European Journal of Chemistry
Journal homepage: www.eurjchem.com

Synthesis, configuration and properties of some new 3,4,5-substituted oxazolidin-2-ones

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ARTICLE INFORMATION

Received: 25 May 2010
Received in revised form: 30 October 2010
Accepted: 08 December 2010
Online: 31 March 2011

KEYWORDS

Oxazolidin-2-ones
Stereochemistry
Configuration
Mannich reaction
Analgesis activity
Drug similarity

ABSTRACT

Novel 3,4,5-substituted-oxazolidin-2-ones containing piperazine, 1-(4-chlorophenyl) piperazine, benzhydrylpiperazine, morpholine and piperidine rings were synthesized via Mannich reaction. The stereochemistry of *syn* and *anti*-isomers was assigned using the observed differences in the chemical shifts of the oxazolidinone ring protons and the values of vicinal coupling constants (^3J) between the two protons of the oxazolidin-2-one ring. For all compounds NOE (Nuclear Overhauser Effect) NMR spectra were measured in order to prove additionally the position of the substituents in the oxazolidin-2-one ring. Some physic chemical, steric and electronic properties of the compounds were determined in order to establish the similarity between the synthesized and reference compounds. The performed computations showed that the *anti*-isomers possessed lower electronic energies in comparison to these of *syn*-compounds. The nucleus-nucleus repulse energies (NRE) and the highest occupied molecular orbital energies (HOMO) of the *anti*-isomers are higher than the HOMO and NRE energies of *syn*-compounds. The Connolly Solvent Accessible Surface Area (SAS) and Connolly Molecular Surface Area (MS) values of *anti*-isomers are lower than these of *syn*-isomers. The same relations were observed for the reference compounds. Probably the differences in the electronic and steric properties are responsible not only for the higher LD_{50} value of the reference *anti*-compound, but also may contribute to the higher toxicity of the prepared *anti*-Mannich bases in comparison to that of the *syn*-diastereoisomers.

1. Introduction

The oxazolidin-2-ones are an important class of heterocyclic compounds, which attract the attention of the investigators with the possibilities they give both for total regio- and stereocontrol in the synthesis of "enantiomerically-pure" compounds like β-hydroxy-carboxylic acids, amino alcohols, aminophosphonic acids and for the modifying of complicated structures of natural origin [1-5]. Moreover, oxazolidin-2-one derivatives are particularly versatile drugs and their study is extremely relevant from a pharmacological point of view. The oxazolidin-2-one compounds represent a new class of antibacterial agents, effective against virtually all important gram-positive pathogens, including methicillin-resistant staphylococci, penicillin-resistant pneumococci, macrolide-resistant streptococci and vancomycin-resistant enterococci. The pharmacokinetic traits of linezolid and eperozolid encouraged the further investigation for improvement of the characteristics of the oxazolidinones in order to eliminate the early resistance development [6-9]. Depending on the placement of the substituents, oxazolidin-2-one derivatives exhibit different activity. The 3-phenyl-oxazolidin-2-one derivatives substituted at position 5 with alcoholic or ethereal group as toloxatone, cimoxatone are proved to be anti-depressant agents, while the 5-amino-3-phenyloxazolidin-2-one derivatives are effective as anti-Parkinson agents (almoxatone, MD 780236) [10-12]. Some oxazolidin-2-ones are useful as selective alpha-7 nicotinic receptor agonist, as centrally acting muscarinic agents and as analgesic agents for the treatment of pain, as sleep aids and agents for treating the symptoms of senile dementia, Alzheimer's disease [13-15].

In this paper, we describe the synthesis of some novel 3,4,5-trisubstituted-oxazolidin-2-ones containing piperazine, benzhydrylpiperazine, morpholine and piperidine rings via Mannich reaction as potential bioactive compound as well as some of their physicochemical, steric and electronic properties in order to estimate the similarity between these new oxazolidinones and our previously reported. The latter compounds were found to possess analgesic activity against acetic acid-induced pain, but different LD_{50} values [16]. The present paper is a continuation of this work and is focused on understanding in more depth the physical properties of 3,4,5-substituted oxazolidin-2-ones as a function of their ring stereochemistry.

The selection of these structures used in this study was done in accordance with the fact that the thiophene heterocycle takes part in the structure of some substances, possessing analgesic activity, and the piperazine, morpholine and piperidine moieties occur as a pharmacophore in many biological active compounds and are essential elements in drug design [17-21].

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.2.1.18-24.125

2. Experimental

Melting points were determined on an Electrothermal AZ 9000 3MK4 apparatus and were uncorrected. The thin layer chromatography (TLC, R_f values) was performed on silica gel 60 plates F_{254} and Al_2O_3-plates (Merck, 0.2 mm thick) using mobile phase benzene:diethyl ether (1:1;), benzene:diethyl ether:ethanol (4:1:3; 4:1:2) and visualization was effected with ultraviolet light. IR spectra were recorded on a Bruker Equinox 55 spectrophotometer as potassium bromide discs. ^{1}H NMR spectra were obtained on a Bruker Avance DRX 250 MHz spectrometer (Bruker, Faelanden, Switzerland) using a dual 5 mm probe head and $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts were expressed relative to tetramethylsilane (TMS) and were reported as δ (ppm). The measurements were carried out at ambient temperature (300 K). Typical conditions for 1-D ^{1}H spectra were: pulse width 30°, FT size 32 K and digital resolution 0.2 Hz per point.

2. Synthesis

Compounds **1**, **2** and **3** were prepared according the procedure reported in [22].

2.1. General procedure for compounds **4** *and* **6**

A three-necked flask equipped with a stirrer, condenser and thermometer was charged with 0.05 mol (*2S,3R*) or (*2R, 3R*)-2-methyl-3-(thien-2-yl)-3-hydroxypropanoyl hydrazide (**2** or **3**), solution of 3.2 mL acetic acid in 100 mL water and 120 mL diethyl ether. To the formed suspension a solution of 3.8 g sodium nitrite in 30 mL water was added drop wise at 15-20 °C by stirring for 20 minutes. The reaction mixture was stirred at the above mentioned temperature up to the full disappearance of the solid phase, about 2 or 3 hours. After neutralization with 10% solution of sodium hydrogen carbonate the aqueous and organic layers were separated. The aqueous layer was extracted several times with benzene and diethyl ether. The collected organic layers were dried with sodium sulphate and after filtering the solution was concentrated under reduced pressure to 20-25 mL of the original volume. 4-Methyl-5-(thien-2-yl)-oxazolidine-2-ones, (**4**, **6**) crystallized after cooling of the remained residue. The compounds were re-crystallized with benzene.

Anti-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-one, **4**: Yield: 70 %. M.p.: 87-90 °C. R_f = 0.66, mobile phase: benzene:diethyl ether (1:1). ^{1}H NMR ($CDCl_3$): 1.33 (3H, CH_3, d, *J*=6.2 Hz); 3.98 (1H, N-CH, dq, *J*=7.7, *J*=6.2 Hz); 5.16 (1H, OCH, d, *J*=7.7), 6.66-6.64 (2H, 2CH, m, thiophene), 6.92 (1H, CH, d, *J*=4.5 Hz, thiophene). ^{13}C NMR ($CDCl_3$): 15.4 (CH_3), 56.5 (NCH), 81.5 (OCH), 125.0, 126.0, 127.2 (3CH, thiophene), 136.8, 137.2 (C, thiophene); 158.7 (C=O). Anal. Calcd. for $C_8H_9NO_2S$: C, 52.44; H, 4.95; N, 7.64; S, 17.50; Found: C, 52.40; H, 4.91; N, 7.69; S, 17.46.

Syn-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-one, **6**: Yield: 65%. M.p.: 101-102 °C. R_f = 0.50, mobile phase: benzene:diethyl ether (1:1), Al_2O_3-plates. ^{1}H NMR ($CDCl_3$): 0.99 (3H, CH_3, d, *J*=6.60 Hz); 4.22 (1H, N-CH, dq, *J*=8.07 Hz, *J*=6.60 Hz), 5.91 (1H, OCH, d, *J*=8.07 Hz), 6.54 (1H, NH, bs), 7.10–6.99 (2H, 2CH, m, thiophene), 7.34 (1H, CH, dd, *J*=1.71 Hz, *J*=4.52 Hz, thiophene). ^{13}C NMR ($CDCl_3$): 16.8 (CH_3), 52.5 (NCH), 78.1 (OCH), 125.8, 126.0, 126.8 (3CH, thiophene), 137.2 (C, thiophene), 159.1 (C=O). Anal. Calcd. for $C_8H_9NO_2S$: C, 52.44; H, 4.95; N, 7.64; S, 17.50; Found: C, 52.38; H, 5.05; N, 7.60; S, 17.37.

2.2. General procedure for compounds 5e and 7e

To a solution of 1 g (5.5 mmol) of the *anti*-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-one **4** or to a solution of 1 g (5.5 mmol) of the *syn*-isomer **6** in 40 mL ethanol were added 0.53 g (2.7 mmol) piperazine hexahydrate and 0.54 mL (6.8 mmol) formaldehyde in form of 35%. The solution was refluxed for 2 hours and after that ethanol was distilled in vacuum to 5-10 mL of original volume. The obtained Mannich base crystallized upon cooling and was re-crystallized with ethanol.

Anti-1,4-bis[(4-methyl-5-thien-2-yl-2-oxo-1,3-oxazolidin-3-yl)methyl] piperazine, **5e**: Yield: 56 %. M.p.: 145-147 °C (Decomp.). R_f = 0.65, mobile phase: bezene:diethyl ether: ethanol (4:1:3), Al_2O_3-plates. ^{1}H NMR ($CDCl_3$): 1.37 (6H, CH_3, d, *J*=6.11 Hz), 2.45–2.67 (8H, 4 NCH_2, m, piperazine), 3.85 (2H, N(*H*CH)N, N(*H*CH)N, d, *J*=12.47 Hz), 3.99 (2H, two N-CH, m, from two oxazolidinone rings), 4.16 (2H, N(HC*H*)N, N(HC*H*)N, d, *J*=12.47 Hz), 5.19 (2H, two OCH, d, *J*=7.34 Hz), 7.02 (2H, SCH-C*H*-CH, dd, *J*=3.42 Hz, *J*=4.89 Hz, from two thiophene rings), 7.14 (2H, SCH-CH-C*H*, dd, *J*=3.42 Hz, *J*=1.22 Hz from two thiophene rings), 7.37 (2H, SCH, dd, *J*=1.22, *J*=4.89 Hz from two thiophene rings). ^{13}C NMR ($CDCl_3$): 17.3 (CH_3), 50.2 (NCH_2, piperazine), 58.6 (NCH, oxazolidinone), 63.9 (NCH_2N), 78.4 (OCH), 126.7, 126. 8, 127.0 (CH, thiophene), 140.0 (C, thiophene), 157.1 (C=O). Anal. Calcd. for $C_{22}H_{28}N_4O_4S_2$: C, 56.30; H, 6.16; N, 11.42; S, 13.07; Found: 56.23; H, 6.19; N, 11.37; S, 13.11.

Syn-1,4-bis[(4-methyl-5-thien-2-yl-2-oxo-1,3-oxazolidin-3-yl)methyl] piperazine, **7e:** Yield: 62 %. M.p.: 196-197 °C (Decomp.). R_f = 0.48, mobile phase: benzene:diethyl ether: ethanol (4:1:3), Al_2O_3-plates. ^{1}H NMR ($CDCl_3$): 0.97 (6H, 2 CH_3, d, *J*=6.60 Hz); 2.59 (8H, 4 N-CH_2, m, piperazine), 3.81 (2H, N(*H*CH)N, N(*H*CH)N, d, *J*=12.62 Hz), 4.23 (4H, N(HC*H*)N, N(HC*H*)N, d, *J*=12.62 Hz; overlapped with two N-CH, dq, *J*=8.07 Hz, *J*=6.60 Hz from the two oxazolidinone rings), 5.82 (2H, two OCH, d, *J*=8.07 Hz), 7.11–6.99 (4H, 2 SCH-CH-C*H* and 2 SCH-C*H*-CH, m, from two thiophene rings), 7.33 (2H, 2 SCH, m, from two thiophene rings). ^{13}C NMR ($CDCl_3$): 13.8 (CH_3), 50.2 (NCH_2, piperazine), 54.9 (NCH, oxazolidinone), 64.0 (NCH_2N), 75.8 (OCH), 125.9, 126.20, 126.8 (CH, thiophene), 137.4 (C, thiophene), 159.4 (C=O). Anal. Calcd. for $C_{22}H_{28}N_4O_4S_2$: C, 56.30; H, 6.16; N, 11.42; S, 13.07; Found: 56.35; H, 6.11; N, 11.47; S, 13.0.

2.3. General procedure for compounds 5a-d and 7a-d

A one necked flask equipped with a stirrer and condenser was charged with 1.2 g (6.6 mmol) of *anti*-4-methyl-5-thien-2-yl-1,3-oxazolidine-2-one **4**, respectively, the *syn*-isomer **6**, 50 mL ethanol, 0.46 mL (5.3 mmol) of the appropriate amine and 0.7 mL (9 mmol) formaldehyde in form of 35% formalin. After refluxing for 2 hours, the solvent was removed by reduced pressure and the remaining light yellow oil crystallized after cooling.

Anti-4-methyl-3-(morpholin-1-ylmethyl)-5-thien-2-yl-1,3-oxazolidin-2-one, **5a**: After removing of the solvent to the remaining oil diethyl ether was added and compound **5a** was crystallized. Re-crystallization was carried out with ethanol. Yield: 62 %. M.p.: 166-169 °C (Decomp.). R_f = 0.61, mobile phase: benzene:diethyl ether:ethanol (4:1:2), Silica gel F_{254}. ^{1}H NMR($CDCl_3$): 1.38 (3H, CH_3, d, *J*=6.24 Hz), 3.1–3.3 (4H, 2 NCH_2,m, morpholine), 3.8–4.0 (4H, 2 OCH_2, m, morpholine), 4.43–4.56 (1H, NCH, m, oxazolidinone), 4.58 (1H, N*H*CHN, d, *J*=13.45 Hz), 4.72 (1H, NHC*H*N, d, *J*=13.45 Hz), 5.55 (1H, OCH, d, J=6.66 Hz), 7.10 (1H, SCH-C*H*-CH, dd, *J*=3.55 Hz, *J*=5.01 Hz), 7.39 (1H, SCH-CH-C*H*, dd, *J*=3.55 Hz, *J*=1.22 Hz), 7.67 (1H, SCH, dd, *J*=1.22 Hz, *J*=5.01 Hz). ^{13}C NMR ($CDCl_3$): 17.0 (CH_3), 43.0 (NCH_2, morpholine), 49.1 (NCH_2, morpholine), 58.4 (NCH, oxazolidin one), 61.2 (NCH_2N), 63.1 (OCH_2, morpholine), 63.6 (OCH_2, morpholine), 78.4 (OCH), 127.7, 128.4, 128.5 (CH, thiophene), 139.2 (C, thiophene), 156.5 (C=O). Anal. Calcd. for $C_{13}H_{18}N_2O_3S$: C, 55.30; H, 6.43; N, 9.92; S, 11.37; Found: C, 55.24; H, 6.48; N, 9.86; S, 11.43.

Syn-4-methyl-3-(morpholin-1-ylmethyl)-5-thien-2-yl-1,3-oxazolidin-2-one, **7a**: After removing the solvent, diethyl ether was added and the compound crystallized. Recrystallization was carried out with ethanol. Yield: 64 %. M.p.: 83-85 °C. R_f = 0.61, mobile phase: benzene:diethyl ether:ethanol (4:1:2), Silica gel F_{254}. ^{1}H NMR ($CDCl_3$): 0.98 (3H, CH_3, d, *J*=6.60 Hz), 2.69–2.49 (4H, 2 NCH_2, m, morpholine), 3.75–3.66 (4H, 2 OCH_2, m, morpholine), 3.79 (1H, N(*H*CH)N, d, *J*=12.47 Hz), 4.21 (1H, N(HC*H*)N, d, *J*=12.47 Hz), 4.25 (1H, NCH, dq, *J*=8.07 Hz, *J*=6.60 Hz), 5.82 (1H, OCH, d, *J*=8.07 Hz), 7.07–6.99 (2H, two CH, m, thiophene), 7.34 (1H, SCH, dd, *J*=2.20 Hz, *J*=4.16 Hz, thiophene). ^{13}C NMR ($CDCl_3$): 13.8 (CH_3), 50.9 (NCH_2, morpholine), 54.9 (NCH, oxazolidinone), 64.4 (NCH_2N), 66.7 (OCH_2, morpholine), 75.8 (OCH), 125.9, 126.2, 126.8 (CH, thiophene), 137.4 (C, thiophene), 157.4 (C=O). Anal. Calcd. for $C_{13}H_{18}N_2O_3S$ C, 55.30; H, 6.43; N, 9.92; S, 11.37; Found: C, 55.35; H, 6.37; N, 9.85; S, 11.29.

Anti-1-{[4-methyl-2-oxo-5-thien-2-yl-1,3-oxazolidin-3-yl]methyl}piperidinium chloride, **5b**: After removing ethanol and water under reduced pressure dry diethyl ether was added to the oil residue. Saturating ether solution with dry HCl gas resulted in the formation of hydrogen chloride of the Mannich base. Yield 68 %, recrystallization with ether:ethanol (2:1). M.p.: 156-158 °C. R_f = 0.5, mobile phase benzene:diethyl ether:ethanol (4:1:3), Silica gel F_{254}. ^{1}H NMR (DMSO-d_6): 1.38 (3H, CH_3, d, *J*=6.60 Hz), 1.60-1.98 (6H, m, 3 CH_2 , piperidine), 2.68-3.10 (2H, m, NCH_2, piperidine), 3.17-3.48 (2H, m, NCH_2, piperidine), 4.43-4.67 (3H, m, NCH_2N, overlapped with NCH from oxazolidinone), 5.56 (1H, OCH, d, *J*=6.11 Hz, oxazolidin one), 7.10 (1H, SCH-C*H*-CH, dd, *J*=3.42 Hz, *J*=5.14 Hz), 7.41 (1H, SCH-CH-C*H*, dd, *J*=3.42 Hz, *J*=1.22 Hz), 7.68 (1H, SCH, dd, *J*=1.22 Hz, *J*=5.14 Hz), 11.29 (1H, NH, bs). ^{13}C NMR (DMSO-d_6): 16.7 (CH_3), 21.4 (CH_2, piperidine), 21.7 (CH_2, piperidine), 48.9 (NCH_2, piperidine), 49.9 (NCH_2, piperidine), 57.8 (NCH, oxazolidinone), 60.5 (NCH_2N), 77.8 (OCH, oxazolidinone), 127.2, 127.8, 128.0 (CH, thiophene). Anal. Calcd. for $C_{14}H_{21}ClN_2O_2S$: C, 53.07; H, 6.68; Cl, 11.19; N, 8.84; S, 10.12; Found: C, 53.11; H, 6.62; Cl, 11.26; N, 8.78; S, 10.17.

Syn-1-{[(4-methyl-2-oxo-5-thien-2-yl-1,3-oxazolidin-3-yl]methyl}piperidinium chloride, **7b**: After removing ethanol and water under reduced pressure dry diethyl ether was added to the oil residue. Saturating of ether solution with dry HCl gas resulted to the formation of hydrogen chloride of Mannich base. Yield 68 %, after recrystallization with ether:ethanol (2:1). M.p.: 181-182 °C. R_f = 0.68, mobile phase benzene:diethyl ether:ethanol (4:1:3), Silica gel F_{254}. ^{1}H NMR (DMSO-d_6): 0.93 (3H, CH_3, d, *J*=6.60 Hz), 1.62-1.96 (6H, m, 3 CH_2 from piperidine), 2.67-3.11 (2H, m, NCH_2, piperidine), 3.31-3.58 (2H, m, NCH_2, piperidine), 4.42-4.54 (1H, N*H*CHN, d, *J*=13.45 Hz), 4.54-4.80 (2H, m, NHC*H*N overlapped with NCH from oxazolidinone), 6.18 (1H, OCH, d, *J*=7.58 Hz), 7.11 (1H, SCH-C*H*-CH, dd, *J*=3.55 Hz, *J*=5.14 Hz), 7.18 (1H, SCH-CH-C*H*, dd, *J*=3.42 Hz, *J*=1.22 Hz), 7.63 (1H, SCH, dd, *J*=1.22 Hz, *J*=5.14 Hz), 11.29 (1H, NH, bs). ^{13}C NMR (DMSO-d_6): 13.7 (CH_3), 22.0 (CH_2, piperidine), 22.1 (CH_2, piperidine), 49.6 (NCH_2, piperidine), 51.5 (NCH_2, piperidine), 55.9 (NCH, oxazolidinone), 61.6 (NCH_2N), 77.0 (OCH, oxazolidinone), 126.2, 126.6, 127.0 (CH, thiophene), 135.7 (C, thiophene), 157.4 (C=O). Anal. Calcd. for $C_{14}H_{21}ClN_2O_2S$: C, 53.07; H, 6.68; Cl, 11.19; N, 8.84; S, 10.12; Found: C, 53.01; H, 6.74; Cl, 11.13; N, 8.77; S, 10.05.

Anti-3-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-one, **5c:** Yield 67 %. M.p.: 130-131 °C, re-crystallization with ethanol. R_f = 0.54, mobile phase: benzene:diethyl ether (4:1), Silica gel F_{254}. ^{1}H NMR ($CDCl_3$): 1.39 (d, 3H, CH_3, *J*=6.2 Hz); 2.72 (m, 4H, 2 NCH_2, piperazine); 3.15 (m, 4H, 2 NCH_2, piperazine); 3.91 (d, 1H, N(*H*CH)N, *J*=12.6 Hz); 4.03 (m, 1H, NCH, qd, *J*=6.2 Hz, *J*=7.2 Hz, oxazolidinone); 4.24 (d, 1H, N(HC*H*)N, *J*=12.6 Hz); 5.20 (d, 1H, CHO, *J*=7.2 Hz, oxazolidinone); 6.83 (m, 2H, CH-Ph, *AA'*BB' system); 7.02 (dd, 1H, SCH-C*H*-CH, *J*=5.1 Hz, *J*=3.7 Hz, thiophene); 7.14 (1H, dd, *J*=1.2 Hz, *J*=3.7 Hz, SCH-CH-C*H*, thiophene), 7.20 (m, 2H, CH-Ph, AA'*BB'* system); 7.37 (dd, 1H, SCH, *J*=1.2 Hz, *J*=5.1 Hz, thiophene). ^{13}C NMR ($CDCl_3$): 17.3 (CH_3), 48.9, 50.3, 58.7, 63.8, 78.5, 117.3 (CH, Ph), 124.6 (C, Ph), 126.8 (CH, thiophene), 126.8 (CH, thiophene), 127.0 (CH, thiophene), 128.9 (CH, Ph), 139.9 (C, thiophene), 149.7 (C, Ph), 157.1 (C=O). Anal. Calcd. for $C_{19}H_{22}ClN_3O_2S$: C, 62.95; H, 6.16; N, 12.23; S, 9.34; Found: C, 62.87; H, 6.12; N, 12.28; S, 9.40.

Syn-3-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-one, **7c**: Yield 64 %. M.p.: 118-120 °C, recrystallization with ethanol. R_f = 0.48, mobile phase: benzene:diethyl ether (4:1), Silica gel F_{254}. ^{1}H NMR ($CDCl_3$): 1.02 (d, 3H, CH_3, *J*=6.60 Hz); 2.75 (m, 4H, 2 NCH_2, piperazin); 3.16 (m, 4H, 2 NCH_2); 3.88 (1H, d, N(*H*CH)N, *J*=12.72 Hz); 4.29 (2H, N(HC*H*)N, d, *J*=12.72 Hz, overlapped with NCH from oxazolidinone); 5.83 (1H, d, CHO, *J*=7.83 Hz, oxazolidinone); 6.84 (m, 2H, CH-Ph, *AA'*BB' system); 7.01-7.06 (2H, m, SCH-C*H*-CH and SCH-CH-C*H*, thiophene); 7.17-7.24 (m, 2H, CH-Ph, AA'*BB'* system); 7.33 (1H, dd, SCH, *J*=2.20 Hz, *J*=4.16 Hz, thiophene). Anal. Calcd. for $C_{19}H_{22}ClN_3O_2S$: C, 62.95; H, 6.16; N, 12.23; S, 9.34; Found: C, 62.89; H, 6.20; N, 12.16; S, 9.38.

Anti-4-methyl-3-[(4-benzhydrylpiperazin-1-yl)methyl]-5-thien-2-yl-1,3-oxazolidin-2-one, **5d**: Yield 70%. M.p.: 133-134 °C, recrystallization with ethanol. R_f = 0.62, mobile phase: benzene:diethyl ether:ethanol (4:1:3), Silica gel F_{254}. ^{1}H NMR ($CDCl_3$): 1.34 (3H, CH_3, d, *J*=6.06 Hz), 2.22-2.41 (8H, 4CH_2, m, piperazine), 3.71 (1H, N(*H*CH)N, d, *J*=12.62 Hz), 4.16-4.01 (3H, N(HC*H*)N, overlapped with C*H*$(Ph)_2$ and N-CH from oxazolidinone), 5.19 (1H, OCH, d, *J*=6.65 Hz), 7.42-6.92 (13H, 2Ph and CH from thiophene). Anal. Calcd. for $C_{26}H_{29}N_3O_2S$: C, 69.77; H, 6.53; N, 9.39; S, 7.16; Found: C, 69.70; H, 6.48; N, 9.29; S, 7.22.

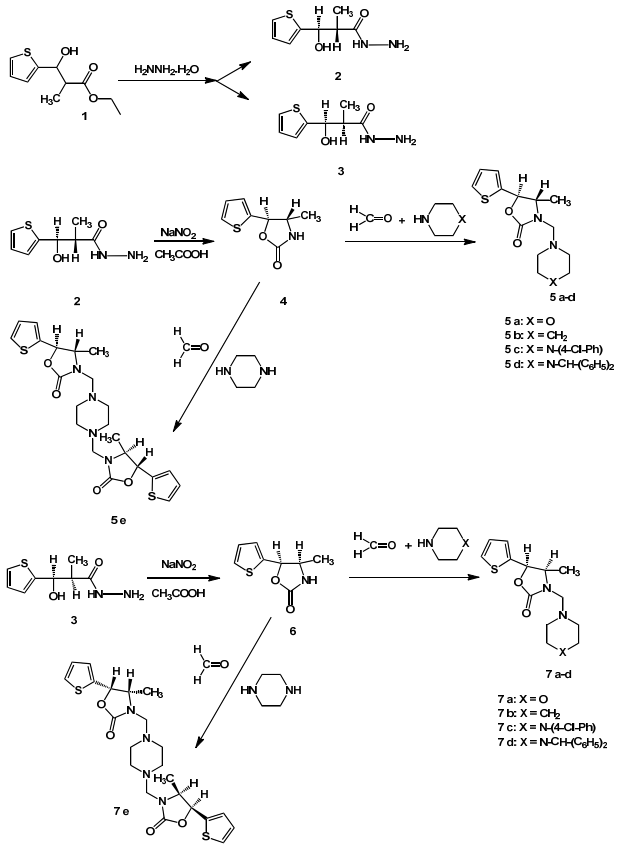
Syn-4-methyl-3-[(4-benzhydrylpiperazin-1-yl)methyl]-5-thien-2-yl-1,3-oxazolidin-2-one, **7d**: Yield 65%. M.p.: 163-164 °C, recrystallization with ethanol. R_f = 0.41, mobile phase: benzene:diethyl ether:ethanol (4:1:3), Silica gel F_{254}. ^{1}H NMR ($CDCl_3$): 0.94 (3H, CH_3, d, *J*=6.46 Hz), 2.61-2.41 (8H, 4CH_2, m, piperazine), 3.79 (1H, N(*H*CH)N, d, *J*=12.62), 4.27-4.16 (3H, N(HC*H*)N, overlapped with C*H*$(Ph)_2$ and N-CH from oxazolidinone), 5.79 (1H, OCH, d, *J*=7.81 Hz), 7.42-7.02 (13H, 2Ph and 3 CH from thiophene). ^{13}C NMR, ($CDCl_3$): 13.8 (CH_3), 50.7 (N-CH_2, piperazine), 51.5 (N-CH_2, piperazine), 55.1 (NCH, oxazolidinone), 64.0 (NCH_2N), 75.7 (CH$(Ph)_2$), 76.0 (OCH), 125.8, 126.1, 126.8 (CH, thiophene), 126.9 (para CH from Ph rings), 127.8 (ortho CH from Ph rings), 128.4 (meta CH from Ph rings), 137.5 (C, thiophene), 142.5 (C form Ph rings), 157.4 (C=O). Anal. Calcd. for $C_{26}H_{29}N_3O_2S$: C, 69.77; H, 6.53; N, 9.39; S, 7.16; Found: C, 69.69; H, 6.60; N, 9.34; S, 7.19.

3. Results and discussion

The Mannich bases of *syn* and *anti*-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-ones were thesized as depicted in Scheme 1. The starting (*2S,3R*) and (*2R,3R*)-3-hydroxy-2-methyl-3-thien-2-ylpropanohydrazides **2** and **3** were prepared in ethanol using hydrazine hydrate and the diastereoisomeric mixture of (*2R, 3R*) and (*2S,3R*)-ethyl 3-hydroxy-2-methyl-3-thien-2-ylpropanoate **1**, obtained through the Reformatsky reaction. The (2*S,3R*) and (*2R,3R*)-3-hydroxy-2-methyl-3-thien-2-ylpropano-hydrazides were separated as we described early [22].

The treatment of the corresponding (2*S,3R*) or (*2R,5R*)-3-hydroxy-2-methyl-3-thien-2-ylpropano-hydrazides with water solution of sodium nitrite in acetic acid/diethyl ether medium via β-hydroxy-azides at 15-20 °C and followed by Curtius rearrangement of β-hydroxy-isocyanates at refluxing of the separated organic solution led to forming the 1,3-oxazolidin-2-one cycle **4** or **6**.

The oxazolidin-2-one **4** or **6**, morpholine, piperidine, benzhydrylpiperazine and 35% formalin were used in the Mannich reaction in ratio 1:0.8:1.3 and by the use of piperazine the ratio was 2:1:2.5. The process was carried out by refluxing in ethanol for 2-3 hours.



Scheme 1

It is known that the nucleophilic properties of the reagents participating in the Mannich reaction are of a significant importance for the successful performing of the synthesis. It was estimated that by the reaction between *anti*- and *syn*-4-methyl-5-thien-2-yl-1,3-oxazolidin-2-ones and the above mentioned secondary cyclic amines it is enough to use a low access of the amines to complete the synthesis successfully. Because the asymmetrical centers were not involved in the reaction, the C-atoms at 4th and 5th position of oxazolidin-2-one cycle in the corresponding compounds **5a-e** and **7a-e** preserved the same configuration as these in the starting oxazolidin-2-ones. Some physical data are given in Table 1.

In order to determine, the geometry and the dihedral angels of compounds **4** and **6**, DFT computations were performed with standard Gaussian 98 program package. We employed the B3LYP hybrid functional with 6-31G basis set. Furthermore for the calculation of some steric, physicochemical and electronic parameters of compounds **5a-e** and **7a-e** Chem3D Ultra, ChemDraw Ultra, Version 11.0.1 was used [23].

Structures of the compounds were confirmed by ^{1}H and ^{13}C NMR spectra. The stereochemistry of *anti*- and *syn*-isomers was assigned using the observed differences in the chemical shifts of some key signals and the values of vicinal coupling constants (^{3}J) between the two protons from the oxazolidin-2-one ring. For all compounds NOE (Nuclear Overhauser Effect) NMR spectra were measured in order to prove additionally the position of the substituents in the oxazolidin-2-one ring.

In five membered rings, due to their higher conformational flexibility, the dihedral angles (Φ) are less well defined and the vicinal coupling constants are not characteristic in general. The conformation of oxazolidinone ring in the studied compounds is relatively fixed due to the presence of two bulky substituents at C4 and C5 atoms. The theoretical values of the dihedral angle defined by H5-C5-C4-H4 atoms of the oxazolidinone ring in the optimized structures of the studied compounds are between 3.4 and 17.3 degrees for *syn*-isomers and between 124.2 and 140.3 degrees for *anti*-compounds, depending on the substituents. The experimental ^{3}J constants of *syn*-derivatives are systematically higher as compared with those in the respective *anti*-compounds. The experimental ^{3}J constant for *syn*-isomers was found to be 7.88 Hz, averaged over six compounds, while for the trans-derivatives the value is 6.77 Hz averaged over five compounds. These results follow the tendency and are in a very good agreement with the ^{3}J values theoretically predicted by Karplus curve simulated using the Karplus equation with coefficients applicable for H-C-C-H fragments and above given values of the dihedral angles [24].

The chemical shift values for the methyl protons in *anti*-compounds are systematically higher (av. value 1.38 ppm) as compared with those for the respective *syn*- isomers (av. value 0.97 ppm). The differences $\Delta\delta(CH_3) = [\delta(CH_3\text{-}anti) - \delta(CH_3\text{-}syn)]$, for a given diastereomeric pair are in the range 0.45 – 0.40 ppm. Figure 1 represents the ^{1}H spectra of the two isomers **7b** and **5b**.

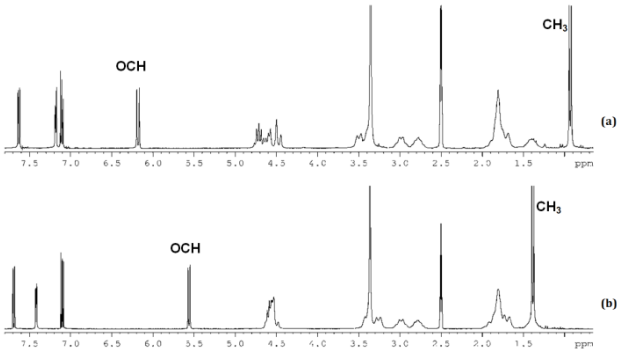


Figure 1. ^{1}H NMR spectra of the two isomers: (a) 7b (*syn*) and (b) 5b (*anti*).

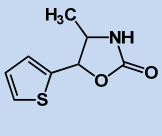
This observation could be explained by the magnetic anisotropy effect of the double bonds from the thiophen ring. In *syn*-compounds the CH_3 group is oriented parallel to the thiophen ring and the protons are in the vicinity of the shielding zone above the double bonds plane. Another characteristic signal is the one for OCH from the oxazolidinone ring, which is systematically deshielded in *syn*-compounds by 0.52 ppm in average – see Figure 1 as an example. This finding is attributed to the γ-effect, which in CH_3–C–C–H molecular fragments leads to downfield shift if the proton and the methyl group are in *antiperiplanar* position, as in the case of *syn*-isomers, and to upfield shift if the proton is in *syn* position with respect to methyl group as in the case of *anti*-isomers.

The above conclusions were based on general considerations of different factors that influence chemical shifts and coupling constants and were made by comparison of these NMR parameters for the two isomers. To give another independent evidence for the structure of the diastereomeric pairs and to additionally prove their stereochemistry we measured the NOE enhancements observed in the NOE difference spectra after the irradiation of some key signals.

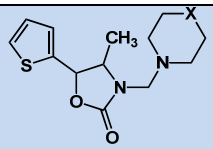
NOE gives information about molecular fragments that are in space proximity and is an effective method to study the relative arrangement of different molecular fragments, e.g. the stereochemistry [25].

Figure 2 represents the optimized structures of the two isomers with some key NOE contacts indicated with arrows. For all *syn*-compounds (**6**, **7a**, **7b**, **7c**, **7d**, **7e**) the irradiation of the OCH proton from the oxazolidinone ring gives a very strong NOE of the signal for the CH-4 proton from the oxazolidinone ring. The irradiation of the CH_3 group leads to NOE for the CH-4 proton attached to the same carbon atom and for one of the

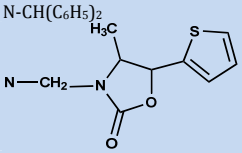
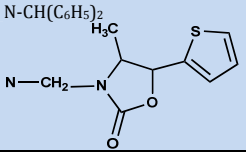
Table 1. Yields and physical data of the synthesized compounds.



4, 6



5 a-e, 7a-e

Compound	Configuration	X	Yield (%)	M.p. (°C)	Formula
4	*anti*	-	70.0	87-90	$C_8H_9NO_2S$
5a	*anti*	O	62.0	166-169	$C_{13}H_{18}N_2O_3S$
5b	*anti*	CH_2	68.0	156-158	$C_{14}H_{21}ClN_2O_2S$
5c	*anti*	N-(4-Cl-Ph)	67.0	130-131	$C_{19}H_{22}ClN_3O_2S$
5d	*anti*	N-CH(C_6H_5)$_2$	70.0	133-134	$C_{26}H_{29}N_3O_2S$
5e	*anti*		56.0	145-147 (Decomp.)	$C_{22}H_{28}N_4O_4S_2$
6	*syn*	-	65.0	101-102	$C_8H_9NO_2S$
7a	*syn*	O	64.0	83-85	$C_{13}H_{18}N_2O_3S$:
7b	*syn*	CH_2	68.0	181-182	$C_{14}H_{21}ClN_2O_2S$
7c	*syn*	N-(4-Cl-Ph)	64.0	118-120	$C_{19}H_{22}ClN_3O_2S$
7d	*syn*	N-CH(C_6H_5)$_2$	65.0	163-165	$C_{26}H_{29}N_3O_2S$
7e	*syn*		62.0	196-197 (Decomp.)	$C_{22}H_{28}N_4O_4S_2$

thiophene protons, however no NOE is observed for the OCH. These observations imply that the CH_3 group and the thiophene ring are in *syn*-orientation. For trans-compounds (**4**, **5a**, **5b**, **5c**, **5d** and **5e**) the irradiation of the proton from OCH gives very strong NOE for the signal of the CH_3 group, while no effect on the other proton from the oxazolidin-2-one ring was observed. An example of NOE's observed for compounds **5b** and **7b** is presented on Figure 3. All the NOE data obtained for the compounds are included in Tables 2 and 3.

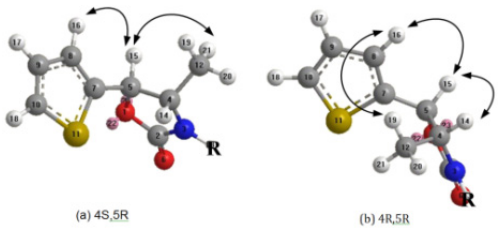


Figure 2. Key NOE contacts observed for: (a) *syn*-compounds and (b) *anti*-compounds.

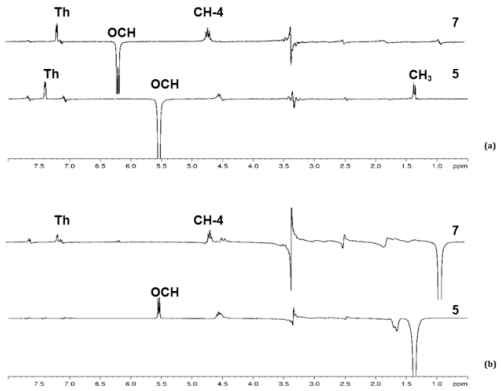


Figure 3. NOE difference spectra of the two isomers: 7 b (*syn*) and 5 b (*anti*): (a) irradiation of OCH proton from oxazolidinone; (b) irradiation of CH_3 group in position 4.

In the case of drug discovery, the likeness in the structures of compounds is a widely used approach, but chemically similar compounds may have different biological actions and different molecules can have similar biological properties [27]. The similarity between compounds can be expressed as a distance in real valued descriptors such as topological, physicochemical or quantum chemical ones [28]. The distance between two points can be a measure for the similarity between two compounds. Therefore the distance d_i of a particular compound "*i*" to the model compound A respectively to B (Figure 4) could be calculated according to equation 1 as described in [29]:

$$d_i^2 = \frac{\sum_{j=1}^{n} \left(1 - \frac{X_{i,j}}{X_{i,M}}\right)^2}{n} \quad (1)$$

where $X_{i,j}$ is the value of molecular descriptor "i" for compound "j", $X_{i,M}$ is the value of the equivalent descriptor "i" for the model compound A respectively for B and "n" is the total number of the considered molecular parameters. Then the similarity of compound "j" to the refer compound A (B) is to be calculated according to equation 2:

$$\text{Similarity (\%)} = (1\text{-}R) \times 100 \quad (2)$$

Where $R = \sqrt{d^2}$ is the quadratic mean and is a measure of a central tendency.

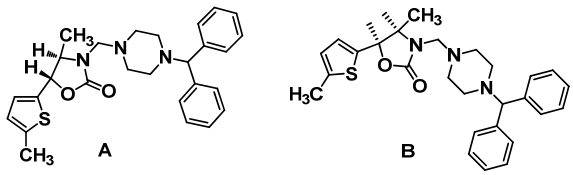


Figure 4. Reference compounds **A** and **B**.

The model compounds A and B were found to possess analgesic activity against acetic acid-induced pain. Compound **A** revealed 96 % and compound **B** 98 % activity, but their LD_{50} values were quite different - 800 mg/kg and 2000 mg/kg, respectively [16]. Therefore it is of interest to determine the similarity between the model compounds and the newly synthesized, and to compare their steric, electronic and physicochemical properties.

Table 2. NOE data (%) for compounds **6** and **7a-e**.

Irradiated signal	NOE % Compound					
	6	**7a**	**7b**	**7c**	**7d**	**7e**
OCH	4.8 – NCH	3.3 – Th 6.2 – NCH	7.3 – Th 13 – NCH	4.5 – Th 8.7 – NCH	6.7 – Th 9.6 – NCH	4.5 – Th 9.4 – NCH
CH_3	3.2 – NCH	2.5 – NCH	3.7 – NCH	N.A.	N.A.	1.5 – Th 4.2 – NCH

N.A.: not available

Table 3. NOE data (%) for compounds **4** and **5a-e**.

Irradiated signal	NOE % Compound					
	4	**5a**	**5b**	**5c**	**5d**	**5e**
OCH	3.3 – CH_3	3.6 – CH_3	4.4 – CH_3	3.5 – CH_3	3.2 – CH_3	3.3 – Th 3.9 – CH_3

For the assessment of the similarity between the synthesized compounds and reference compounds **A** and **B**, we used 4 steric molecular surface descriptors, 6 electronic and 3 physicochemical molecular parameters. The steric and molecular surface descriptors are as follows:

ClsC-(Cluster Count)-Topology Indice; - **SAS** (Connolly Solvent Accessible Surface Area) - Å², the focus of the center of a spherical probe (representing the solvent) as it is rolled over the molecular model; - **MS** (Connolly Molecular Surface Area) - Å², the contact surface created when a sphere (representing the solvent) is rolled over the molecular model; - **SEV** (Connolly Solvent-Excluded Volume) - Å ³, the volume contained within the contact molecular surface created when a sphere (representing the solvent) is rolled over the molecular model.

The followed global physicochemical parameters were calculated: - **log P** (hydrophobic parameter - octanol-water partition coefficient); - **MR** (Molar refractivity) - Kcal/mol; - **E** (Total energy) - Kcal/mol.

The quantum chemical descriptors used by determination of similarity were: - **HOMO**-energy (The Highest Occupied Molecular Orbital energy) - eV; - **LUMO** - energy (The Lowest Occupied Molecular Orbital energy) - eV; - **ElcE** (Electronic energy) - eV; - **TotE** (Total energy) - (eV); -**DPL** (Dipole) - (Debye); - **NRE** nucleus-nucleus repulse energy - (eV).

After the computation of descriptors, it was established that the likeness between **5d**, respectively, **7d** and the reference compounds had the highest value - 90.53 and 94.53 %, followed by **5e** - 80.4 and **7e** 86.01 as well as by **5c** and **7c** 80.54 and 76.30 % respectively, as it can be seen from the data given in Table 4.

The values of the descriptors and the calculation of Equation 1 for compound **5d** are as follows:

$$d^2 = [(1\text{-}ClC_{5d}/ClC_A)^2 + (1 - SAS_{5d}/SAS_A)^2 + (1- MS_{5d}/MS_A)^2 + (1 - SEV_{5d}/SEV_A)^2 + (1 - DPL_{5d}/DPL_A)^2 + (1 - ElcE_{5d}/ElcE_A)^2 + (1- HOMO_{5d}/HOMO_A)^2 + (1- LUMO_{5d}/LUMO_A)^2 + (1 - LogP_{5d}/LogP_A)^2 + (1 - MR_{5d}/MR_A)^2 + (1- E_{5d}/E_A)^2 + (1- NRE_{5d}/NRE_A)^2 + (1- TotE_{5d}/TotE_A)^2]/13 =$$

$$[(1\text{-}32/33)^2 + (1- 629.609/646.879)^2 + (1 - 410.135/425.959)^2 + (1 - 383.254/401.380)^2 + (1- 5.2213/4.4871)^2 + (1 - 45972.9/49035.5)^2 + (1 - 9.19889/8.99577)^2 + (1 - 0.143672/0.112407)^2 + (1 - 5.346/5.682)^2 + (1 - 129.21/134.32)^2 + (1 - 129.338/127.465)^2 + (1 - 40790.9/43697.8)^2 + (1 - 5182/5338)^2]/13 = 0.09473$$

Because the frontier molecular orbital are involved in the activity properties of the compounds, the highest occupied molecular orbital (HOMO) energy and the lowest unoccupied molecular orbital (LUMO) energy is discussed. The HOMO energy relates to the ionization potential and the reactivity of a molecule as a nucleophile. The LUMO energy represents the electron affinity of a molecule or its reactivity as an electrophile.The performed computation showed lower electronic energies (ElcE) of the *anti*-isomers in comparison to those of the *syn*-diastereoisomers, but regarding to the HOMO energies and the repulse energies (NRE), the *anti*-isomers possessed higher values than *syn*-diastereoisomers. If the steric descriptors are taken in consideration it is pointed out that the **SAS** and **MS** values of *anti*-isomers are lower than these of *syn*-isomers. The same relations are observed for compound **A** and compound **B**. Probably, the differences in the steric and electronic properties of the compounds are responsible for the different LD_{50} values of the both isomers. It may be expected that the new synthesized compounds would act in a similar way. The biological study of the reported compounds is in progress.

Table 4. Calculated value of the distances and the similarity of the compounds.

Compound	d^2	R	Similarity %
5a	0.1587	0.3983	61.16
7a	0.1788	0.4229	57,71
5b	0,1331	0.3649	63,51
7b	0,3581	0.3581	64,19
5c	0,0379	0.1946	80,54
7c	0,0562	0.2370	76.30
5d	0,0090	0.0947	90.53
7d	0,0030	0.0547	94.53
5e	0,0399	0.1996	80.04
7e	0,0196	0.1399	86.01

Acknowledgement

Thanks are due to National Science Fund/Ministry of Education and Science grant X1408 and Science Fund/Chemical Technology and Metallurgy University grant 10580 for the financial support of this work.

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