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PREPARATION OF SOME PYRIDYL-SUBSTITUTED SCHIFF BASES AND THEIR LABELLING WITH Tc-99m

ABSTRACT

This work presents synthesis and characterization of the some pyridyl-substituted Schiff bases and their labeling with Tc-99m. The eleven heterocyclic Schiff bases have been synthesized from the condensation reactions of pyridine-2-aldehyde with derivative of aminophenol, aniline and aminopyridine compounds. Structure of all synthesized compounds have been determined by using the data obtained from their ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass (MS) spectral results. Schiff base derivatives of pyridine-2-aldehyde were labeled with Tc-99m and their radiochemical purity was calculated by using chromatographic methods (TLC and HPLC); and obtained chromatograms have been evaluated by TLC scintigraphy system and gamma counters.

Keywords: Schiff Base, Pyridine-2-aldehyde, Tc-99m, Labeling, Radiopharmaceutical

BAZI PİRİDİL-SÜBSTİTÜE SCHIFF BAZLARININ HAZIRLANMASI VE Tc-99m İLE ETİKETLENMESİ

ÖZET

Bu çalışmada bazı piridil-substitue Schiff bazlarının sentezi, karakterizasyonu ve Tc-99m ile etiketlenmesi sunulmaktadır. Piridin-2-aldehit bileşiğinin, aminofenol, anilin ve aminopiridin türevi bileşikler ile kondenzasyon reaksiyonlarından onbir adet heterosiklik Schiff bazı sentezlenmiştir. Tüm bu bileşiklerin yapıları, ultraviyole (UV), infrared (IR), nükleer magnetik rezonans (NMR) ve kütle spektral (MS) verilerinden yararlanılarak belirlenmiştir. Schiff bazı türevleri Tc-99m ile etiketlenmiş ve radyokimyasal saflık kromatografik yöntemler kullanılarak (TLC, HPLC) hesaplanmış ve oluşturulan kromatogramlar TLC görüntüleme ve gama sayıcı cihazları yardımıyla değerlendirilmiştir.

Anahtar Kelimeler: Schiff Bazı, Piridin-2-aldehit, Tc-99m, Etiketleme, Radyofarmasötik

1. INTRODUCTION (GİRİŞ)

Compounds with the structure of –C=N- (Azomethine group) are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups. Schiff bases are important class of compounds in medicinal and pharmaceutical fields. They show biological applications including antibacterial, antifungal and antitumor activity [1, 2, 3 and 4]. Similarly pyridine derivatives are prepared for a long time for a variety of biological activities such as CNS depressant, anticancerous, antibiotic, antihistaminic, anticonvulsants and many others.

Radiolabeled bio-molecules are potentially useful tools for cancer diagnosis and therapy. Radiometals such as Tc-99m, have physical properties, that are well suited for tumor imaging. Many efforts have been made to develop diagnostic pharmaceuticals of Tc-99m labelled small molecular complexes because of the superior medicoimaging characteristics (Biological $t_{1/2}$, low energy content) and the availability of radionuclide [5]. Since the small size of the complex is very important for the retention of the bioactivity [6], one of the strategies in the investigation is to explore novel complexes with small size, multifunctional ligands that possess specific bioactivities. Some applications of these Schiff bases with favorable cell membrane permeability have been exploited in cancer multidrug resistance [5].

Based on the mentioned properties of Schiff bases, we report herein their synthesis, spectroscopic characterization and labeling with Tc-99m. In this work, we have used substituted aromatic amines to prepare Schiff base compounds as reported in literature [7, 8, 9, 10, 11, 12, 13 and 14]. Presence of –C=N- and other functional groups forms more stable complexes compared to Schiff bases having only –C=N- coordinating moiety. For nuclear medicine applications, derivatives of pyridine-2-carboxaldehyde with substituted aromatic amines have been synthesized. Their synthesis and radiolabeling are described herein.

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

Radiopharmaceuticals are known as pharmaceutical dosages that include a radionuclide (radioactive moiety) in their structure and they are used in nuclear medicine for diagnosis and treatment of diseases. When the structures of radiopharmaceuticals are investigated, it is seen that they may generally have phosphor, nitrogen, sulphur atoms or carbocyclic acid groups.

It is quite important to develop and produce radiopharmaceuticals for the purpose of both diagnosis and treatment. On the other hand, it is also observed that labeling studies of some Schiff bases, which are used in health area because of their antihistaminic, anticancer, antimicrobial, antibacterial, antifungal activities, with a radioisotope is gaining interest to supply them to nuclear medicine in recent years [15].

Therefore, in the light of literature researches, this study has been started by the aim of giving some remarkable contributions to the class of that kind of compounds by synthesizing pyridyl-substituted Schiff bases (one of them is new) having heteroatom in their structure, and then by labeling of them.

The research consists of two steps. In the first step, pyridyl-substituted Schiff bases have been synthesized. At the second step, labeling of all of these compounds with Tc-99m have been accomplished in high yields (>%90).

3. MATERIALS AND METHODS (MATERYAL VE METOD)

All chemicals used in the present study are of analytical grade purchased from Sigma, Aldrich and Merck Chemical Co. All the solvents were used after distillation. Melting points were determined on a Gallenkamp melting point apparatus. The UV spectra were measured with Philips PU 8700 UV/VIS spectrophotometer. IR spectra were recorded on the FT-IR Perkin Elmer Spectrum One spectrophotometer by ATR technique. NMR spectra were obtained by using Varian Mercury-VX 400 BB model (400 MHz) and Bruker Avance III model (500 MHz) NMR spectrometer. Compound was dissolved in $CDCl_3$ and chemical shifts were referenced to TMS. The MS spectra were recorded from Agilent GC/MS 6890/5973 spectrometer. Radiocomplexation and radiochemical purity were checked by instant strip chromatography (Silica gel impregnated paper chromatography) with ITLC-SG (Gelman Sciences, Ann arbor, MI, USA). ITLC spectra were obtained by Bioscan AR-2000 radio-TLC Imaging Scanner instrument.

3.1. Preparation of Schiff Bases (Schiff Bazlarının Hazırlanması)

The Schiff base was prepared by mixing the solution of pyridine-2-aldehyde (0.535 g, 5.0 mmoles) in anhydrous ethanol (5.0 mL) with 5.0 mmoles of other moiety (Amine compound) in the same volume of anhydrous ethanol. The mixture was then refluxed with stirring for 6 h. The reaction solvent was removed by rotary evaporator. The product was extracted several times with petroleum ether (60-80°) from the oily residue. The collected petroleum ether phase was kept in the freezer for the crystallization of the product and dried at room temperature with 63-86% yield. Structure of the synthesized Schiff bases was given at Table 1. Spectral data of Schiff bases was given at Table 2.

The IR spectrum of the Schiff bases shows very strong intensity absorption band at 1645-1590 cm^{-1} assigned to CN stretching mode. The presence of aromatic rings has been identified by their characteristic ring vibrations at 1450-1400, 1100-1090 and 760-720 cm^{-1} regions. The absence of bands characteristic of ν(CO) and primary amine ν(NH) confirms the formation of the proposed Schiff base framework.

The structure of the new compound 11 was characterized by NMR spectroscopy. ^{1}H, ^{13}C, COSY, HSQC, HMBC and APT techniques of NMR spectroscopy were used for the identification of the structure of the compound 11. The ^{1}H-NMR and ^{13}C-NMR spectral data of the compound are given at Table 2. The peak integrations in the ^{1}H-NMR spectrum correspond to the expected number of protons. The influences seen in the COSY, HSQC and HMBC spectra were compatible with the structure and given at Table-3. The quaterner carbons of the compound were seen in the APT spectrum.

Table 1. Structures of the Synthesized Schiff Bases and Reaction Yields
(Tablo 1. Sentezlenen Schiff Bazlarının Yapıları ve Reaksiyon Verimleri)

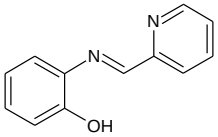
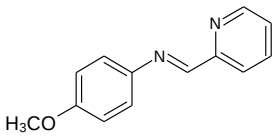
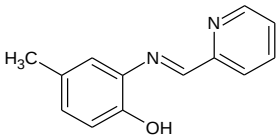
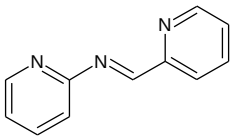
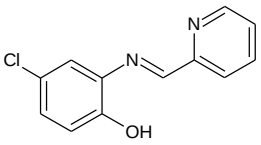
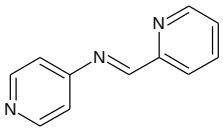
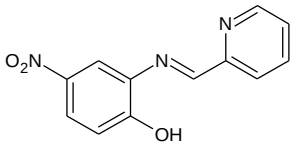
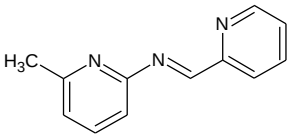
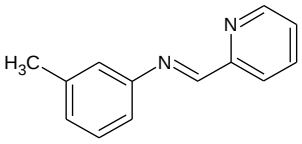
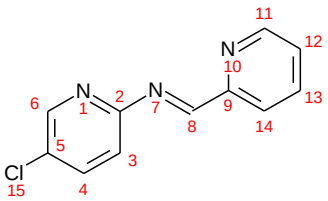
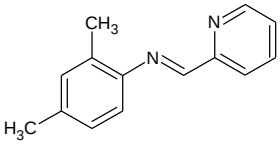
Amine	Pyridine-2-aldehyde	Amine	Pyridine-2-aldehyde
2-Aminophenol	 (1) %83	4-Methoxy-aniline	 (7) %75
2-Amino-4-methylphenol	 (2) %78	2-Amino-pyridine	 (8) %72
2-Amino-4-chlorophenol	 (3) %63	4-Amino-pyridine	 (9) %76
2-Amino-4-nitrophenol	 (4) %86	2-Amino-6-methyl-pyridine	 (10) %69
3-Methyl-aniline	 (5) %71	2-Amino-5-chloro-pyridine	 (11) %66 (new)
2,4-Dimethyl-aniline	 (6) %75		

Table 2. Spectral Analysis of Schiff Bases
(Tablo 2. Schiff Bazlarının Spektral Analizi)

Name of the compound	Spectral data
2-[(E)-(pyridin-2-ylmethylidene)amino] phenol (1)	MP: 101°C; UV-vis: 279.0 and 361.0 nm; IR(KBr): 3362, 3047, 1627, 1564, 1355 cm^{-1}; MS m/z : 198 (M^+), 181 (M-OH), 169 (M-HCN), 120 (M-C_5H_4N).
4-Methyl-2-[(E)-(pyridin-2-ylmethylidene)amino] phenol (2)	MP: 81°C; UV-vis: 276.0 and 372.0 nm; IR(KBr): 3352, 3048, 1625, 1587, 1356 cm^{-1}; MS m/z : 212 (M^+), 195 (M-OH), 183 (M-HCN), 134 (M-C_5H_4N).
4-Chloro-2-[(E)-(pyridin-2-ylmethylidene)amino] phenol (3)	MP: 136°C; UV-vis: 280.0 and 370.0 nm; IR(KBr): 3382, 3050, 1626, 1584, 1367 cm^{-1}; MS m/z : 232 (M^+), 215 (M-OH), 203 (M-HCN), 197 (M-Cl), 154 (M-C_5H_4N).
4-Nitro-2-[(E)-(pyridin-2-ylmethylidene)amino] phenol (4)	MP: 197°C; UV-vis: 280.0 and 351.0 nm; IR(KBr): 3368, 3098, 1621, 1568, 1353 cm^{-1}; MS m/z : 241 (M-2), 225 (M-OH), 195 (M-NO_2), 167 (M-C_5H_4N).
3-Methyl-N-[(E)-pyridin-2-ylmethylidene]aniline (5)	MP: 49°C; UV-vis: 278.0 and 336.0 nm; IR(KBr): 3053, 2917, 1628, 1564, 1355 cm^{-1}; MS m/z : 196 (M^+), 181 (M-CH_3), 168 (M-HCN), 118 (M-C_5H_4N).
2,4-Dimethyl-N-[(E)-pyridin-2-ylmethylidene]aniline (6)	MP: 69°C; UV-vis: 337.0 nm; IR(KBr): 3054, 2949, 1627, 1563, 1347 cm^{-1}; MS m/z : 210 (M^+), 195 (M-CH_3), 182 (M-HCN), 132 (M-C_5H_4N).
4-Methoxy-N-[(E)-pyridin-2-ylmethylidene]aniline (7)	MP: 43°C; UV-vis: 272.0 and 343.0 nm; IR(KBr): 3048, 2929, 1625, 1563, 1343 cm^{-1}; MS m/z : 212 (M^+), 197 (M-CH_3), 185 (M-HCN), 134 (M-C_5H_4N).
N-[(E)-pyridin-2-ylmethylidene]pyridin-2-amine (8)	MP: 117°C; UV-vis: 272.0 and 279.0 nm; IR(KBr): 3052, 1598, 1571, 1529, 1325 cm^{-1}; MS m/z :183 (M^+), 182 (M-1), 156 (M-HCN), 105(M-C_5H_4N).
N-[(E)-pyridin-2-ylmethylidene]pyridin-4-amine (9)	MP: 136°C; UV-vis: 276.0 and 279.0 nm; IR(KBr): 3074, 1645, 1593, 1587, 1337 cm^{-1}; MS m/z :183 (M^+), 182 (M-1), 156 (M-HCN), 105(M-C_5H_4N).
6-Methyl-N-[(E)-pyridin-2-ylmethylidene]pyridin-2-amine (10)	MP: 109°C; UV-vis: 271.0 and 279.0 nm; IR(KBr): 3013, 2921, 1597, 1531, 1335 cm^{-1}; MS m/z : 197 (M^+), 182 (M-CH_3), 169 (M-HCN), 119 (M-Ar), 105(M-C_5H_4N), 78 (ArH^+).
5-Chloro-N-[(E)-pyridin-2-ylmethylidene]pyridin-2-amine (11)	MP: 115°C; UV-vis: 273.0 and 305.0 nm; IR(KBr): 3078, 1589, 1575, 1517, 1314 cm^{-1}; ^1H NMR($CDCl_3$):δ 6.77 (1H, d, J=7.4 Hz, H-3), 7.25 (1H, dd, J=7.4; 1.2 Hz, H-14), 7.26 (1H, dd, J=7.4; 1.2 Hz, H-11), 7.55 (1H, brd, J=7.8 Hz, H-4), 7.69 (2H, td, J=7.8;1.2 Hz, H-12 and H-13), 8.57 (1H, brd, J=1.6 Hz, H-6), 9.20 (1H, s, H-8) ppm. ^{13}C NMR($CDCl_3$): δ 120.95(C-3), 121.74(C-14), 127.89(C-4), 137.09(C-11), 137.48(C-12), 137.96(C-13), 145.60(C-9), 145.98(C-2), 148.83(C-5), 150.25(C-6), 156.52(C-8) ppm. MS m/z : 217 (M^+), 190 (M-HCN), 182 (M-Cl), 112($C_5H_4NCl^+$), 105 (M-C_5H_4NCl).

Table 3. NMR Spectral Data of Compound 11
(Tablo 3. Bileşik 11'in NMR Spektral Verileri)

H	^{1}H-NMR	COSY	HSQC	HMBC	C	APT
2	---	---	---	---	2	145.98
3	6.77 (1H, d)J=7.4 Hz	H-4	C-3	C-5	3	120.95
4	7.55 (1H, brd) J=7.8 Hz	H-3	C-4	C-2 C-6	4	127.89
5	---	---	---	---	5	148.83
6	8.57 (1H, brd) J=1.6 Hz	---	C-6	C-2 C-4	6	150.25
8	9.20 (1H, s)	---	C-8	C-2 C-14	8	156.52
9	---	---	---	---	9	145.60
11	7.26 (1H, dd) J=7.4; 1.2 Hz	H-12	C-11	C-9 C-13	11	137.09
12	7.69 (2H, td)J=7.8; 1.2 Hz	H-11 H-13	C-12	C-14	12	137.48
13	7.69 (2H, td)J=7.8; 1.2 Hz	H-12 H-14	C-13	C-9 C-11	13	137.96
14	7.25 (1H, dd) J=7.4; 1.2 Hz	H-13	C-14	C-12	14	121.74

3.2. Radiolabelling of Schiff Bases with Tc-99m (Schiff Bazlarının Tc-99m İle Radyoetiketlenmesi)

5.0 mg of the Schiff base compounds were dissolved in ethanol (1.0 mL) and 1.0 mL solution of $SnCl_2.2H_2O$ (0.4 mg/mL) was added and then taken in a shielded vial. The pH of the solution was adjust within the range of 6,5 – 7.0 by NaOH solution. To the resulting solution, generator elution [99mTc]TcO_4 solution (8-10 mCi) was added and the reaction mixture was incubated at room temperature or heated in water bath by mixing to facilitate complete complexation. Reaction time, temperature and labeling yields for each compound were giving at Table 4.

The complexation yield and radiochemical purity of 99mTc-Schiff base complexes were determined by ITLC (instant thin layer chromatography, Gelman SG, 1x10 cm strips). The presence of free [99mTc]TcO_4 and colloidal [99mTc]TcO_2 was assessed with acetone and saline. The R_f values are given below.

Saline:
R_f = 0 for 99mTc-Schiff base complexes and colloidal [99mTc]TcO_2.
R_f = 0.9 – 1.0 for free [99mTc]TcO_4.

Acetone:
R_f = 0 for colloidal [99mTc]TcO_2
R_f = 0.9 – 1.0 for 99mTc-Schiff base complexes and [99mTc]TcO_4.

Obtained chromatograms have been evaluated by TLC scintigraphy system and then each ITLC was cut into 1.0 cm segments and counts of each segment were taken by gamma counters. ITLC spectrum for Tc-99m complex of compound 11 is given at Figure 1.

Several experiments were carried out by varying the molar ratios of Schiff base to Tc-99m, temperature, reaction time and pH of the reaction mixture, to optimize the labeling reaction conditions to obtain quantitative yields. Effect of reaction time to labeling yield was given at Figure 2.

Table 4. Labeling Reaction Values of Schiff Base Compounds
(Tablo 4. Schiff Bazı Bileşiklerinin Etiketlenme Reaksiyon Değerleri)

Compound	Reaction Temperature (°C)	Reaction Time (minute)	Labelling Yield (%)
1	Room Temp.	45	96
2	Room Temp.	30	95
3	Room Temp.	60	93
4	Room Temp.	30	95
5	60	60	98
6	60	60	97
7	60	60	96
8	60	60	91
9	60	60	90
10	60	60	96
11	80	90	91

Figure 1. ITLC Spectrum for 99mTc-compound 11 (saline)
(Şekil 1. 99mTc-Bileşik 11 için ITLC Spektrumu (salin))

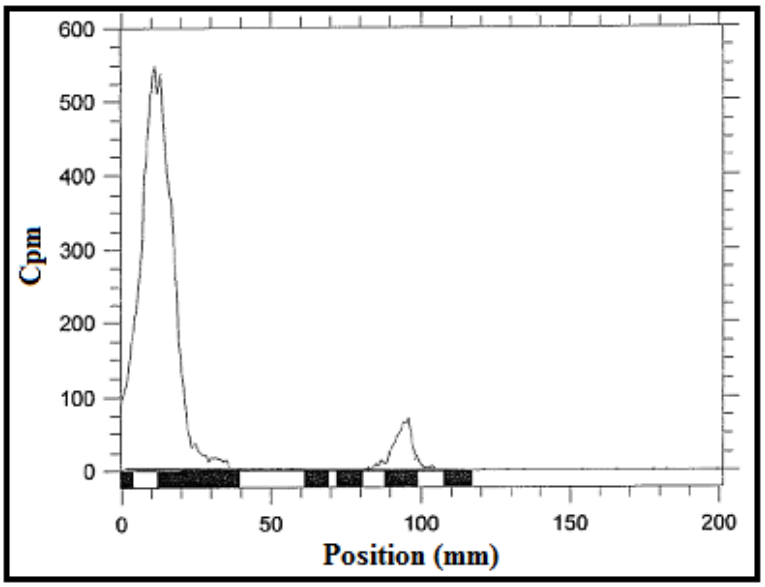
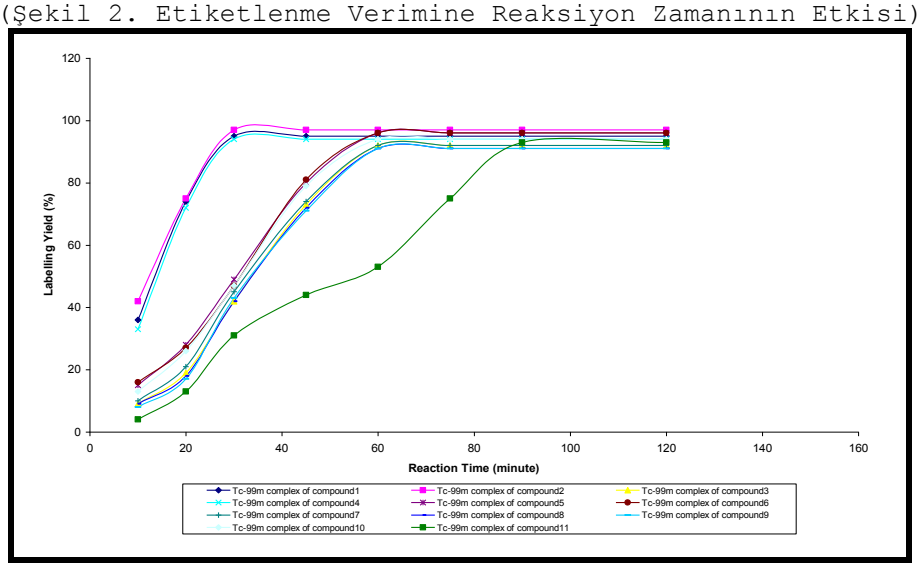


Figure 2. Effect of Reaction Time to Labelling Yield
(Şekil 2. Etiketlenme Verimine Reaksiyon Zamanının Etkisi)



4. CONCLUSIONS (SONUÇLAR)

The investigation of the present work is the synthesis of Schiff bases which could be used in health area because of their antihistaminic, anticancer, antimicrobial, antibacterial, antifungal activities and then labeling of them with Tc-99m to consider the complexation capacity and stability.

Synthesis of Schiff bases have been achieved in high yields starting from the condensation reaction of pyridine-2-aldehyde and derivatives of aminophenol, aniline and aminopyridine analogues as reported in literature. The synthesized ligands were checked by comparing the TLC with the starting materials, which resulted in a single spot different from the starting materials. IR studies of each compound confirms the formation of –C=N- bonds as well as lack of –C=O- from original aldehydic compounds. For the Schiff base compounds (1-10) which have been previously synthesized, UV, IR and GC-MS spectra were taken to determine the purity and identification of the structure. The structure of the new Schiff base (11) was characterized by UV, IR, GC-MS and NMR spectroscopy. ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC and APT techniques of NMR spectroscopy were used for the identification of the structure of the compound 11.

The synthesized Schiff base derivatives of pyridine-2-aldehyde were labeled with Tc-99m. Labeling yield and radiochemical purity were calculated by using chromatographic methods and obtained chromatograms have been evaluated by TLC scintigraphy system and gamma counters. Preliminary complexation of synthesized compounds with Tc-99m was found to give sufficiently stable complexes under physiological conditions.

The preliminary studies with these novel Schiff base ligands are encouraging to carry out further in vivo experiments for targeted imaging of human tumor. The therapeutic potential of these complexes can further extend by applying these in different animal models and cell lines.

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