SYNTHESIS AND STUDY OF NEW N-SUBSTITUTED CARBAZOLE AS SOME ANTIFUNGAL AGENTS

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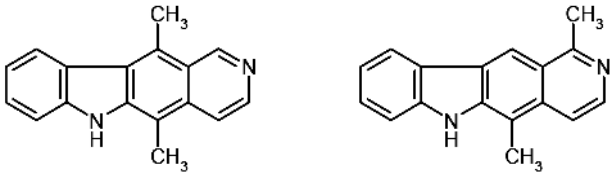
Abstract

Carbazole (1) upon condensation with ethylchloroacetate followed by treatment with hydrazine hydrate yielded 2-(9*H*-carbazol-9-yl) acetohydrazide (3). Treatment of compound (3) with sodium nitrite resulted in the formation of diazonium salt (4). Reaction of (4) with ethylacetoacetate and acetylacetone afforded ethyl 2-[3-(9*H*-carbazol-9-ylacetyl) triazanylidene]-3-oxobutanoate (5) and 3-[3-(9*H*-carbazol-9-ylacetyl)triazanylidene] pentane-2,4-dione (6), respectively. Reaction of (5) and (6) with phenylhydrazine and urea yielded 4-[3-(9*H*-carbazol-9-ylacetyl)triazanylidene]-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (7) and 5-[3-(9*H*-carbazol-9-ylacetyl) triazanylidene]-4,6-dimethylpyrimidin-2(5*H*)-one (8), respectively. The newly synthesized compounds were characterized by IR, ^{1}H-NMR and ^{13}C-NMR analysis. Potential antifungal effects of the synthesized compounds were investigated using the *Candida albicans* and *Aspergillus niger* strains. The newly synthesized compounds exhibited promising activities, particularly compounds (7, 8 and 9).

Introduction

The clinical need for therapeutic agents which restore or enhance a response in immuno compromised patients such as that which occurs in viral infections, cancer, autoimmune disease and acquired immune deficiency syndrome (AIDS) has led to the search for novel immuno stimulants[(1)]. Carbazole, an important class of indole alkaloids has gained much importance in recent years due to their diverse biological activities[(2-5)]. The therapeutic prominence of the carbazole derivatives was well established[(6,7)]. Also, pyrido carbazoles were reported to elicit anticancer and anti-HIV properties[(8-11)] and hence they have gained an important place in medicinal chemistry[(12)]. The discovery of the antineoplastic activity of the naturally occurring alkaloid *ellipticine* and its isomer *olivacine* Fig.(1) has stimulated considerable research efforts in the field of carbazole systems[(13)]. A variety of carbazoles and its annulated derivatives represent DNA ligands with pronounced antitumor activity[(14)].

With the aim to construct new systems with the carbazole nucleus, I turned my attention to synthesizing N-acetohydrazide carbazole that would be an important synthon for the synthesizing of new heterocycles.



Ellipticine **Olivacine**

Fig.(1) : Structures of the alkaloids, ellipticine and Olivacine.[(15)]

Experimental

Instruments:

Melting points were determined on GallenKamp melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded on Shimadzu FTIR-8300 spectrometer as KBr disc; results are given in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, in DMSO-d_6 for all compounds on a Bruker AMX-400 NMR spectrometer. The chemical shifts are reported in part per million (ppm) downfield from internal tetramethylsilane (TMS) (chemical shift in δ values). ^{1}H-, ^{13}C-NMR were made at the Organic Chemical Technology Department, Technical University of Budapest (Budapest, Hungary).

Chemicals:

Carbazole (**1**)

This material was supply from Aldrich Chemical Company as synthetic material and used directly without further purification.

Synthesis of ethyl 9*H*-carbazol-9-ylacetate (**2**)

Ethylchloroacetate (0.01mol, 1.06ml) was added dropwise to a stirred solution of carbazole (1) (0.01mol, 1.67g), KOH (0.01mol, 0.56g) in 20ml abs. ethanol. The reaction mixture was refluxed for 8hrs, then the reaction mixture was filtered and the filtrate poured on crushed ice. The resulting product was recrystallized from ethanol. M.P. 167-169˚C, Yield 78%.

Synthesis of 2-(9*H*-carbazol-9-yl) acetohydrazide (**3**)

A mixture of ester (2) (0.01mol, 2.37g) and hydrazine hydrate (0.015mol, 1.5ml) was refluxed for 4hrs, abs. ethanol (20ml) was added and refluxed for further 8hrs[16].Then the excess solvent was evaporated under reduced pressure. The reaction mixture was filtered and the final product was recrystallized from ethanol. M.P. 202-204˚C, Yield 85%.

Synthesis of [(9*H*-carbazol-9-ylacetyl) amino] diazonium chloride (**4**)

A mixture of 2-(9*H*-carbazol-9-yl) acetohydrazide (3) (0.01mol, 2.50g) in conc. HCl (3ml) was cooled to 0-5˚C in ice bath, cooled sodium nitrite solution (0.01mol in 5ml water) added to it dropwise during 7-10min. The reaction mixture was stirred for 30min.

Synthesis of ethyl 2-[3-(9*H*-carbazol-9-ylacetyl)triazanylidene]-3-oxobut- noate (**5**)

An ice-cooled mixture of ethylaceto-acetate (0.01mol, 1.27ml) and anhyd- rous sodium acetate (0.01mol, 0.82g) in abs. ethanol was added dropwise with stirring to a solution of diazonium salt (4). The stirring was continued for 30min and the reaction mixture then left under stirring for 2hrs at room temperature. The solid product was collected and recrystallized from chloroform. M.P. 138-140˚C, Yield 67%.

Synthesis of 3-[3-(9*H*-carbazol-9-ylacetyl) triazanylidene]pentane-2,4-dione (**6**)

The same method described for the synthesis of compound (5) but using acetyl acetone instead of ethylaceto- acetate (0.01mol, 1.03ml). M.P. 155-157˚C, Yield 73%.

Synthesis of 4-[3-(9*H*-carbazol-9-yl acetyl) triazanylidene]-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (**7**)

A solution of compound (5) (0.01mol, 3.80g) in glacial acetic acid (15ml) was added to phenylhydrazine (0.01mol, 0.98ml) and anhydrous sodium acetate (0.01mol, 0.82g). The reaction mixture was heated under reflux for 12hrs. Then the mixture was poured into ice-cold water and stored in a refrigerator. The crude product which separated was washed with water, dried and recrystallized from ethanol. M.P. 187-189˚C, Yield 87%.

Synthesis of 5-[3-(9*H*-carbazol-9-ylacetyl) triazanylidene]-4,6-dimethyl pyrimidin-2(5*H*)-one (**8**)

A mixture of compound (**7**) (0.01mol, 3.66g) and urea (0.01mol, 0.60g) in abs. ethanol (15ml) was heated under reflux for 15hrs. After cooling to room temperature, crushed ice was added and the mixture was stirred for 1hrs. The separated product was collected by filtration and recrystallized from ethanol. M.P. 220-222˚C, Yield 65%.

Synthesis of 5-(9*H*-carbazol-9-yl methyl)-1,3,4-oxadiazole-2-thiol (**9**)

To a solution of 2-(9H-carbazol-9-yl) acetohydrazide (3) (0.02mol, 4.40g) in abs. ethanol (15ml) at 0˚C, KOH (0.02mol, 1.12g) and CS_2 (0.04mol, 2.40ml)[17] were added respectively. The mixture was held at reflux for 10hrs or until evolution of H_2S gas has been ceased. The solvent was evaporated in under reduced pressure, the residue dissolved in ice-water and acidified with conc. HCl. The precipitate was filtered off and recrystallized from chloroform. M.P. 160-162˚C, Yield 75%.

Antifungal Studies:

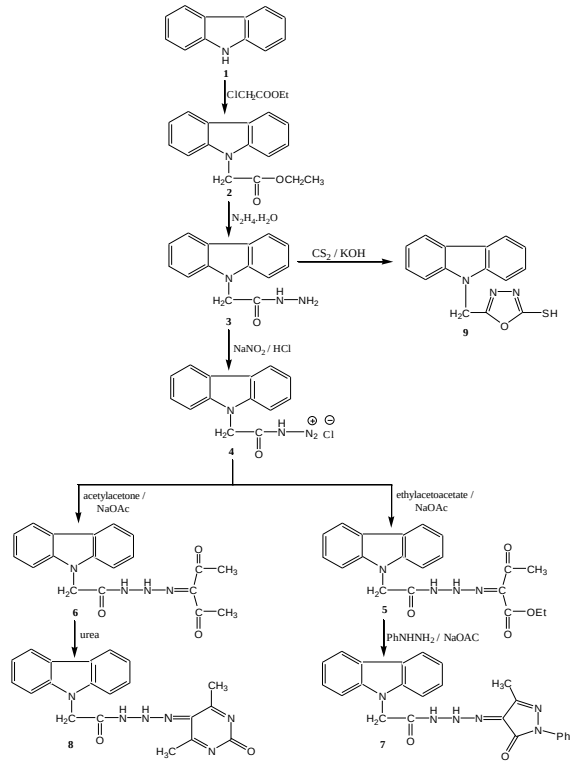
In this work, all synthesized compounds were assayed for their antifungal activity *in vitro* against two strains of fungi: Yeast-like fungi (*Candida albicans*) and moulds (*Aspergillus niger*). Prepared agar and petri dishes were sterilized by autoclaving for 15min at 121˚C. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled

with 100µl of the prepared compounds; DMSO was used as a solvent. These plates were incubated at 37˚C for 48hrs in case of *Candida albicans* and for 72hrs for the *Aspergillus niger*. The inhibition zones caused by the various compounds were examined.

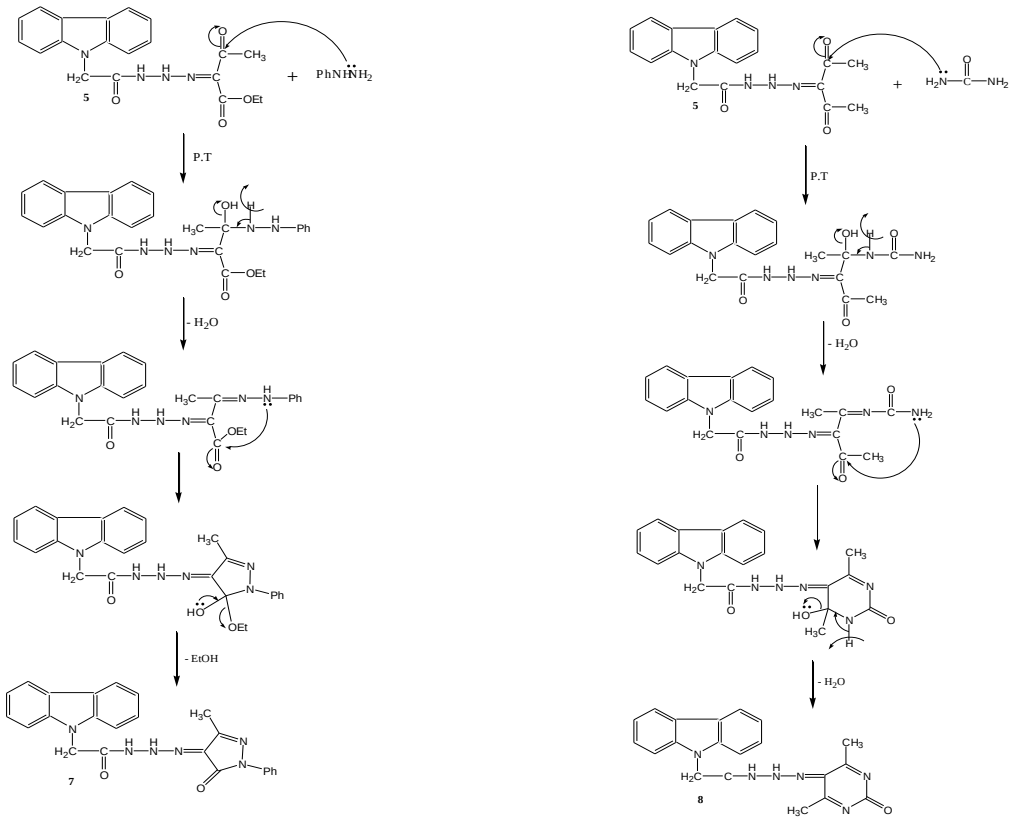
Results and Discussion

In this article, i reported the synthesis of carbazole derivatives from diazonium salts. 2-(9*H*-carbazol-9-yl) acetohydrazide (3) was obtained through the condensation of carbazole (1) with ethylchloroacetate to yield compound (2) which is then converted to compound (3) via its reaction with hydrazine hydrate. Treatment of (3) with sodium nitrite in the presence of HCl yield [(9*H*-carbazol-9-ylacetyl) amino]diazonium chloride (4) which ready for reacted with ethylaceto- acetate to give ethyl 2-[3-(9*H*-carbazol -9-ylacetyl) triazanylidene]-3-oxobutn- oate (5). The FTIR spectrum of compound (5) showed strong band corresponding to C=O stretching vibration of ester group at 1705cm^{-1}, the intense C=O band of ester group is due to the force constant of the carbonyl bond is increase by the electron-attracting nature of the adjacent oxygen atom (inductive effect). ^{1}H-NMR spectrum of this compound showed a singlet at δ1.57ppm corresponding to the methyl protons. The ethyl group protons appeared as a multiplet at δ1.65 and 1.72 ppm. The carbonyl carbons of compound (5) appeared at δ168.1, 170.5 and 172.3ppm in ^{13}C-NMR spectrum. Other characteristic absorptions data are recorded in Table (2). On the other hand, diazonium salt (4) was also ready for reacted with acetyl acetone to afford 3-[3-(9*H*-carbazol-9-ylacetyl) triazanylidene] pentane-2,4-dione (6). FTIR spectrum of this compound showed strong bands at 3200, 1725 and 1625cm^{-1} due to the stretching vibration of N-H, two C=O diketone and C=N groups, respectively, while ^{1}H-NMR spectrum of compound (6) showed characteristic signal at δ1.59ppm which was integrated for the six protons of two -CH_3 groups. Signals due to C=O groups appeared at δ170.5, 172.3 and 173.5ppm in ^{13}C-NMR spectrum of this compound. Table (2) shows more characteristic evidence for the compound (6) formation. Compounds (5) and (6) were reacted with phenylhydrazine in glacial acetic acid and urea in abs. ethanol to give [3-(9*H*-carbazol-9-yl acetyl)triazanylid- ene]-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (7) and -[3 - (9*H* – carbazol – 9 - ylacetyl) triazanylidene]-4,6-dimethylpyrimidin-2(5*H*)-one (8), respectively. The most characteristic evidence, in the FTIR spectrum, for the formation of compound (7) is the disappearance of band 1705cm^{-1} due to the ester group with the appearance of band at 1645cm^{-1} due to the C=O stretching vibration of amide group, the C=O absorption of amides occurs at lower frequencies than "normal" carbonyl absorption due to the electron-donating nature of nitrogen atom by resonance effect. ^{1}H-NMR spectrum of compound (7) showed multiple signals at δ7.01-7.89ppm due to the thirteen protons of aromatic rings. Furthermore, two carbonyl carbons appeared at δ169.3 and δ170.4ppm in the ^{13}C-NMR spectrum of compound (7). More data information is written in Table (2).

The formation of pyrimidine ring in compound (8) was detected by the appearance of amide carbonyl band at 1650cm^{-1} in its FTIR spectrum. ^{13}C-NMR spectrum of compound (8) showed two carbonyl signals at δ170.6 and 173.4ppm. ^{1}H-NMR data of this compound was in agreement with IR and ^{13}C-NMR data. Finally, reaction of acid hydrazide (3) with CS_2 produced 5-(9*H*-carbazol-9-yl methyl)-1,3,4-oxadiazole-2-thiol (9). The S-H signal, of compound (9), appeared at 2550 and δ5.32ppm in FTIR and ^{1}H-NMR spectra, respectively. On the other hand there is no signal due to C=O group in both FTIR and ^{13}C-NMR spectra, Table (2). All synthesis steps of this article are reported in Scheme (I). The mechanisms of reaction for the formation of compounds (7) and (8) are shown in Scheme (II).



Scheme (I): Synthetic pathway for the preparation of target compounds (2-9)



Scheme (II): Mechanism of the reaction for the formation of compounds (7 & 8)[18]

Antifungal Studies

From the data obtained, it can found clearly that all compounds exhibited good inhibitory activity against tested pathogenic micro-organism. Compounds (7), (8) and (9) showed higher activity than other, Table (1).

Table (1)
Antifungal activities of compounds (1-9).

Comp. No.	Microorganisms	
	Candida albicans	Aspergillus niger
1	+	+
2	+	+
3	+	+
5	+	++
6	++	+
7	+++	+++
8	+++	+++
9	+++	+++

+ = slightly active
++ = moderately active
+++ = highly active

Table (2)
Spectral data for compounds (2-9).

Comp.	FT-IR	^{1}H-NMR	^{13}C-NMR

Comp. No.	FT-IR	^{1}H-NMR	^{13}C-NMR
No.			
7	3325, 3237(*v* asym, sym NH_2), 3185(*v*N-H), 3085 (*v*C-H aromatic), 2905, 2871 (*v*C-H aliphatic), 1650(*v*C=O amide)	2.11(s, 2H, CH_2), 6.88-7.49 (m, 8H, Ar-**H**), 8.20, 8.65 (2s, 2H, 2N**H**) (D_2O exchange, disappear)	51.8(1C, CH_2), 128.1-133.2 (12C, aromatic carbons), 169.3, 170.3 (1C, C=O)
8	3220(*v*N-H), 3080 (*v*C-H aromatic), 2950, 2830 (*v*C-H aliphatic), 1650(*v*C=O amide)	1.62, 1.78(2s, 6H, 2C**H**$_3$), 2.21(s, 2H, C**H**$_2$), 7.15-7.76 (m, 8H, Ar-**H**), 8.12-8.58 (2s, 2H, 2N**H**) (D_2O exchange, disappear)	16.2(1C, **C**H_3), 51.6(1C, **C**H_2), 118.2, 120.1(2C, 2**C**=N), 130.5-137.3(12C, aromatic carbons), 170.6, 173.4 (2C, 2C=O)
9	3070(*v*C-H aromatic), 2988, 2864 (*v*C-H aliphatic), 2550 (*v*S-H), 1620(*v*C=N), 1266(*v*C-O-C), 1368 (*v*C=S)	1.67(s, 1H, CH_2), 5.32 (s, 1H, S-**H**), 11.57(s, 3H, CH_3), 1.65 (t, 3H, CH_3), 1.72 (q, 4H, 2CH_2), 2.28(s, 2H, CH_2), 6.89-7.70 (m, 8H, Ar-**H**)	53.4(1C, **C**H_2), 122.3, 16.7, 18.2(2C, -$CH_2$$CH_3$), 123.4 (2C, 2**C**=N), 53.0(1C, CH_2), 116.6(1C, C=N), 129.5-136.7 (12C, aromatic carbons), 168.1, 170.5, 172.3(3C, C=O)
5	3189(*v*N-H), 3100 (*v*C-H aromatic), 2965, 2870 (*v*C-H aliphatic), 1645 (*v*C=O amide), 3185(*v*N-H), 3085 (*v*C-H aromatic), 2905, 2871 (*v*C-H aliphatic), 1650(*v*C=O amide), 3167(*v*N-H), 3080(*v*C-H aromatic), 2900, 2880(*v* C-H aliphatic), 1643 (*v* C=O amide), 3070(*v*C-H aromatic), 2988, 3188(*v*N-H), 3030 (*v*C-H aromatic), 2981, 2850 (*v*C-H aliphatic), 1720(*v*C=O ketone), 1705 (*v*C=O ester), 1620 (*v*C=N)	7.11-7.85(m, 6H, Ar-H), 1.65(s, 3H, C**H**$_3$), 2.02 (s, 2H, C**H**$_2$), 7.01-7.89 (m, 13H, Ar-**H**), 8.20, 8.65 (2s, 2H, 2N**H**) (D_2O exchange, disappear), 8.22(s, 1H, NH) (D_2O exchange, disappear), 9.71(s, 2H, NH_2) (D_2O exchange, disappear), 1.62, 1.78(2s, 6H, 2C**H**$_3$), 2.21(s, 2H, C**H**$_2$), 7.15-7.76 (m, 8H, Ar-**H**), 8.12-8.58 (2s, 2H, 2N**H**) (D_2O exchange, disappear)	132.3(12C, aromatic carbons), 166.1(1C, C=O) 18.7(1C, CH_3), 52.9(1C, CH_2), 118.1(1C, **C**=N), 128.8-134.4 (18C, aromatic carbons), 169.3, 170.4 (2C,2C=O) 16.2(1C, **C**H_3), 51.6(1C, **C**H_2), 118.2, 120.1(2C, 2**C**=N), 130.5-137.3(12C, aromatic carbons), 170.6, 173.4 (2C, 2C=O) 53.4(1C, **C**H_2), 122.3, 16.7, 18.2(2C, -$CH_2$$CH_3$), 123.4 (2C, 2**C**=N), 53.0(1C, CH_2), 116.6(1C, C=N), 129.5-136.7 (12C, aromatic carbons), 168.1, 170.5, 172.3(3C, C=O)
6	3200(*v*N-H), 3025 (*v*C-H aromatic), 2931, 2820 (*v*C-H aliphatic), 1725 (*v*C=O diketone), 1625 (*v*C=N)	1.59(s, 6H, 2CH_3), 2.05 (s, 2H, CH_2), 7.07-8.10 (m, 8H, Ar-H), 8.22, 8.45 (2s, 2H, 2NH) (D_2O exchange, disappear)	16.6(1C, CH_3), 52.5(1C, CH_2), 117.8(1C, C=N), 130.5-138.1 (18C, aromatic carbons), 170.5, 172.3, 173.5(3C,3C=O)

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الخلاصة

ان تكثيف الكاربازول مع أثيل كلوروأستيت ومن ثم معاملة الناتج مع الهيدرازين المائي أعطى المركب (3) . عومل المركب (3) مع نتريت الصوديوم ليؤدي الى تكوين أملاح الدايازونيوم (4). تفاعل المركب (4) مع أثيل أسيتو أستيت و أستيل أسيتون أنتج المركبان (5) و (6)على التوالي. ان تفاعل المركبان (5) و (6) مع الفنيل هيدرازين واليوريا أعطى (7) و (8) على التوالي. لقد تم تشخيص جميع المركبات المحضرة بتقنيات الـ IR، ^{1}H-NMR ، ^{13}C-NMR . قد تم كذلك دراسة الفعالية البايولوجية لجميع المركبات المحضرة ضد فطريات الـ *Candida albicans* و الـ *Aspergillus niger* ، لقد أظهرت جميع المركبات فعالية جيدة ضد الفطريات المستخدمة وقد كانت المركبات (7)، (8) ، (9) هم الأكثر فعالية على وجة الخصوص.