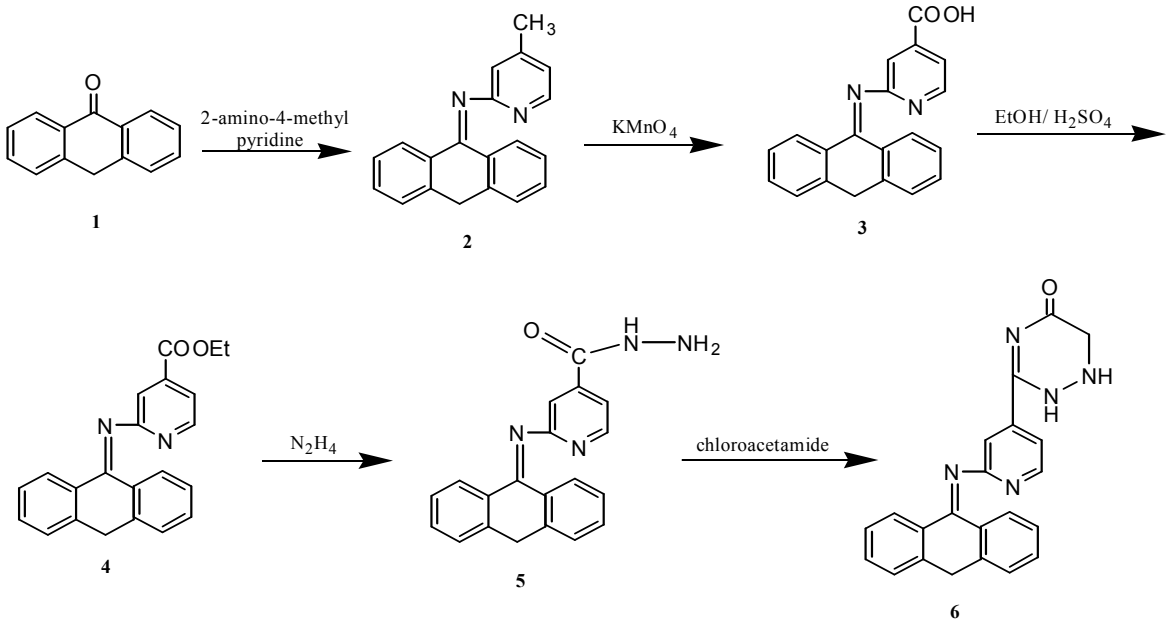
Instrumentation

The percentage composition of the elements (CHN) for the compounds was determined using an elemental analyzer CHN Model Fison EA 1108 by using sulphoylaminde as a standard. The infrared spectra were recorded as potassium bromide discs using a Perkin-Elmer spectrophotometer GX. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded using the JEOL JNM-ECP 400 spectrometer.

Results and Discussion

Synthesis and physical properties

The 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2H)-one)- pyridine **6** was prepared by the reaction of anthron **1** with 2-amino-4-methylpyridine in glacial acetic acid to give N-anthracen-9(10H)-ylidene-4-methylpyridine-2-amine **2.** Oxidation of compound **2** using $KMnO_4$ gave 2-(anthracen-9(10H)-ylideneamino)-4-carboxylic acidpyridine **3** which converted to the target product through its reaction with EtOH and hydrazine hydrate, respectively, Scheme 1:



Scheme 1

The purity of the synthesized compounds was checked by TLC using silica gel-G as adsorbent. Further evidence for the characterization of the synthesized compounds was obtained from C, H and N analysis, which are in agreement with the calculated values, Table 1.

Infra-Red spectroscopy

The FTIR spectrum of N-anthracen-9(10H)-ylidenehistidine **2** showed disappearance of ketone C=O bands at 1715 cm^{-1} which confirm the conversion of compound **2** to 2-(anthracen-9(10H)-ylideneamino)-4-carboxylic acidpyridine **3**. On the other hand, the FTIR spectrum of compound **3** has carboxylic acid C=O stretching vibration at 1735 cm^{-1} [13]. In the spectra of ethyl 2-(anthracen-9(10H)-ylideneamino)pyridine -4-carboxylate **4**, 2-(anthracen-9(10H)-ylideneamino)pyridine-4-carbohydrazide **5** and 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2 H)-one)pyridine **6**, the bands at 1723, 3324-3256 and 1685 cm^{-1} were assigned to the stretching of ester C=O, -$NHNH_2$ and amide C=O groups, respectively. Table 2 lists the stretching frequency (υ) for some of the characteristics groups exhibited by the synthesized compounds.

Nuclear magnetic resonance

The ^{1H}NMR spectra for all compounds were recorded in [$_2H^6$] DMSO using tetramethysilane as the internal standard. The data are compiled in Table 3. The conclusion drawn from ^{1H}NMR studies of the synthesized compounds lend further support to suggested formation of 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2 H)-one)pyridine **6**. The most characteristic evidence support the formation of compound **6** was the two singlet peaks at δ 8.40 and 8.53 ppm due to the N-H protons, which further characterized by D_2O exchange. Furthermore, there are a multiple signals of the aromatic protons resonances at 6.42-7.89 ppm [14].

Table 1. Physical data for the synthesized compounds

Comp.	Color	% Yield	M. P. °C	Molecular Formula	Found (Calcd.) %		
					C	H	N
1	Brown	-	152-154	$C_{18}H_{24}O$	84.99 (84.32)	8.72 (9.44)	-
2	Yellow	77	92-94	$C_{25}H_{34}N_2$	83.43 (82.82)	10.01 (9.45)	8.56 (7.73)
3	White	83	126-128	$C_{25}H_{32}N_2O_2$	77.56 (76.49)	9.13 (8.22)	6.32 (7.14)
4	Light pink	72	102-104	$C_{27}H_{36}N_2O_2$	76.38 (77.10)	9.24 (8.60)	7.14 (6.66)
5	Brown	90	188-190	$C_{25}H_{34}N_4O$	72.29 (73.85)	7.39 (8.41)	12.92 (13.78)
6	White	87	89-91	$C_{29}H_{43}N_5O$	73.41 (72.92)	10.31 (9.07)	13.92 (14.66)

Table 2. Characteristic absorption bands of the synthesized compounds

Comp.	O-H	-$NHNH_2$	N-H	Aromatic protons	Aliphatic protons	C=O	C=N
1	-	-	-	3056	-	1715	-
2	-	-	-	3069	2943, 2857	-	1610
3	3421	-	-	3054	-	1735	1611
4	-	-	-	3067	2952, 2864	1723	1612
5	-	3324-3256	3172	3063	-	1680	1610
6	-	-	3176	3060	-	1685	1613

Table 3. ^{1H}NMR data (δ, ppm) of all compounds prepared

Comp.	-C<u>H</u>$_3$	-C<u>H</u>$_2$-	Aromatic protons	N-H	-NH$_2$	O-H
1	-	-	6.77-7.98	-	-	-
2	1.34	-	6.56-7.74	-	-	-
3	-	-	6.58-7.83	-	-	9.54
4	1.52	2.03	6.57-7.75	-	-	-
5	-	-	6.54-7.62	8.42	8.89	-
6	-	-	6.56-7.70	8.40, 8.53	-	-

Table 4 shows the most relevant ^{13C}NMR data. Due to scan solubility of the synthesized compounds, their spectra were recorded in [$_2$H^6] DMSO. The –CH$_3$ peak of N-anthracen-9(10H)-ylidene-4-methylpyridine-2-amine **2** appeared at 12.63 ppm. Furthermore, the C=O resonances group of 2-(anthracen-9(10H)-ylideneamino)-4-carboxylic acidpyridine **3**, ethyl 2-(anthracen-9(10H)-ylideneamino)pyridine -4-carboxylate **4**, 2-(anthracen-9(10H)-ylideneamino)pyridine-4-carbohydrazide **5** and 2-(4-(anthracen-9(10H)-ylideneamino)-4-(1,6-dihydro-1,2,4-triazin-5(2 H)-one)pyridine **6** appeared at 173.46, 171.25, 170.26 and 169.83 ppm, respectively [15].

Antimicrobial activity

All the compounds **1-6** were in *vitro* screened for their antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* PTCC 1023) and Gram-negative (*Escherichia coil* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027) bacteria by the drug diffusion method [16]. The zone of inhibition was measured in mm and was compared with standard drug. DMSO was used as a blank and Streptomycin was used as antibacterial standard. All the compounds were tested at 100 µg/ml and 250 µg/ml concentration. The data are summarized in Table 5, and show that all compounds display certain antibacterial activity.

Table 4. ^{13C}NMR data (δ, ppm) of all compounds prepared

Comp.	-CH$_3$	-C<u>H</u>$_2$-	-C<u>=</u>N-	Aromatic carbons	C=O
1	-	13.47	-	134.16-142.85	170.12
2	12.63	13.31	40.15	133.67-143.29	-
3	-	13.42	41.13	132.68-142.20	173.46
4	12.54	13.38, 13.89	40.89	133.53-144.21	171.25
5	-	13.43	40.76	134.39-143.22	170.26
6	-	13.46	40.78	132.65-142.89	169.83

Table 5. Antibacterial activity of all compounds prepared

Comp.	Zone of inhibition in mm											
	S. aureus		*S. epidermidis*		*B. subtilis*		*K. pneumoniae*		*E. coli*		*P. aeruginosa*	
	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
1	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+++	++	++	++	++	+++	++	++	++
3	++	+++	+	+	+	+++	++	++	+	+	++	++
4	+	+	++	+	++	+	+	+	++	+++	+++	+
5	++	+	+	+++	+++	++	+	+		+	+	+
6	+++	+++	++	++	++	+++	+++	++	+	+	+	+++
Streptomycin	+	+	++	+	+	+	+	++	+	+	+	+

+++ = high activity, ++ = moderate activity, + = low activity, - = no activity

Conclusions

In conclusion, we have described the synthesis and antibacterial activities of a new heterocyclic compounds **2-6**. These compounds showed in *vitro* growth inhibitory activity against the tested organisms comparable or higher than Streptomycin. The biological data revealed that with slight modifications in the structure one can plan for the drug design.

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