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2,3-BENZO[e]OKSAZEPİN TÜREVLERİNİN SENTEZLERİ VE ANTİMİKROBİYAL AKTİVİTE AÇISINDAN TARANMALARI

SYNTHESIS AND ANTIMICROBIAL SCREENING OF 2,3-BENZO[e]OXAZEPINE ANALOGS

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SUMMARY

N-oxidation and subsequent ring enlargement through the Meisenheimer Rearrangement of the properly substituted tetrahydroisoquinolines yielded nine novel 7,8-dimethoxy-3-methyl-1-(substitutedphenyl)-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine derivatives. Characterization of the final products have been done by UV, EIMS, ^{1}H and ^{13}C NMR spectroscopy. These compounds, together with seven more analogs which have been obtained previously by the authors, have been screened for their antimicrobial activity, using the microdilution technique. The results indicate that none of the compounds possesses any significant antibacterial or antifungal activity against the microorganisms tested in this study.

Key words: 2,3-benzo[e]oxazepines, 1,2,3,4-tetrahydroisoquinoline N-oxides, Meisenheimer Rearrangement, ring enlargement, antimicrobial activity

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ÖZET

Uygun bir şekilde sübstitüe olmuş tetrahidroizokinolinlerin N-oksidasyonunu takibeden Meisenheimer Çevrilmesi ile halka genişlemesi sonucunda dokuz adet yeni 7,8-dimetoksi-3-metil-1-(sübstitüefenil)-1,2,3,4-tetrahidro-2,3-benzo[e]oksazepin türevi kazanılmıştır. Son ürünlerin yapıları, UV, Eİ-kütle, ^{1}H ve ^{13}C NMR spektroskopileri yardımıyla aydınlatılmıştır. Elde edilen bu bileşikler, daha önce yazarlar tarafından elde edilmiş bulunan yedi analogla birlikte, mikrodilüsyon tekniği kullanılmak suretiyle antimikrobiyal etkileri açısından incelenmişlerdir. Sonuçlar, incelenen bileşiklerden hiçbirisinin bu çalışmada denenen mikroorganizmalara karşı belirgin bir antibakteriyel ya da antifungal aktivite göstermediğini ortaya koymuştur.

INTRODUCTION

2,3-benzo[e]oxazepine derivatives are shown to display sedative, anxiolytic, antiinflammatory and muscle-relaxant activities (*1-3*). A number of routes have been described in literature for the synthesis of compounds having this fused heterocyclic nucleus (*1-3*), one of which is the Meisenheimer Rearrangement (*4*). This reaction involves a thermal rearrangement of an N-oxide to yield a trisubstituted hydroxylamine. An interesting application of this reaction results in a ring enlargement to yield a 1,2-oxaza system, provided that the starting N-oxide moiety is incorporated in a ring (*5-7*). Bremner and coworkers, who have investigated in detail the chemistry and the mechanism of this reaction, utilized 1,2,3,4-tetrahydroisoqiunoline N-oxides to obtain various tetrahydro-2,3-benzo[e]oxazepines (*5*). They have also established that the ring expansion competes with a Cope Elimination unless the C-1 position is substituted with an aryl moiety (*5*).

In a previous communication, we have reported the synthesis of some 2,3-benzoxazepine derivatives with potential pharmacological activity using a four-step sequence, where the final step was an application of the Meisenheimer Rearrangement. (*8*). As a part of a wider program designed to investigate the structure-activity relationship of 2,3-benzo[e]oxazepine derivatives, further 7,8-dimethoxy-3-methyl-1-phenyl-1,3,4,5-tetrahydro-2,3-benzo [e]oxazepines with variable substituents on 1-aryl moiety are synthesized. Special care is taken to design such derivatives where the substituents display diverse but orderly electronic and lipophilic characteristics, aiming at a meaningful interpretation of the data obtained from the pharmacological screening results. In this study, we also report the antimicrobial screening results of the 7,8-dimethoxy-3-methyl-1-(substitutedphenyl)-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine derivatives **1-9**, as well as of the analogous compounds **10-16** synthesized by the authors during their previously published work (*8, 9*).

EXPERIMENTAL SECTION

Chemical Studies

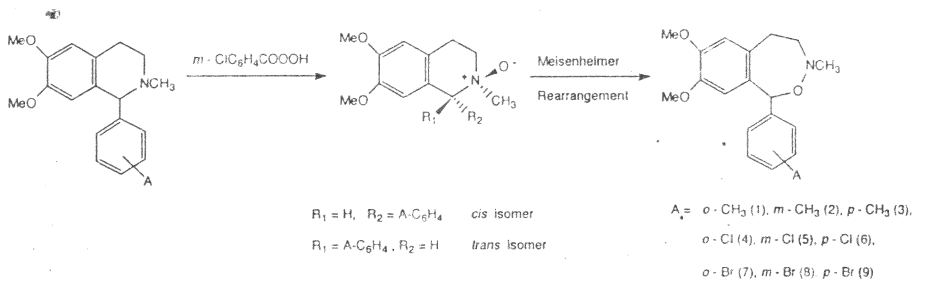
Material and Analytical Instrumentation

TLC is carried out on precoated silica gel 60 HF_{254} (Merck) plates. Silica gel 60 (Merck) is used for column chromatography. UV spectra are recorded on Shimadzu UV-160A Spectrophotometer in methanolic solutions. ^{1}H and ^{13}C NMR spectra are taken in $CDCl_3$ solutions on Bruker AC-300 NMR Spectrometer at 300 MHz and 50 MHz, respectively. Electron impact mass analyses are recorded on Finnagan-SSQ Spectrometer at 70 eV. Chemicals used in synthesis are purchased from Aldrich Company. All solvents are of Merck quality.

Conversion of Tetrahydroisoquinolines to 2,3-Benzo [e]oxazepines

The 1,2,3,4-tetrahydroisoquinolines utilized in this study have been synthesized and authenticated in a former work by the authors (*10*).

The corresponding tetrahydroisoquinoline (2 mmol) in $CHCl_3$ (20 ml) was placed in a mixture of ice and sodium chloride. *m*-Chloroperbenzoic acid (3 mmol) in $CHCl_3$ (20 ml) was added dropwise with stirring in 30 minutes. The reaction mixture was stirred in ice for another hour. It was washed twice with a 5% aqueous solution of $NaHCO_3$ and then with water. The organic phase was dried over anhydrous Na_2SO_4 and distilled *in vacuo* to dryness. The crude product was dissolved in CH_3CN (10 ml) and refluxed for 9 hours. The residue obtained by distillation of CH_3CN *in vacuo* was subjected to column chromatography [n-hekzan :benzene: acetone: ammonia (25%) (40:50:10:0.25)]. The faster moving band furnished the desired 2,3-benzo[e]oxazepine derivativs as a viscous oil.



The final products thus obtained are

7,8-dimethoxy-1-(*o*-tolyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]-oxazepine (**1**),

7,8-dimethoxy-1-(*m*-tolyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo [e]-oxazepine (**2**),

7,8-dimethoxy-1-(*p*-tolyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]-oxazepine (**3**),

7,8-dimethoxy-1-(*o*-chlorophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**4**),

7,8-dimethoxy-1-(*m*-chlorophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**5**),

7,8-dimethoxy-1-(*p*-chlorophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**6**),

7,8-dimethoxy-1-(*o*-bromophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**7**),

7,8-dimethoxy-1-(*m*-bromophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**8**),

7,8-dimethoxy-1-(*p*-bromophenyl)-3-methyl-1,3,4,5-tetrahydro-2,3-benzo[e]oxazepine (**9**).

Microbiological Studies

Material

The following Gram (-) and Gram (+) bacteria were used for the antibacterial activity:

Bacillus subtilis ATCC 6633

Staphylococcus aureus ATCC 25922

Escherichia coli ATCC 25923

Pseudomonas aeruginosa ATCC 27853

The following yeast-like fungi, isolated in the Hacettepe University, Faculty of Medicine, Department of Microbiology, were used for the antifungal activity test:

Candida albicans

Candida stellatoidea

Candida parapsilosis

Candida pseudotropicalis s.

The microorganism suspensions used for inoculation were prepared at 10^6 Cfu/ml concentration by diluting of the fresh cultures at Mc Farland 0.5 density (10^8 Cfu). It was known that there were $5x10^4$ Cfu/ml microorganism in each well after inoculation.

Mueller-Hilton Broth (Oxooid) liquid nutrient medium was used for diluting the microorganism suspensions and for a two-fold dilution of the compounds. Sabouraud liquid medium (Oxoid) was used for yeast-like fungi for the same purposes.

96-Well Falcon^R microplates were used for the microdilution metod. Brinkman transferpette^R was used for the two-fold dilution of the compounds in the wells.

Procedure

Microdilution method was employed for both the antibacterial and the antifungal tests (*11*) . The 2,3-benzo[e]oxazepine derivatives as well as the standarts, ampicillin sodium and clotrimazole, were dissolved in DMSO at 800 μg/ml concentrations.

The solutions of each compound at 400, 200.....3.12 μg/ml concentrations were prepared in the wells of the microplates by diluting with the aforementioned media (12). Suspensions of the microorganisms at 10 Cfu/ml concentration were inoculated to the two-fold diluted solutions of the compounds. Consequently, the microorganism concentrations in each well were approximately $5x10^4$ Cfu/ml. DMSO-microorganism mixture, pure microorganisms and pure media were used as control wells.

Microplates were then covered and incubated at 360°C for 24 to 48 hours. Wet cotton-wool was placed in the incubation chamber in order to avoid evaporation and to preserve sufficient humidity. At the end of the incubation period, the concentrations of the the compounds in the wells where no growth was observed were assesed as the minimum inhibotory concentrations (MIC) of the compounds. There was no inhibitory activity in the wells containing DMSO.

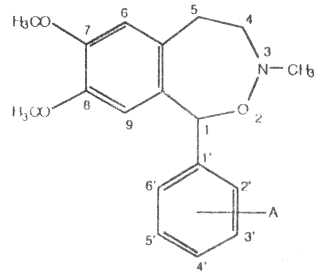
RESULTS

The yields, UV and MS data of Compounds **1-9** are summarized in Table 1. ^{1}H NMR and ^{13}C NMR data are given in Tables 2 and 3, respectively. The MIC values of Compounds **1-16** and of the controls are given as $\mu g/ml$ concentration in Table 4.

Table 1. The yields, UV and Mass Spectral Data of Compounds 1-9

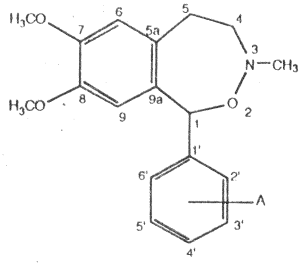
Comp No:	Yield g. (%)	UV λ MeOH (log ε) max nm	EIMS M/Z (% intensity)
1	0.155 (18.4)	285 (3.53) 240 sh (3.89)	313(M^+,100), 296(42), 239(81), 223(24)
2	0.744 (10.2)	283 (3.72) 236 sh (4.07)	313(M^+,86), 296(55), 267(25), 254(58), 253(50), 240(20), 239(100), 238(20), 223(64), 208(23), 165(28)
3	0.093 (14)	283 (3.59)	313(M^+,27), 296(23), 239(100), 223(32), 208(25), 165(25), 126(21), 105(46), 91(28), 69(26) 65(20), 55(25)
4	0.199 (28.8)	283 (3.55), 239 sh (3.90)	335(M+2, 31), 333(M^+,100),316(48), 287(21), 239(85), 208(24), 191(39), 165(20)
5	0.183 (27.4)	286 (3.46) 238 sh (3.82)	335(M+2, 15), 333(M^+,45), 316(27), 274(27), 239(100), 208(25), 191(25)
6	0.179 (27)	284 (3.45) 239 (3.87)	335(M+2, 7), 333(M^+,27), 316(22), 242(26), 239(100), 208(39), 191(38), 165(42), 152(24), 139(20), 111(20), 77(30), 75(23), 63(20)
7	0.206 (27.3)	284 (3.52) 240 sh (3.84)	379(M+2, 28), 377(M^+,28), 362(21), 360(21), 240(21), 239(100), 238(22), 208(35), 191(28), 165(26)
8	0.305 (40.3)	285 (3.92) 240 sh (3.72)	379(M+2, 79), 377(M^+,79), 362(42), 360(43), 331(20), 320(24), 239(100), 208(40), 191(36), 165(28)
9	0.146 (19.3)	284 (3.51) 242 sh (3.85) 230 sh (4.13)	379(M+2, 30), 377(M^+,30), 362(21), 360(22), 240(23), 239(100), 208(38), 191(26)

Table 2. ^{1}H NMR Data of Coumpounds 1 - 9



Comp. No	A	7-OCH$_3$	8-OCH$_3$	H-1	N-CH$_3$	H-4	H-5	H-6	H-9	H-2'	H-3'	H-4'	H-5'	H-6'
1	2.50	3.91	3.68	6.31	2.77	2.78 m 3.46 m	2.90 m 3.20 m	6.47	6.31	-	6.95 m	7.22 m	7.12 m	7.22 m
2	2.45	4.00	3.83	6.14	2.87	2.82 m 3.67 m	3.06 m 3.30 m	6.83	6.52	7.17 m	-	7.35 m	7.35 m	7.17 m
3	2.34	3.89	3.71	6.03	2.75	2.70 m 3.15 m	2.91 m 3.15 m	6.71	6.41	7.19 d j_o 8.0	7.15 d	-	7.15 d j_o 8.0	7.19 d
4	-	3.80	3.59	6.24	2.68	2.73 m 3.36 m	2.84m 3.12m	6.64	6.40	-	7.33 m	7.06-7.18 m	7.06-7.18 m	7.06-7.18 m
5	-	3.89	3.73	6.02	2.75	2.76 m 3.49 m	2.76 m 2.95 m	6.72	6.37	7.27-7.31 m	-	7.27-7.31 m	7.19 m	7.27-7.31 m
6	-	3.90	3.72	6.03	2.75	2.78 m 3.46 m	2.94 m 3.16 m	6.73	6.37	7.32 dd j_o 8.5 j_m 2.0	7.24 dd	-	7.24 dd j_o 8.5 j_m 2.0	7.32 dd
7	-	3.90	3.69	6.34	2.79	2.59 m 3.46 m	2.59 m 3.22 m	6.74	6.45	-	7.62 dd j_o 7.7 j_m 1.04	7.25 ddd j_o 7.4 j_m 1.3	7.16 m	7.16 m
8	-	3.89	3.73	6.01	2.75	2.75 m 3.48 m	2.95 m 3.18 m	6.72	6.37	7.46 m	-	7.43 m	7.22 m	7.22 m
9	-	3.89	3.72	6.01	2.74	2.78 m 3.46 m	2.94 m 3.17 m	6.72	6.35	7.18 d j_o 8.4	7.48 d	-	7.48 d j_o 8.4	7.18 d

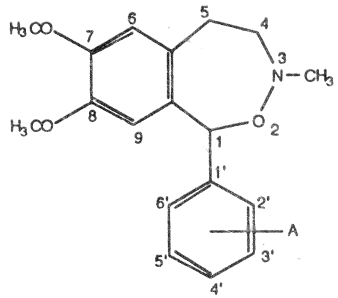
Table 3. ^{13}C NMR Data of Compounds **1 - 9**



	1	2	4	5	6	7	8	9
C-1	84.39	83.93	83.45	87.03	86.89	85.83	87.03	86.90
C-4	66.05	59.62	60.05	59.94	59.86	60.01	59.96	59.95
C-5	33.00	32.61	33.17	32.74	32.73	33.06	32.76	32.81
C-5a	129.84	128.62	129.72	128.94	128.53	129.55	128.90	128.98
C-6	113.71	118.68	113.86	113.88	113.85	113.70	113.90	113.86
C-7	147.43*	147.26*	147.40*	147.40*	147.46*	147.41*	147.46*	147.55*
C-8	147.43*	147.34*	147.50*	147.50*	147.36*	147.49*	147.60*	147.33*
C-9	110.68	110.87	110.48	110.86	110.76	110.51	110.80	110.84
C-9a	132.60	132.84	132.38	132.06	132.27	132.28	132.04	132.26
C-1'	138.02	140.20	137.85	142.33	138.84	139.40	142.62	139.39
C-2'	136.81	128.29#	133.71	128.16	128.5	124.25	131.30#	130.00
C-3'	130.34	138.02	129.46#	134.27	113.79#	132.80	122.55	131.55#
C-4'	126.00#	128.88#	130.19#	128.16#	133.78	130.40#	131.10#	122.07
C-5'	125.90#	128.98#	126.91	129.67#	113.73#	127.53	130.04#	131.55#
C-6'	128.00	125.36	129.22#	126.42	129.60	129.60#	126.92	130.00#
7-OCH_3	55.80+	55.73+	55.80+	55.84+	55.70+	55.81+	55.87+	55.85+
8-OCH_3	55.98+	55.90+	55.94+	55.96+	55.90+	55.92+	55.98+	55.98+
N-CH_3	46.40	46.39	46.46	46.49	46.47	46.13	46.10	46.53
Ar-CH_3	18.90	21.38	-	-	-	-	-	-

4'-Methyl derivative **3** was not available when the ^{13}C NMR were being recorded.
Similar symbols stand for interchangeable values.

Table 4. The Minimum Inhibitory Concentrations for Antimicrobial Activity (μg/ml) of Compounds 1 - 16



Comp. No	A	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida stellatoidea*	*Candida parapsilosis*	*Candida pseudotropicalis s.*
1	*o*-CH_3	100	100	200	200	100	100	100	100
2	*m*-CH_3	200	200	200	200	50	50	50	50
3	*p*-CH_3	200	200	200	200	200	200	200	200
4	*o*-Cl	100	100	100	100	100	100	100	100
5	*m*-Cl	100	100	100	100	100	100	100	100
6	*p*-Cl	100	100	200	200	100	100	100	100
7	*o*-Br	200	200	200	200	100	100	100	100
8	*m*-Br	100	100	200	200	100	100	100	100
9	*p*-Br	100	100	200	200	100	100	100	100
10[a]	*o*-OCH_3	200	200	200	200	100	100	100	100
11[a]	*m*-OCH_3	200	200	200	200	100	100	100	100
12[a]	*p*-OCH_3	200	200	200	200	100	100	100	100
13[b]	*o*-NO_2	200	200	200	200	100	100	100	100
14[b]	*m*-NO_2	200	200	200	200	100	100	100	100
15[b]	*p*-NO_2	100	100	200	200	100	100	100	100
16[a]	H	200	200	200	200	100	100	100	100
Ampicillin Sodium		3.13	3.13	3.13	6.25	6.25	-	-	-
Clotrimazole		-	-	-	-	-	3.13	3.13	3.13

[a] obtained in *(8)*

[b] obtained in *(9)*

DISCUSSION

In this study, nine novel 2,3-benzo[e]oxazepine derivatives have been synthesized by a two-step synthesis involving an N-oxidation followed by a ring expansion. Thus, a properly substituted 1,2,3,4-tetrahydroisoquinoline is initially treated with *m*-chloroperbenzoic acid. The formation of the expected N-oxide derivative is established with the help of TLC as well as by ^1H NMR spectrum of the crude product. It is observed that he desired N-oxides are obtained always as a mixture of *cis* and *trans* isomers but in varying ratios for individual compounds (*5*, *8*, *9*). Easily recognizable features of the ^1H NMR spectrum are the prominent downfield shifts of the N-methyl signal (about 0.7-0.8 ppm) and of the H-1 resonance (about 1.1-1.4 ppm) with respect to the tertiary counterpart, indicating the formation of the desired N-oxide.

Since it has been demonstrated that similar qualitative and quantitative results are obtained from pyrolysis of either a pure isomer or a mixture of isomers (*5*), separation of the isomers prior to thermolysis is not attempted. Thus, refluxing of the crude work-up product of the N-oxide in acetonitrile results in a ring expansion to yield the corresponding 2,3-benzo[e]oxazepine derivative. It has been postulated that this reaction proceeds *via* a homolytic cleavage of the C_1-N bond, which is demonstrated to occur more effectively when C_1 is doubly benzylic, as is the case in the present study. Subsequent formation of a C-O bond results in the corresponding fused oxaza systems (*5*).

The ^1H and ^{13}C NMR spectra of **1-9** provide precise information for the formation of the expected 2,3-benzo[e]oxazepine analogs. One of

the prominent features in the ^{1}H NMR spectra of the compounds is the significant downfield shift of the H-1 as compared to that of the N-oxide counterparts (approximately 0.5 ppm), which is due to the additional deshielding effect of the proximal oxygen atom. Another salient feature is the magnitude of the chemical shift of N-methyl protons (approximately δ 2.7), which has undergone an upfield shift of about 0.4-0.5 ppm with respect to that of the starting N-oxide, clearly indicating the formation of the 2,3-benzo[e]oxazepine nucleus *via* ring expansion. As expected, the chemical shifts of the remaining protons, both of the 2,3-benzo[e]oxazepine moiety and of the lower pendant aromatic ring, resemble closely those of the corresponding 1,2,3,4-tetrahydroisoquinolines as well as of the N-oxides.

The multiplicities of the eighteen carbons (nineteen for Compounds **1** and **2**) accounted for in the ^{13}C NMR spectra are determined by DEPT experiments. The most characteristic chemical shift, which provides proof to the presence of the 2,3-benzo[e]oxazepine nucleus, appears at about δ 81-83. This resonance belongs to C-1, which is significantly deshielded due to the proximal oxygen atom and the benzene ring.

The structures are further verified by the low resolution EIMS of the compounds, where the m/z values of molecular ion peaks are in complete agreement with the calculated molecular weights for individual compounds. The relative intensity of the molecular ion peak ranges between 30-100%, with no clearly observed correlation to the identity and the position of the substituents. However, the ion at m/z 239 is often the base peak, or else a very prominent peak in the EIMS of all of the Compounds **1-9**. Some variations in other peaks probably result from the different substituents of the 1-aryl moiety.

The antimicrobial activity screening results indicate that none of the Compounds **1-16** possesses significant antibacterial or antifungal activity against the microorganisms tested in this study.

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