

Synthesis and HIV-1 Reverse Transcriptase Inhibitor Activity of Some 2,5,6-Substituted Benzoxazole, Benzimidazole, Benzothiazole and Oxazolo(4,5-b)pyridine Derivatives

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Summary

In this study, the synthesis of some benzoxazoles and their analogues were described and their antiviral activities were studied together with the previously synthesized 2,5,6-trisubstituted benzoxazole, benzothiazole, benzimidazole and oxazolo(4,5-b)pyridine derivatives. The reverse transcriptase (RT) inhibitory activity of these compounds was determined using a commercial kit and assay system which utilizes the scintillation proximity assay principle. The results are

concentration at which the compound inhibits RT activity by 50 %). The compounds inhibited the in vitro binding of thymidine to the RT enzyme exhibiting IC_{50} values between $6.3 \times 10^5 \mu\text{mol/l}$ -0.34 $\mu\text{mol/l}$ and their activities were compared to some standard drugs such as 3'-azido-2',3'-dideoxythymidine triphosphate and dideoxythymidine triphosphate.

Zusammenfassung

Synthese von 2,5,6-substituierten Benzoxazol-, Benzimidazol-, Benzothiazol- und Oxazolo(4,5-b)Pyridin-Derivaten und Bestimmung ihrer HIV-1 Reverse Transkriptase-inhibitorischen Wirkung

In der vorliegenden Studie wird die Synthese einiger Benzoxazole und ihrer Derivate sowie ihre antivirale Aktivität zusammen mit der Wirkung der bereits früher synthetisierten 2,5,6-trisubstituierten Benzoxazol-, Benzothiazol-, Benzimidazol- und Oxazolo(4,5-b)Pyridin-Derivate beschrieben. Die inhibitorischen Wirkungen dieser Verbindungen auf die Reverse Transkriptase (RT) wur-

den mit einem kommerziell erhältlichen Kit bestimmt, welches das Szintillations-Annäherungsprinzip verwendet. Die Ergebnisse werden in Form von IC_{50} -Werten (d. h. diejenige Konzentration, bei der die Verbindungen die Aktivität der Reversen Transkriptase um 50 % herabsetzen) präsentiert. Die Verbindungen hemmen in vitro die Bindung von Thymidin an die RT mit IC_{50} -Werten zwischen $6.3 \times 10^5 \mu\text{mol/l}$ und 0.34 (mol/l). Die Wirkungen wurden mit denjenigen der Standardsubstanzen 3'-Azido-2',3'-dideoxythymidintriphosphat und Dideoxythymidintriphosphat verglichen.

Key words

- Antiviral drugs
- Benzimidazoles
- Benzothiazoles
- Benzoxazoles
- HIV-1 reverse transcriptase, enzyme inhibitors
- Oxazolo(4,5-b)pyridines

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1. Introduction

According to an estimation by the World Health Organization (WHO) 30–40 million people had been infected with HIV and 10 million people had developed AIDS until 1999 [1, 2]. Efforts to eradicate the disease have intensified in the last 10 years and, as a consequence, massive resources focused on both the study of HIV and the development of antiretroviral agents. The discovery that the virus requires the catalytic activity of several unique enzymes for its life cycle made them ideal therapeutic targets. Research strategies have mostly targeted the inhibition of reverse transcriptase (RT), a multi-purpose enzyme, responsible for the synthesis of double-stranded viral DNA from proviral RNA for subsequent incorporation into the host cell chromosome, therefore being essential for the replication of HIV [1, 2].

DNA synthesis by the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) is distinguished by low fidelity and an increased frequency of extension of mismatched primer termini. HIV-1 RT also incorporates chain-terminating nucleotide analogues more readily than cellular DNA polymerases, suggesting that the enzyme can tolerate structural variability at the 3' position of the nucleotide substrate [3]. This feature has made the enzyme a target for inhibition by nucleoside analogues.

Knowing RT inhibitors concern either nucleoside or non-nucleoside classes, the non-nucleoside inhibitors (NNRTIs) bind to an allosteric site on the enzyme at a close distance from its polymerase active site. They, in contrast with nucleoside analogues, are highly specific

for HIV-1, do not bind to HIV-2 RT and are effective without toxic effects at relatively high concentrations [1].

Recent observations suggested that substituted benzimidazoles, benzoxazoles, benzothiazoles and related fused heterocycles indicated potential antitumor, antiviral and antibiotic activities as the new topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors with lower toxicities in the chemotherapeutic approach [1, 3–9]. A benzoxazole derivative, 3-(4,7-dichlorobenzoxazol-2-ylmethylamino)-5-ethyl-6-methylpyridin-2(1H)-one (L-697,661) (Fig. 1) was observed as a specific non-nucleoside reverse transcriptase inhibitor for the human immunodeficiency virus HIV-1 type and their use in combined therapy with zidovudine achieved a marked decrease of viraemia in some primary HIV infected patients. Moreover, the carbonucleoside carbovir had been shown to act as a potent and selective anti-HIV agent and 2-mercapto 1-(β -4-pyridethyl)benzimidazole (MPB) (Fig. 1) inhibited the incorporation of tritiated uridine and thymidine into RNA and DNA in cell cultures [7–11].

In this study, the synthesis of some benzoxazoles and related heterocyclic compounds are described and their antiviral activity against HIV-1 RT are studied together with the previously synthesized fused heterocyclic derivatives (Fig. 2) [12–21]. Moreover their antiviral affinities are compared to the standard drugs 3'-azido-2',3'-dideoxythymidine triphosphate (AZT-TP) and dideoxythymidine triphosphate (ddT-TP).

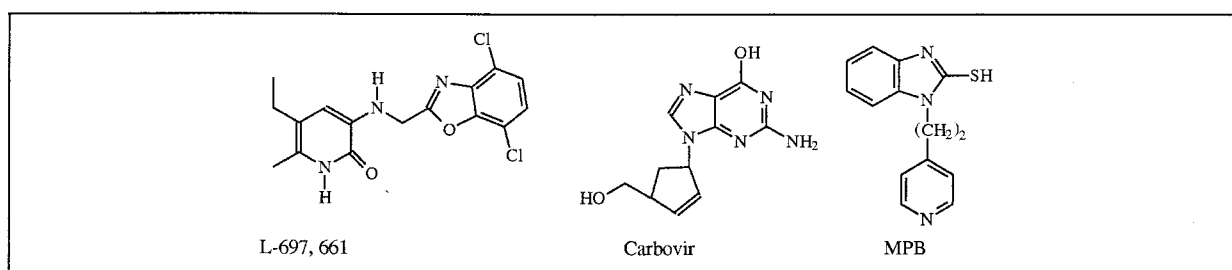


Fig. 1: Structure of L-697,661, carbovir and MPB.

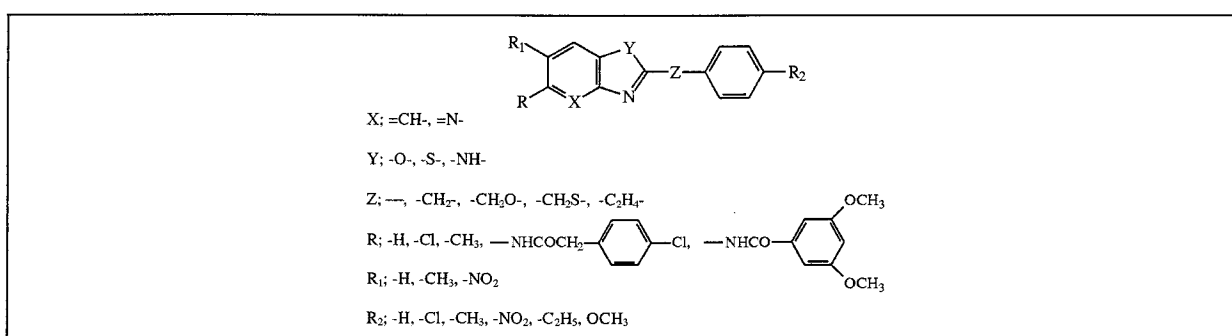
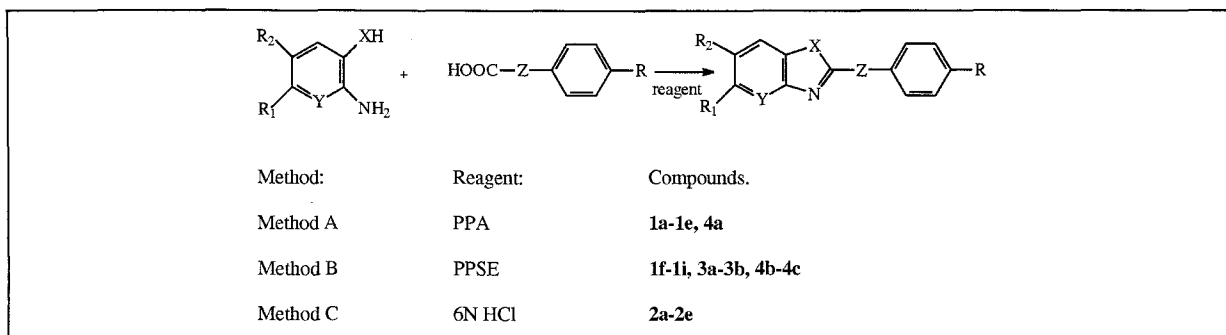


Fig. 2: Compounds subjected to antiviral activity tests.



Scheme 1: General synthesis of the compounds 1a-1e, 4a, 1f-1i, 3a-3b, 4b-4c, 2a-2e.

The syntheses of benzoxazoles **1a-1i**, benzimidazoles **2a-2e**, benzothiazoles **3a-3b** and oxazolo(4,5-b)pyridines **4a-4c** were performed through heating carboxylic acids with appropriate *o*-substituted anilines by means of several dehydrating agents in a one step procedure.

Polyphosphoric acid (PPA) or trimethylsilyl polyphosphate ester (PPSE) were used as the cyclodehydration reagent in the ring closure reactions of the compounds [12, 15-17, 22]. During the synthesis of benzimidazole derivatives **2a-2e**, aqueous hydrochloric acid was used as the condensation reagent according to the well known Phillips method [23].

On the other hand, the synthesis of the compounds **1j-1k** was performed by reacting 5-amino-2-phenyl- or 5-amino-2-(*p*-ethylphenyl)benzoxazoles with appropriate carboxylic acid chlorides obtained by treating carboxylic acids with thionyl chloride (Method D) [21]. 5-Amino-2-phenyl- or 5-amino-2-(*p*-ethylphenyl)benzoxazoles were obtained by heating benzoic acids or *p*-ethylbenzoic acids with 2,4-diaminophenol in PPA (polyphosphoric acid) as the cyclodehydration reagent in a one step procedure [21].

The compounds **1f**, **1g**, **1i**, **2d**, **4b**, **4c** were prepared as new products. The structure of these compounds was supported by spectral data. The IR and ¹H-NMR spectra are in agreement with the proposed structures. Physical and spectral data of the new synthesized compounds are reported in the experimental section.

2. Materials and methods

2.1. Chemistry

Melting points were determined with a Büchi SMP-20 melting point apparatus (Büchi Laboratoriumstechnik, Flawil, Switzerland) and are uncorrected. All the instrumental analyses were performed at the Lab. of Scientific and Technical Research Council of Turkey (Tübitak, Ankara, Turkey), with a Shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) (IR spectra were recorded as potassium bromide discs), a Bruker AC 400 NMR spectrometer (Bruker, Rheinstetten, Karlsruhe, Germany) (the ¹H-NMR spectra were measured in DMSO-*d*₆ and CDCl₃, all chemical shifts were reported as δ (ppm) values). Elementary

analyses were performed on a LECO CHN 932 analyzer (Leco, St. Joseph, USA) and satisfactory results ± 0.4 % of calculated values (C, H, N) were obtained. For the chromatographic analysis Merck Silica Gel 60 (230-400 mesh ASTM) was used. The chemical reagents used in the synthesis were purchased from E. Merck (Darmstadt, Germany), Sigma (Deisenhofen, Germany) and Aldrich (Milwaukee, USA). AZT-TP and ddT-TP used in the antiviral activity test were obtained from Invivogen (San Diego, CA, USA) and Sigma (Deisenhofen, Germany).

2.1.1. Method A (Compounds 1a-1e, 4a)

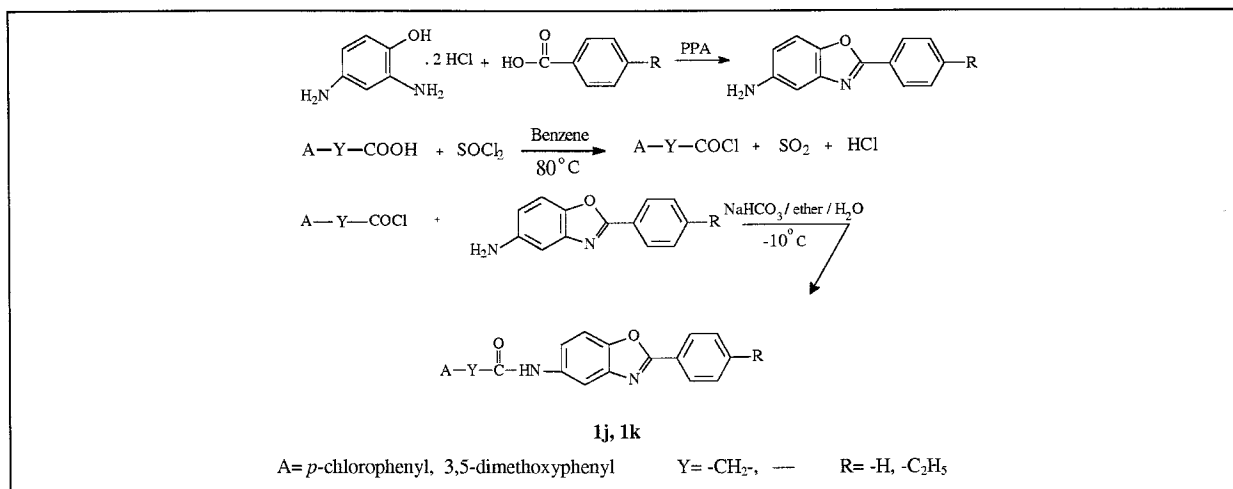
A mixture of 2-substituted anilines (0.01 mol) and appropriate acids (0.015 mol) was heated over 100 °C in PPA (12 g) with stirring for 2.5 h. At the end of the reaction period, the residue was poured into ice-water and neutralized with excess of % 10 NaOH solution extracted with benzene, the benzene solution was dried over anhydrous sodium sulphate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of solvent in vacuo, the crude product was obtained and recrystallized (Scheme 1).

2.1.2. Method B (Compounds 1f-1i, 3a-3b, 4b-4c)

A mixture of corresponding carboxylic acids (5 mmol) and appropriate 4- and/or 5-disubstituted 2-aminophenols or 2-amino-3-hydroxypyridine (6.9 mmol) was heated under reflux with stirring at various temperatures and time in 15 ml PPSE. At the end of the reaction period, the mixture was taken to 30 ml dichloromethane and neutralized with 50 ml 1N NaOH solution. The organic layer was separated and the aqueous solution extracted with 3 x 25 ml portions of CH₂Cl₂. The combined extracts were dried on Na₂SO₄, filtered and the solvent was removed with rotary evaporator. The residue was purified by flash chromatography, eluting with benzene and the obtained product was recrystallized (Scheme 1).

2.1.2.1. 5-Chloro-6-nitro-2-phenoxyethylbenzoxazole (1f)

Prepared by heating a mixture of 1.30 g 2-amino-4-chloro-5-nitrophenol and 0.76 g at 140 °C for 3 h according to General Method B. Recrystallized from ethanol to give the compound **1f**: 0.64 g (42.1 % yield); m.p. 82-83 °C; TLC (only CHCl₃) R_f = 0.78; IR (KBr pellet) 3125, 2900, 1605-1570, 1540, 1445, 1340, 1255-1060, 935-700 cm⁻¹; UV (MeOH) λ_{max} 216.4, 238.6, 268.6; ¹H-NMR (CDCl₃); δ 8.10 (s, 1H, C-7 H), 7.90 (s, 1H, C-4 H),



Scheme 2: General synthesis of the compounds 1j, 1k.

7.50–7.00 (m, 5H, phenyl protons), 5.30 (s, 2H, CH₂). Anal. (C₁₄H₉N₂O₄Cl) %C 55.17 (54.98), %H 2.95(2.98), %N 9.19(9.23).

2.1.2.2. 5-Chloro-2-(*p*-chlorophenoxymethyl)benzoxazole (1g)

Prepared by heating a mixture of 0.99 g 2-amino-4-chlorophenol and 0.93 g at 100 °C for 4.5 h according to General Method B. Recrystallized from EtOH to give the compound **1g**: 0.44 g (30.0 %) yield; m.p. 97–98 °C; TLC (CH₂Cl₂/hexane, 5:1) R_f = 0.89; IR (KBr pellet) 3120, 2960–2900, 1605–1495, 1455, 1290–1045, 945–660 cm⁻¹; UV (MeOH) λ_{max} 212.2 (s), 228.0, 280.8 nm; ¹H-NMR (CDCl₃) δ 7.75–7.22 (m, 5H, C-4 H, C-6 H, C-7 H, C-3' H and C-5' H), 7.03–6.90 (dd, 2H, C-2' H and C-6' H, J_{2',3'} and J_{5',6'} = 9.12 Hz), 5.28 (s, 2H, CH₂). Anal. (C₁₄H₉NO₂Cl₂) %C 57.14 (57.22), %H 3.06 (3.09), %N 4.76 (4.74).

2.1.2.3. 5-Chloro-6-nitro-2-phenylthiomethylbenzoxazole (1i)

Prepared by heating a mixture of 1.30 g 2-amino-4-chloro-5-nitrophenol and 0.84 g at 130 °C for 2.5 h according to General Method B. Recrystallized from CH₂Cl₂ and hexane to give the compound **1i**: 0.57 g (35.62 %) yield; m.p. 78–79 °C; TLC (only CHCl₃) R_f = 0.75; IR (KBr pellet) 3125, 2970, 1630–1570, 1540, 1445, 1345, 1260–1000, 960–695 cm⁻¹; UV λ_{max} 213.4, 243.8, 284.0 nm; ¹H-NMR (CDCl₃) δ 8.05 (s, 1H, C-7 H), 7.80 (s, 1H, C-4 H), 7.40–7.20 (s, 5H, phenyl protons), 4.30 (s, 2H, CH₂). Anal. (C₁₄H₉N₂O₃SCl) %C 52.41 (52.22), %H 2.80 (2.79), %N 8.73 (8.76).

2.1.2.4. 2-Phenoxymethylloxazolo(4,5-b)pyridine (4b)

Prepared by heating a mixture of 0.75 g 2-amino-3-hydroxypyridine and 0.76 g at 170 °C for 4 h according to General Method B. Recrystallized from benzene and petroleum ether to give the compound **4b**: 0.40 g (35.39 %) yield; m.p. 75–76 °C; TLC (CHCl₃/MeOH, 4:0.1) R_f = 0.73; IR (KBr pellet) 3100, 2940, 1600–1500, 1445, 1245–1070, 945–700 cm⁻¹; UV λ_{max} 218.2, 277.0, 286.0 (s) nm; ¹H-NMR (CDCl₃) δ 8.50–7.80 (m, 2H, C-5 H and C-7 H), 7.30–6.98 (m, 6H, C-6 H and phenyl protons), 5.37 (s, 2H, CH₂). Anal. (C₁₃H₁₀N₂O₂) %C 69.02 (68.82), %H 4.42 (4.40), %N 12.38 (12.44).

2.1.2.5. 2-(*p*-Chlorophenoxymethyl)oxazolo(4,5-b)pyridine (4c)

Prepared by heating a mixture of 0.75 g 2-amino-3-hydroxypyridine and 0.93 g at 160 °C for 4 h according to General Method B. Recrystallized from benzene and petroleum ether to give the compound **4c**: 0.65 g (50.38 %) yield; m.p. 118–119 °C; TLC (CHCl₃/MeOH, 4:0.1) R_f = 0.74; IR (KBr pellet) 3100, 2950, 1625–1500, 1410, 1260–1080, 969–660 cm⁻¹; UV λ_{max} 209.2 (s), 226.0, 2279.2, 285.4 (s) nm; ¹H-NMR (CDCl₃) δ 8.64–7.80 (m, 2H, C-5 H and C-7 H), 7.37 (s, 1H, C-6 H), 7.32–7.20 (dd, 2H, C-3' H and C-5' H, J_{3',2'} and J_{5',6'} = 9 Hz), 7.04–6.93 (dd, 2H, C-2' H and C-6' H, J_{2',3'} and J_{6',5'} = 9 Hz), 5.36 (s, 2H, CH₂). Anal. (C₁₃H₉N₂O₂Cl) %C 59.88 (60.12), %H 3.45 (3.47), %N 10.74 (10.68).

2.1.3. Preparation of PPSE

A mixture of phosphorus pentoxide (10 g, 35 mmol), hexamethyldisiloxane (25 ml, 132.5 mmol), and 50 ml 1,2-dichlorobenzene was refluxed for 5 min under nitrogen atmosphere until the solution became clear and the obtained colorless syrup like solution was used as the cyclodehydration reagent in the ring closure procedure of the synthesized compounds.

2.1.4. Method C (Compounds 2a–2e)

A mixture of corresponding carboxylic acids (10 mmol) and appropriate 5-substituted-2-phenylendiamines (10 mmol) was boiled under reflux with stirring for various time in 15 ml 6N HCl. At the end of the reaction period, the mixture was neutralized with excess of NaHCO₃. The collected precipitate washed (water), dried in vacuum, purified by flash chromatography, eluting with CHCl₃ and recrystallized (Scheme 1).

2.1.4.1. 5-Chloro-2-phenylthiomethylbenzimidazole (2d)

Prepared from heating a mixture of 1.42 g 4-chloro-2-phenylenediamine and 1.68 g at 100 °C for 6 h according to General Method C. Recrystallized from EtOH and water to give the compound **2d**: 1.10 g (40.14 %) yield; m.p. 138–139 °C; TLC (CHCl₃/MeOH/petroleum ether 4:0.3:1) R_f = 0.79; IR (KBr pellet) 3200–2650, 1600–1530, 1440, 1300–1020, 940–695 cm⁻¹; UV

λ_{max} 218.4, 250.2, 285.4 nm; $^1\text{H-NMR}$ (CDCl_3) δ 7.60–6.80 (m, 8H, C-4 H, C-6 H, C-7 H and phenyl protons), 4.34 (s, 2H, CH_2). Anal. ($\text{C}_{14}\text{H}_{11}\text{N}_2\text{SCl}$) %C 61.20 (61.44), %H 4.00 (4.04), %N 10.20 (10.24).

2.1.5. Method D (Compounds 1j–1k)

Appropriate carboxylic acid (5/10 mmol) and thionyl chloride (1.5 ml) were refluxed in benzene (5 ml) at 80 °C for 3 h. Excess thionyl chloride was then removed in vacuo. The residue was dissolved in ether (10 ml) and solution added during 1 h to a stirred, ice-cooled mixture of 5-amino-2-phenylbenzoxazole or 5-amino-2-(p-ethylphenyl)benzoxazole (5/10 mmol), sodium bicarbonate (10/20 mmol), diethylether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2N HCl, water, respectively, and finally with ether to give **1j–1k**. The product was recrystallized from ethanol-water mixture and needles are dried in vacuo (Scheme 2).

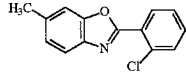
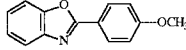
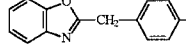
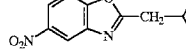
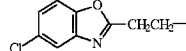
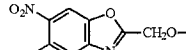
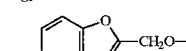
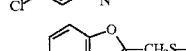
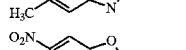
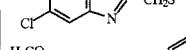
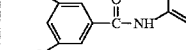
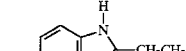
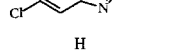
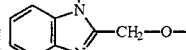
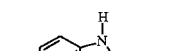
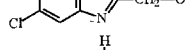
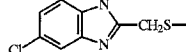
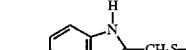
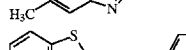
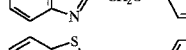
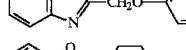
2.2. Antiviral activity against HIV-1 RT

IC_{50} values of RT activity inhibiting affinities of the compounds were determined using a commercial kit (RETRO SYS RT Activity Kit, Innovagen AB, Lund, Sweden) in accordance with the manufacturer's instructions. Briefly, the substance to be analysed are serially diluted and added to the plate with reaction mixture. After 30 min of preincubation at 33 °C, the reaction is started by the addition of a standardized amount of RT which incorporates bromodeoxyuridine (BrdUMP) depending on the level of inhibition. The reaction is stopped by washing the plate and the product quantified by the addition of RT product tracer which binds to the incorporated BrdUMP. After removing the excess tracer the amount of bound tracer is determined by an alkaline phosphatase/ p-nitrophenylphosphate (pNPP) colour reaction. After correction for background signal, the measured residual RT activity for each substance dilution is calculated as a percentage of the measured RT activity in absence of inhibiting substances. The inhibitory effect of each substance is expressed as an IC_{50} value (i.e. the concentration at which 50 % of the RT activity is inhibited).

3. Results

The synthesis of some benzoxazoles and their analogues are described and their antiviral activity are studied together with the previously synthesized 2,5,6-trisubstituted benzoxazoles, benzothiazoles, benzimidazoles and oxazolo(4,5-b)pyridine derivatives. The activity results are summarized in Table 1. The RT inhibitory activity of the compounds was determined using a commercial kit, which utilizes the scintillation proximity assay principle, as described in Methods. The results are presented as the IC_{50} values (i.e. the concentration at which the compound inhibits RT by 50 %). The antiviral activities of the compounds in comparison to some control drugs are shown in Table 1 indicating that the compounds inhibit in vitro binding of thymidine with RT enzyme exhibiting IC_{50} values between $6.3 \times 10^5 \mu\text{mol/l}$ – $0.34 \mu\text{mol/l}$.

Table 1: The in vitro activity of the compounds 1a–k, 2a–e, 3a, b, 4a–c and standard drugs (MIC in $\mu\text{g/ml}$).

Comp. No.	Compounds	IC_{50} ($\mu\text{mol/l}$)
1a		4.6
1b		4.1×10^5
1c		1.77
1d		6×10^4
1e		1.6
1f		1.5
1g		9×10^4
1h		4.15×10^3
1i		5.4×10^5
1j		2.6
1k		6.3×10^5
2a		2×10^5
2b		10.1
2c		1.8
2d		1.2
2e		8×10^4
3a		3×10^5
3b		0.34
4a		4.79
4b		4.67
4c		4.75
AZT-TP ^{a)}		0.003
DdT-TP ^{b)}		0.075

^{a)} 3'-Azido-2',3'-dideoxythymidine triphosphate; ^{b)} dideoxythymidine triphosphate.

4. Discussion

2,5,6-Trisubstituted benzoxazoles, benzothiazoles, benzimidazoles and oxazolo(4,5-b)pyridine derivatives provided a wide range of antiviral activity against HIV-1 RT as shown in Table 1. 2-(p-Chlorophenoxymethyl)-benzothiazole **3b** was found to be more active than the other compounds having an IC_{50} value of 0.34 $\mu\text{mol/l}$. However, all the tested compounds showed lower potency than the reference drugs AZT-TP and ddTTP having IC_{50} values of 0.003 $\mu\text{mol/l}$ and 0.075 $\mu\text{mol/l}$, respectively. Compound **3a** without having a chlorine atom at the para position of the 2-phenyl moiety on the benzothiazole ring showed less activity than **3b** with an IC_{50} value of 3x105 $\mu\text{mol/l}$.

In addition, some compounds exhibited better inhibitory activity than the other tested compounds such as 2-(p-methoxybenzyl)benzoxazole **1c**, 5-chloro-2-(2-phenylethyl)benzoxazole **1e**, 5-chloro-6-nitro-2-(phenoxymethyl)benzoxazole **1f**, 5-chloro-2-(p-chlorophenoxymethyl) benzimidazole **2c** and 5-chloro-2-(phenylthiomethyl)benzimidazole **2d**, having IC_{50} values of 1.77, 1.60, 1.50, 1.80 and 1.20 $\mu\text{mol/l}$, respectively. However, all the tested compounds showed lower potency than the reference drugs AZT-TP and ddTTP having IC_{50} values of 0.003 and 0.075 $\mu\text{mol/l}$, respectively.

As a conclusion, it could be stated that it is worthy to further study the benzoxazole, benzimidazole, benzothiazole and oxazolo(4,5-b)pyridine heterocyclic ring systems as they can be the new drug candidates for the treatment of HIV by inhibiting HIV-1 RT enzyme.

5. References

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