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A one pot synthesis and evaluation of 13-oxo-quino[3,4-*b*]carbazol-*N*-oxides as antimicrobial agents

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1-Oxo-1,2,3,4-tetrahydrocarbazoles (**1a-e**) upon mixed aldol condensation with *o*-nitrobenzaldehyde (**2**) yielded 13-oxo-quino[3,4-*b*]carbazol-*N*-oxides (**3a-e**). All the prepared compounds were characterized by elemental and spectral analysis. A plausible mechanism for the formation of the final products is proposed. The title compounds proved to have great potentialities as antibacterial and antifungal agents due to the presence of the *N*-oxide group. Particularly, the chloro substituted derivative, **3d**, showed excellent antimicrobial activity.

Keywords: 1-oxo-1,2,3,4-tetrahydrocarbazoles, *o*-nitrobenzaldehyde, aldol condensation, 13-oxo-quino[3,4-*b*]carbazol-*N*-oxide, nitrones, mechanism, antibacterial, antifungal

In recent years, carbazole nucleus has attracted considerable interest owing to its significant biological activities (1–3). The discovery of the antineoplastic activity of the ellitipcine (5,11-dimethyl-6*H*-pyrido[3,4-*b*]carbazole) and its isomer olivacine has stimulated endeavors to find new pathways for the synthesis of pyridocarbazoles. The pharmacological profile of carbazole alkaloids was atested by the development of new deriv atives, such as elliptinium and datelliptium, which are presently used as drug molecules (4). Moreover, pyridocarbazoles are well known anticancer (5–7) and anti-HIV agents (8). Based on the above facts, our aim was to design a simple synthetic route for quino[3,4-*b*] -carbazoles, which can also be considered as benzo fused pyridocarbazoles from 1-oxo-1,2,3,4-tetrahydrocarbazoles (**1**). Having succeeded in our attempt, we tested the titled compounds for their antibacterial and antifungal activities.

EXPERIMENTAL

Melting points were determined with a Boetius micro heating table (Biby Sterlin, UK) and are uncorrected. IR spectra were recorded on a Shimadzu FTIR-8201 PC spectrophotometer (Shimadzu, Japan) using potassium bromide. ^{1}H NMR spectra were recorded

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on a Varian AMX 400 FT-NMR spectrometer (Varian Australia PTY, Australia) using tetramethylsilane as internal reference in DMSO-d_6. Mass spectra were recorded on a Jeol--JMS-D-300 mass spectrometer (70 eV) (Jeol, Japan). Microanalyses were done on a Perkin Elmer Model 240 CHN analyzer (*Perkin-Elmer, USA*). The purity of the products was tested by TLC using glass plates coated with silica gel G (HiMedia Laboratories, India). Petroleum ether and ethyl acetate were used as the developing solvents.

Characterization data for the prepared compounds are recorded in Tables I and II.

*Table I. Physical characterization of 13-oxo-quino[3,4-b]carbazol-*N*-oxide derivatives (**3a-e**)*

Compound No.	Yield (%)	Reaction solvent	M.p. (°C)	Molecular formula (M_r)	Elemental analysis (%) Calculated/Fonud		
					C	H	N
3a	71	MeOH	255–257 decomposes	$C_{20}H_{14}N_2O_2$ (314.20)	76.44 76.49	4.46 4.50	8.91 8.89
3b	79	MeOH	243–245 decomposes	$C_{20}H_{14}N_2O_2$ (314.20)	76.44 76.40	4.46 4.52	8.91 8.93
3c	67	MeOH	162–165	$C_{20}H_{14}N_2O_2$ (314.20)	76.44 76.45	4.46 4.47	8.91 8.87
3d	63	MeOH	276–178	$C_{19}H_{11}N_2O_2Cl$ (334.64)	68.19 68.20	3.29 3.33	8.37 8.40
3e	60	MeOH	228–231	$C_{19}H_{12}N_2O_2$ (300.19)	76.01 75.97	4.00 4.03	9.33 9.30

*Synthesis of 13-oxo-quino[3,4-b]carbazol-*N*-oxides (**3a-e**). General method*

1-oxo-1,2,3,4-tetrahydrocarbazole (9) (**1**, 0.001 mol) was dissolved in 4% potassium hydroxide solution in methanol and was refluxed for 0.5 h. Then, *o*-nitrobenzaldehyde (0.001 mol) was added to the hot mixture in portions. The reaction mixture was further refluxed for 2 h. The completion of the reaction was followed by TLC. At the end of the reaction time, the excess of methanol was removed by distillation and the mixture was poured into crushed ice and neutralized with ice cold 2 mol L^{-1} HCl. The crude mixture was then extracted with ethyl acetate (3x50 mL) and thoroughly washed with water. On removal of the solvent, the brown residue was purified using column chromatography on silica gel with petroleum ether: ethyl acetate (85:15) as eluant, to yield 13-oxo-quino[3,4-*b*]carbazol-*N*-oxide as orange coloured powder, which recrystallized as pale orange prisms.

The same reaction pathway was extended to other derivatives, **1b-e**, to afford the corresponding *N*-oxides (**3b-e**) (Scheme 1).

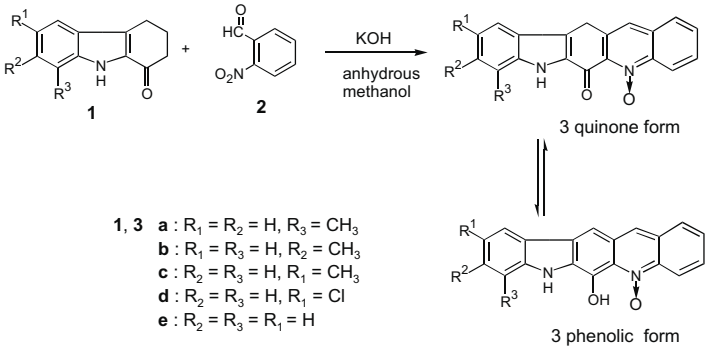
*Table II. Spectroscopic data for 13-oxo-quino[3,4-b]carbazol-*N*-oxide derivatives (**3a-e**)*

Compound No.	IR (cm^{-1})	^1H NMR signals (δ ppm)	m/z (M^+)	Quinoid/phenolic form ratio
3a	3450 (OH) 3284 (NH) 1690 (C=O) 1533 (C=N) 1321 (N→O)	2.63 (s, 3H, C_{11}–CH_3), 7.26–7.33 (m, 2H, C_2–H, C_3–H), 7.83–7.99 (m, 2H, C_4–H, C_5–H), 8.01–8.34 (m, 4H, C_6–H, C_8–H, C_9–H, C_{10}–H), 8.36 (d, 2H, C_7–2H, *J* = 8.10 Hz), 9.15 (b s, 1H, NH), 13.24 (b s, C_{13}–OH of the phenolic form)	314	1:1
3b	3485 (OH) 3255 (NH) 1685 (C=O) 1510 (C=N) 1328 (N→O)	3.09 (s, 3H, C_{10}–CH_3), 7.02–7.44 (m, 2H, C_2–H, C_3–H), 7.82–8.02 (m, 2H, C_4–H, C_5–H), 8.21–8.36 (m, 4H, C_6–H, C_8–H, C_9–H, C_{11}–H), 8.15 (d, 2H, C_7–2H, *J* = 8.08 Hz), 9.16 (b s, 1H, NH), 13.32 (b s, C_{13}–OH of the phenolic form)	314	1:1
3c	3420 (OH) 3220 (NH) 1672 (C=O) 1522 (C=N) 1325 (N→O)	2.51 (s, 3H, C_9–CH_3), 7.21–7.52 (m, 2H, C_2–H, C_3–H), 7.69–8.10 (m, 2H, C_4–H, C_5–H), 8.30 (d, 2H, C_7–2H, *J* = 8.09 Hz), 8.33–8.63 (m, 4H, C_6–H, C_8–H, C_{10}–H, C_{11}–H), 9.13 (b s, 1H, NH), 13.20 (b s, C_{13}–OH of the phenolic form)	314	2:1
3d	3505 (OH) 3290 (NH) 1726 (C=O) 1637 (C=N) 1329 (N→O)	6.52–6.74 (m, 2H, C_2–H, C_3–H), 7.04–7.63 (m, 2H, C_4–H, C_5–H), 7.68 (d, 2H, C_7–2H, *J* = 8.26 Hz), 7.83–8.04 (m, 4H, C_6–H, C_8–H, C_{10}–H, C_{11}–H), 8.87 (b s, 1H, NH), 13.01 (b s, C_{13}–OH of the phenolic form)	334	1:1
3e	3490 (OH) 3298 (NH) 1679 (C=O) 1525 (C=N) 1327 (N→O)	6.61–6.76 (m, 2H, C_2–H, C_3–H), 7.42–7.62 (m, 5H, C_4–H, C_5–H, C_6–H, C_7–2H), 7.84–8.30 (m, 4H, C_8–H, C_9–H, C_{10}–H, C_{11}–H), 9.13 (b s, 1H, NH), 13.00 (b s, C_{13}–OH of the phenolic form)	300	1:1

Antimicrobial studies

Antibacterial activity. – All the newly synthesized compounds (**3a–e**), were screened for their *in vitro* antibacterial activities against *Escherischia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027) and *Bacillus subtilis* (ATCC 6633) according to the disc diffusion method (2). The minimal inhibitory concentration (*MIC*) values were determined by a serial dilution technique. Furacin was used as a standard drug for comparison in antibacterial screening studies. The results are presented in (Table III).

Antifungal activity. – The antifungal screening studies of compounds **3a–e** were performed by the standard agar disc diffusion method (11). Seven-day old cultures of *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231), *Altenaria macrospora* and *Fusarium oxysporum* (isolated from rotten fruits) were used as test organisms. They were



Sheme 1

*Table III. Antibacterial activity data of 13-oxo-quino[3,4-b]carbazol-*N*-oxides (**3a-e**)*

Compound[a]	*MIC* (μg mL^{-1})			
	Escherischia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
3a	10.0	15.0	15.0	12.5
3b	10.0	15.0	15.0	20.0
3c	10.0	15.0	15.0	12.5
3d	6.0	12.5	12.5	12.5
3e	10.0	15.0	25.0	15.0
Furacin (standard)	6.0	12.5	12.5	12.5

[a] DMSO: negative control

grown on a potato dextrose agar medium. The *MIC* values were determined by the serial dilution technique. The growth of the microorganisms was followed visually and the lowest concentration that inhibited the growth of the microorganisms for 24 hours at 37 °C was taken as the *MIC* in μg mL^{-1}. The standard drug used in antifungal screening studies was carbendazim. The results are presented in Table IV. Solutions of the standards, furacin and carbendazim were prepared using dimethylsulphoxide.

Dimethylsulphoxide (DMSO) was used for dissolving test compounds and standard substances. Control experiments using dimethylsulphoxide were done for both the antibacterial and antifungal activity studies.

Table IV. Antifungal screening data of 13-oxo-quino[3,4-b]carbazol-N-oxides (3a-e)

Compound[a]	*MIC* (μg mL^{-1})			
	Aspergillus niger	*Candida albicans*	*Altenaria macrospora*	*Fusarium oxysporum*
3a	50	50	75	150
3b	150	100	150	150
3c	50	50	75	150
3d	25	25	40	30
3e	200	150	200	200
Carbendazim (standard)	25	25	35	30

[a] DMSO: negative control

RESULTS AND DISCUSSION

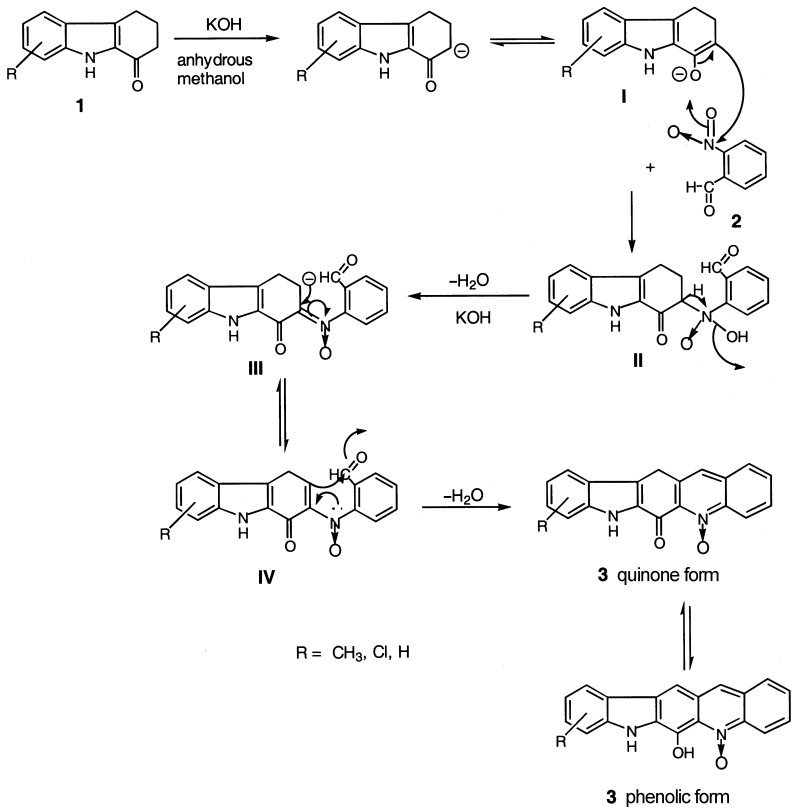
Treatment of 8-methyl-1-oxo-1,2,3,4-tetrahydrocarbazole (**1a**) with *o*-nitrobenzaldehyde (**2**) in 4% methanolic KOH furnished the desired product **3a**. From the IR spectrum, it was inferred that compound **3a** existed in the quinoid form as well as in the phenolic form. The IR spectrum showed strong bands corresponding to C=O group of quinoid at 1690 cm^{-1} and the C=N stretching absorption at 1533 cm^{-1}. A strong band at 1321 cm^{-1} clearly attested the presence of N-O group. The strong bands at 3450 cm^{-1} and 3284 cm^{-1} were due to the OH group of the phenolic form and NH group, respectively. ^{1}H NMR spectrum showed a singlet at δ 2.63 ppm corresponding to the methyl protons at C_{11} position. The C_2-H and C_3-H protons appeared as a multiplet in the region δ 7.26 to δ 7.33 ppm. The C_4-H and C_5-H protons appeared as a multiplet in the region δ 7.83 to δ 7.99 ppm and the C_6-H, C_8-H, C_9-H and C_{10}-H protons appeared as a multiplet in the region δ 7.83 to δ 7.99 ppm. A broad singlet at δ 9.15 ppm indicated the presence of NH proton. The C_7-H proton appeared as a doublet at δ 8.36 ppm with J = 8.10 Hz. The presence of a broad singlet at δ 13.24 ppm in the ^{1}H NMR spectrum clearly indicated that compound **3a** existed in the phenolic form (C_{13}-OH group). From the proton integration of ^{1}H NMR, the ratio of the quinoid form (C_7-2H) and phenolic form (C_{13}-OH and C_7-H) was found to be 1:1 (Table II). The mass spectrum showed the presence of a molecular ion peak at ($M^{+\cdot}$) 314 with other major fragmentation peaks at m/z 313, 273, 257 and 176. Elemental analysis agreed well with the molecular formula $C_{20}H_{14}N_2O_2$. From the spectral data and the elemental analysis, compound **3a** was proven to be 11-methyl-13-oxo-quino-[3,4-*b*]carbazol-*N*-oxide.

1-Oxo-1,2,3,4-tetrahydrocarbazole (**1**) is known to undergo aldol condensation at the α-position to give 2-benzylidine-1-oxo-1,2,3,4-tetrahydrocarbazole (10). The formation of **3** therefore suggests a condensation of the enolate ion of 1-oxo-1,2,3,4-tetrahydrocarbazole (**1**) at the nitrogen atom of *o*-nitrobenzaldehyde (**2**), leading to a nitrone intermediate **III** through intermediate **II**. The nitrone intermediate **III** regiospecifically loses a proton at

the third position in order to function like an enamine derivative **IV**. Then the C_3-carbon of **IV** attacks intramolecularly the aldehydic carbon to give the final product **3**. Product **3** is found to be an equilibrium mixture of quinoid and phenolic forms (Scheme 2).

The antibacterial screening studies indicate that compound **3d**, carrying the chloro group, showed excellent antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis*, namely it was as active as furacin. Compounds **3a** and **3c** showed somewhat lower activity against all the bacteria tested. Activity of compounds **3b** and **3c** was additionally lowered against *B. subtilis* and *P. aeruginosa*, respectively.

The antifugal screening studies revealed that **3d** exhibited excellent antifungal activity against all the fungi tested in comparison with the fungicide carbendazim. Such high activity might be due to the chloro group at the 9th position along with the *N-oxide* group in the compound. Compounds **3a** and **3c** with the methyl group showed marked activity against *A. niger*, *C. albicans* and *A. macrospora*. The activities of other compounds were markedly lower than that of carbendazim.



Sheme 2

The solvent used as control was found to be inactive against all the microorganisms chosen for antibacterial and antifungal studies.

From our earlier report (2) it is known that the chloro substituted carbazole derivatives have shown enhanced pharmacological properties. The presence of *N*-oxide along with that of the chloro group might be the reason for the stronger activity of compound **3d** than the methyl substituted compounds. The antimicrobial activity was found directly proportional to the concentrations of the test compound.

CONCLUSIONS

In this report, an easy method to synthesize quino[3,4-*b*]carbazol-*N*-oxides in good yield has been presented. All the compounds were tested for their *in vitro* antibacterial and antifungal activity and the chloro substituted compound has shown significant activity comparable with that of the conventional antibaterial and antifungal standards. The activity may be due to the presence of the *N*-oxide functional group along with the chloro group and this compound may need further pharmacological studies by suitably substituting the *N*-oxide group.

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REFERENCES

1. R. Balamurali and K. J. Rajendra Prasad, A facile synthesis of quino[2,3-*a*]carbazoles, *Z. Naturforch*. **54b** (1999) 1618–1620.
2. R. Balamurali and K. J. Rajendra Prasad, Synthesis, characterization and pharmacological activities of 5,6,11,12-tetrahydroindolo[2,3-*a*]carbazole derivatives, *Il Farmaco* **56** (2001) 229–232.
3. M. Sekar and K. J. Rajendra Prasad, Synthesis and biological activity of 1,6-dihydro-6-oxo-5*H*-benzo[*b*]carbazoles, *Indian J. Chem*. **34b** (1995) 731–733.
4. N. Haider, Pyridazine-fused carbazoles, reactivity and antitumor activity, *J. Heterocyclic Chem*. **39** (2002) 511–521.
5. N. Haider, R. Jabara, F. Khadami and R. Wanko, Synthesis of pyridazino[4,5-*b*]carbazoles as potential antitumor agents, *Heterocycles* **48** (1998) 1609–1622.
6. V. M. Hedin, T. Tabka, L. Poulain, T. Godard, M. Lachevrel, C. Saturnino, J. C. Lancelot, J. Y. Le Talaer and P. Gauduchon, Biological properties of 5,11-dimethyl-6*H*-pyrido[3,2-*b*]carbazoles: a new class of potent antitumour drugs, *Anti-Cancer Drug Des.* **15** (2000) 109–118.
7. C. B. de Koning, J. P. Michael and A. L. Rosseau, A versatile synthesis of benzo[*a*]carbazoles and pyrido[2,3-*a*]carbazoles, *J. Chem. Soc. Perkin Trans* **1** (2000) 1705–1713.
8. K. Hirata, C. Ito, H. Furukawa, M. Itogiawa, L. Mark Cosentino and K. H. Lee, Substituted 7*H*-pyrido[4,3-*c*]carbazoles with potential anti-HIV activity, *Bioorg. Med. Chem. Lett*. **9** (1999) 199–122.
9. D. Sowmitharan and K. J. Rajendra Prasad, Synthesis of 1-hydroxycarbazoles and mukonine isomers, *Heterocycles* **24** (1986) 711–717.

10. R. Balamurali and K. J. Rajendra Prasad, Synthesis of tetracyclic carbazole derivatives, *Heterocyc. Commun.* **7** (2001) 149–154.
11. T. Aboul-Fadi, M. A. Hussein, A. Nasser El-Shorbogi and A. Rouf Khallil, New 2*H*-tetrahydro--1,3,5-thiadiazine-2-thiones incorporating glycine and glycinamide as potential antifungal agents, *Arch. Pharm.* **9** (2002) 438–442.

S A Ž E T A K

Sinteza i biološko vrednovanje 13-okso-kino[3,4-*b*]karbazol-*N*-oksida kao potencijalnih antimikrobnih tvari

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1-Okso-1,2,3,4-tetrahidrokarbazoli (**1a**–**e**) mješanom aldolnom kondenzacijom s *o*-nitrobenzaldehidom (**2**) daju 13-okso-kino[3,4-*b*]karbazol-*N*-okside (**3a-e**). Struktura sintetiziranih spojeva određena je elementarnom analizom i spektroskopski. Predložen je mehanizam nastajanja produkata. Testirano je antibakterijsko i antimikotsko djelovanje sintetiziranih spojeva. Zahvaljujući prisutnosti *N*-oksidne skupine svi spojevi imaju veliki potencijal kao antimikrobna sredstva, anaročito kloro derivat **3d**.

Ključne riječi: 1-okso-1,2,3,4-tetrahidrokarbazoli, *o*-nitrobenzaldehid, aldolna kondenzacija, 13-okso--kino[3,4-*b*]karbazol-*N*-oksid, nitroni, mehanizam, antibakterijsko djelovanje, antimikotsko djelovanje

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