Synthesis and Characterizations of New 1,3-Oxazine Derivatives

S. ARDA OZTURKCAN*, KADIR TURHAN AND ZUHAL TURGUT
Yildiz Technical University, Faculty of Science, Department of Chemistry, 34210, Davutpasa Esenler-Istanbul, Turkey.
turkcana@hotmail.com*

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Summary: In this study, Betti's classical procedure, a Mannich-type aminoalkylation reaction of 2- naphthol with various aldehydes was applied in the presence of ammonia and then 1,3-disubstituted-2,3-dihydro-1*H*-naphtoxazines were synthesized. The structures of the obtained new compounds have been clarified by spectroscopic methods and confirmed with elemental analysis results.
From the synthesized compounds, 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine was investigation of the effects of solvent and concentration with UV-Visible Spectrophotometer. Additionally, this compound was analyzed the mutagenic and anti-mutagenic properties.

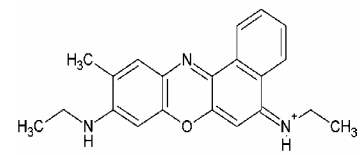
Introduction

The development of simple synthetic routes to widely used organic compounds using readily available reagents is one of the main objectives of organic synthesis. Nitrogen heterocycles are of special interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activity [1-4]. The synthesis of 1,3-oxazines has attracted attention in the past because of their potential as antibiotics, antitumor agents, analgesics, and anticonvulsants. 1,3-Oxazines have generated great interest as anti-psychotic agents and as possible effectors for serotonin and dopamine receptors. In addition, benzo-1,3-oxazines are known to be biologically active as anti-malarial, anti-anginal, anti-hypertensive and potent anti-rheumatic agents [5]. Oxazines are an important group of organic dyes which are generally π-conjugated systems, with interesting photo-physical and lasing properties [6].

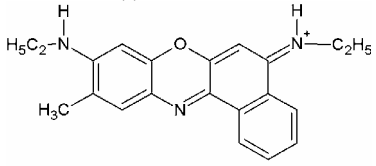
Aromatic oxazines were first synthesized in 1944 by Holly and Cope through Mannich reactions from phenols, formaldehyde, and amines [7, 8].

Several methods for the preparations of 1,3-oxazine derivatives have previously been reported [9, 10]. The ring-chain tautomeric inter-conversion of N-unsubstituted 1,3-N-O-heterocycles and the corresponding hydroxylalkylimines can often be exploited advantageously in different areas of organic synthesis and also in physical, medicinal and peptide chemistry [11, 12]. Hence, the synthesis of these derivatives is of considerable interest.

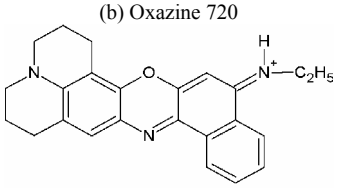
Nowadays oxazine are the subject of many literature and patent sources and are used as dye material, anti-corrosion chemicals, and are often used in certain steps of synthesis of dye and drug dye materials [13]. For example; Oxazine 170 (a), Oxazine 720 (b) and Oxazine 750 (c) are the compounds for the most common used dye material (Fig. 1) [14, 15].



(a) Oxazine 170



(b) Oxazine 720



(c) Oxazine 750

Fig. 1: Most common used oxazine dye compounds.

Zakerhamidi and co-worker's studies, the aggregative properties of Oxazine 720 and Oxazine 750 dyes in polyacrylamide hydrogels with different compositional percentage of structural species were studied using optical spectroscopy. As the result, Oxazine 720 and Oxazine 750 aggregated form in all percentages of polyacrylamide are lower than those in aqueous medium [16, 17].

In this study, Mannich type aminoalkylation was processed by applying classical Betti's reaction

*To whom all correspondence should be addressed.

[18] and many type of oxazine synthesis by this reaction (Fig. 2) [2].

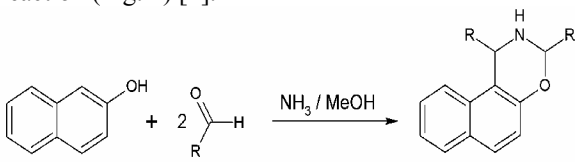


Fig. 2: General reaction of 1,3-oxazine derivatives.

The structures of the obtained new compounds have been clarified by spectroscopic methods and confirmed with elemental analysis results. From the synthesized compounds, 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine was investigation of the effects of solvent and concentration with UV-Visible Spectrophotometer. Additionally, this compound was analyzed the mutagenic and anti-mutagenic properties.

Results and Discussion

The characteristic C=O bands of the aldehydes disappeared and absorption bands corresponding to NH groups were observed between 3275-3340 cm^{-1} in the IR spectra of the compound, while NCH proton singlet between 6.2 and 6.5 ppm were observed in the ^{1}H NMR spectra.

The structure of cream color of oxazine derivatives 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine was explained by the help of FTIR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy (Fig. 3).

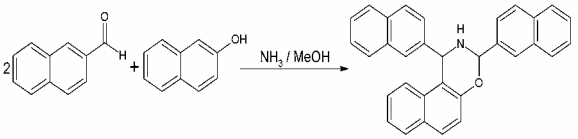


Fig. 3: Reaction of 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine.

The solubility of the oxazine compound 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine in methyl alcohol, chloroform, dichloromethane, ethyl acetate, hexane solvents was tested with UV-Vis Spectrophotometer at certain wavelength. The wavelength scanning for the compound at its different concentrations and the linear concentration range was examined; reasonable results were obtained especially with dichloromethane.

The effect of the different solvent was investigated with UV-Visible Spectrophotometer and the results are shown in Fig. 4. Methanol, chloroform, dichloromethane, ethyl acetate and hexane were tested as solvent for the newly synthesized compound at the concentration of 10 ppm and it was found that dichloromethane was effectively dissolved the compound.

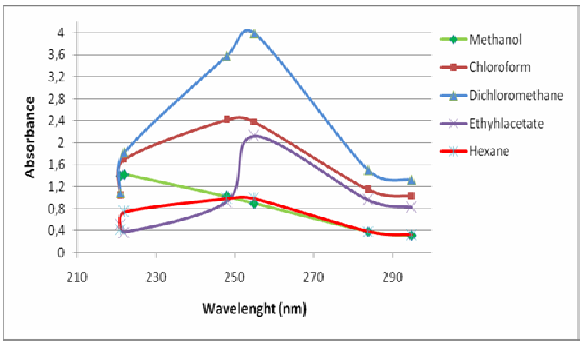


Fig. 4: The effect of different solvents for the new synthesized 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine compound at the concentration of 10 ppm.

The wavelength scanning at the different concentration of compound was performed with UV-Visible Spectrophotometer and the results are shown in Fig. 5. The maximum absorbance for newly synthesized oxazine compound was observed at 254 nm.

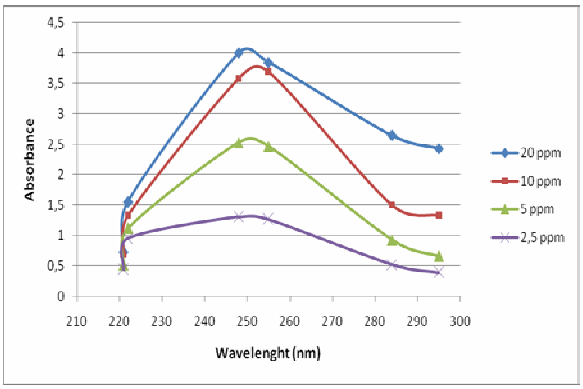


Fig. 5: The wavelength scanning at different concentration for the newly synthesized 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine compound in dichloromethane.

The linear concentration range for 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine compound was tested and the results are shown in Fig. 6. In the concentration range of 2.5-20 ppm newly synthesized oxazine compound obeys the Beer Lambert Law at 254 nm.

Additionally, synthesized 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3] oxazine was analyzed the mutagenic and anti-mutagenic properties. This compound tested at three different concentrations including 0.01, 0.1 and 1 µg/plate didn't show mutagenic effect in mutagenicity assays performed with *S. typhimurium* TA1535, and *E. coli* WP2*uvrA*. The results were summarized in Table-1.

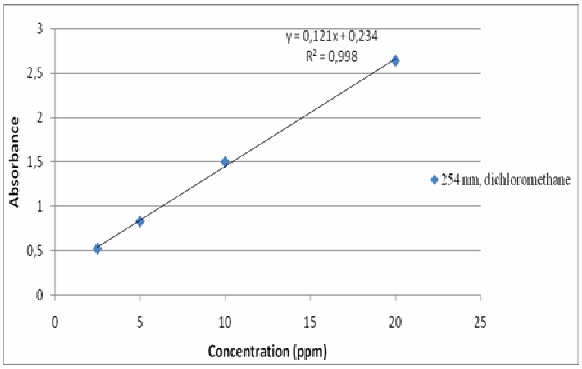


Fig. 6: Linear concentration range for the newly synthesized 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine compound at 254 nm.

Otherwise, in the antimutagenicity assays, we have shown that the compound has antimutagenic activity in TA1535 strain of *S. typhimurium* at three concentrations (0.01, 0.1 and 1 µg/plate).

Experimental

Reagents

All chemicals were purchased from Merck, Fluka and Aldrich.

Instrumental

TLC was carried out on silica gel 60 F_{254} precoated plates and visualized with "Camag UV light" (254/366 nm). Column chromatography was performed on silica gel 60, 70–230 mesh. FT-IR spectra were recorded on a Philips PU 9714 ATR spectrophotometer, and using "Perkin-Elmer Spectrum One" program. ^{1}H NMR and ^{13}C NMR (500 MHz) spectra were recorded on "Inova 500" spectrometer using TMS as an internal standard in $CDCl_3$ or DMSO. All spectroscopic data of the products were identical to data from authentic samples. High resolution Mass Spectra were obtained using "Finnigan Trace DSQ" instrument. The UV scans of the samples were done using UV-vis spectrophotometer at 200 nm to 800 nm. Melting points were determined with Gallenkamp melting point apparatus and were uncorrected.

Synthesis Method

Aldehyde (2 mmol) (furan-2-, furan-3-, thiophene-3-, pyridine-4- and naphthalene-2-carbaldehydes) and %25 methanolic ammonia solution (0.5 ml) were added to a solution of 2-naphthol (1 mmol) in absolute MeOH (0.5 ml). The mixture was left to stand in cooler for 2 days, and then the crystalline product was formed and separated out. The crude crystals were filtered off, washed with cold MeOH (2 x 2 ml) and purified by column chromatography, eluting with the indicated solvents.

Characterization

The structure of the obtained new compound has been clarified by spectroscopic methods (FTIR, ^{1}H NMR, ^{13}C NMR and MS) and elemental analysis after the purification processes.

Table-1: The mutagenicity and antimutagenicity assay results of new compound ($C_{32}H_{23}NO$) for *E. coli* WP2*uvrA* and *S. typhimurium* TA1535 bacterial tester strains at µM concentrations.

	Test Items Concentration (µg/plate)		Number of revertants			
			S. typhimurium TA1535		*E. coli* WP2	
			Mean ± S.E.	Mutat. %	Mean ± S.E.	Mutat. %
	MNNG (µl/plate)					413.00 ± 2.04
	NaN_3 (µl/plate)		426.00 ± 2.48			
	DMSO (µl/plate)	100	18.00 ± 0.91	------	16.00± 0.40ö	
Mutagenicity	$C_{32}H_{23}NO$	0.01	15.00 ± 1.47	-	16.00 ± 1.68	-
		0.1	12.00 ± 1.82	-	17.50 ± 0.40	-
		1	15.50 ± 0.91	-	16.50 ± 0.91	-
Antimutagenicity		0.01	307.50 ± 1.08	27.81	214.00 ± 2.94	48.18*
		0.1	249.50 ± 2.08	41.43*	241.50 ± 3.70	41.52*
		1	238.00 ± 2.19	44.13*	279.00 ± 2.48	32.44

1,3-Di(2-furyl)-2,3-dihydro-1H-naphto[1,2-e][1,3]oxazine: yield %61, cream crystal, m.p=103,4-104,5 ^{0}C; IR (KBr) : υ = 3315 (N-H), 3122 ve 3056 (C-H), 1622, 1598 (C=C), 1435, 1401 ve 1356 (C-H) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) : δ = 5.7 (s, 1H, NCH(Ar), 6.4 (s, 1H, O-CH(Ar)), 6.5-7.8 (m, 12H, aromatic) ppm; ^{13}C NMR ($CDCl^3$, 500 MHz) : δ = 50.91, 107.10, 109.33, 112.26, 115.86, 118.07, 119.48, 120.26, 120.32, 121.32, 122.53, 125.68, 127.54, 127.61, 127.77, 128.64, 129.08, 129.26, 130.87, 141.65, 141.76; MS (EI, 70 eV) m/z : 317 (M^+, 100), 223 ($C_{15}H_{13}NO$, 13), 95 (C_5H_5NO, 3) Anal. Calc. for $C_{20}H_{15}NO_3$ C=75.70, H= 4.76, N= 4.41 Found: C= 75.67, H= 4.85, N= 4,33.

1,3-Di(3-furyl)-2,3-dihydro-1H-naphto[1,2-e][1,3]oxazine: yield %59, cream crystal, m.p.=128,2-129,8 ^{0}C; IR (KBr) : υ = 3340 (N-H), 3130 (C-H), 1619, 1597 (C=C), 1433, 1393 ve 1362 (C-H), 1228 (C-O) cm-1; ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 5.7 (s, 1H, NCH(Ar), 6.5 (s, 1H, O-CH(Ar)), 6.8-7.8 (m, 12H, aromatic) ppm; ^{13}C NMR ($CDCl_3$, 300 MHz) : δ = 40.96, 60.67, 102.03, 103.86, 109.24, 113.48, 116.93, 117.62, 119.25, 120.91, 122.75, 123.15, 123.41 123.98, 125.51, 134,41, 136.99, 137.44, 137.78; MS (EI, 70 eV) m/z : 317 (M^+, 100), 223 ($C_{15}H_{13}NO$, 8), 96 (C_5H_7NO, 4) Anal. Calc. for $C_{20}H_{15}NO_3$ C=75.70, H= 4.76, N= 4.41 Found: C= 75.97, H= 4.75, N= 4,43.

1,3-Di(3-tiyophenyl)-2,3-dihydro-1H-naphto[1,2-e][1,3]oxazine: yield %57, cream crystal, m.p.=132,0-134,0 ^{0}C; IR (KBr) : υ = 3323 (N-H), 3053 (C-H), 1621, 1597 (C=C), 1434, 1399 ve 1386 (C-H), 1232 (C-S) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 5.6 (s, 1H, NCH(Ar), 6.4 (s, 1H, O-CH(Ar)), 6.7-7.8 (m, 12H, aromatic) ppm; ^{13}C NMR ($CDCl_3$, 300 MHz) : δ = 50.21, 115.30, 119.34, 120.33, 122.40, 122.51, 122.86, 123.36, 125.11, 126.02, 126.40, 126.64, 127.28, 127.98, 128.53, 129.23, 131.52, 140.75, 144.24; MS (EI, 70 eV) m/z : 349 (M^+, 100), 239($C_{15}H_{13}NS$, 39), 156 ($C_{11}H_9N$, 2) Anal. Calc. for $C_{20}H_{15}NOS_2$ C=68.74, H= 4.33, N= 4.01 Found: C= 67.67, H= 4.25, N= 4,00.

1,3-Di(4-pyridinyl)-2,3-dihydro-1H-naphto[1,2-e][1,3]oxazine: yield %49, *y*ellow crystal, m.p.=184,7-186,3 ^{0}C; IR (KBr) : υ = 3324 (N-H), 3055 ve 3034 (C-H), 1621, 1596 (C=C), 1426 ve 1397 (C-H), 1233 (C-N) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) : δ = 5.6 (s, 1H, NCH(Ar), 6.5 (s, 1H, O-CH(Ar)), 7.2-8.7 (m, 14H, aromatic) ppm; Anal. Calc. for $C_{22}H_{17}N_3O$ C=77.86, H= 5.05, N= 12.38 Found: C= 76.97, H= 5.05, N= 12,43.

1,3-Di(2-naphthyl)-2,3-dihydro-1H-naphto[1,2-e][1,3]oxazine: yield %74, cream crystal, m.p.=134,2-137,1 ^{0}C; IR (KBr) : υ = 3275 (N-H), 3056 (=C-H), 1623, 1597, 1574 (C=C), 1437 and 1363 (C-H); ^{1}H NMR ($CDCl_3$, 300 MHz) : δ = 6.24 (s, 1H, NCH(Ar), 6.25 (s, 1H, O-CH(Ar), 7.15-8.75 (m, 20H, aromatic) ppm; ^{13}C NMR ($CDCl_3$, 300 MHz) : δ = 50.86, 92.87, 109.55, 114.62, 119.44, 124.69, 125.52, 126.03, 126.10, 126.31, 127.37, 127.62, 127.72, 128.25, 128.49, 128.75, 129.14, 129.56, 129.64, 130.92, 133.10, 133.21, 133.48, 133.74, 134.61, 134.97, 139.27 MS (EI, 70 eV) m/z: 437 (M^+, 2), 327 ($C_{23}H_{21}NO$, 3), 326 ($C_{23}H_{20}NO$, 25), 325 ($C_{23}H_{19}NO$, 100), 171 ($C_{12}H_{13}N$, 11), 156 ($C_{11}H_{11}N$, 2) Anal. Calc. for $C_{32}H_{23}NO$ C=87.84, H= 5.30, N= 3.20 Found: C= 87.97, H= 5.27, N= 3,23.

Mutagenicity and Antimutagenicity Tests

The bacterial mutagenicity and antimutagenicity assays were performed according to described [18]. The known mutagens NaN_3 (in distilled water - 1 µg/plate) for *S. typhimurium* TA1535, and MNNG (in 10% DMSO - 1 µg/plate) for *E. coli* WP2*uvrA* were used as positive controls and 10% DMSO was used as negative control in these studies.

In the mutagenicity test performed with TA1535 strain of *S. typhimurium*, 100 µl of the overnight bacterial culture, 50 µl test compounds at different concentrations (0.01, 0.1, 1 µg/plate in 10% DMSO), and 500 µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37ºC for 48 or 72 h.

In the antimutagenicity test performed with same strains, 100µl of the overnight bacterial culture, 50 µl mutagen, 50 µl test compounds at different concentrations (0.01, 0.1, 1 µg/plate in 10% DMSO), and 500 µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48 or 72 h.

The procedures of mutagenicity and antimutagenicity assays described above for the *Salmonella* assay are all applicable to the *E. coli* WP2 reverse mutation assay. The only procedural difference is the addition of limited tryptophan (0.01 mM) instead of histidine to the top agar [19].

The plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays [20].

In mutagenicity assays, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative (solvent) control. A sample was considered mutagenic when were observed a dose-response relationship and a two-fold increase in the number of mutants with at least one concentration was observed [21-24].

In antimutagenicity assays, the inhibition of mutagenicity was calculated by using the following equation (M: number of revertants/plate induced by mutagen alone, S_0: number of spontaneous revertants, S_1: number of revertants/plate induced by the extract plus the mutagen): % Inhibition = $[(M-S_1)-(M-S_0)]\times100$; 20–40% inhibition was defined as moderate antimutagenicity; 40% or more inhibition as strong antimutagenicity; and 20% inhibition as no antimutagenicity [25-27].

Conclusion

In this study, Betti's classical procedure, a Mannich-type aminoalkylation reaction of 2-naphthol with various was applied in the presence of ammonia and then 1,3-disubstituted-2,3-dihydro-1*H*-naphtoxazines were synthesized.

A new material 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine was synthesized, characterized and investigated mutagenic/anti-mutagenic properties in this study.

The effect of the different solvent for dissolving the newly synthesized oxazine compound was investigated with UV-Visible Spectrophotometer and it was observed that dichloromethane can effectively dissolve the compound. The wavelength scanning was performed for the different concentrations of the compound and the maximum absorbance was found at 254 nm. The linear concentration range for this compound was tested at the wavelength of 254 nm and it was determined that in the range of 2.5-20 ppm this newly synthesized oxazine compound obeys the Beer Lambert law.

The biologically active results obtained from the mutagenicity and anti-mutagenicity assays showed that 1,3-Di(2-naphthyl)-2,3-dihydro-1*H*-naphto[1,2-e][1,3]oxazine has anti-mutagenic activity in *S. typhimurium* TA1535, and *E. coli* WP2*uvrA* strains at three concentrations.

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