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HETEROCYCLIC SYNTHESIS USING NITRILIMINES: PART 18. SYNTHESIS OF NEW FUSED HETEROCYCLES CONTAINING TRIAZINONE MOIETY

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Abstract

A series of new 4,5-dihydro-6-oxo-1,2,4-triazino[4,5-b]1,2,3,4-tetrahydro-β-carbolines were synthesized by the reaction of methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate and the appropriate hydrazonoyl halide in presence of triethylamine. The structures of the title compounds have been established by their elemental analyses and spectroscopecal data. The microbial features of some of the synthesized compounds were studied by a known method.

Keywords: 4,5-dihydro-6-oxo-1,2,4-triazino[4,5-b]1,2,3,4-tetrahydro-β-carboline, cyclocondensation, methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate, nitrilimines

1. Introduction

β-Carbolines are tricyclic nitrogen heterocycles formed in plants and animals by the Maillard reaction through the condensation of amino acids and reducing sugars or aldehydes [1-4] and associated with a broad spectrum of biological activities and pharmaceutical properties including sedative, anxiolytic, hypnotic, anticonvulsant, antitumor, antiviral, antiparasitic, and antimicrobial activities [5-11] as well as phosphodiesterase (PDE5) inhibitors [12] and hypotensive agents [13-15]. β-carbolines found in some plants are known as the harmala alkaloids; e.g. harmaline in Peganum harmala or Banisteriopsis caapi. Some β-carbolines have been detected in the tissues and fluids of mammals, including human tissues such as eye lens. The photophysical and photochemical properties of some β-carbolines have been studied in the literature [16-18]. Some of the β-carbolines have been shown to be phototoxic to bacteria and insects, and the others are possess neurotoxic and antioxidant properties [19-21] and inhibiting the precipitation of proteins. Tetrahydro-β-carboline core, present in numerous alkaloids (*Vinca*-, *Rauwolfia*-, *Harman* alkaloids) and synthetic products with valuable biological properties, is accessible from tryptamine *via* the well-known Pictet-Spengler [22] and Bischler-Napieralski reactions. Some annulated tetrahydro-β-carbolines were reported to be potent benzodiazepine antagonists or tyrosine kinase inhibitors [23-24]. Furthermore, 1,2,3,4-Tetrahydro-β-carboline has been found to be a potent inhibitor of lipid peroxidation and cyanide intoxication in mice [25]. A multicomponent pathway, using masked nucleophile containing aldehydes, was proposed for the preparation of new heterocycle fused tetrahydro-β-carbolines. Following a chemoselective deprotection, an internal nucleophilic ring opening of the Meldrum's acid moiety could be envisaged and the resulting carboxylic acid function was to be relayed toward the targeted tryptamine and β-carboline compounds. Recently, fused β-carbolines namely [1,3,5]triazino[1',2':1,6]pyrido[3,4-b]-indol-4-ones were achieved by one-pot synthesis involving condensation of 3-amino-β-carbolines with ethoxycarbonyl isothiocyanate followed by amination of the resulting thioureas and finally thermal ring closure of the resulting guanidines to fused β-carbolines [26].

2. Experiment

Melting points were determined on a Stuart Electrothermal Apparatus and are uncorrected. The IR spectra were obtained by using Satellite 3000 Mid infrared spectrophotometer in potassium bromide pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer (400.13 MHz) at room temperature in CDCl3 if not noted otherwise stated, using tetramethylsilane (TMS) as an internal reference. All chemical shifts) were reported in ppm from TMS. Electron impact (EI) mass spectra were measured on Finnigan MAT 8200 and 8400 Mass spectrometers at 70

Table 1. Physical Data, Molecular Ion Peaks and Elemental Analysis for Compounds 6a-l

Comp. No.	Mol. Formula (MW)	Yield (%)	Melting Point (^oC)	Analysis, Calculated/Found (%)			[$M^{+\cdot}$]
				C	H	N	
6a	$C_{26}H_{21}N_5O_2$ (435.49)	70	243-245	71.71 (71.50)	4.86 (4.70)	16.08 (15.95)	435
6b	$C_{26}H_{20}ClN_5O_2$ (469.93)	72	204-206	66.45 (66.60)	4.29 (4.15)	14.90 (15.05)	469/471
6c	$C_{26}H_{20}BrN_5O_2$ (514.39)	71	213-215	60.71 (71.50)	3.92 (4.70)	13.61 (15.95)	514/516
6d	$C_{26}H_{20}FN_5O_2$ (453.48)	73	231-233	68.87 (68.60)	4.45 (4.60)	15.44 (15.30)	453/455
6e	$C_{27}H_{23}N_5O_2$ (449.52)	72	219-221	72.14 (71.90)	5.16 (5.00)	15.58 (15.70)	449
6f	$C_{26}H_{20}N_4O_2$ (420.47)	68	187-189	74.27 (74.10)	4.79 (4.90)	13.32 (13.15)	420
6g	$C_{26}H_{19}ClN_4O_2$ (454.92)	65	212-214	68.65 (68.50)	4.12 (4.30)	12.32 (12.45)	454/456
6h	$C_{24}H_{17}ClN_4O_3$ (444.88)	67	193-195	64.80 (64.95)	3.85 (4.70)	12.59 (12.45)	444/446
6i	$C_{24}H_{17}ClN_5O_2S$ (460.95)	69	188-190	62.54 (62.70)	3.72 (3.60)	12.15 (11.00)	460/462
6j	$C_{30}H_{22}N_4O_2$ (470.54)	63	241-243	76.58 (76.40)	4.71 (4.85)	11.91 (12.05)	470
6k	$C_{30}H_{21}ClN_4O_2$ (504.98)	65	217-219	71.36 (71.55)	4.19 (4.30)	11.09 (10.95)	504/506
6l	$C_{31}H_{24}N_4O_2$ (484.56)	61	230-232	76.84 (76.65)	4.99 (5.15)	11.56 (11.40)	484

eV. Elemental analysis are performed at Cairo University, Egypt and the results agreed with the calculated values within experimental errors. The methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate **3** [27] and hydrazonoyl halides **4** [28-30], used in this study, were prepared according to previously described procedures.

Synthesis of 4,5-dihydro-6-oxo-1,2,4-triazino[4,5-b]1,2,3,4-tetrahydro-β-carbolines **6a-l**. To a stirred solution of the appropriate hydrazonoyl halides (0.012 mol) in chloroform (50 mL) was added a solution of methyl ester hydrochloride (2.5 g, 0.01 mol) in methanol (40 mL). To the resulting reaction mixture, cooled in an ice-salt bath (-5-0 ^oC), was dropwise added triethylamine (0.05 mol). After addition was complete, stirring was continued for 1 h at 0 ^oC, and then at room temperature for 6-8 h. The solvent was removed under reduced pressure, and the residue was washed with water. The resulting solid product was collected and recrystallized from methanol or ethanol to give the desired products 6a-l. The physical and analytical data of the title compounds are provided in Table 1.

3. Results and Discussion

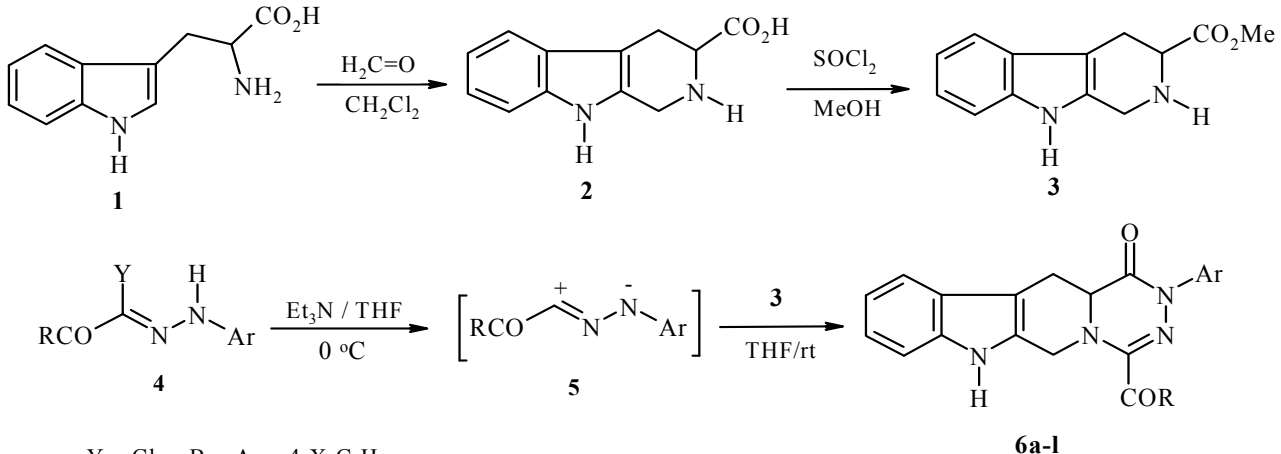
Cyclocondensation reactions of nitrilimines with 2-hydrazinoacetate or with α-amino esters represent an important synthetic route to prepare substituted 1,2,4-triazin-6-ones [27,31-33]. 1,2,4-Triazin-6-ones and their 4,5-dihydro analogues, condensed with heterocycles, such as pyrazole and indole, have been reported [12,34-35]. 1,2,4-Triazino-β-carbolines from nitrilimines was first explored by El-Abadelah in 1997 [36]. In the present study, the 4,5-dihydro-6-oxo-1,2,4-triazino[4,5-b]1,2,3,4-tetrahydro-β-carbolines **6a-l** were synthesized *via* one-pot reaction between methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylate **3** and appropriate nitrilimines **5**, generated *in situ* from the respective hydrazonoyl halides **4** in the presence of triethylamine as a base (Figure 1). 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid **2** was prepared by Mannich reaction from L-

tryptophane **1** and formaldehyde following the procedure of Harvey *et al.* [37]. The carboxylic acid **2** was converted to the corresponding methyl ester hydrochloride **3** by treatment with thionyl chloride in methanol, following a reported method [27] for esterification of related α-amino acids (Figure 1). The assignment of structures **6a-l** is based on their analytical and spectroscopic data. Physical properties, molecular ion peaks and elemental analysis are presented in Table 1. No investigations were carried out concerning optical purity and activity.

Spectroscopic data analysis. The electron impact (EI) mass spectra of compounds 6a-l displayed the correct molecular ions ($M^{+\cdot}$) in accordance with the suggested structures (Table 1). Their IR spectra of in KBr showed strong absorption bands of N-H in the 3370-3350 cm^{-1} region, a lactam carbonyl band in the 1680-1670 region, at 1680-1670 cm^{-1} for R-C=O, and at 1625-1600 cm^{-1} was assignable to C=N. The ^{1}H NMR spectra of compounds **6a-l** showed all the signals of the proposed structures, indicating that, all compounds have N-H proton of the ring appeared as singlet at 8.0-7.90 ppm and the methine proton at position 12a appeared as two doublets in the range 4.50-4.40 ppm due to its coupling with the two diastereotopic protons at position 12 (J= 5 Hz). Correspondingly, the later H-12 protons appeared as two doublets of doublets due to further geminal coupling (J = 1.4 Hz). The diastereotopic H-6 methylene protons are also mutually coupled to each other (J = 1.5 Hz) and hence are displayed as two doublets. The ^{1}HNMR spectral data of compounds **6a-l** are given in Table 2.

The ^{13}CNMR spectra of compounds 6a-l displayed the characteristic signals of the suggested structures. The signal of the lactam appeared in the range of 162-160 ppm, and that of the carbonyl carbon of R-C=O group resonated in the range of 1660-1640 ppm. The signal at 142-140 ppm, is attributed to C=N of the triazinone ring. The ^{13}C NMR spectral data of compounds 6a-l are given in Table 3.

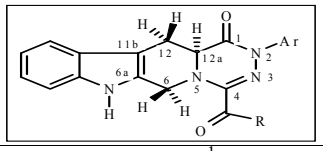
Antimicrobial activity. Six of the newly synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as *Euterococci, Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp*, *Proteus spp*, and fungi such as *Aspergillus niger*, *Candida albicans*, employing the nutrient agar disc diffusion method [38-40] at 10 mg/mL concentration in dimethyl formamide (DMF) by measuring the average diameter of the inhibition zone in mm (Table 4). The data reported in Table 1 represent average of three experiments. The results showed that all the tested compounds exhibited a marked degree of activity against bacteria and fungi compared with well known antibacterial and antifungal substances such as tetracycline and fluconazole. According to NCCLS (2004), zones of inhibition for tetracycline and fluconazole <14 mm were considered resistant, between 15 and 18 mm were considered weakly sensitive and >19 mm were considered sensitive. Also, the results showed the degree of inhibition varied with the tested compounds (Table 4).



	a	b	c	d	e	f	g	h	i	j	k	l
R	PhNH	PhNH	PhNH	PhNH	PhNH	Ph	Ph	2-C_4H_3O	2-C_4H_3S	2-$C_{10}H_7$	2-$C_{10}H_7$	2-$C_{10}H_7$
X	H	Cl	Br	F	Me	H	Cl	Cl	Cl	H	Cl	Me

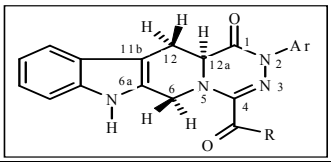
Figure 1. Synthetic Pathway to 1,2,4-triazino[4,5-b]1,2,3,4-tetrahydro-β-carbolines 6a-l

Table 2. Proton Chemical Shift (δ/ppm) for Selected Protons of Compounds 6a-l



Compound No.	^{1}H NMR							
	H-12		H-12a	H-6		NH_{ring}	H Arom.	NH_{amide}
6a	3.10 (dd, 1H)	3.38 (dd, 1H)	4.38 (2d, 1H)	4.47 (d, 1H)	5.64 (d, 1H)	7.94 (s, 1H)	7.58-7.14 (m, 14H)	9.84 (s, 1H)
6b	3.11 (dd, 1H)	3.37 (dd, 1H)	4.39 (2d, 1H)	4.45 (d, 1H)	5.67 (d, 1H)	7.96 (s, 1H)	7.60-7.20 (m, 13H)	9.86 (s, 1H)
6c	3.10 (dd, 1H)	3.36 (dd, 1H)	4.38 (2d, 1H)	4.48 (d, 1H)	5.70 (d, 1H)	8.00 (s, 1H)	7.83-7.11 (m, 13H)	9.82 (s, 1H)
6d	3.12 (dd, 1H)	3.40 (dd, 1H)	4.40 (2d, 1H)	4.47 (d, 1H)	5.65 (d, 1H)	7.98 (s, 1H)	7.76-7.14 (m, 13H)	9.84 (s, 1H)
6e	3.11 (dd, 1H)	3.39 (dd, 1H)	4.38 (2d, 1H)	4.46 (d, 1H)	5.68 (d, 1H)	7.96 (s, 1H)	7.55-7.12 (m, 13H)	9.86 (s, 1H)
6f	3.06 (dd, 1H)	3.34 (dd, 1H)	4.36 (2d, 1H)	4.50 (d, 1H)	5.52 (d, 1H)	7.94 (s, 1H)	7.78-7.06 (m, 14H)	---
6g	3.08 (dd, 1H)	3.35 (dd, 1H)	4.37 (2d, 1H)	4.52 (d, 1H)	5.54 (d, 1H)	7.95 (s, 1H)	7.80-7.09 (m, 13H)	---
6h	3.06 (dd, 1H)	3.40 (dd, 1H)	4.39 (2d, 1H)	4.51 (d, 1H)	5.36 (d, 1H)	7.92 (s, 1H)	7.82-7.11 (m, 12H)	---
6i	3.12 (dd, 1H)	3.40 (dd, 1H)	4.40 (2d, 1H)	4.49 (d, 1H)	5.37 (d, 1H)	7.93 (s, 1H)	7.85-7.10 (m, 12H)	---
6j	3.13 (dd, 1H)	3.41 (dd, 1H)	4.42 (2d, 1H)	4.52 (d, 1H)	5.24 (d, 1H)	7.94 (s, 1H)	8.52-7.25 (m, 16H)	---
6k	3.09 (dd, 1H)	3.38 (dd, 1H)	4.43 (2d, 1H)	4.53 (d, 1H)	5.26 (d, 1H)	7.95 (s, 1H)	8.63-7.16 (m, 15H)	---
6l	3.08 (dd, 1H)	3.37 (dd, 1H)	4.42 (2d, 1H)	4.52 (d, 1H)	5.21 (d, 1H)	7.94 (s, 1H)	8.43-7.19 (m, 15H)	---

Table 3. Carbon-13 Chemical Shift (δ/ppm) for Selected Carbons of Compounds 6a-l



Compound No.	^{13}C NMR							
	RC=O	C-1	C-4	C-6	C-6a	C-11b	C-12	C-12a
6a	159.6	161.5	140.8	44.4	129.6	106.9	24.6	56.7
6b	159.7	161.1	140.7	44.8	129.4	106.7	24.9	56.6
6c	159.5	161.3	140.8	44.6	129.3	106.9	24.7	56.8
6d	159.6	161.2	140.9	44.7	129.7	107.2	24.9	56.9
6e	159.7	161.4	140.7	44.8	129.3	106.7	24.8	56.7
6f	185.2	160.7	141.2	44.0	128.8	106.8	24.6	56.2
6g	185.4	160.8	141.0	44.1	128.7	106.9	24.9	56.3
6h	174.6	162.2	142.2	44.5	130.2	107.4	25.3	56.6
6i	176.2	162.1	141.8	44.7	130.3	107.5	25.1	56.8
6j	187.4	161.8	142.3	45.3	131.4	106.5	24.9	57.0
6k	187.3	161.9	142.1	45.6	131.7	106.8	25.2	57.4
6l	187.4	161.6	141.6	45.2	131.1	106.6	24.7	57.1

Table 4. Antimicrobial Screening Results of the Tested Compounds*

Compound No.	Antibacterial activity					Antifungal activity	
	Euterococci	*E. coli*	*S. aureus*	*Klebsiella spp*	*Proteus spp*	*C. albicans*	*A. niger*
6b	16	17	17	13	6	15	14
6d	13	18	15	11	10	17	18
6g	19	15	11	14	16	18	16
6h	18	16	17	18	9	16	12
5i	16	19	16	19	11	19	11
5k	13	12	14	16	7	16	9
DMF	-	-	-	-	-	-	-

*Zone of inhibition in mm

4. Conclusion

In conclusion, the cyclocondensation of several nitrilimines with β-carboline leads to formation of triazine-β-carboline derivatives, and some of them proved to have potent antibacterial and antifungal activity. The results confirm that the antimicrobial activity is strongly dependent on the nature of the substituents at N-2 and C-4 of the 1,2,4-triazinone ring.

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