Isothiazole derivatives showed differential pharmacological activities such as anti-inflammatory (1, 2), antiviral (3, 4), analgesic and antibacterial (5) and immunomodulatory ones (6-8).

Our earlier investigations revealed that the presence of ñCH=N- (Schiff’s base) moiety was crucial for the pharmacological activity of isothiazole derivatives (9-11). Considering this fact we decided to use ethyl ester of 5-hydrazino-3-methyl-4-isothiazolecarboxylic acid and ethyl ester of N'=1-{4-aminophenyl}-ethylidenohydrazino]-3-methyl-4-isothiazolecarboxylic acid (9, 12), synthesized by us previously, for the synthesis of Schiff’s bases and hydrazones with sugars as carbonyl component. Substituted urea derivatives, as a consequence of reaction with appropriate isocyanates, were also obtained.

The isothiazole derivatives were subjected to evaluation of their immunological activities. More specifically, the compounds were tested for their ability to affect the humoral immune response to sheep red blood cells in vitro, the proliferative response of mouse splenocytes to concanavalin A and pokeweed mitogen, the immunotropic activities of the new isothiazole derivatives and potential application of the compounds in therapy are discussed.

Keywords: ethyl 5-hydrazino-3-methyl-4-isothiazolecarboxylate hydrazone derivatives, mice, humoral immune response, mitogens, TNF-α, IL-6

SYNTHESIS AND IMMUNOMODULATORY ACTIVITIES OF NEW 5-HYDRAZINO-3-METHYL-4-ISOTHIAZOLECARBOXYLIC ACID ETHYL ESTERS

URSZULA LIPNICKA1*, MARCIN MĄCZYŃSKI1, JOLANTA ARTYM1 and Michał ZIMECKI1

1 Wrocław Medical University, Faculty of Pharmacy, Department of Organic Chemistry, Grodzka 9, 50-137 Wrocław, Poland;
2 Institute of Immunology And Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland

Abstract: Several new derivatives of 5-hydrazino-3-methyl-4-isothiazolecarboxylic ethyl esters were synthesized. Using 4-aminacetophenone, the hydrazine group was transformed in position 5 in the hydrazone which reacted with the isocyanates, aldehydes and sugars. Thirteen newly synthesized compounds were tested for their ability to affect the immunological response in vitro in several rodent models. The immunoregulatory properties of the compounds were differential and dose-dependent. The strongest activity was exhibited by 5-{N'ñ[1-4]-4-[3-{(methoxyphenyl)-ureido]-phenylethyldene]-hydrazino]-3-methyl-4-isothiazolecarboxylic acid ethyl ester (compound 3a). The compound strongly inhibited the secondary, humoral immune response to sheep erythrocytes and the proliferative response of mouse splenocytes to concanavalin A and pokeweed mitogen. The immunotropic activities of the new isothiazole derivatives and potential application of the compounds in therapy are discussed.

EXPERIMENTAL

Immunology

Material and Methods

Animals

CBA mice, 12-weeks old, derived from the breeding centre in Ilkowice/Kraków, 2-month old Wistar rats were from the Animal Facility of The Medical University in Wrocław. The animals were fed a commercial pelleted food and water ad libitum. The local Ethics Committee approved the study.

Reagents and reference compounds

Sheep red blood cells (SRBC) were delivered by Wrocław University of Life and Environmental Sciences, Concanaevalin A (ConA), pokeweed mito-
Preparation of the compounds for the in vitro experiments

The preparations were initially dissolved in DMSO and subsequently in the culture medium. For a control, appropriate dilutions of DMSO were applied, corresponding to respective preparation concentrations.

Preparation of the compounds for the in vitro experiments

The secondary humoral immune response to SRBC
Mice were primed with 0.2 mL of 1% SRBC suspension, intraperitoneally. After 4 days, the splenocytes were isolated and a single cell suspension was prepared in a medium (referred later as the culture medium) consisting of RPMI 1640, supplemented with 10% fetal calf serum, glutamine, sodium pyruvate and antibiotics. The cells were incubated in 24-well culture plates (5◊10^6/mL/well) with addition of 50 µL of 0.005% SRBC. After 4 days, the number of antibody forming cell number (AFC) in the cultures was determined (13). The results are shown as the mean values of AFC number from 4 wells ± standard error, calculated per 10^6 viable cells.

The proliferative response of splenocytes to mitogens
Spleens were pressed through a plastic screen into a pre-cooled Hanks’ medium. Erythrocytes were lysed using 0.84% NH₄Cl, the lymphocytes were washed 3 times with Hanks’ medium and re-suspended in the culture medium. The cells were then distributed into 100 µL aliquots (2◊10^5 cells) in 96-flat-bottom well plates. ConA and PWM were used at a concentration of 2.5 µg/mL. Control cultures contained solvent (DMSO) at appropriate dilutions. After 3-day culture, cell proliferation was determined using MTT colorimetric method (14).

Induction of cytokines in the culture of rat peritoneal cells
Peritoneal cavities of rats were lavaged with 10 mL of Hanks’ medium, the cells were washed twice with Hanks’ medium, counted and re-suspended in the culture medium at a density of 5×10^6/mL. The cells were distributed to 24-well culture plates in 1 mL aliquots. The compounds were added at a concentration of 10 µg/mL and LPS at a dose of 5 µg/mL. After 24 h culture in a cell culture incubator the supernatants were harvested for cytokine determination.

Determination of tumor necrosis factor alpha and interleukin-6 activities
The activities of cytokines were measured by bioassays. TNF-α concentration was determined using a highly sensitive WEHI 164.13 clone (ATCC CRL 1751) (15). IL-6 production was determined using 7TD1 indicator cells (16).

Statistics
The results are presented as the mean values ± standard error (SE). The Levene’s and Brown-Forsyth’s tests were used for determination of the homogeneity of variance between groups. When the variance was homogenous, analysis of variance (One Way ANOVA) was applied, followed by post hoc comparison with the Tukey’s test to estimate the significance of the difference between groups.
Nonparametric data were evaluated by the Kruskal-Wallis’ analysis of variance, as indicated in the text. Significance was determined at p = 0.05. The statistical analysis was performed using STATISTICA 6.1 for Windows.

Chemistry
The melting points of all new compounds were measured in a Boetius hot stage (VEB Kombinat Negema Wägetechnik Rapido, Germany) and were uncorrected. Elemental analysis, carried out at the Microlaboratory of the Pharmacy Department, Wrocław Medical University, showed that all analytical results were in accord with the calculated ones within ± 0.5%.

The following equipment was employed: IR spectra – Specord M 80 apparatus, in KBr discs. 1H NMR spectra – 300 MHz Bruker (Germany). The chemical shifts are expressed in δ (ppm) using TMS (tetramethylsilane) as an internal standard, coupling constants are given in Hz. TLC was performed with Polygram SIL G/UV254 plates (Macherey-Nagel, Düren, Germany, using toluene : methanol (9:1, v/v) as a mobile phase. Detection of the compounds on the chromatograms was performed with iodine vapor.

General method of synthesis of Schiff’s bases (2a-c)
0.01 mole of compound 1 in 70 mL of anhydrous ethanol and 0.01 mole of respective aldehyde was warmed up for 4 h. The course of the reaction was controlled by TLC. Then, the reaction mixture was left for 12 h at room temperature.
General methods of synthesis of 3(a-d) derivatives

0.005 mole of compound 1 in 35 mL of anhydrous mixture CH₃COOC₂H₅ : DMF (1:1, v/v) and 0.0055 mole of respective isocyanate (RNCO) were heated under reflux for about 4 h. The mixture was cooled, the precipitate was filtered, washed with ethanol (2 mL) and crystallized from ethanol or EtOH/DMF.

General method of synthesis of hydrazones (5a-e)

Compound 4 (0.01 mole) was dissolved in 80 mL of ethanol, treated with a solution of sugar (0.01 mole in water) and few drops of glacial acetic acid. The reaction mixture was refluxed for 4 h. An excess of the solvent was removed under reduced pressure. The residue was triturated with 20 mL of ether, the solid product was filtered, dried and crystallized from ethanol.

Figure 1. Immunoregulatory effects of the compounds on the secondary humoral response of mouse splenocytes to SRBC. The compounds were added to the splenocyte cultures at 1 (A) and 10 (B) µg/mL. After four days the number of antibody-forming cells was determined.

Statistics: A: Control vs. DMSO NS; DMSO vs. levamisole NS; DMSO vs. CsA p = 0.0001; DMSO vs. 3a p = 0.0002; DMSO vs. 3b p = 0.0036; DMSO vs. 3c p = 0.0415; DMSO vs. 3d NS; DMSO vs. 2a p = 0.0001; DMSO vs. 2b p = 0.0001; DMSO vs. 2c p = 0.0001; DMSO vs. 2d p = 0.0001; DMSO vs. 5a NS; DMSO vs. 5b NS; DMSO vs. 5c p = 0.0036; DMSO vs. 5d NS; DMSO vs. 5e p = 0.0065 (ANOVA); B: Control vs. DMSO NS; DMSO vs. levamisole NS; DMSO vs. CsA p = 0.0002; DMSO vs. 3a p = 0.0466; DMSO vs. 3b NS; DMSO vs. 3c NS; DMSO vs. 3d NS; DMSO vs. 3e p = 0.0001; DMSO vs. 2a p = 0.0001; DMSO vs. 2b p = 0.0001; DMSO vs. 2c NS; DMSO vs. 2d p = 0.0001; DMSO vs. 5a NS; DMSO vs. 5b NS; DMSO vs. 5c NS; DMSO vs. 5d NS; DMSO vs. 5e NS (ANOVA). NS = not significant.
Figure 2. Effects of the compounds on the proliferative response of mouse splenocytes to concanavalin A. The compounds were added to the cultures at concentrations of 0.1 (A), 1 (B) and 10 (C) µg/mL. Statistics: A: all comparisons NS (ANOVA); B: DMSO vs. 3a \( p = 0.0001 \); other comparisons NS (ANOVA); C: Control vs. DMSO NS; DMSO vs. levamisole NS; DMSO vs. 3a \( p = 0.0000 \); DMSO vs. 3b NS; DMSO vs. 3c \( p = 0.0001 \); DMSO vs. 3d \( p = 0.0020 \); DMSO vs. 2a \( p = 0.0027 \); DMSO vs. 2b \( p = 0.0003 \); DMSO vs. 2c NS; DMSO vs. 2d \( p = 0.0010 \); DMSO vs. 5a NS; DMSO vs. 5b NS; DMSO vs. 5c NS; DMSO vs. 5d NS; DMSO vs. 5e \( p = 0.0179 \) (ANOVA). NS – not significant.
Figure 3. Effects of the compounds on the proliferative response of mouse splenocytes to pokeweed mitogen. The compounds were added to the cultures at concentrations of 0.1 (A), 1 (B) and 10 (C) µg/mL. Statistics: A: DMSO vs. 3a p = 0.0156; DMSO vs. 3c p = 0.0207; other comparisons NS (ANOVA); B: Control vs. DMSO NS; DMSO vs. levamisole NS; DMSO vs. 3a p = 0.0016; DMSO vs. 3b p = 0.0180; DMSO vs. 3c p = 0.0198; DMSO vs. 3d NS; DMSO vs. 2a NS; DMSO vs. 2b NS; DMSO vs. 2c NS; DMSO vs. 2d NS; DMSO vs. 5a NS; DMSO vs. 5b NS; DMSO vs. 5c p = 0.0237; DMSO vs. 5d NS; DMSO vs. 5e p = 0.0301 (ANOVA); C: Control vs. DMSO NS; DMSO vs. levamisole NS; DMSO vs. 3a p = 0.0000; DMSO vs. 3b p = 0.0120; DMSO vs. 3c p = 0.0008; DMSO vs. 3d p = 0.0100; DMSO vs. 2a NS; DMSO vs. 2b p = 0.0090; DMSO vs. 2c NS; DMSO vs. 2d NS; DMSO vs. 5a NS; DMSO vs. 5b NS; DMSO vs. 5c NS; DMSO vs. 5d NS; DMSO vs. 5e p = 0.0310 (ANOVA). NS = not significant.
RESULTS AND DISCUSSION

Immunology

Effects of the compounds on the secondary humoral immune response in vitro to SRBC

The compounds, at concentrations of 0.1 and 10 µg/mL, were tested for their potential immunoregulatory activity in the in vitro model of the secondary humoral immune response to SRBC. The results (Figure 1A) revealed differential effects of the compounds. Compounds 3a-3d and 5a-e were moderately inhibitory whereas 2a-2d were stimulatory at 1 µg/mL. The inhibitory effects of 3a, 3b, 3c, 5c and 5e were statistically significant, similarly as the stimulatory effects of compounds 2a, 2b, 2c and 2d. At a dose of 10 µg/mL (Figure 1B), 3a was moderately inhibitory, on the other hand, 2a, 2b and 2d exhibited remarkable stimulatory activities (3.5, 5.0 and 7.9-fold stimulation over the control response, respectively). Whereas CsA exhibited expected strong inhibitory action, levamisole did not elevate the immune response.
Table 1. Analytical data for the obtained compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula – molecular weight (g/mol)</th>
<th>Elemental analysis Calculated/Found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{12}H_{22}N_{2}O_{5}S – 407</td>
<td>C%      H%      N%</td>
</tr>
<tr>
<td>2a</td>
<td>61.91/62.05</td>
<td>5.15/5.40 17.19/16.82</td>
</tr>
<tr>
<td>2b</td>
<td>C_{12}H_{23}ClN_{2}O_{5}S – 440.5</td>
<td>59.93/59.21 4.76/5.02 12.71/12.30</td>
</tr>
<tr>
<td>2c</td>
<td>C_{12}H_{23}N_{2}O_{5}S – 422</td>
<td>62.55/61.98 5.21/5.15 13.27/13.70</td>
</tr>
<tr>
<td>2d</td>
<td>C_{12}H_{23}N_{2}O_{5}S – 406</td>
<td>65.02/65.50 5.14/4.98 13.79/13.55</td>
</tr>
<tr>
<td>3a</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 469</td>
<td>59.10/58.67 5.35/5.63 14.99/14.80</td>
</tr>
<tr>
<td>3b</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 437</td>
<td>60.41/61.07 5.26/5.50 16.02/16.39</td>
</tr>
<tr>
<td>3c</td>
<td>C_{12}H_{23}N_{2}O_{5}S – 504.97</td>
<td>54.65/54.12 4.36/4.63 13.86/14.01</td>
</tr>
<tr>
<td>3d</td>
<td>C_{12}H_{23}ClN_{2}O_{5}S – 423.5</td>
<td>51.01/50.95 5.19/5.35 16.53/16.28</td>
</tr>
<tr>
<td>3a</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 363</td>
<td>42.98/42.65 5.78/5.52 11.57/11.98</td>
</tr>
<tr>
<td>3b</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 363</td>
<td>42.98/42.59 5.78/5.63 11.57/12.00</td>
</tr>
<tr>
<td>3c</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 363</td>
<td>42.98/43.02 5.78/5.87 11.57/11.42</td>
</tr>
<tr>
<td>3d</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 363</td>
<td>42.98/42.49 5.78/5.98 11.57/11.77</td>
</tr>
<tr>
<td>3e</td>
<td>C_{12}H_{22}N_{2}O_{5}S – 333</td>
<td>43.24/43.56 5.71/5.84 12.61/12.60</td>
</tr>
</tbody>
</table>

Effects of the compounds on the proliferative response of mouse splenocytes to mitogens

In the next step we analyzed the effects of the compounds on the proliferative response of mouse splenocytes to concanavalin A (ConA), a T-cell mitogen, and pokeweed mitogen (PWM), a B-cell mitogen (Figures 2A-C). It appeared that the inhibitory actions of the compounds were strongly dose-dependent. Whereas no effects on the proliferative response to ConA were observed at 0.1 µg/mL concentration, at 1 µg/mL dose only 3a compound was significantly inhibitory. However, at 10 µg/mL dose, a statistically significant inhibitory activities were registered for compounds 3a, 3c, 3d, 3b, and 3e. An exceptionally strong suppression of proliferation was noted in cases of compounds 3a and 3c (Figure 2A-C).

A similar but not identical activity pattern was observed for the effects on the proliferative response of splenocytes to PWM (Figure 3A-C). The inhibition was also distinctly dose-dependent. Interestingly, at 0.1 µg/mL dose, a small, stimulatory effect was seen for compounds 3b and 3c. At 1 µg/mL dose, four compounds demonstrated small inhibitory activity (3a, 3b, 5b and 5d), whereas at 10 µg/mL a very strong suppression of the proliferative response was found for compounds 3a and 3c, a moderate for 2b and 3d and a negligible one for 3b and 5e. Levamisole was without any effect on the proliferative response of splenocytes induced by ConA or PWM.

Effects of the compounds on lipopolysaccharide-induced production of TNF-alpha and interleukin-6 by rat peritoneal cells

For these assays we selected several active compounds and, for control, compound 5b which was inactive in the previously described models. As a reference, drug leflunomide was chosen. The compounds were applied at concentrations of 10 µg/mL. The results (Figure 4) showed that among the studied compounds only 3c expressed significant stimulatory action on TNF-alpha production. Leflunomide, at this dose, was not suppressive.

On the other hand, compounds 3a and 3e were to some degree inhibitory with regard to LPS-induced IL-6 production (Figure 5) although these effects were not statistically significant. The suppressive action of leflunomide was marked but not statistically significant.

In summary, several compounds exhibited very interesting, strong, directional actions, which are of potential therapeutic value. Compound 3a is a hydrazone and represents a substituted derivative of urea. The residue at the urea nitrogen atom is a phenyl, substituted at position 4 with methoxy group, containing two free electron pairs at the oxygen atom. Compound 3a, at higher doses, demonstrated very strong, antiproliferative actions against T and B cells. That property was correlated with its ability to inhibit the humoral immune response in vitro which was not, however, so profound as in the
Scheme 1.
case of CsA, suggesting a lower toxicity of 3a in comparison to CsA. Until now we have not found such a strong, antiproliferative action among other, strongly suppressive synthetic compounds, for example isoxazoles (17). Compound 3c, similarly as compound 2, also contains a phenyl group at the urea nitrogen atom, substituted at position 3 with electronegative trifluoromethyl group. This compound, characterized by the ability to strongly stimulate TNF-alpha production, could be of potential value in the search for new anticancer drugs. On the other hand, compound 3d has a chloroethyl group at the urea nitrogen atom, where the carbon atom is characterized by sp3 hybridization. That structural configuration would be probably the cause of low activity of compound 3 since, as we demonstrated previously (11), the pharmacological activity can be only ensured by existence of sp2 carbon hybridization. Lastly, compound 2d, the exceptionally strong stimulator of the immune response, may be of potential interest in treatment of immunodeficiencies or in immunocompromised patients, as recently suggested for an isoxazole derivative RM-11 (18).

In conclusion, the isoxazole system may serve as a rich source of new compounds with differential properties in the immunological tests and potential therapeutic value.

Chemistry

The starting materials 5-hydrazino-3-methyl-4-isothiazolecarboxylic acid ethyl esters 4 were synthesized by treating 5-chloro-3-methyl-4-isothiazolecarboxylic acid ethyl ester with hydrazine hydrate (12) (Scheme 1). Compound 4 was treated with p-aminoacetophenone in the presence of acetic acid (6) (Scheme 1). Compound 5-{Ní-[1-(2-chlorophenyl)-methylidene]-hydrazino}-3-methyl-4-isothiazolecarboxylic acid ethyl ester (2a)

M.p. 206-207°C, yield 78.13%. IR (KBr, cm-1): 1700 (>C=O), 1600 (Ar), 1550 (C≡N), 1260 (-C=CH2). 1H NMR (DMSO, δ ppm): 1.1-1.3 (t, J = 7.5 Hz, 3H, CH2-CH3), 2.23-2.40 (s, 6H, CH2-C≡N), 4.23-4.33 (q, J = 7.5 Hz, 2H, CH2-CH2), 7.38-7.42 (d, J = 8.3 Hz, 2H, Ar), 7.48-7.51 (d, J = 8.5 Hz, 2H, C5H5-N), 7.79-7.81 (d, J = 8.3 Hz, 2H, Ar), 8.7-8.76 (d, J = 8.5 Hz, 2H, C1H1-N), 8.82 (s, 1H, CH≡N), 10.05 (s, 1H, NH-N=).

5-{N′-[1-(4-[[1-(4-pyridin-4-yl)-methylidene]-amino}phenyl]-ethylidene]-hydrazino)-3-methyl-4-isothiazolecarboxylic acid ethyl ester (2b)

M.p. 176-177°C, yield 60.87%. IR (EtOH/DMF), δ ppm: 1.31-1.37 (t, J = 7.5 Hz, 3H, CH2-CH3), 2.23-2.40 (s, 3H, CH2-C≡N), 3.28 (s, 3H, CH3-C≡N), 4.26-4.31 (q, J = 7.5 Hz, 2H, CH2-CH2), 6.58-7.25 (m, 4H, C4H4-C≡Cl), 7.48-7.53 (d, J = 8.3 Hz, 2H, Ar); 7.8-7.87 (d, J = 8.3 Hz, 2H, Ar), 8.88 (s, 1H, CH≡N), 10.05 (s, 1H, NH-N=).

5-{N′-[1-(4-[[1-(2-chlorophenyl)-methylidene]- amino}phenyl]-ethylidene]-hydrazino)-3-methyl-4-isothiazolecarboxylic acid ethyl ester (2c)

M.p. 177-178.5°C, yield 60.87%. IR (KBr, cm-1): 3100-3250 (-OH), 1695 (C=O), 1610 (Ar), 1550 (C≡N), 1260 (-C=CH2). 1H NMR (DMSO, δ ppm): 1.29-1.33 (t, J = 7.5 Hz, 3H, CH2-CH3), 2.36 (s, 3H, CH3-C≡N), 3.3 (s, 3H, CH3-C≡N), 4.2-4.33 (q, J = 7.5 Hz, 2H, CH2-CH2), 6.84-6.89 (m, 4H, Ar), 7.2-7.64 (dd, J = 8.8 Hz, 4H, C4H4-CH2=), 8.73 (s, 1H, CH≡N); 10.05 (s, 1H, NH-N=), 11.2 (s, 1H, ArOH).

5-{N′-[1-(4-[[benzylideneaminophenyl]-ethylidene]- hydrazino)-3-methyl-4-isothiazolecarboxylic acid ethyl ester (2d)

M.p. 174-175°C, yield 62.8%. IR (KBr, cm-1): 1600 (C=O), 1600 (Ar), 1550 (C≡N), 1255 (-C=CH2).
5-N-[1-{4-[3-(4-methoxyphenyl)-ureido]-phenyl}-ethylidene]-hydrazino]-3-methyl-4-isothiazolecarboxylic acid ethyl ester (3a)

M.p. 205-206°C, yield 89.4% IR (KBr, cm⁻¹): 3280 (CONH), 3250-2950 (CH), 1720 (COOC₂H₅), 1670 (CONH), 1550 (C=O), 1160 (C-O). ¹H NMR (DMSO, δ ppm): 1.32-1.37 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.26-2.48 (s, 6H, CH₂-C=O), 3.33 (s, 3H, CH₂-O-), 4.25-4.32 (q, J = 7.5 Hz, 2H, -CH₂-CH₂-), 6.95-6.99 (d, J = 8.3 Hz, 2H, Ar), 7.41-7.46 (d, J = 8.3 Hz, 2H, Ar), 7.67-7.72 (d, J = 8.4 Hz, 2H, Ar); 8.7, 8.9 (s, 1H, -NH-CONH-), 10.12 (s, 1H, NH-N=).

5-N-[1-{4-[3-(phenylureido)-phenyl}-ethylidene]-hydrazino]-3-methyl-4-isothiazolecarboxylic acid ethyl ester (3b)

M.p. 257-258°C, yield 81.7% (DMF). IR (KBr, cm⁻¹): 3290 (CONH), 1730 (COOC₂H₅), 1670 (CONH), 1570 and 1600 (Ar), 1520 (C=O). ¹H NMR (DMSO, δ ppm): 1.33-1.37 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.31-2.43 (s, 6H, CH₂C=O), 4.23-4.28 (q, J = 7.5 Hz, 2H, -CH₂-CH₂-), 7.49-7.52, 7.7-7.79 (dd, J = 8.3 Hz, 4H, Ar), 8.3, 8.5 (s, 2H, -NH-CONH-); 10.16 (s, 1H, NH-N=).

5-N-[1-{4-[3-(trifluoromethylphenyl)-ureido]-phenyl}-ethylidene]-hydrazino]-3-methyl-4-isothiazolecarboxylic acid ethyl ester (3c)

M.p. 265-266°C, yield 50.1%. IR (KBr, cm⁻¹): 3300 (CONH), 1720 (COOC₂H₅), 1670 (CONH), 1580 (Ar), 1550 (C=O). ¹H NMR (DMSO, δ ppm): 1.32-1.37 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.32 (s, 6H, CH₂-C=O), 4.27-4.34 (q, J = 7.5 Hz, 2H, -CH₂-CH₂-), 6.9-7.1 (m, 3H, Ar), 7.64-7.67 (d, J = 8.3 Hz, 2H, Ar), 7.72-7.75 (d, J = 8.3 Hz, 2H, Ar); 8.0 (s, 1H, Ar); 9.0-9.1 (s, 2H, NHCONH-), 10.1 (s, 1H, NH-N=).

5-N-[1-{4-[3-(2-chloroethyl)-ureido]-phenyl}-ethylidene]-hydrazino]-3-methyl-4-isothiazolecarboxylic acid ethyl ester (3d)

M.p. 218-219°C, yield 38.3%. IR (KBr, cm⁻¹): 3320 (CONH), 3000-2950 (C-H), 1725 (COOC₂H₅), 1680 (CONH), 1590 (Ar), 1550 (C=O). ¹H NMR (DMSO, δ ppm): 1.31-1.35 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.35 (s, 6H, CH₂-C=O); 4.27-4.31 (q, J = 7.5 Hz, 2H, -CH₂-CH₂-), 6.3-6.7 (m, 4H, -CH₂-CH₂-), 7.58-7.62 (d, J = 8.35 Hz, 2H, Ar), 7.78-7.80 (d, J = 8.35 Hz, 2H, Ar), 8.5, 8.8 (s, 2H, NHCONH-), 10.1 9.9 (s, 1H, NH-N=).

3-Methyl-5-[N'-2-(1,3,4,5,6-pentahydroxyhexyli-dene)-hydrazino]-4-isothiazolecarboxylic acid ethyl ester (5a) with fructose

M.p. 196-198°C, yield 56.3%. IR (KBr, cm⁻¹): 3450-3330 (OH), 3230 (NH), 2950 (CH), 1720 (C=O), 1550 (C=N), 1160 (C-O). ¹H NMR (DMSO, δ ppm): 1.31-1.35 (t, 3H, CH₃-CH₂-), 2.35 (s, 3H, CH₂-C=O), 4.27-4.31 (q, J = 7.5 Hz, 2H, -CH₂-CH₂-), 3.78 (m, 3H, 5'H, 2 × 6'H), 3.92 (s, 2H, 2 × 1'H), 4.2-4.6 (m, 7H, CHOH and -CH₂-CH₂-), 8.8 (s, 1H, NH).

3-Methyl-5-[N'-2-(1,3,4,5,6-pentahydroxyhexyli-dene)-hydrazino]-4-isothiazolecarboxylic acid ethyl ester (with galactose 5b)

M.p. 237-239°C, yield 49.6%. IR (KBr, cm⁻¹): 3450-3320 (OH), 3230 (NH), 2920 (CH), 1725 (C=O), 1560 (C=O), 1120 (C-O). ¹H NMR (DMSO, δ ppm): 1.33-1.35 (t, 3H, CH₃-CH₂-), 2.35 (s, 3H, CH₂-C=O), 3.75 (m, 3H, 5'H, 2 × 6'H), 4.1-4.6 (m, 10H, CHOH and CH₂-CH₂-), 5.2 (d, J = 6 Hz, 1H, -CH₂-); 8.7 (s, 1H, NH).

3-Methyl-5-[N'-2-(1,3,4,5,6-pentahydroxyhexyli-dene)-hydrazino]-4-isothiazolecarboxylic acid ethyl ester (with glucose 5c)

M.p. 208-209°C, yield 45.5%. IR (KBr, cm⁻¹): 3550-3360 (OH), 3200-3160 (NH), 2930 (CH), 1725 (C=O), 1210 (C-O). ¹H NMR (DMSO, δ ppm): 1.3 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.4 (s, 3H, CH₂-C=O), 3.73 (m, 3H, 5'H, 2 × 6'H), 3.9-4.6 (m, 10H, -CHOH and CH₂CH₂-), 5.4 (d, J = 6 Hz, 1H, -CH₂-), 8.5 (s, 1H, NH).

3-Methyl-5-[N'-2-(1,3,4,5,6-pentahydroxyhexyli-dene)-hydrazino]-4-isothiazolecarboxylic acid ethyl ester (with mannose 5d)

M.p. 210-211°C, yield 42.3%. IR (KBr, cm⁻¹): 3500-3400 (OH), 3180-3200 (NH), 2930 (CH), 1720 (C=O), 1215 (C-O). ¹H NMR (DMSO, δ ppm): 1.29 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.35 (s, 3H, CH₂-C=O), 3.7 (m, 3H, 5'H, 2 × 6'H), 4.0-4.55 (m, 10H, -CHOH and CH₂-CH₂-), 5.4 (d, J = 6 Hz, 1H, -CH₂-), 8.6 (s, 1H, NH).

3-Methyl-5-[N'-2-(1,3,4,5,6-pentahydroxyhexyli-dene)-hydrazino]-4-isothiazolecarboxylic acid ethyl ester (with ribose 5e)

M.p. 168-170°C, yield 60.3%. IR (KBr, cm⁻¹): 3350-3500 (OH), 3200 (NH), 2960
(CH), 1720 (C=O), 1550 (C=N). 'H NMR (DMSO, δ ppm): 1.35 (t, J = 7.5 Hz, 3H, CH₃-CH₂-), 2.42 (s, 3H, CH₃=C=N), 3.65 (m, 3H, -CHOH), 4.2-4.6 (m, 6H, CH₂-CH₃ and 4-OH); 5.4 (d, J = 6 Hz, 1H, -CH₂-); 8.8 (s, 1H, NH).

REFERENCES


Received: 07. 01. 2009