Synthesis, characterization and comparative study the microbial activity of some heterocyclic compounds containing oxazole and benzothiazole moieties

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Abstract

New derivatives of five member heterocyclic compounds containing oxazole and benzothiazole rings are reported. These compounds have been characterized by elemental analysis, FT-IR and \textsuperscript{1}H NMR spectroscopy. This study was designed to show the microbial activity difference for two types of five member heterocyclic rings. The compounds were screened for antibacterial activity against \textit{Escherichia coli}, \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa} in nutrient agar medium, and for antifungal activity against \textit{Aspergillus niger} and \textit{Candida albicans} in Sabouraud’s dextrose agar medium. The results show that the derivatives containing benzothiazole moiety are more active than the derivatives containing oxazole moiety.

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1. Introduction

Chemistry of heterocyclic compounds is one of the leading lines of investigations in the organic chemistry. Heterocyclic compounds are widely distributed in nature and are essential for life. They play a vital role in the metabolism of all living cells. There are vast numbers of pharmacologically active heterocyclic compounds, many of which are a regular clinical use. Nitrogen, sulphur and oxygen containing five member heterocyclic compounds have occupied enormous significance in the field of drug discovery process. We report herein the synthesis and comparative the microbial activities for two types of five member heterocyclic derivatives (oxazole and benzothiazole).

Oxazoles play a vital role in the manufacture of various biologically active drugs as brain-derived neurotrophic factor inducers (Maekawa et al., 2003), analgesic (Serrano et al., 1995), trypanocidal activity (Pinto et al., 1997), antimitotic agents with pro-apoptotic activity (Uckun, 2001), antifungal activity (Kunes et al., 2001), anti-inflammatory (Kaspady et al., 2009), antidepressant (Elmegeed et al., 2010), antitumor (Liu et al., 2009), antimicrobial, antidiabetic and antiobesity. (Mesaik et al., 2004; Khan et al., 2006).
On the other hand, benzothiazoles are heterocyclic compounds with multiple applications and, although they have been known from long ago to be biologically active (Kaur et al., 2010; Prabhu et al., 2011; Yadav et al., 2011). It is bicyclic ring system with diverse chemical reactivity and broad spectrum of biological activities such as antimicrobial, antitumor, anti-inflammatory, antilishmanial and antifungal (Latrofa et al., 2005; Shi et al., 2001; Paramashivappa et al., 2003; Delmas et al., 2004; Sonwane et al., 2008). They show, for example, very intensive antitumor activity, especially the phenyl-substituted benzothiazole (Bradshaw et al., 2002). 2-substituted-6-nitro- and 6-aminobenzothiazole (Delmas et al., 2002), fluoro benzothiazoles (Pattan et al., 2002), and Schiff bases derived from benzothiazoles (Mahmood-ul-Hasan et al., 2002) show microbiological activity.

In continuation of the work on the synthesis of biologically important heterocyclic compounds (Tomi et al., 2011; Tomi et al., in press), herein is reported the synthesis and biological activities of some oxazole and benzothiazole derivatives.

2. Experimental

All the chemicals used were procured from Sigma–Aldrich and Fluka, and used without further purification. Melting points were determined in open capillary tubes on Electrothermal capillary apparatus and are uncorrected. Elemental analysis (C, H, and N) were carried out using a Perkin–Elmer model 2400 instrument, IR spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu 8400S) using KBr disc method. 1H NMR spectra were recorded in DMSO-6 on Bruker spectrometer model ultra shield at 300 MHz using TMS as an internal reference standard.

2.1. 4-Methoxy-hippuric acid (IA)

Glycine (1.5 g, 0.02 mol) in 1N sodium hydroxide solution (20 mL) was cooled at 0–5 °C and the cold solution was added dropwise to a solution of 4-methoxybenzyl chloride (3.41 g, 0.02 mol) in chloroform (30 mL). The mixture reaction was continued under stirring for an additional 1 h. The aqueous layer was separated and acidified with 2N hydrochloric acid. The product IA was collected by filtration and recrystallized from ethanol as colorless needles. Yield (83%); mp: 178–180 °C; FT-IR (KBr disk, cm−1) 3366 (N–H), 3190–2534 (O–H, carboxylic), 2933, 2847 (C–H aliph.) 1743 (C=O ketone), 1641 (C=O amide); 1H NMR (DMSO-d6, 300 MHz, δ) 8.65–8.63 (t, 1H, NH), 7.86–7.79 (dd, 2H, Ar.H), 7.03–7.00 (dd, 2H, Ar.H of phenyl attached OCH3), 3.70 (s, 3H, OCH3). Anal. Calcd. For C10H11NO4 (209.06 g/mol): C, 57.41; H, 5.30; N, 8.63 (t, 1H, NH), 7.86–7.79 (dd, 2H, Ar.H), 6.98–6.95 (dd, 2H, Ar.H of phenyl attached OCH3), 2933, 2847 (C–H aliph.) 1743 (C=O ketone), 1641 (C=O amide); 1H NMR (DMSO-d6, 300 MHz, δ) 8.70–8.59 (t, 1H, NH), 7.93–7.91 (dd, 2H, Ar.H of phenyl attached CH3), 7.74–7.72 (dd, 2H, Ar.H of phenyl attached OCH3), 7.36–7.34 (dd, 2H, Ar.H of phenyl attached CH3), 6.83–6.81 (dd, 2H, Ar.H of phenyl attached OCH3), 4.10–4.08 (d, 2H, CH2), 3.93 (s, 3H, OCH3). Found: C, 57.42; H, 5.26; N, 8.59.

2.2. 2-(4-Methoxyphenyl)-5-(4-hydroxyphenyl)-1,3-oxazole (IA)

The hippuric acid (IA) was treated with equimolar quantity of ethyl chlorofomate in the presence of N-methylmorpholine in methylene chloroform at room temperature to afford the corresponding azlactone (2A) as off white needles. Yield (80%); mp: 86–88 °C; FT-IR (KBr disk, cm−1) 2944, 2843 (C–H aliph.), 1785 (C=O), 1633 (C=O); 1H NMR (DMSO-d6, 300 MHz, δ) 7.85–7.76 (dd, 2H, Ar.H), 7.07–7.01 (dd, 2H, Ar.H), 4.36 (s, 2H, CH2), 3.87 (s, 3H, OCH3). Anal. Calcd. For C16H13NO3 (257.27 g/mol): C, 62.82; H, 4.74; N, 7.33. Found: C, 62.76; H, 4.69; N, 7.36.

2.3. 2-Aza-1-(4-methoxyphenyl)-4-toluel-1,4-butanediones (3A)

The azlactone (2A) (0.96 g, 0.005 mol) in (25 mL) of toluene in excess was treated portionwise with (2.00 g, 0.015 mol) of anhydrous aluminum chloride at room temperature. After the addition, the reaction mixture was continued under stirring for 24 h. The reaction mixture was then poured over crushed ice with hydrochloric acid and the organic component was extracted with methylene chloride, washed with water and dried. The solvent was removed to yield the crude azadiketones (3A), which was crystallized from ethanol as deep gray powder. Yield (71%); mp: >250 °C; FT-IR (KBr disk, cm−1) 3282 (N–C), 2924, 2854 (C–H aliph.), 1649 (C=O ketone), 1634 (C=O amide); 1H NMR (DMSO-d6, 300 MHz, δ) 8.70–8.59 (t, 1H, NH), 7.93–7.91 (dd, 2H, Ar.H of phenyl attached CH3), 7.74–7.72 (dd, 2H, Ar.H of phenyl attached OCH3), 7.36–7.34 (dd, 2H, Ar.H of phenyl attached CH3), 6.83–6.81 (dd, 2H, Ar.H of phenyl attached OCH3), 4.10–4.08 (d, 2H, CH2), 3.93 (s, 3H, OCH3). Anal. Calcd. For C16H17NO3 (283.12 g/mol): C, 72.07; H, 6.05; N, 4.94. Found: C, 72.01; H, 6.10; N, 4.89.

2.4. 2-(4-Methylphenyl)-5-(4-methoxyphenyl)-1,3-oxazole (4A)

The compound (3A) (2.83 g, 0.01 mol) was refluxed with (10 mL) phosphorus oxychloride for 48 h. and the reaction mixture was then treated with ice water and the precipitate was washed with 10% sodium bicarbonate solution and water, dried and recrystallized in ethanol to afford the crude oxazole (4A) in 77% yield, mp: 145–147 °C; FT-IR (KBr disk, cm−1) 2944, 2854 (C–H aliph.), 1641 (C=O), 1244, 1078 (C–O–C); 1H NMR (DMSO-d6, 300 MHz, δ) 8.07–8.05 (dd, 2H, Ar.H of phenyl attached CH3), 7.73–7.71 (dd, 2H, Ar.H of phenyl attached OCH3), 7.32–7.34 (dd, 2H, Ar.H of phenyl attached CH3), 7.60 (s, 1H, C=O of oxazole moiety), 6.92–6.90 (dd, 2H, Ar.H of phenyl attached OCH3), 3.89 (s, 3H, OCH3). Anal. Calcd. For C16H17NO3 (283.12 g/mol): C, 72.07; H, 6.05; N, 4.94. Found: C, 72.01; H, 6.10; N, 4.89.

2.5. 2-(4-Methylphenyl)-5-(4-hydroxyphenyl)-1,3-oxazole (5A)

To Compound (4A) (0.63 g, 0.00238 mol) suspended in dry benzene (25 mL), (1.00 g, 0.0075 mol) of anhydrous aluminum chloride was added. The reaction mixture was refluxed for 24 h. The solvent was evaporated and the residue was poured into ice-water. The solid was collected and purified by dissolving in (30 mL) of 10% sodium hydroxide solution. The remaining solid was filtered and the filtrate was neutralized with 10% hydrochloric acid. The crude product precipitate during the neutralization washed with water several times and dried to give the desired Compound (5A). Yield (71%); mp: >250 °C (decom.); FT-IR (KBr disk, cm−1) 3479–3178 (broad O–H), 2922, 2852 (C–H aliph.), 1649 (C=O), 1240, 1057 (C–O–C); 1H NMR (DMSO-d6, 300 MHz, δ) 7.79–7.71 (dd, 2H, Ar.H of...

2.6. 2-(4-Methylphenyl)-5-(4-alkoxyphenyl)-1,3-oxazole (6Aₙ)

A mixture of compound (5A) (0.06 g, 0.00024 mol) and anhydrous potassium carbonate (0.033 g, 0.00024 mol) was dissolved in acetone (10 mL). n-Alkyl bromide or iodide (0.0003 mol) was added to this mixture dropwise and refluxed for 24 h, then it was added to ice-cold water. The crude solid product was washed with 5% aqueous sodium bicarbonate solution and water several times. The products obtained were dried and recrystallized from ethanol. ¹H NMR (DMSO-d₆, 300 MHz, δ) (6Aₙ) 7.99–7.92 (dd, 2H, Ar.H of phenyl attached CH₃), 7.71–7.67 (dd, 2H, Ar.H of phenyl attached OCH₃), 7.26–7.21 (s, 1H, –C–H of oxazole moiety), 6.83–6.79 (dd, 2H, Ar.H of phenyl attached OCH₃), 3.93 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 1.47–1.69 (m, 2H, CH₂ attached OCH₃), 1.33–1.29 (m, 2H, CH₂ attached CH₃), 0.94–0.91 (t, 3H, CH₃).

2.7. 2-(4-hydroxyphenylazo)-benzothiazole (1Bₙ)

2-Aminobenzothiazole (0.27 g, 0.00178 mol) was dissolved by heating and stirring in (8 mL) of 85% phosphoric acid. The solution was cooled to 0 °C in an ice bath, and then concentrated nitric acid (4 mL) and a solution of sodium nitrite (0.13 g, 0.00187 mol) in (2 mL) of water was added. The mixture was stirred vigorously and maintained at below 5 °C for 10 min. Afterwards a solution of phenol (0.17 g, 0.00178 mol) in (0.5 mL) water was added dropwise with stirring. The mixture was poured into cold water (100 mL). The precipitate solid was filtered, washed several times with water and recrystallized from ethanol. Yield (59%); mp: 269–272 °C; FT-IR (film, cm⁻¹) 3410–3183 (broad O–H), 3064 (C–H arom.), 1724 (C=O carboxylic), 1629 (C=O carbonylic), 1580 (N=O), 644 (C–S–S); ¹H NMR (DMSO-d₆, 300 MHz, δ) 11.09 (s, 1H, OH carboxylic), 8.26–8.22 (d, 1H, (a) H of benzo-thiazole), 8.11–8.08 (d, 1H, (b) H of benzothiazole), 7.61–7.55 (t, 2H, (b) and (c) H of benzothiazole), 7.21–7.16 (dd, 2H, Ar.H of phenyl attached OCH₂COOH), 6.82–6.76 (dd, 2H, Ar.H of phenyl attached OCH₂COOH), 4.81 (s, 2H, CH₂). Anal. Calcd. For C₂₁H₁₇NO₅ (313.05 g/mol): C, 57.50; H, 3.54; N, 13.41. Found: C, 57.59; H, 3.61; N, 13.51.

2.9. 2-(4-Acetylchloridephenylazo)-benzothiazole (3Bₙ)

Compound (2B) (0.31 g, 0.001 mol) was refluxed with thionyl chloride (5 mL) in presence of 1 drop of DMF. Unreacted thionyl chloride was removed under reduced pressure to obtained oily acid chloride (3B). This compound is very active and sensitive to the moisture, for this reason is not possible to obtain their physical properties and spectral analysis and used without purification.

2.10. 2-(4-Substitutedacetylbenzoyloxy)-phenylazo]-benzothiazole (4Bₐ₋ₗₓ,ₜₓ)

To a stirred solution of various phenols (0.001 mol) in (5 mL) of dried pyridine, the compound (3B) in (5 mL) of dried pyridine was added dropwise at 0 °C. After the addition had been completed, the resulting mixture was stirred overnight at room temperature then poured into (50 mL) of 10% HCl solution. The precipitate was filtered, washed with solution of 10% NaHCO₃ and then with water several times, dried and recrystallized from ethanol. ¹H NMR (DMSO-d₆, 300 MHz, δ) (4Bₐ₋ₗₓ,ₜₓ) 8.28–8.24 (d, 1H, (a) H of benzo-thiazole), 8.09–8.06 (d, 1H, (d) H of benzothiazole), 7.52–7.48 (t, 2H, (b) and (c) H of benzothiazole), 6.99–6.95 (dd, 2H, Ar.H of phenyl attached OCH₃), 6.73–6.69 (dd, 2H, Ar.H of phenyl attached OCH₃), 1.89–1.85 (d, 2H, Ar.H of phenyl attached OCH₃), 0.94–0.91 (t, 3H, CH₃).

3. Results and discussion

3.1. Synthesis

The key intermediates involved in the synthesis of desired oxazoles (6Aₙ) are described in Scheme 1.

All the newly synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of their elemental analysis and spectral (FT-IR and ¹H NMR) data. The characterization data of all compounds are given in the experimental section.

The compound 1A was synthesized by the reaction of 4-methoxybenzoyl chloride with glycine according to Steiger’s procedure (Steiger, 1944; Schiketanz et al., 2002) to afford the corresponding hippuric acid 1A. The FT-IR spectrum of this product indicated the absence of absorption bands due to NH₂ group of glycine and the presence of N–H and C=O absorption bands at 3366 and 1625 cm⁻¹ respectively. Also the ¹H NMR spectrum of this compound showed a triplet at δ 8.65–8.63 ppm integrating for proton of the NH and a doublet at δ 3.86–3.84 ppm integrating for protons of the CH₃ group. The compound 1A was dehydrated to the respective azlactone 2A. The disappearance of triplet peak in ¹H NMR spectrum at δ 8.65–8.63 ppm in compound 1A, assigned to the N–H group.

I.H.R. Tomi et al.
and appearance a strong band in FT-IR spectrum at 1785 cm\(^{-1}\) of the carbonyl group in azlactones in compound 2A are good evidence for the structure given to the product. The azlactone 2A was then reacted with toluene under Friedel–Crafts reaction conditions using anhydrous aluminum chloride in the presence of excess reactant as a solvent. The FT-IR spectrum of this compound 3A showed two sharp absorption bands, the first appears at 1691 cm\(^{-1}\) and is attributed to carbonyl function of the ketone and the other, observed at 1634 cm\(^{-1}\), was assigned to a carbonyl stretching frequency corresponding to the amide carbonyl. Also the \(^1\)H NMR spectrum of this compound showed the peaks at \(\delta\) 8.70–8.59, 4.10–4.08, and 2.38 ppm. These peaks in \(^1\)H NMR spectrum and two carbonyl bands in FT-IR spectrum of compound 3A were utilized to confirm the structure of this compound. After workup, the intermediate 3A was then dehydrated in the presence of phosphorous oxychloride to afford the corresponding oxazole 4A. The mechanism of dehydration (Padmavathi et al., 2008) in presence of POCl\(_3\) is depicted in the following steps: (Scheme 2).

The disappearance of two carbonyl bands in FT-IR spectrum and appearance the singlet peak at \(\delta\) 7.60 ppm in \(^1\)H NMR spectrum that is assigned to the C–H of oxazole ring for compound 4A are the good prove to obtained this compound. The compound 4A was demethylated with anhydrous aluminum chloride.
in dry benzene to give the desired 2-(4-Methylphenyl)-5-(4-hydroxyphenyl)-1,3-oxazole (5A). The $^1$H NMR spectrum of compound 5A showed the hydroxyl group at $\delta$ 5.22 ppm. The compounds 6A$_4$, 6A$_6$ and 6A$_8$ were synthesized by alkylation reaction of compound 5A with $n$-bromo- or iodoalkane in dry acetone with presence of anhydrous potassium carbonate. Table 1 shows the physical properties, elemental analysis and FT-IR spectral data of these compounds. The disappearance of peak at $\delta$ 5.22 ppm in compound 5A, assigned to the hydroxyl group and appearance the peaks of alkyl groups (OCH$_2$, CH$_2$ and CH$_3$) in compound 6A$_4$ are good evidence for the structure given to the product.

The designated of benzothiazole derivatives were synthesized according to Scheme 3.

The azo compound 1B was prepared by coupling between diazonium salt of the 2-aminobenzothiazole with phenol. The FT-IR spectrum of this compound showed absorption broad peak at 3410–3183 cm$^{-1}$ due to the intermolecularly hydrogen bonded of hydroxyl group. The compound 2B was synthesized by alkylation reaction of compound 1B with 1-chloro acetic acid in dry acetone with presence of anhydrous potassium carbonate.

The sharp peak at 1724 cm$^{-1}$ in FT-IR spectrum and singlet peak at $\delta$ 11.09 ppm in $^1$H NMR spectrum of compound 2B are the good prove to formation the acid compound, this compound was treated with thionyl chloride in presence of DMF to afforded the corresponding acid chloride 3B, this compound is very sensitive to the moisture, for this cause is not possible to obtain their FT-IR and $^1$H NMR spectra. Finally, this compound 3B was condensed with one equivalent of various phenols in dry pyridine as solvent and acid acceptor to yield new compounds 4B$_{x,y,z}$. The physical properties, ele-

Table 1 The physical properties, elemental analysis and FT-IR spectral data of compounds 6A$_n$.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar mass g/mol</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
<th>Analysis C, H and N (%) found/(calcd.)</th>
<th>FT-IR characteristic bands ($\nu$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6A$_4$</td>
<td>307.16</td>
<td>139–140</td>
<td>74</td>
<td>C: 78.15 (78.24), H: 6.89 (6.94), N: 4.56 (4.62)</td>
<td>C–H aliph. 2926, 1642, 1251, 1035</td>
</tr>
<tr>
<td>6A$_7$</td>
<td>335.19</td>
<td>129–131</td>
<td>77</td>
<td>C: 78.77 (78.69), H: 7.51 (7.43), N: 4.18 (4.23)</td>
<td>C–H aliph. 2928, 1635, 1253, 1031</td>
</tr>
<tr>
<td>6A$_8$</td>
<td>363.22</td>
<td>123–125</td>
<td>81</td>
<td>C: 79.30 (79.39), H: 8.04 (7.98), N: 3.85 (3.93)</td>
<td>C–H aliph. 2924, 1639, 1257, 1039</td>
</tr>
</tbody>
</table>

Table 2 The physical properties, elemental analysis and FT-IR spectral data of compounds 4B$_{x,y,z}$.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar mass g/mol</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
<th>Analysis C, H and N (%) found/(calcd.)</th>
<th>FT-IR characteristic bands ($\nu$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4B$_{x}$, G = CN</td>
<td>414.08</td>
<td>168–169</td>
<td>65</td>
<td>C: 63.76 (63.81), H: 3.40 (3.46), N: 13.52 (13.44)</td>
<td>C–H arom. 3066, 1714, 2228</td>
</tr>
<tr>
<td>4B$_{y}$, G = NO$_2$</td>
<td>434.07</td>
<td>181–184</td>
<td>51</td>
<td>C: 58.06 (58.11), H: 3.25 (3.31), N: 12.90 (12.84)</td>
<td>C–H arom. 3061, 1719, 1491, 1351</td>
</tr>
<tr>
<td>4B$_{z}$, G = OCH$_3$</td>
<td>419.09</td>
<td>219–221</td>
<td>74</td>
<td>C: 63.00 (62.97), H: 4.09 (4.15), N: 10.02 (10.10)</td>
<td>C–H arom. 3059, 1720, 2933, 2861</td>
</tr>
</tbody>
</table>
mental analysis and FT-IR spectral data of all compounds (4B) are summarized in Table 2. In the FT-IR spectra of these esters, a band near 1714 cm⁻¹ was shown that is assigned the carbonyl of these esters. The ¹H NMR spectrum of compound 4B, for example of esters compounds showed the peak at δ 3.77 ppm for the hydrogen of lateral methoxy group, are good evidence for the structure given to the product.

3.2. Biological evaluation

All the compounds have been screened for both antibacterial and antifungal activities using cup-plate agar diffusion method (Barry, 1976) by measuring the inhibition zone in mm. Ofloxacin (50 μg/mL) was used as standard drug for antibacterial activity, and ketoconazole (50 μg/mL) as a standard drug for antifungal activity. The compounds were screened for antibacterial activity against Escherichia coli, Staphylococcus aureus and Pseudomonas aerugenosa in nutrient agar medium, and for antifungal activity against Aspergillus niger and Candida albicans in Sabouraud’s dextrose agar medium. These sterilized agar media were poured into Petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds (50 μg/mL) were placed serially in the cavities with the help of micropipette and allowed to diffusion for 1 h, DMSO was used as solvent for all compounds, and as control. These plates were incubated at 37 °C for 24 h and 28 °C for 48 h, for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured and percent inhibition of the compounds was calculated. The results are summarized in Table 3.

The benzothiazole derivatives 4Bx,y,z showed good antibacterial activity against E. coli, S. aureus and P. aerugenosa when compared with the oxazole derivatives 6An. The compounds 4By and 4Bz showed potent activity against E. coli and P. aerugenosa respectively, when compared with standard drug. Rest of the compounds showed moderate to good antibacterial activity. The oxazole derivatives 6An were also found to be effective against microorganisms at the same concentration. The compound 6A8 showed maximum antibacterial activity against P. aerugenosa, whereas compounds 6A4 and 6A8 showed the same inhibition against E. coli.

The pattern of result of the antifungal activity of the test compounds was the same from their antibacterial activity. Rest of the benzothiazole derivatives showed moderate to good antifungal activity, whereas the oxazole derivatives showed moderate to low antifungal activity against both A. niger and C. albicans. Thus, it is concluded from the screening results that benzothiazole derivatives were most effective against all microorganisms at a concentration of 50 μg/mL (Gajdos et al., 2009).

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